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(54) METHODS OF IDENTIFYING TARGET POLYPEPTIDES

(76) Inventors: **David C. Anderson**, San Bruno, CA (US); **Mark K. Bennett**, Moraga, CA (US); **Todd M. Kinsella**, Fayetteville, NC (US); **Esteban Masuda**, Menlo Park, CA (US)

Correspondence Address:
DORSEY & WHITNEY LLP
INTELLECTUAL PROPERTY DEPARTMENT
4 EMBARCADERO CENTER
SUITE 3400
SAN FRANCISCO, CA 94111 (US)

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(57) **ABSTRACT**

The present invention provides methods for identifying and/or rank ordering target polypeptides that interact with a candidate polypeptide. Potential targets for the candidate polypeptide are identified using an interaction assay. A set of mutants are derived from the candidate polypeptide and a functional profile is obtained for the set of mutants and the candidate polypeptide. Also, for each identified potential target, an interaction profile is obtained for the set of mutants and the candidate polypeptide. The interaction profiles are compared to the functional profile and based on the degree of correspondence between the profiles, the potential targets are identified and/or rank ordered as target polypeptides that interact to the candidate polypeptide.

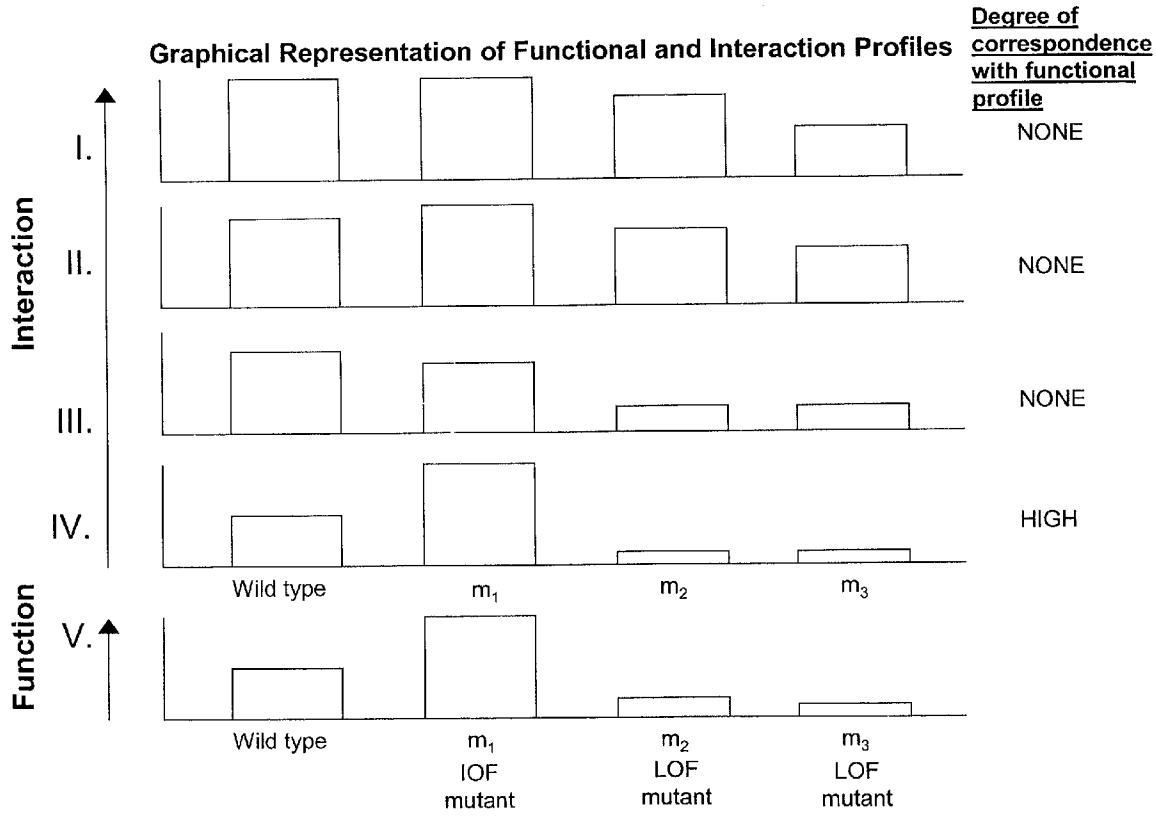


FIG. 1

Graphical Representation of Functional and Interaction Profiles

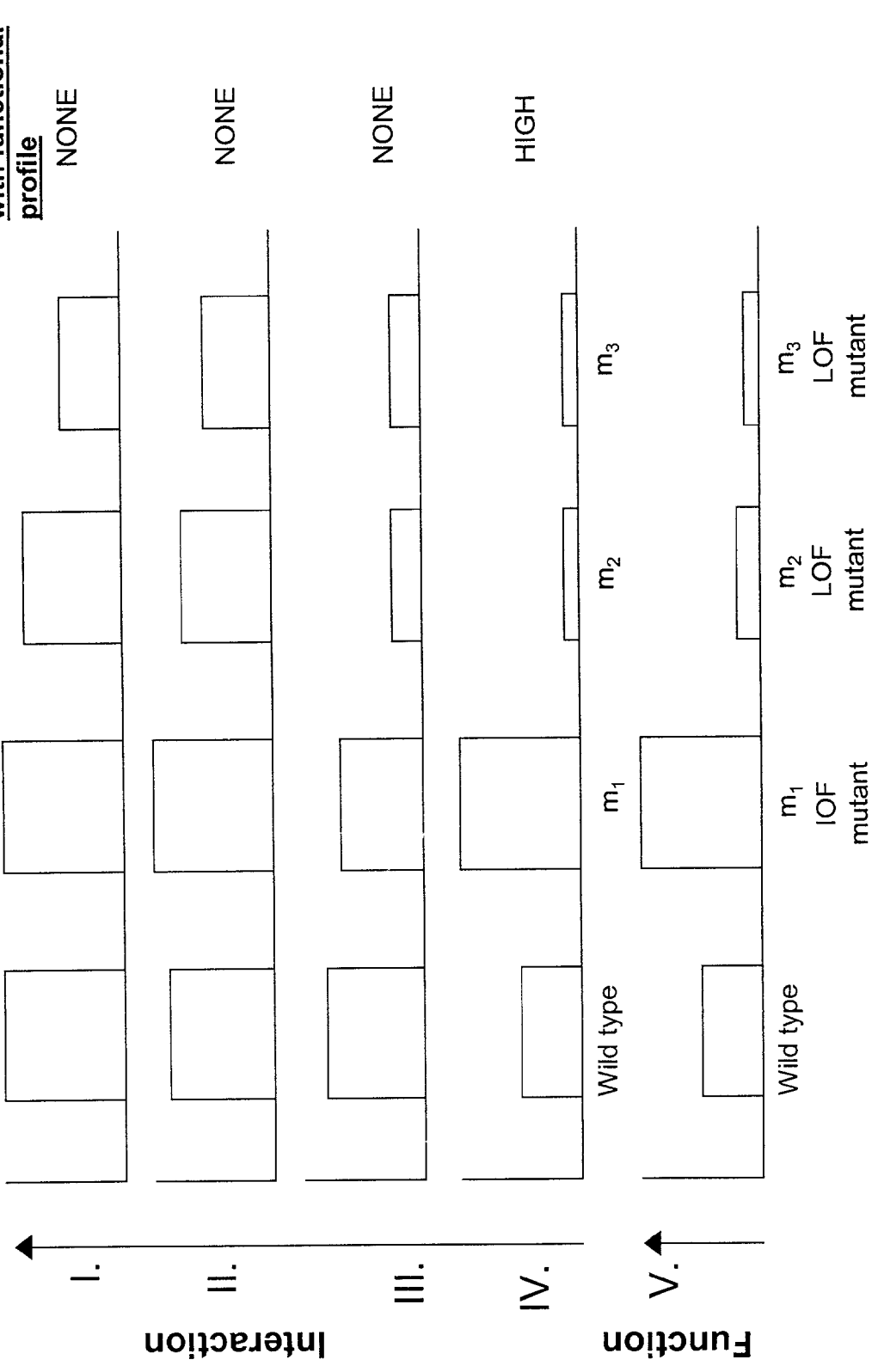


FIG. 2

Categorical Representation of Functional and Interaction Profiles

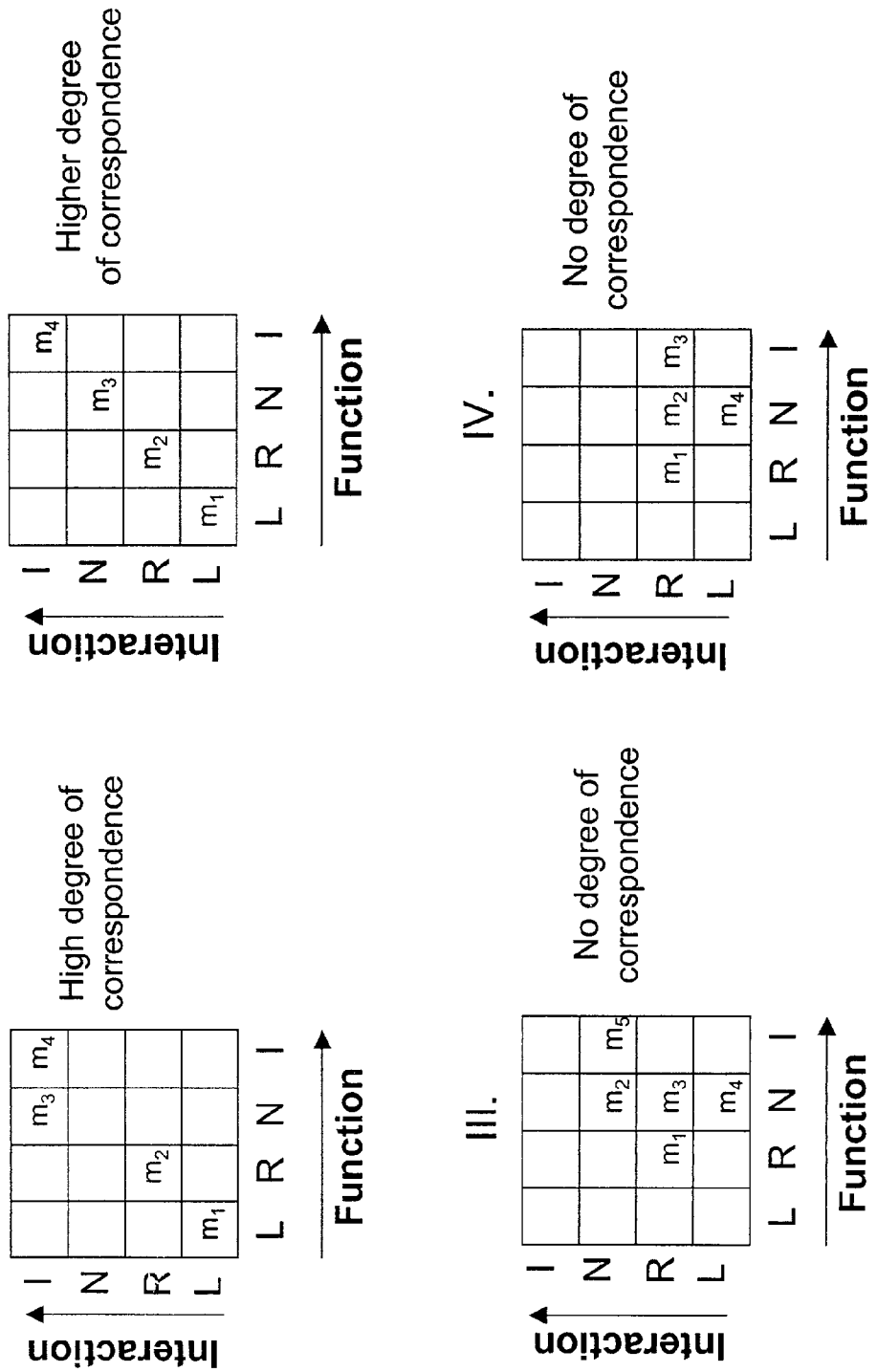


FIG. 3A

Alanine Mutagenesis

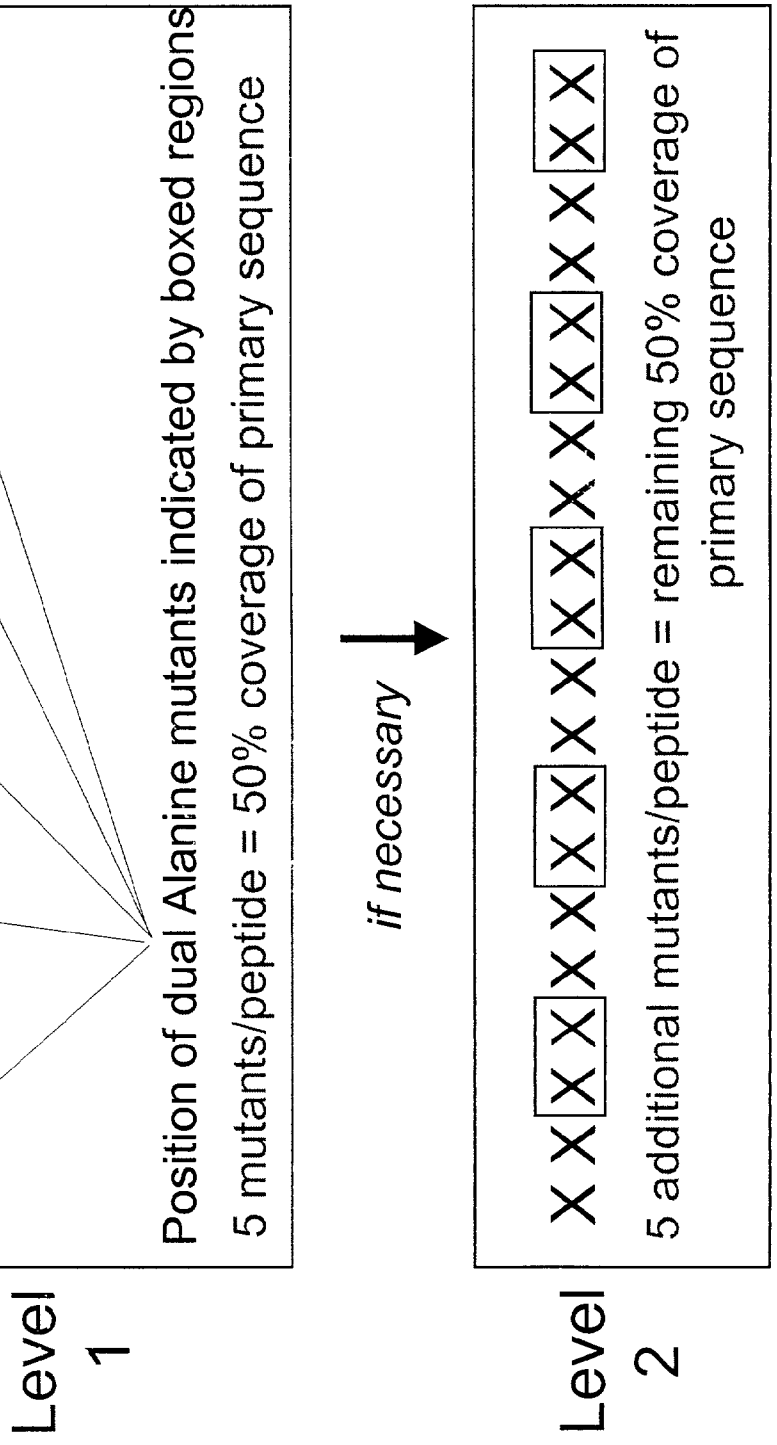


FIG. 3B
Distribution of Alanine Mutations

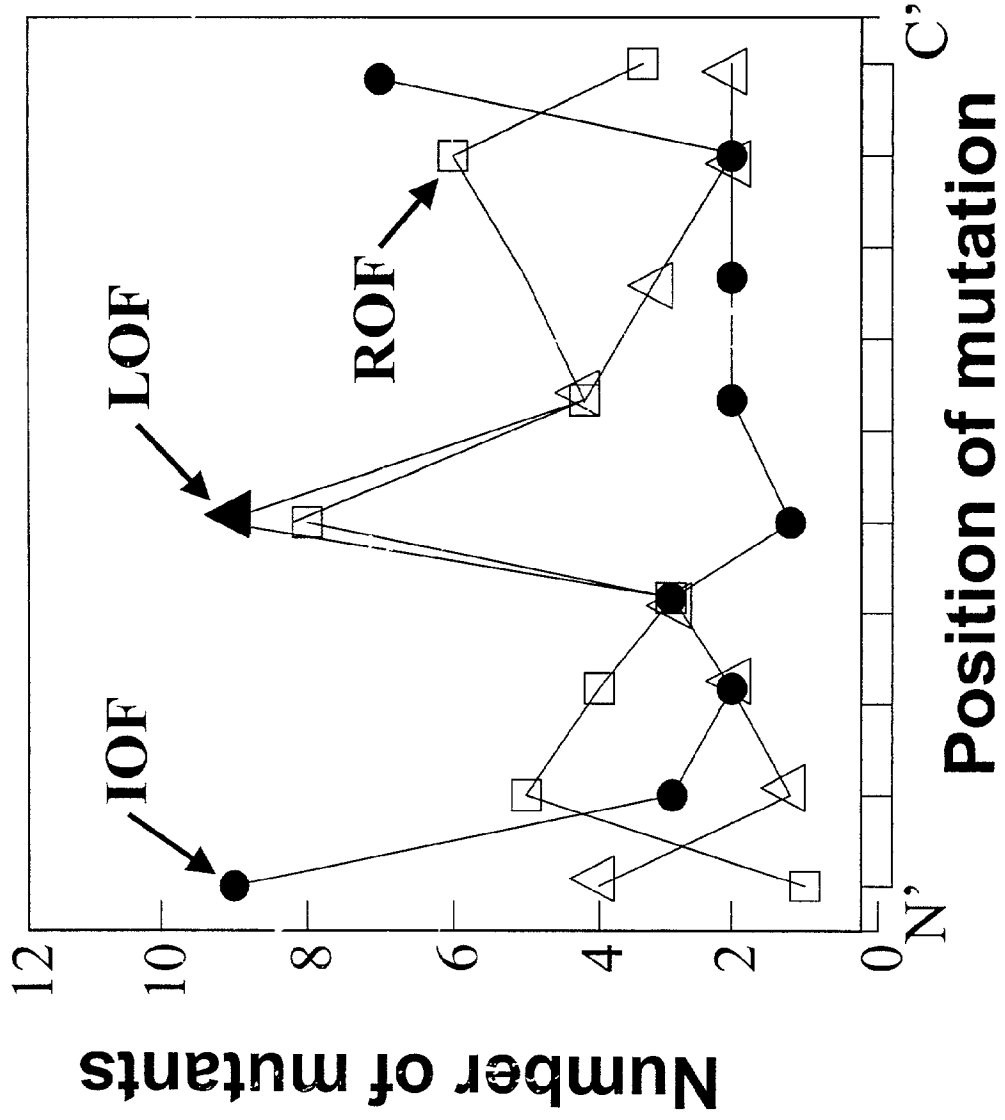


FIG. 4

Categorization of Mutants: Correlation Along the Diagonal

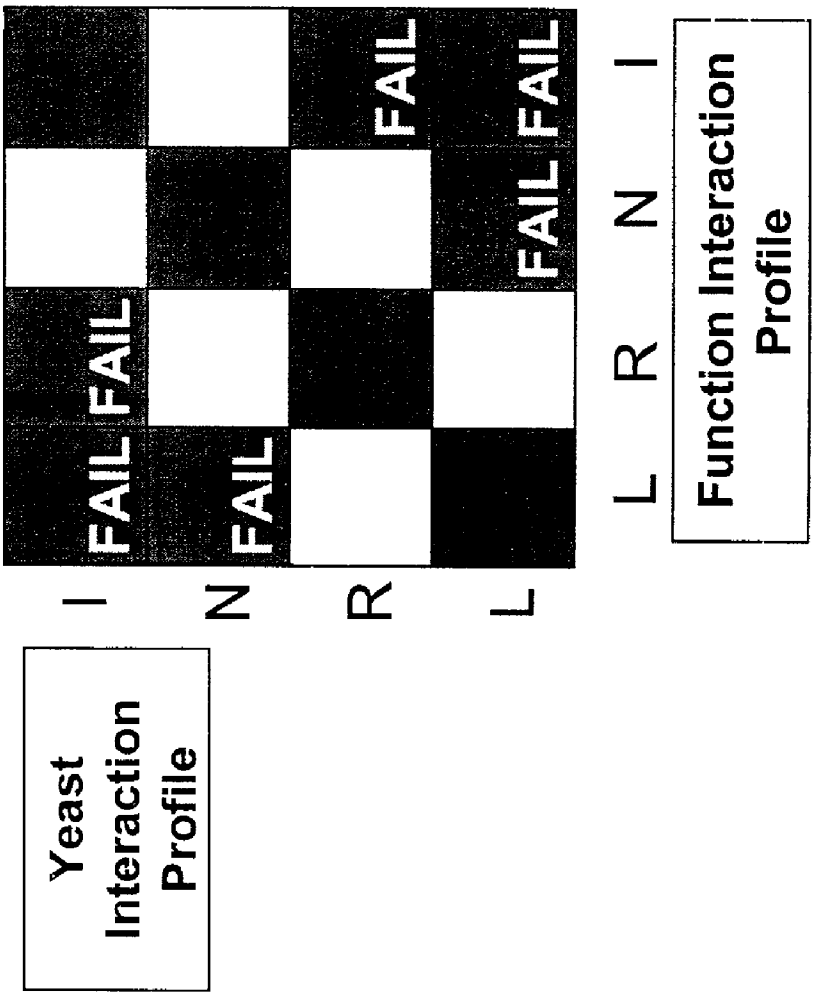


FIG. 5

Interaction Profile: Selection Criteria

A: Reduction of Interaction (ROI)	**B:** Loss of Interaction (LOI)	**C:**Increase of Interaction (IOI)	**D:** Neutral (N)
Statistical reduction (p value)	Statistical reduction (p value) **AND** greater than 50% reduction	Increase in affinity of 20% **OR** statistical increase over wild type	Change in affinity less than or equal to 20% **OR** statistically insignificant change over wild type
Restrictions:	Must not have a B that fails unless countered by 2 other B passes	Must not have an A that fails unless countered by 2 passes of A, B, or C	Must not have both an A and a B that fails
Exceptions:	Can have a C that fails as long as 2 pass A or B and no A or B fail	Can have a D that fails as long as 2 pass A, B, or C	
Failure is defined as a one-step shift along the linear progression from B to A to D to C; a two-step shift (i.e. from B to D) eliminates a candidate from consideration			
When more than one YTH cDNA displays a passing mutant profile, hits are ranked by best profile and bioinformatic analysis			

FIG. 6

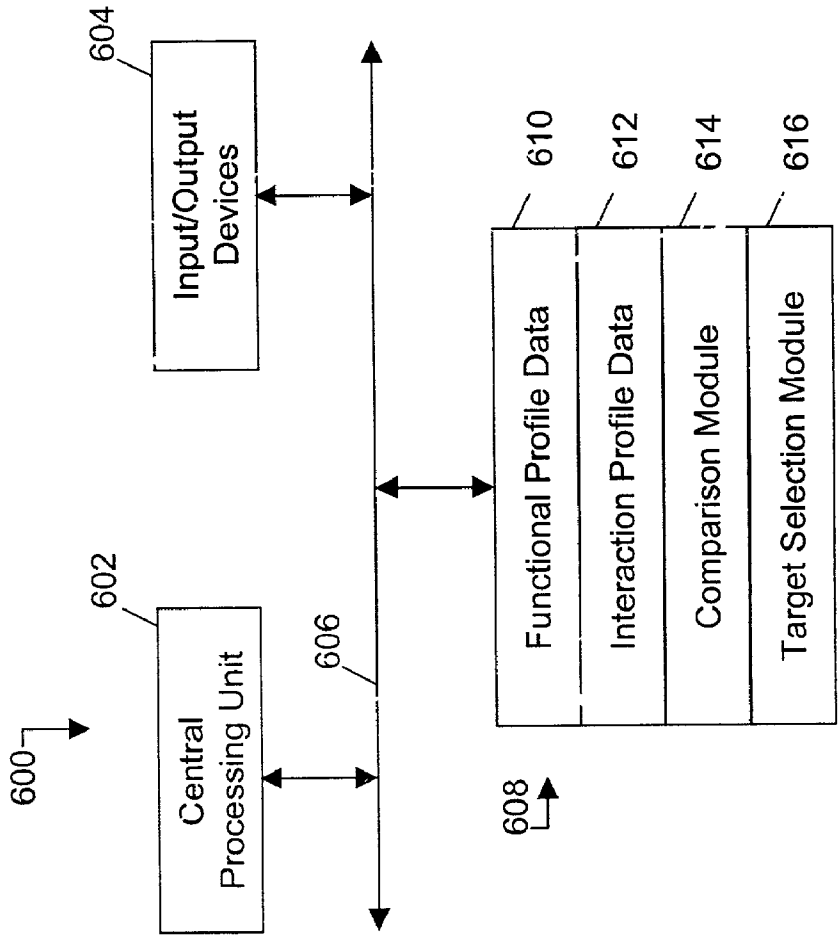

600
602
Central Processing Unit
604
Input/Output Devices
606
608
610
Functional Profile Data
612
Interaction Profile Data
614
Comparison Module
616
Target Selection Module

FIG. 7

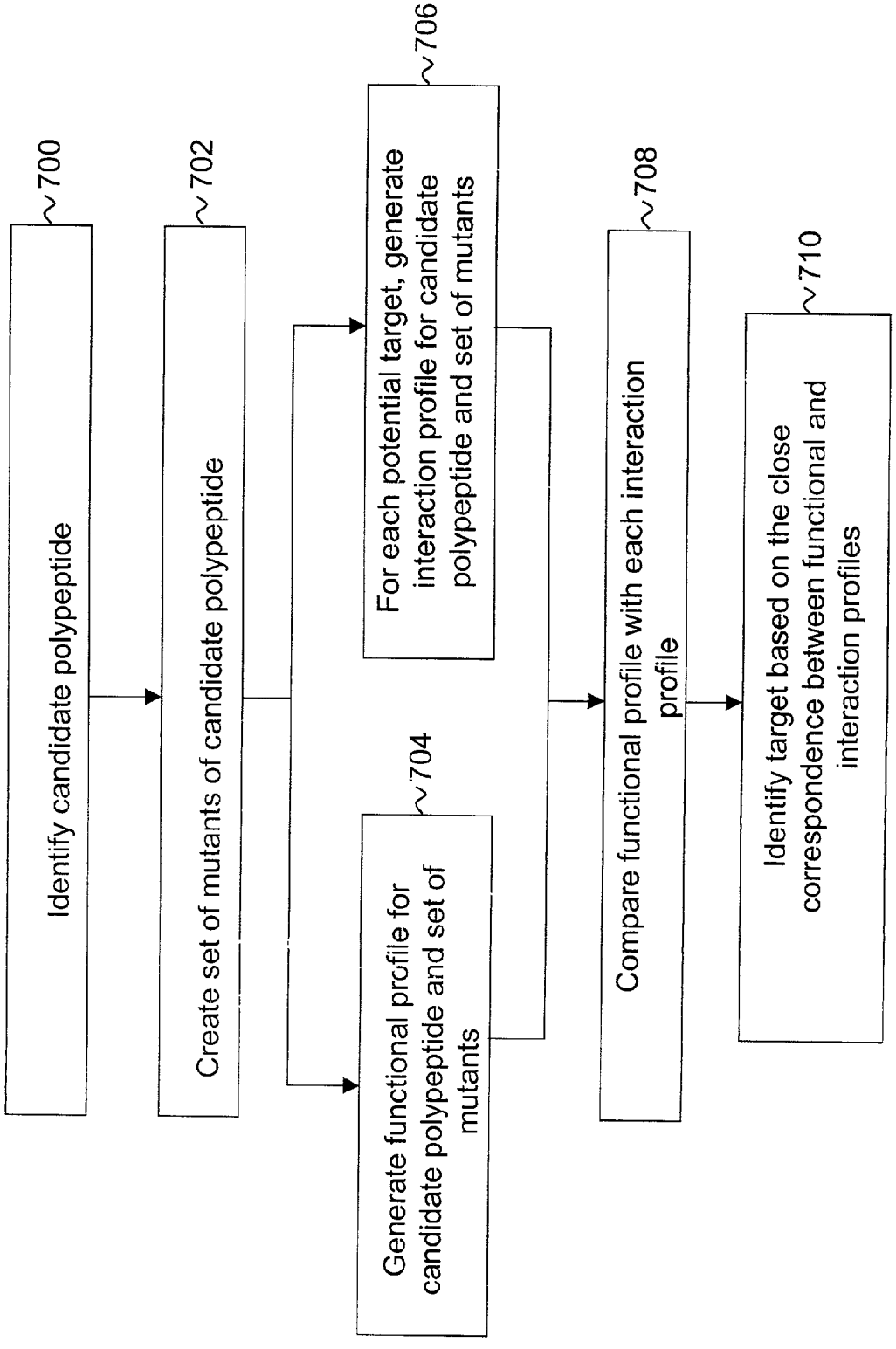
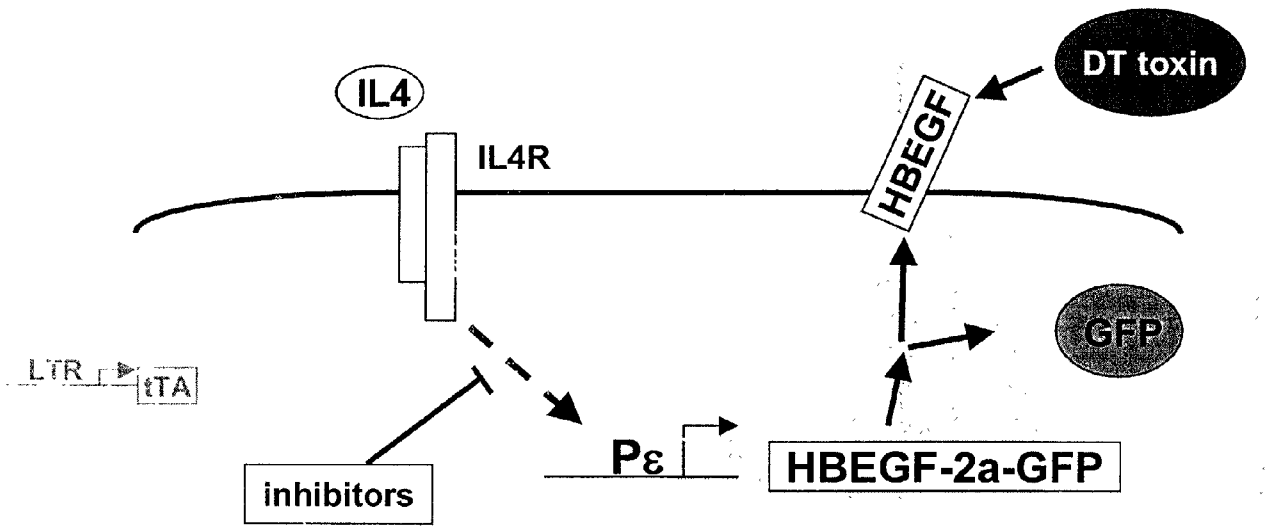

700
Identify candidate polypeptide
702
Create set of mutants of candidate polypeptide
704
Generate functional profile for candidate polypeptide and set of mutants
706
For each potential target, generate interaction profile for candidate polypeptide and set of mutants
708
Compare functional profile with each interaction profile
710
Identify target based on the close correspondence between functional and interaction profiles

FIG. 8A

The DT Toxin Selection of the A5T4 Reporter Line



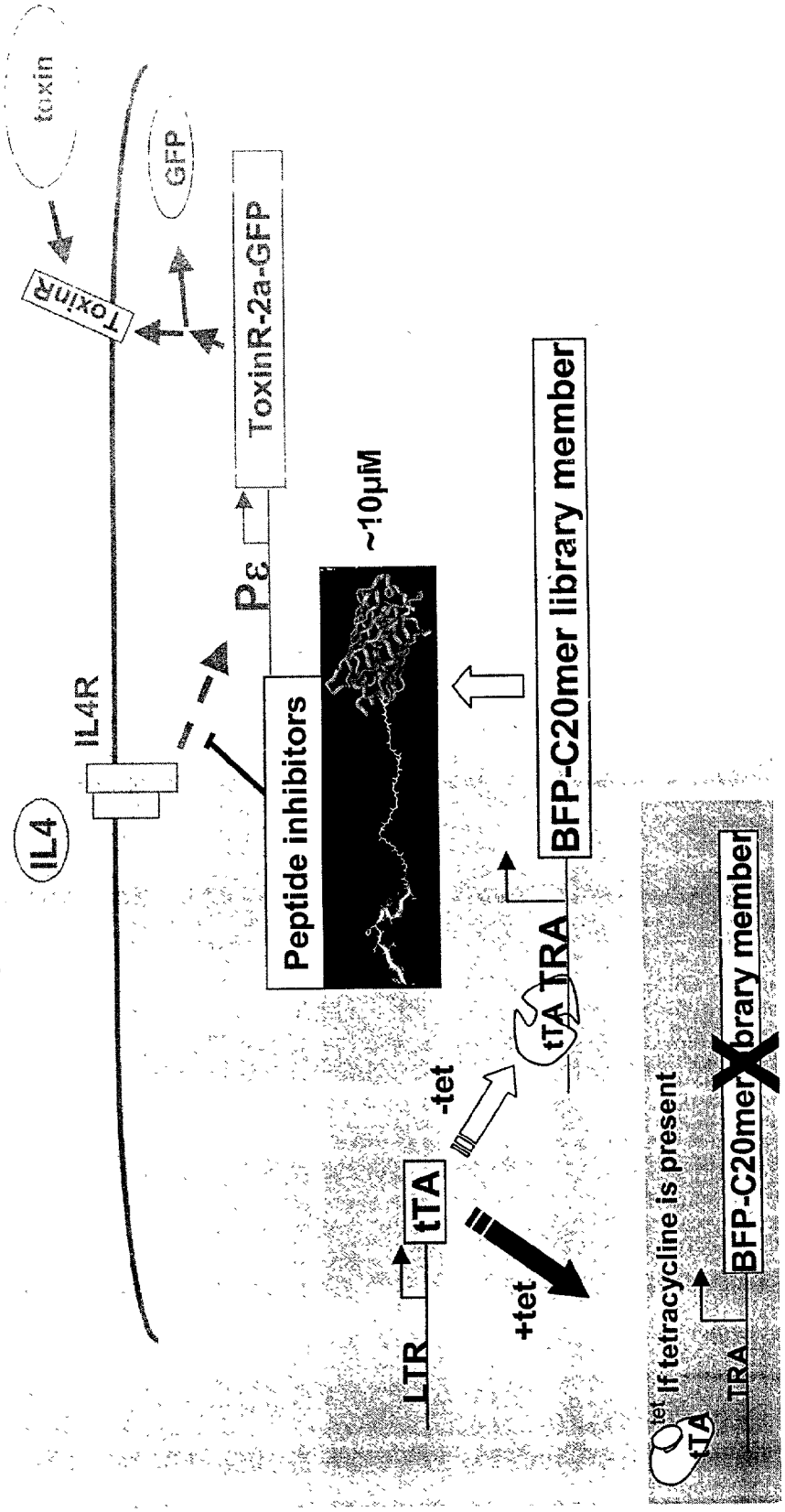
- **The A5T4 cell line generated has a dual-function reporter driven by the germline ε promoter:**

  1. **HBEGF functions as the receptor for the diphtheria (DT) toxin and confers high sensitivity to DT toxin killing following IL4 induction (for use in survival screening strategies)**
  2. **GFP to monitor IL4 induction of the Pε by fluorescence (FACS)**

- **The A5T4 screening line consists of the B cell line BJAB engineered to contain the above dual-function HBEGF2a/GFP reporter.**

FIG. 8B

The Tet/Dox Controlled Peptide Expression System of Reporter Line A5T4



- The A5T-4 cell line was further engineered to express the tetracycline-regulated transactivator (tTA) allowing for regulation of peptide library expression
- The 20mer peptides are expressed as carboxy-terminal fusions to a BFP scaffold

FIG. 9

Outline of Enrichment and Screening Procedure

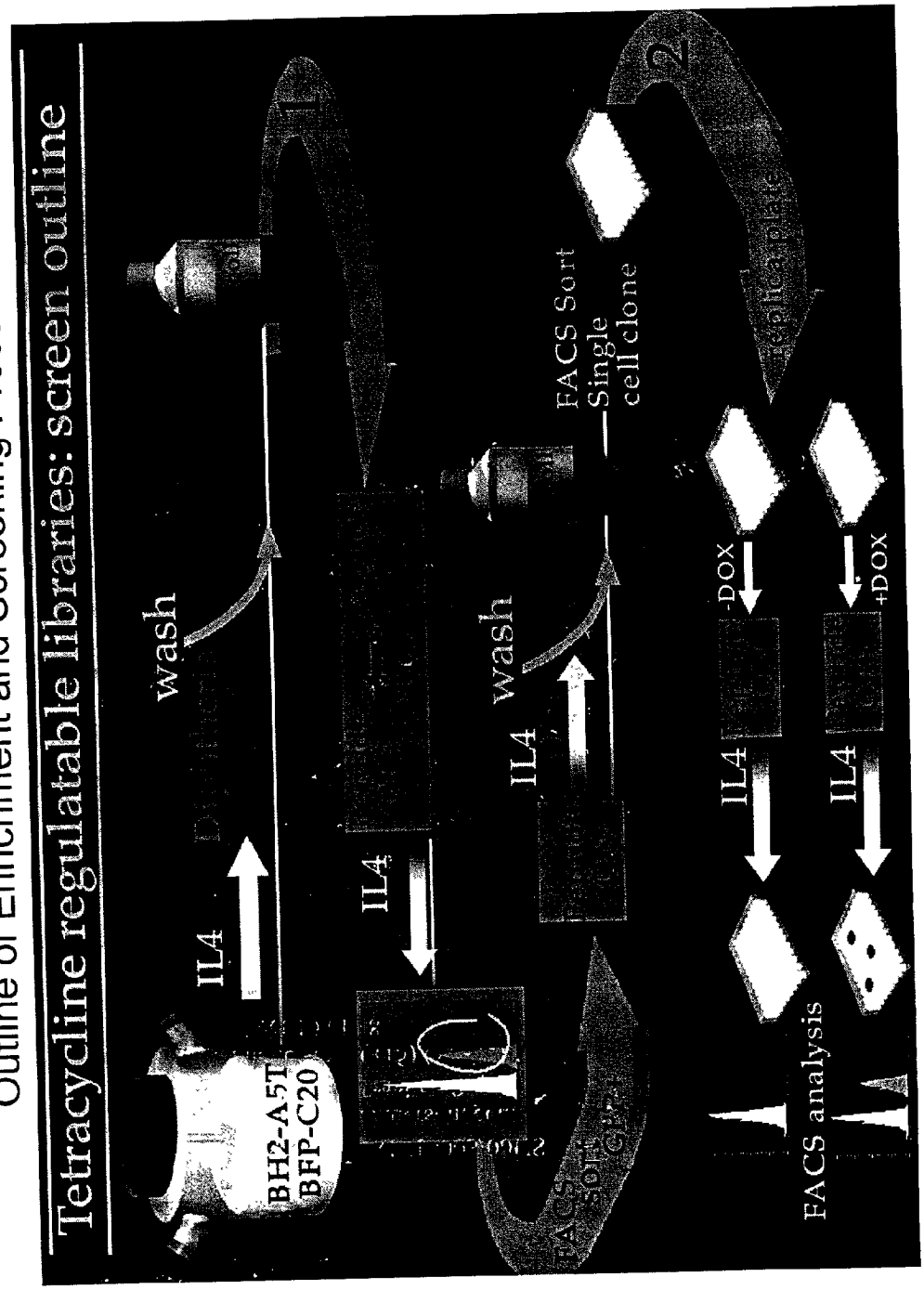


FIG. 10

DNA Transfer: *CL06wt Peptide Transfers Phenotype*

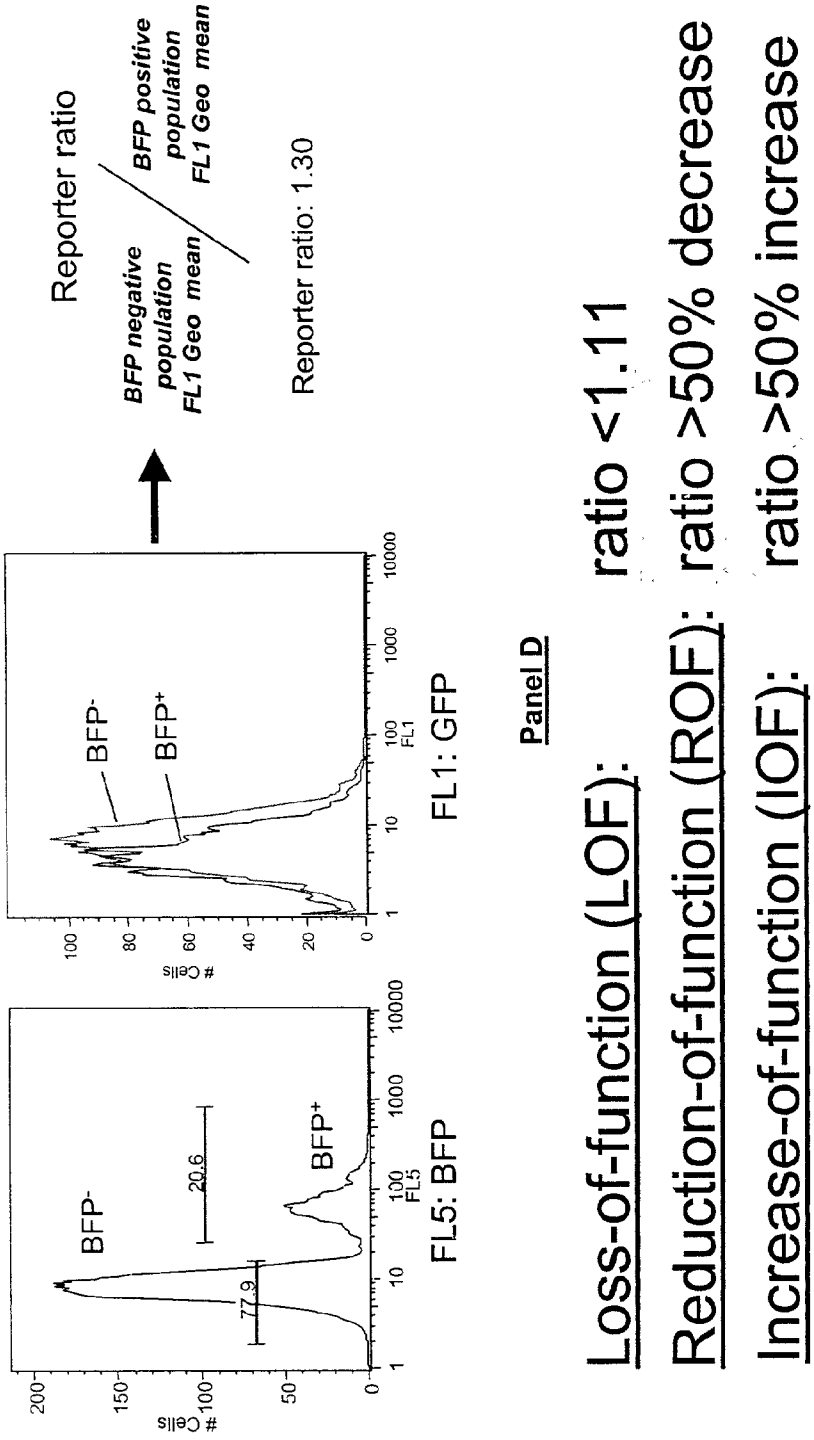
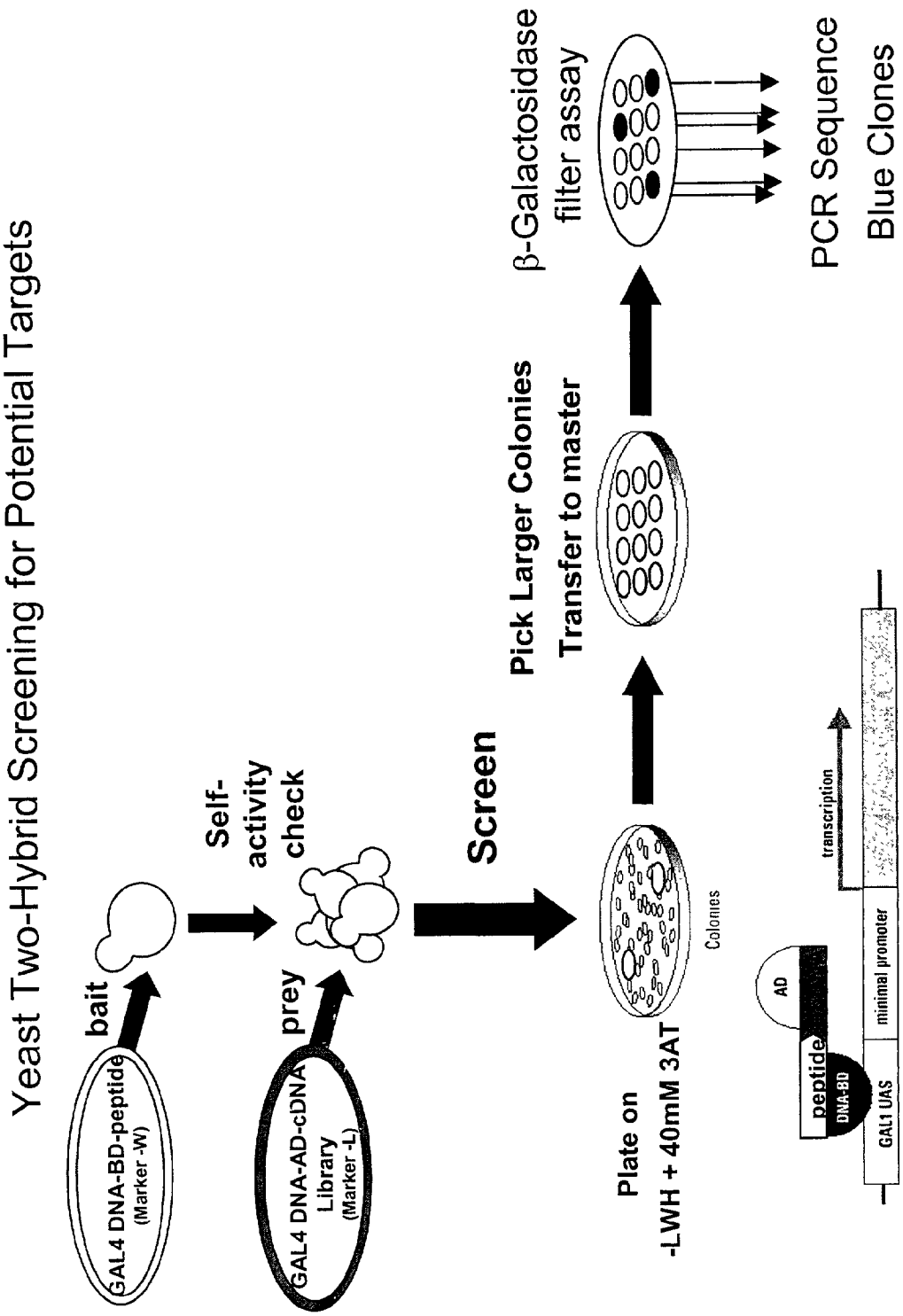


FIG. 11A


Yeast Two-Hybrid Screening for Potential Targets
bait
GAL4 DNA-BD-peptide
(Marker -W)
Self-
activity
check
prey
GAL4 DNA-AD-cDNA
Library
(Marker -L)
Screen
Plate on
-LWH + 40mM 3AT
Colonies
Pick Larger Colonies
Transfer to master
β-Galactosidase
filter assay
PCR Sequence
Blue Clones
transcription
AD
peptide
DNA-BD
GAL1 UAS
minimal promoter

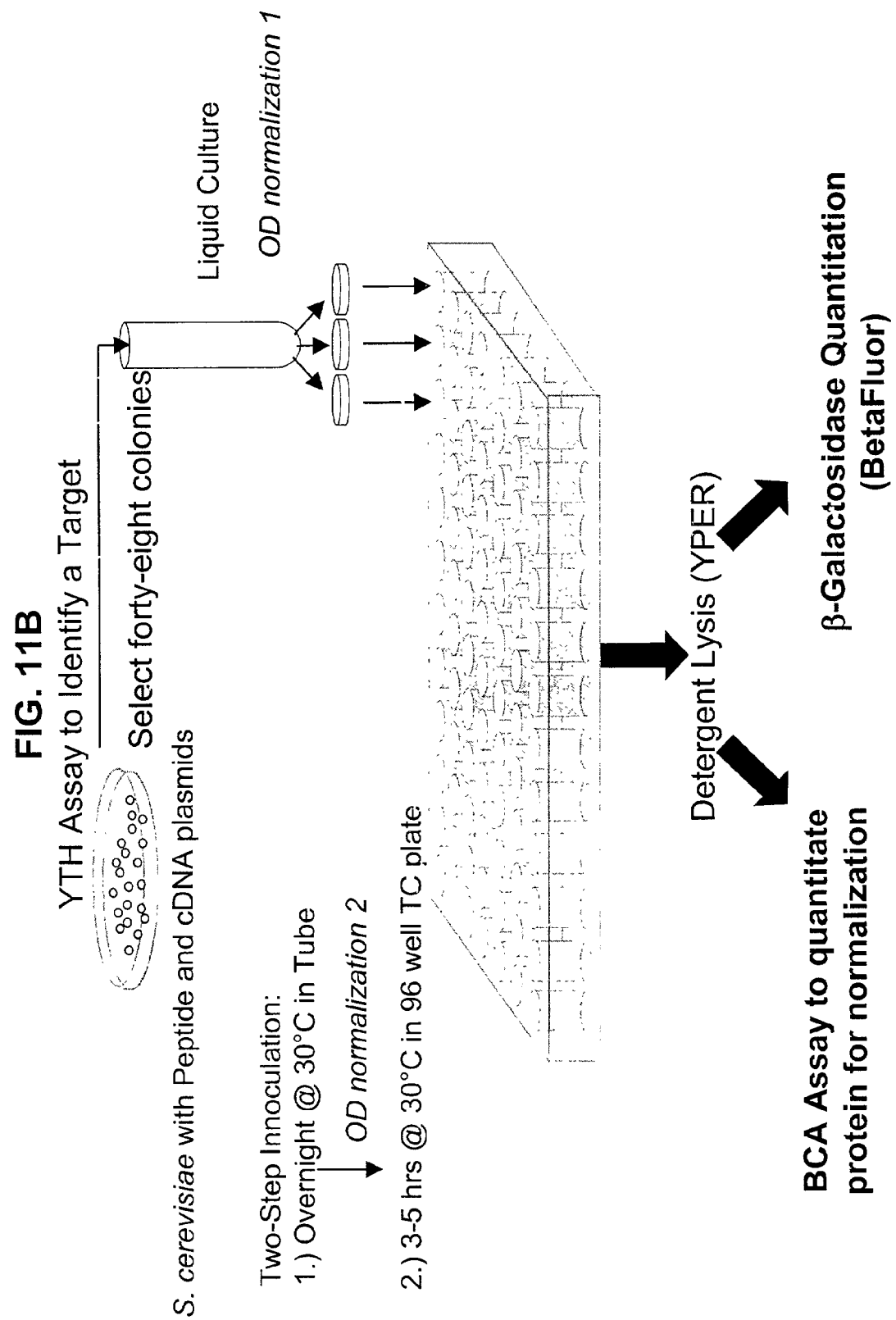

YTH Assay to Identify a Target
S. cerevisiae with Peptide and cDNA plasmids
Select forty-eight colonies
Liquid Culture
OD normalization 1
Two-Step Innoculation:
1.) Overnight @ 30°C in Tube
OD normalization 2
2.) 3-5 hrs @ 30°C in 96 well TC plate
Detergent Lysis (YPER)
β-Galactosidase Quantitation
(BetaFluor)
BCA Assay to quantitate
protein for normalization

FIG. 11B

FIG. 12
Reconfirmation Strategy

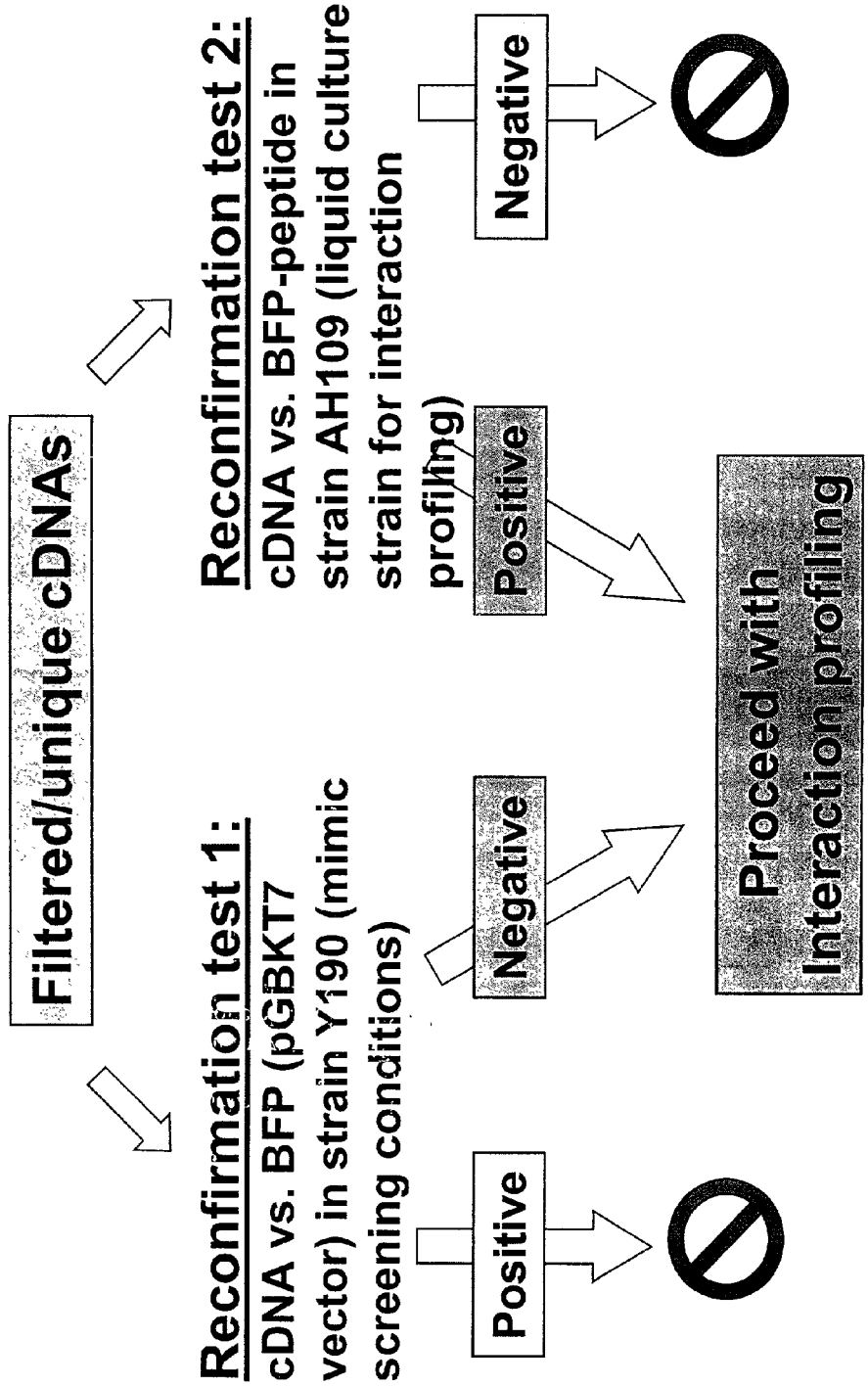


FIG. 13

YTH Interaction Profile: CL06wt peptide Binds hRXR-beta

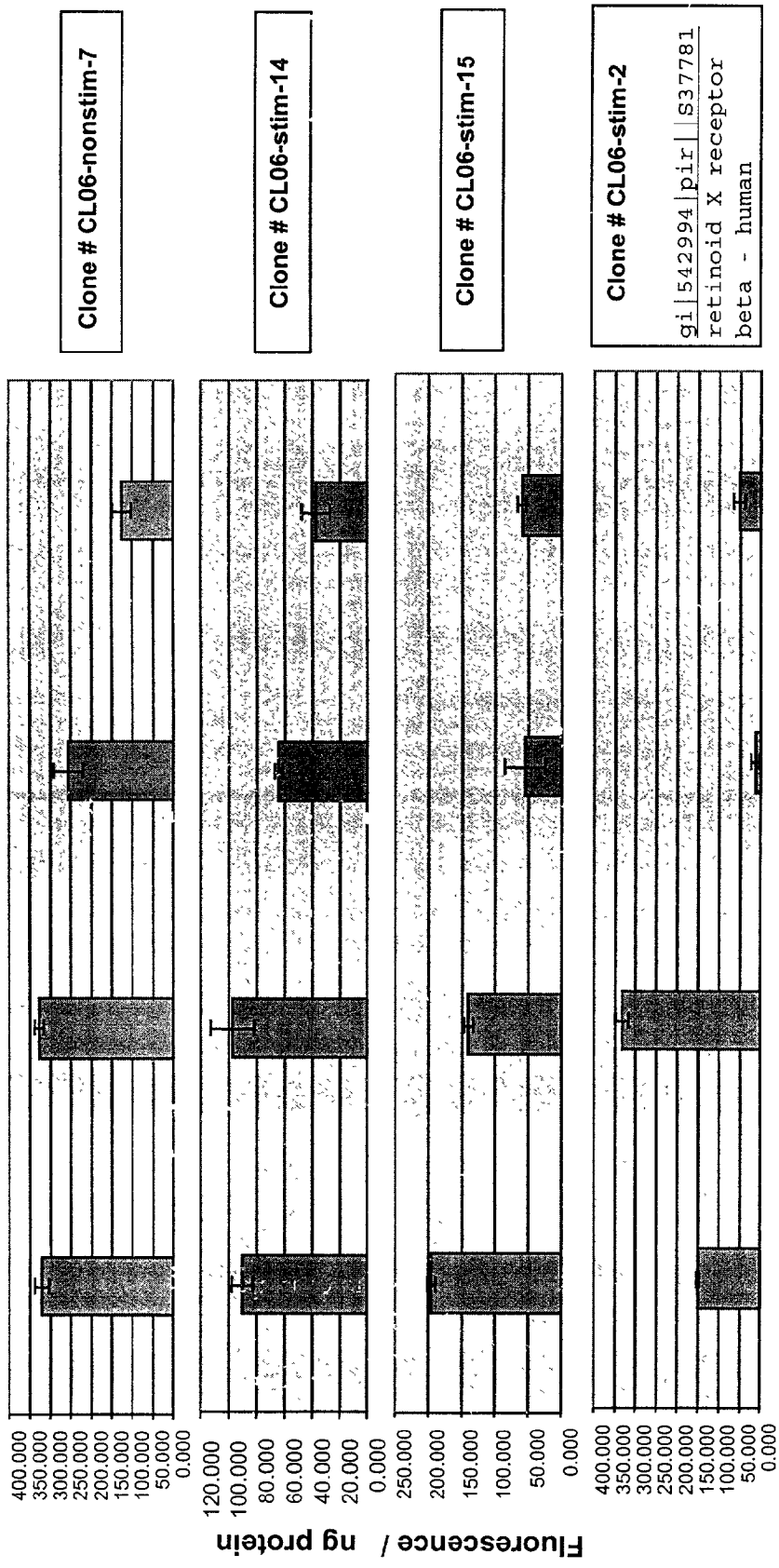


FIG. 14

Graphic Profiles: CL06wt peptide Binds hRXR-beta

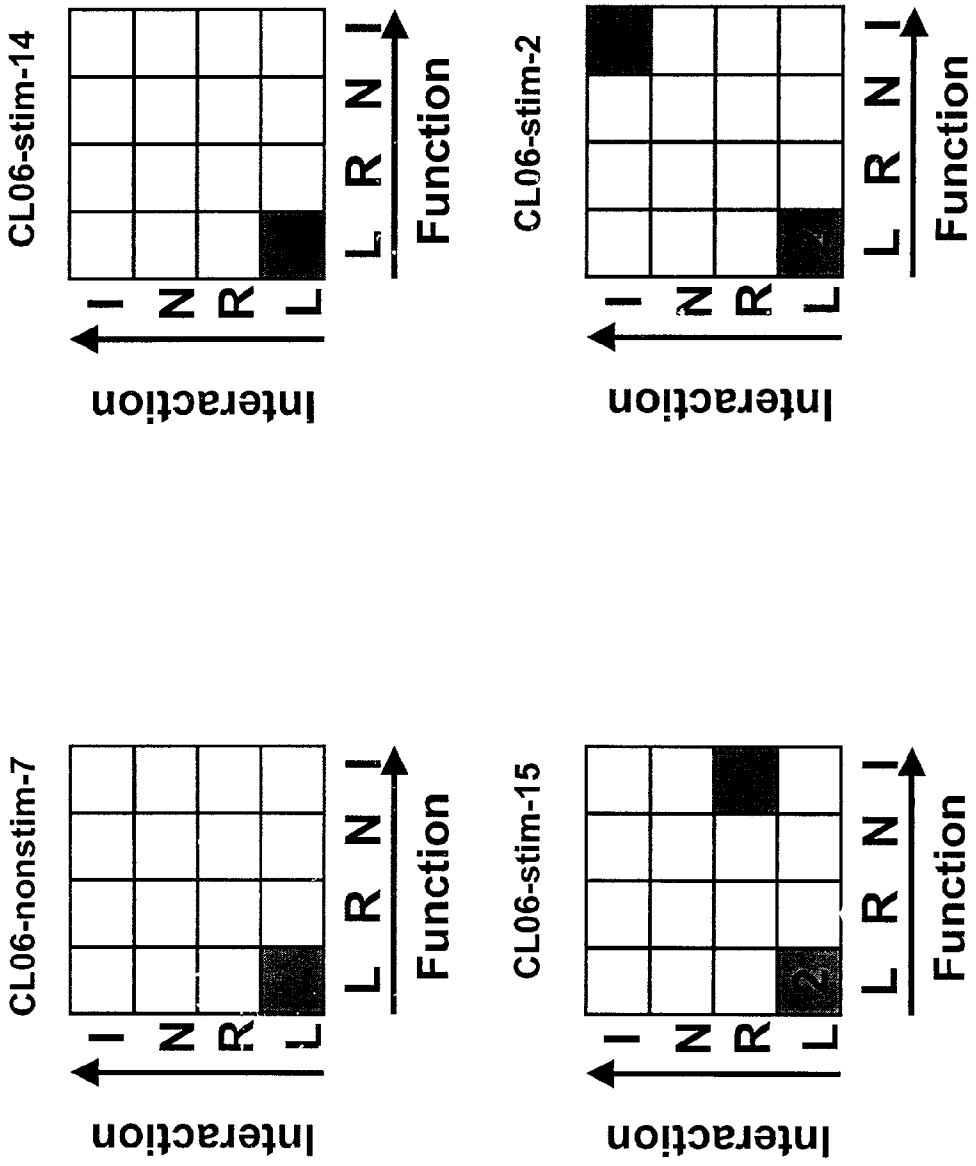
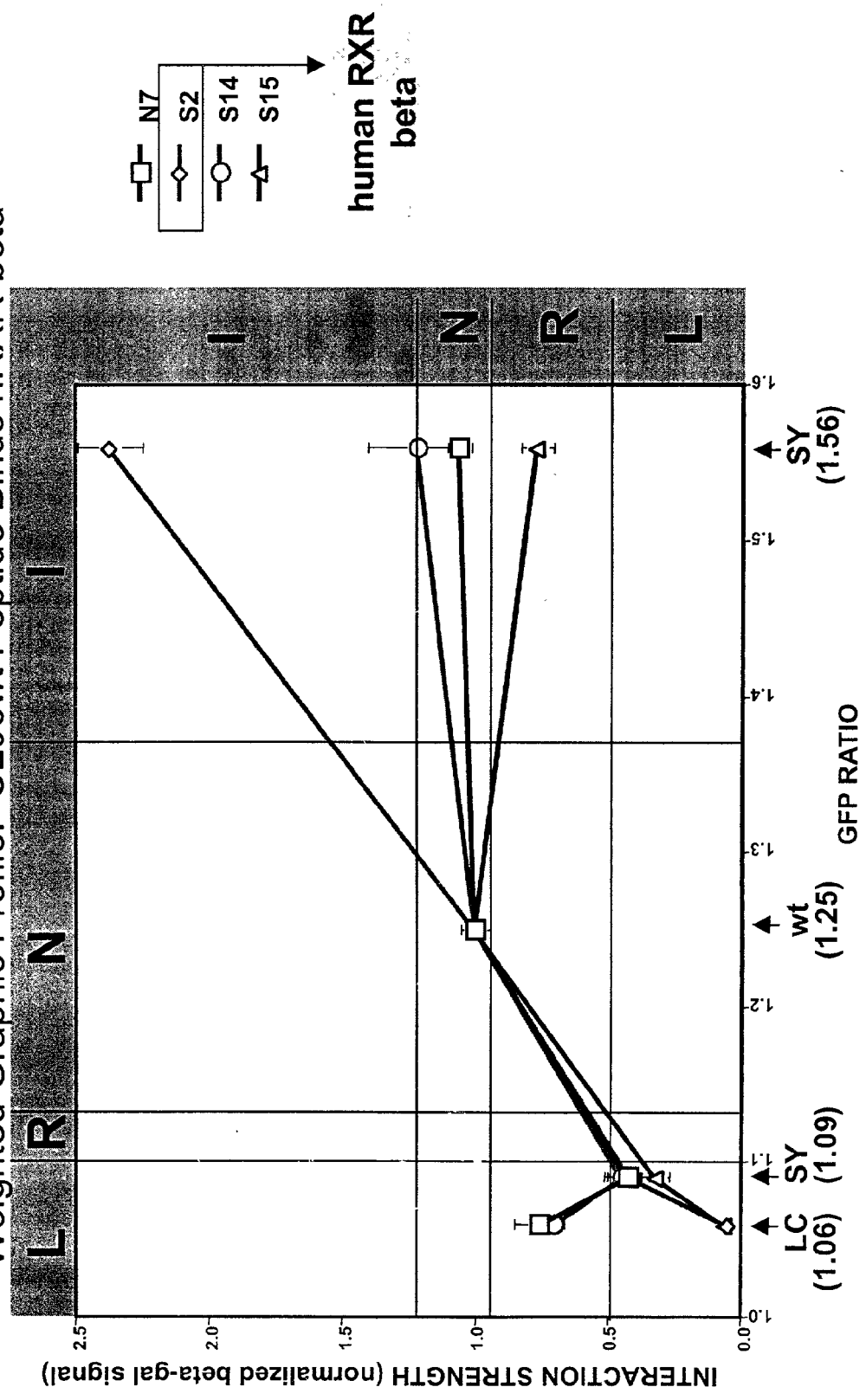


FIG. 15


Weighted Graphic Profile: CL06wt Peptide Binds hRXR-beta
N7
S2
S14
S15
human RXR beta
L
R
N
I
INTERACTION STRENGTH (normalized beta-gal signal)
GFP RATIO
LC (1.06)
SY (1.09)
wt (1.25)
SY (1.56)

METHODS OF IDENTIFYING TARGET POLYPEPTIDES

1. FIELD OF THE INVENTION

[0001] The present invention relates to methods for identifying and/or rank ordering target polypeptides that interact with a candidate polypeptide. Specifically, the methods identify and/or rank order target polypeptides by performing functional and interaction assays and comparing the functional and interaction profiles obtained from these assays.

2. BACKGROUND OF THE INVENTION

[0002] Proteins and protein-protein interactions play a central role in various essential biological processes. For example, these interactions are evident in the interaction of hormones with their respective receptors, in the intracellular and extracellular signalling events mediated by proteins, in enzyme substrate interactions, in intracellular protein trafficking, in the formation of complex structures like ribosomes, viral coat proteins, and filaments, and in antigen-antibody interactions.

[0003] Abnormal or disease states can be the direct result of aberrant protein-protein interactions. For example, oncoproteins can cause cancer by interacting with and activating proteins responsible for cell division. Protein-protein interactions are also central to the mechanism of a virus recognizing its receptor on the cell surface as a prelude to infection. Thus, identification of proteins that interact with each other not only leads to a broader understanding of protein-protein interactions, but also aids in the design of inhibitors of aberrant protein-protein interactions.

[0004] Both biochemical and genetic methods have been traditionally used to study interactions between polypeptides. Newer genomics approaches are also used to study these interactions. The biochemical techniques include chemical cross-linking, co-immunoprecipitation and co-fractionation and -purification. However, these biochemical methods are laborious and slow, often involving painstaking isolation, purification, sequencing and further biochemical characterization of the proteins being tested for interaction.

[0005] As an alternative to the biochemical approaches, genetic approaches to detect interactions between polypeptides have gained popularity as these methods allow the rapid detection of the polypeptides involved in the interactions.

[0006] An example of a genetic system to detect polypeptide interactions is the "Two-Hybrid" system to detect polypeptide interactions in the yeast *Saccharomyces cerevisiae* (Fields and Song, 1989, Nature 340:245-246; U.S. Pat. No. 5,283,173 by Fields and Song). This assay utilizes the reconstitution of a transcriptional activator like GAL4 (Johnston, 1987, Microbiol. Rev. 51:458-476) through the interaction of two polypeptide domains that have been fused to the two functional units of the transcriptional activator: the DNA-binding domain and the activation domain. This is possible due to the bipartite nature of certain transcription factors like GAL4. Being characterized as bipartite signifies that the DNA-binding and activation functions reside in separate domains and can function in trans (Keegan et al., 1986, Science 231:699-704). The reconstitution of the transcriptional activator is monitored by the activation of a reporter gene like the lacZ gene that is under the influence of a promoter that contains a binding site (Upstream Activating Sequence or UAS) for the DNA-binding domain of the transcriptional activator. This method is most commonly used either to detect an interaction between two known polypeptides (Fields and Song, 1989, Nature 340:245-246) or to identify interacting polypeptides from a population that would bind to a known polypeptide (Durfee et al., 1993, Genes Dev. 7:555-569; Gyuris et al., 1993, Cell 75:791-803; Harper et al., 1993, Cell 75:805-816; Vojtek et al., 1993, Cell 74:205-**214).**

[0007] Other examples of genetic systems to detect polypeptide interactions include different variations of the "Interaction-Trap system" (Gyuris et al., 1993, Cell 75:791-803; Durfee et al., 1993, Genes Dev. 7:555-569; Kishore and Shah, 1988, Annu. Rev. Biochem. 57:627-663), "Contingent Replication Assay" (Nallur et al., 1993, Nucleic Acids Res. 21:3867-3873; Vasavada et al., 1991, Proc. Natl. Acad. Sci. USA 88:10686-10690), and "Karyoplasmic Interaction Selection Strategy" (Fearon et al., 1992, Proc. Natl. Acad. Sci. USA 89:7958-**7962).**

[0008] Several newer genomics techniques have also been used to identify polypeptides that interact with each other. One manner of identifying target polypeptides involves the comparison of amino acid sequences. In the candidate polypeptide, the putative sequences that would bind to interacting polypeptides (putative binding domains) are identified. Next, numerous amino acid sequences in protein databases are scanned to identify polypeptides with sequences that would potentially interact with the binding domains of the candidate polypeptide.

[0009] However, the traditional biochemical and genetic techniques and the newer genomics approaches have several drawbacks. For example, these techniques produce a high rate of false positives. That is, numerous putative target polypeptides are identified for a particular candidate polypeptide, however these prior art techniques provide no means to verify which putative target polypeptide would interact with the candidate polypeptide in a biologically relevant environment, like the cellular environment. Also, once a target polypeptide has been identified, the prior art techniques provide no means to identify the function performed by the interaction of the target polypeptide and candidate polypeptide. One of the objectives of the present invention is to devise techniques that are superior to the prior art techniques in identifying the target polypeptides that interact to particular candidate polypeptides.

[0010] Overall, the methods of the present invention provide means to identify target polypeptides that interact with particular candidate polypeptides, while at the same time avoid some of the drawbacks of the prior art techniques.

3. SUMMARY OF THE INVENTION

[0011] The present invention provides methods for identifying and/or rank ordering target polypeptides that interact with candidate polypeptides.

[0012] In its broadest sense, the method involves comparing interaction profiles of a set of polypeptides with a functional profile of the set of polypeptides to identify those interaction profiles that correspond to the functional profile.

The set of polypeptides comprises a candidate polypeptide for which interaction partner is desired and a set of mutants derived therefrom. In one embodiment, the set of polypeptides includes, in addition to the candidate polypeptide, at least one mutant from each of four different categories: (i) a neutral mutant (N); (ii) a reduction-of-function mutant (ROF or R); (iii) a loss-of-function mutant (LOF or L); and (iv) an increase-of-function (IOF or I) mutant. The functional profile comprises quantitative functional information for each polypeptide of the set, which is typically obtained by assaying each polypeptide of the set in the same functional assay. The interaction profiles comprise quantitative interaction information for each polypeptide of the set, which is typically obtained by assaying each polypeptide in an interaction assay that assesses the ability of the polypeptide to interact with a potential target polypeptide. The degree of correspondence observed between the interaction profiles and functional profiles can be used to rank-order the potential target polypeptides and/or to identify a target polypeptide that interacts with the candidate polypeptide. For rank-ordering, the potential target polypeptides are ordered based upon the degree of correspondence between their respective interaction profiles and functional profile, from highest correspondence to lowest correspondence. The rank-ordering represents the likelihood that a particular potential polypeptide represents an actual target polypeptide for the candidate polypeptide in a biological system. Target polypeptides are identified by selecting those interaction profiles that correspond with the functional profile to a specified degree.

[0013] The functional and interaction profiles can be displayed and compared for correspondence in a variety of different ways. For example, in one embodiment, the profiles are displayed as bar graphs (e.g., polypeptide vs. function and polypeptide vs. interaction) and compared for correspondence. In another embodiment, each mutant polypeptide of the set is categorized as a neutral (N_f), reduction of function (R_f), loss of function (L_f), or increase of function (I_f) mutant based on its functional activity as compared to that of the candidate polypeptide. For each interaction profile, each mutant polypeptide of the set is classified as a neutral (N_I), reduction of interaction (R_I), loss of interaction (L_I), or increase of interaction (I_I) mutant based upon its interaction activity as compared to that of the candidate polypeptide. The functional and interaction profiles are then compared for correspondence, either by visual inspection or with the aid of a computer.

[0014] The methods of the invention may be used in a variety of contexts to rank-order potential target polypeptides that interact with a candidate polypeptide of interest and/or to identify target polypeptides that interact with a candidate polypeptide of interest and provides significant advantages over current techniques. The traditional techniques have several drawbacks, the main one being that many of the identified potential target polypeptides are not biologically relevant in a cellular environment. These traditional techniques have the capability to identify polypeptides that interact with each other, however, more often than not the identified interacting polypeptides do not perform any biologically relevant function in a cellular environment. By comparing the interaction profiles with functional profiles, the current invention not only identifies polypeptides that interact with each other, but also identifies potential functions performed by this interaction in a cellular environment.

4. BRIEF DESCRIPTION OF THE FIGURES

[0015] **FIG. 1** illustrates an embodiment of the method of the invention employing graphed functional and interaction profiles;

[0016] **FIG. 2** illustrates an embodiment of the method of the invention employing categorized functional and interaction profiles;

[0017] **FIG. 3A** provides a graphic representation of the dual alanine mutagenesis technique;

[0018] **FIG. 3B** illustrates the relationship between the frequency of mutants and the distribution of the alanine mutations;

[0019] **FIG. 4** illustrates a graphical representation of the interaction and functional profiles in which the mutants have been categorized;

[0020] **FIG. 5** provides selection criteria for the interaction profiling method used to confirm h-RXRβ as a target polypeptide for the CL06 compound of the invention;

[0021] **FIG. 6** depicts an apparatus for determining the correspondence between interaction and functional profiles;

[0022] **FIG. 7** is a flowchart illustrating an embodiment of a method of the invention;

[0023] **FIG. 8A** illustrates the diphtheria toxin (DT) selection of reporter cell line A5T4;

[0024] **FIG. 8B** illustrates the tetracycline/doxycycline controlled peptide expression system of reporter cell line A5T4;

[0025] **FIG. 9** illustrates the enrichment and screening procedure used to identify certain active CL06 compounds of the invention;

[0026] **FIG. 10** provides DNA transfer data for peptide CL06wt (Panels A and B), an illustration of the calculation of a reporter ratio (Panel C), and criteria for functional categorization of mutants (Panel D);

[0027] **FIG. 11A** illustrates a yeast two hybrid (YTH) assay used to screen for potential target polypeptides for the CL06 compounds of the invention;

[0028] **FIG. 11B** illustrates a yeast two hybrid (YTH) assay used to identify h-RXRβ as a target polypeptide for the CL06 compounds of the invention;

[0029] **FIG. 12** illustrates the strategies for reconfirming potential targets identified in the YTH assay depicted in **FIG. 11A**;

[0030] **FIG. 13** provides interaction/functional graphical profiles for peptide CL06wt and mutants derived therefrom;

[0031] **FIG. 14** provides graphic interaction/functional profiles for peptide CL06wt and mutants derived therefrom; and

[0032] **FIG. 15** provides weighted graphic interaction/functional profiles for peptide CL06wt and mutants derived therefrom.

5. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0033] 5.1 Abbreviations

[0034] The abbreviations used for the genetically encoded amino acids are conventional and are as follows:

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	B
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

[0035] When the three-letter abbreviations are used, unless specifically preceded by an "L" or a "D," the amino acid may be in either the L- or D-configuration about α-carbon (C_α). For example, whereas "Ala" designates alanine without specifying the configuration about the α-carbon, "D-Ala" and "L-Ala" designate D-alanine and L-alanine, respectively. When the one-letter abbreviations are used, upper case letters designate amino acids in the L-configuration about the α-carbon and lower case letters designate amino acids in the D-configuration about the α-carbon. For example, "A" designates L-alanine and "a" designates D-alanine. When polypeptide sequences are presented as a string of one-letter or three-letter abbreviations (or mixtures thereof), the sequences are presented in the N->C direction in accordance with common convention.

[0036] 5.2 Definitions

[0037] As used herein, the following terms shall have the following meaning:

[0038] "Polypeptide" refers to an organic compound comprising two or more amino acids covalently joined by peptide bonds. The term includes organic compounds commonly known in the art as peptides, oligopeptides, polypeptides, and proteins. The polypeptides may include non-natural amino acids and any of the modifications such as glycosylation and phosphorylation and additional amino and carboxyl groups as are known in the art. Polypeptides include organic compounds with any degree of secondary, tertiary, and/or quaternary structure.

[0039] "Candidate polypeptide" refers to a polypeptide that is being studied to identify the target polypeptides that interact with it in an extra-cellular or intra-cellular environment.

[0040] The terms "potential target" and "potential target polypeptide" as used herein mean a polypeptide that has been identified as a polypeptide with which the candidate polypeptide has some likelihood of interacting in an extra-cellular or intra-cellular environment.

[0041] The terms "target" and "target polypeptide" as used herein mean a polypeptide that has been identified as a polypeptide with which the candidate polypeptide interacts in an extra-cellular or intra-cellular environment.

[0042] 5.3 Methods of the Invention

[0043] The present invention provides methods for rank-ordering and/or identifying target polypeptides that interact with a candidate polypeptide. The method involves comparing a functional profile for a set of polypeptides including the candidate polypeptide with interaction profiles for the set of polypeptides and identifying those interaction profiles that correspond to the functional profile. Based upon the degree of correspondence, the target polypeptides can then be rank-ordered and/or identified as target polypeptides that interact with the candidate polypeptide.

[0044] Candidate polypeptides for which target polypeptides may be rank-ordered or identified according to the invention may include virtually any polypeptide known or found to have a function of interest. For example, the candidate polypeptide may be an orphan receptor or a polypeptide identified in a functional screening assay.

[0045] 5.4 Functional Profiles

[0046] In the methods of the present invention, the functional profiles can be obtained by using information known in the art or with functional data obtained by performing functional assays. In addition, the functional assays can be used to identify candidate polypeptides to be studied. Candidate polypeptides may also be selected using information known in the art.

[0047] Functional assays are assays in which one or more biological activities can be evaluated. The biological activities that can be evaluated include interactions with receptors, induction of cellular proliferation or differentiation, synthesis or activation of polypeptides, activation of signaling pathways, or phosphorylation or other modifications of polypeptides. Typically, the functional assays used will have the capacity to quantify the biological activity being studied.

[0048] Numerous functional assays suitable for the present invention are known in the art. For example, see Lorens et al., 2001, *Current Opinion in Biotechnology*, 12:613-621. One example of a functional assay is the assay described in Berger et al., 2001, *PNAS*, 98:130-135. In this assay the biological activity being evaluated is the expression of the BRCA1 gene. The human ovarian cancer-derived cells used in this assay stably express green fluorescence protein (GFP) as a marker under the control of the BRCA1 promoter. The biological activity, i.e., the expression of BRCA1 gene, can be quantified by measuring the level of GFP expression.

[0049] Typically, the functional assay is used in combination with one of the known library screen methodologies to identify the candidate polypeptides. The library screen techniques allow the introduction of genes or polypeptides into the cells of the functional assay to elucidate the regulation of the biological activity being studied. In particular, retroviral library techniques are suitable for use in the present invention. Examples of retroviral libraries are described in Lorens

et al., 2001, *Current Opinion in Biotechnology*, 12:613-621; U.S. Pat. No. 6,153,380; WO 97/27213; and WO 01/34806. Other non-limiting examples of recombinant library methodologies that may be used in connection with the assays of the invention are described in U.S. Pat. Nos. 6,156,571; 6,107,059 and 5,733,731, the disclosures of which are incorporated herein by reference.

[**0050**] In Berger et al., 2001, *PNAS*, 98:130-135, a retroviral ribozyme gene library was used in combination with the BRCA1 functional assay. The gene library was introduced into the human cancer-derived cells expressing GFP as the marker under the control of the BRCA1 promoter. In this manner, specific ribozyme genes from the library that regulate BRCA 1 promoter were identified. Particular ribozymes that increase or decrease BRCA1 expression were identified by quantifying the level of GFP expression.

[**0051**] In one embodiment of the invention, the interleukin-4 (IL-4) activity assay is used as a functional assay to identify candidate polypeptides. This assay employs cell lines that express the IL-4 receptor and have been transformed by a recombinant vector comprising a reporter gene operatively linked to an IL-4 inducible element. When IL-4 binds to the IL-4 receptor the IL-4 signaling cascade is activated, which in turn leads to the activation of the IL-4 inducible element leading to expression of the reporter gene. The reporter gene is typically a gene that encodes a protein that produces an observable signal, such as fluorescent protein. The level of reporter gene product expressed is proportional to the activation of the IL-4 inducible element. The IL-4 inducible element may be a germline ϵ promoter. Particularly useful IL-4 activity assays are described in U.S. Pat. No. 5,958,707, WO 99/58663, and WO 01/34086.

[**0052**] Retroviral libraries, such as those described in Lorens et al., 2001, *Current Opinion in Biotechnology*, 12:613-621; U.S. Pat. No. 6,153,380; WO 97/27213; and WO 01/34806, can be used in combination with the IL-4 activity assay to identify candidate polypeptides. Using such retroviral methods, very large libraries of candidate polypeptides can be constructed (e.g., 10^6-10^{10} peptides) and screened. The cells employed in the IL-activity assay can be infected with retrovirus such that different populations of cells are produced that express the individual polypeptides in the retroviral library. The polypeptides in the retroviral peptide library are thus evaluated for their involvement in the IL-4 signaling cascade. By measuring the level of reporter gene expressed in response to the activation of the IL-4 signaling cascade, one or more of the polypeptides in the retroviral library can be identified as the polypeptide that regulates IL-4 activity. If it is desired to identify a candidate polypeptide that inhibits the activation of the IL-4 induced signaling cascade, then polypeptides from the retroviral peptide library that cause decreased expression of the reporter gene in response to IL-4 are selected as the candidate polypeptide.

[**0053**] As mentioned above, the functional assay can be used to develop a functional profile of the candidate polypeptide and mutants derived therefrom (described in detail below). A functional profile is a set of functional values, each functional value corresponding to the biological activity of the candidate polypeptide and the individual mutants from the set of mutants. The value can be a number or a non-numerical category. For example, if the IL-4 activity assay is used, the value could be the level of reporter gene expressed in response to stimulation by IL-4. In the IL-4 activity assay the expression of the reporter gene can be linked to the germline ϵ promoter, then the biological activity of the candidate polypeptide would be linked to the IL-4 signaling cascade. Candidate polypeptides that inhibit IL-4 induced transcription will yield reduced amounts of reporter gene product as compared to control cells that do not express the candidate polypeptide. Thus, measurements of reporter gene products provide a biological activity for the candidate polypeptide, i.e. inhibition or activation of IL-4 induced transcription. These biological activities obtained from the functional assays, for example, measurements of reporter gene product from IL-activity assay, can be used to develop the functional profiles.

[**0054**] When the IL-4 activity assay is used to obtain a functional profile for a candidate polypeptide and mutants derived therefrom, different cell populations are created with each population expressing either the mutants or the candidate polypeptide. For each cell population, the level of reporter gene product expressed is measured following exposure of the cell to IL-4. The level of reporter gene product obtained for each cell population is used to develop the functional profile. In this case, the functional profile would consist of a set of measurements of the reporter gene product, i.e., functional values, each measurement corresponding to the candidate polypeptide and mutants derived therefrom.

[**0055**] For purposes of the present invention, the functional profiles can be depicted using numerous different methods. Two methods for depicting the functional profiles are shown in **FIGS. 1 and 2**. In **FIG. 1**, the functional profile for the candidate polypeptide is depicted in a graphical manner (see panel V of **FIG. 1**). The biological activities of the candidate polypeptide and its mutants, obtained from the functional assays, are plotted as a bar graph. **FIG. 2** shows a second method for depicting the functional profile. In this method, the biological activities of the candidate polypeptide and its mutants are obtained using a functional assay. The biological activity of each mutant is compared to the candidate polypeptide and based on this comparison the mutants are categorized as L, R, N, and I. It is this information that is used to plot **FIG. 2**. Further details regarding **FIG. 2** are provided below.

[**0056**] 5.5 Interaction Profiles

[**0057**] In the methods of the present invention, interaction profiles can be obtained using information known in the art or with interaction data obtained by performing interaction assays. In addition, an interaction assay can be used to screen for potential targets for a candidate polypeptide. In one embodiment of the invention, potential targets for a candidate polypeptide may be identified using information known in the art.

[**0058**] Interaction assays are assays used to detect the interaction of two or more polypeptides. The term "interaction" and its grammatical equivalents as used herein means the process of two or more things, for example polypeptides, acting on each other. Interaction assays with the ability to quantify the interaction among two or more polypeptides are particularly useful in the present invention. The interaction among the polypeptides may lead to the modification of function of one or more polypeptides involved in the inter-

action. Numerous interaction assays are known in the art. These assays can include either in vivo or in vitro assays.

[0059] One example of an interaction assay is a transcription activation assay such as the yeast two-hybrid (YTH) assay. The yeast two-hybrid system and variations are described in Fields and Song, 1989, *Nature*340, 245-246; U.S. Pat. No. 5,283,173, Fearon et al., 1992, *Proc. Natl. Acad. Sci., USA* 89:7958-7962; and Osborne et al., 1995, *Biotechnology* 13:1474-1478. Its variations like the one-hybrid systems, three-hybrid systems, reverse two-hybrid systems, split-hybrid systems, alternative n-hybrid systems, and small molecule-based hybrid systems can also be used to practice the present invention. See Drees, 1999, *Current Opin. Chem. Biol.*, 3, 64-70; Vidal et al., 1999, *Nucleic Acids Research*, 27, 919-929; *Current protocols in Molecular Biology*, 1996, eds. Ausubel et al., Wiley, New York; Huang et al., 1997, *Proc. Natl. Acad. Sci.* (*USA*), 94, 13396-13401; Yang et al., 1995, *Nucleic Acids Res.*, 23, 1152-1156; Colas et al., 1996, *Nature*, 380, 548-550; Xu et al., 1997, *Proc. Natl. Acad. Sci.* (*USA*), 94, 12473-12478. These references are incorporated herein by reference in their entirety.

[0060] The conceptual basis for a transcription activation assay is predicated on the modular nature of transcription factors, which consist of functionally separable DNA-binding and trans-activation domains. When expressed as separate proteins, these two domains fail to mediate gene transcription. However, the ability to activate transcription can be restored if the DNA-binding domain and the trans-activation domain are bridged together through a protein-protein interaction. These domains can be bridged, for example, by expressing the DNA-binding domain and trans-activation domain as fusion proteins (hybrids), where the proteins that are appended to these domains can interact with each other. The protein-protein interaction of the hybrids can bring the DNA-binding and trans-activation domains together to create a transcriptionally competent complex.

[0061] The transcriptional assays can also be performed using mammalian host cells as described in U.S. Pat. No. 6,114,111. This reference is incorporated herein by reference in its entirety.

[0062] Other examples of interaction assays include phage display systems, coprecipitation, fluorescence resonance energy transfer (FRET), far-western blot, optical biosensor methods (e.g., surface plasmon resonance), analytical ultracentrifugation, titration microcalorimetry, size exclusion chromatography, equilibrium dialysis, fluorescence polarization, mass spectrometry, and light scattering. See Chapters 19 and 20, *Current Protocols in Protein Science*, C D, Winter 2002 update, John Wiley and Sons, Inc. The bioluminescence resonance energy transfer (BRET) technique is also useful as an interaction assay. The BRET technique is based on energy transfer from a bioluminescent donor to a fluorescent acceptor protein. Further details regarding the BRET technique are provided in Cheng et al., 2001, *J Biol Chem.*, 276(51):48040-7 and Angers et al., 2000, *Proc Natl Acad Sci U S A.*, 97(7):3684-9. Another useful assay is the cDNA phage display technique. See Sche et al., 1999, *Chem Biol.*, 6(10):707-16.

[0063] The interaction assay can be used to screen for potential targets for a candidate polypeptide. For example, the yeast two hybrid assay can be used for this purpose. The candidate polypeptide would be expressed as a fusion protein with either the DNA-binding domain or the trans-activation domain. Depending on the fusion protein formed for the candidate polypeptide, each polypeptide from a library of polypeptides would be expressed as fusion proteins with either the DNA-binding domain or the trans-activation domain. A polypeptide from the library is identified as a potential target polypeptide if it forms a transcriptionally competent complex with the candidate polypeptide fusion protein.

[0064] In the present invention, an interaction assay can also be used to develop interaction profiles. The interaction assay is used to quantify the ability of one or more polypeptides to interact with each other and the information obtained from this quantification process is used to develop the interaction profile. For example, interaction assays can be used to quantify the ability of the candidate polypeptide and mutants derived therefrom to interact with a potential target polypeptide and to develop interaction profiles suitable for the present invention. An interaction profile is a set of interaction values, each interaction value quantifying the ability of two or more polypeptides to interact with each other. Specifically, in the present invention, an interaction value quantifies the ability of individual mutants and the candidate polypeptide to interact with a particular potential target polypeptide. This value can be a number or a non-numerical category. One example of an interaction value is the level of reporter gene product expressed in a transcriptional activation assay.

[0065] In one embodiment of the invention, the yeast two hybrid assay is used to develop an interaction profile. For an interaction profile of a candidate polypeptide and its mutants for a particular potential target polypeptide, the candidate polypeptide and its mutants are expressed as a fusion protein with either the DNA-binding domain or the trans-activation domain. Depending on the fusion protein formed for the candidate polypeptide and its mutants, the target polypeptide is expressed as a fusion protein with either the DNA-binding domain or the trans-activation domain. Interaction values for the interaction profile are obtained from the level of reporter gene product expressed. The level of reporter gene product expressed is related to the formation of the transcriptionally competent complex by the candidate polypeptide and its mutants with the target polypeptide. Thus, the level of the reporter gene product measured reflects the interaction of the candidate polypeptide and its mutants to the potential target polypeptide.

[0066] For purposes of the present invention, the interaction profiles can be depicted using numerous different methods. Two methods for depicting the interaction profiles are depicted in **FIGS. 1 and 2**. In **FIG. 1**, the interaction profiles are depicted in a graphical manner (see panels I-IV of **FIG. 1**). The interaction values of the candidate polypeptide and its mutants, obtained from the interaction assays, are plotted as a bar graph. **FIG. 2** shows a second method for depicting the interaction profile. In this method, first the interaction values of the candidate polypeptide and its mutants for a particular potential target polypeptide are obtained using an interaction assay. For each potential target polypeptide, the interaction values of each mutant is compared to the candidate polypeptide and based on this comparison the mutants are classified as L, R, N, and I. It is this information that is used to plot **FIG. 2**. Further details regarding **FIG. 2** are provided below.

[0067] The interactions being evaluated in the interaction assays can be either direct or indirect. Direct polypeptide interactions involve the direct action of two or more polypeptides on each other. In this direct interaction, a modification of function of one or more polypeptides involved in the interaction may be observed. Indirect polypeptide interactions include polypeptides that modify each other's functions, but do not directly act on each other.

[0068] For both direct and indirect interactions, the interaction value can reflect the level of function of one or more polypeptides directly or indirectly involved in the interaction. For example, direct interaction of a neurotransmitter with its receptor can cause the opening of an ion channel located in the receptor. In direct interactions of this form, the interaction value can reflect the activity of the ion channel. The yeast two hybrid assay is an example of an interaction assay suitable to evaluate direct interactions. The IL-4 activity assay, described above, is an example of an assay that can be used to obtain interaction values of an indirect interaction. In the IL-4 activity assay, IL-4 indirectly interacts with the germline ϵ promoter, which in turn causes the expression of a reporter gene product, for example, a blue or green fluorescent polypeptide. The interaction value obtained from this assay is the level of the reporter gene product expressed, which in turn is related to the indirect interaction of IL-4 with the germline ϵ promoter.

[0069] As mentioned above, an interaction assay can provide interaction values that reflect the interaction of two or more polypeptides or the function of one or more polypeptides directly or indirectly involved in the interaction. In the present invention, typically a functional profile is compared to interaction profiles to identify the targets of candidate polypeptides. Hence, the interaction values used in the methods of the present invention will typically reflect the direct interaction of two or more polypeptides.

[0070] 5.6 Mutants

[0071] As mentioned above, in the present invention a set of mutants of the candidate polypeptide is used. The set of mutants contains one or more mutants. A mutant is a polypeptide that has a sequence with at least one amino acid different from the sequence of the wild type candidate polypeptide.

[0072] The mutants may be created using numerous mutagenesis techniques known in the art. Mutagenesis techniques for the replacement, insertion, or deletion of one or more selected amino acid residues are well known to one skilled in the art (e.g., U.S. Pat. No. 4,518,584, the disclosure of which is hereby incorporated by reference). There are two principal variables in the construction of each amino acid sequence variant, the location of the mutation site and the nature of the mutation. In designing each mutant, the location of each mutation site and the nature of each mutation will depend on the biochemical characteristic(s) to be modified. Each mutation site can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections, depending upon the results achieved, (2) deleting the target amino acid residue or (3) inserting one or more amino acid residues adjacent to the located site.

[0073] Typically, the nature of the mutations performed will not disrupt the structure of the polypeptide, i.e., the mutants will have a structure similar to the wild type candidate polypeptide. In one aspect of the invention, the location of the mutations is the site that is suspected to interact with the target polypeptides.

[0074] The mutants can be substitution mutants, deletion mutants, or insertion mutants. Substitution mutants are mutants wherein at least one amino acid residue is removed and a different residue is inserted in its place. Amino acid sequences substituted generally range from about 1 to 30 amino acid residues, typically from about 1 to 20 amino acid residues, more typically from about 1 to 10 amino acid residues and most typically from about 1 to 5 residues. The substitutions can be in contiguous or non-contiguous residues. Deletion mutants are mutants wherein at least one amino acid residue is removed. The deleted amino acid residues can be contiguous or non-contiguous residues. Amino acid sequence deletions generally range from about 1 to 30 amino acid residues, typically from about 1 to 20 amino acid residues, more typically from about 1 to 10 amino acid residues and most typically from about 1 to 5 residues. Amino-terminal, carboxy-terminal and internal intrasequence deletions are contemplated. Insertion mutants may include insertions of an amino- and/or carboxyl-terminal fusion ranging in length from one residue to one hundred or more residues, as well as internal intrasequence insertions of single or multiple amino acid residues. Internal additions may range generally from about 1 to 20 amino acid residues, preferably from about 1 to 10 amino acid residues, more preferably from about 1 to 5 amino acid residues, and most preferably from about 1 to 3 amino acid residues.

[0075] Amino acid sequence variations of the wild type candidate polypeptide are typically prepared by mutations in the DNA. At the genetic level, the mutants ordinarily are prepared by site-directed mutagenesis of nucleotides in the DNA encoding the wild type candidate polypeptide, thereby producing DNA encoding the mutant, and thereafter expressing the DNA in recombinant cell culture.

[0076] In one aspect of the invention, the site for introducing an amino acid sequence variation may be predetermined, but the mutation per se may not be predetermined. For example, random mutagenesis may be conducted at the target codon or region and the expressed mutants screened for the optimal combination of desired activity. In another aspect of the invention, substitution mutations may be made at predetermined sites in the DNA of the candidate wild type polypeptide, for example, site-specific mutagenesis. In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by publications such as Adelman et al., DNA 2, 183 (1983), the disclosure of which is incorporated herein by reference.

[0077] In one embodiment of the invention, the mutants are characterized by functional assays. Performance of the functional assay provides information regarding the biological activity exhibited by the mutants relative to the wild type candidate polypeptide. The biological activities evaluated can include activities like interactions to a receptor or induction of cellular proliferation or differentiation. Typically, in the functional assay the biological activities of the mutants and wild type candidate polypeptide are measured using conventional techniques. For example see Lorens et al., 2001, *Current Opinion in Biotechnology*, 12:613-621. Based on the biological activity of the mutants relative to the

candidate polypeptide, the mutants may be classified as loss of function (L or L_f), reduction of function (R or R_f), increase of function (I or I_f), and neutral (N or N_f) mutants. The loss of function mutants are characterized by almost no biological activity compared to the wild type candidate polypeptide. The biological activity of the loss of function mutants is not statistically different from a known inactive polypeptide. The reduction of function mutants are characterized by a decrease in biological activity compared to the wild type candidate polypeptide. The biological activity of the reduction of function mutants is statistically lower than the wild type candidate polypeptide and is also statistically greater than the loss of function mutants. Also, the R_f mutant has statistically detectable function. The increase of function mutants are characterized by an increase in biological activity compared to the wild type candidate polypeptide. The biological activity of the increase of function mutants is statistically greater than the wild type candidate polypeptide. The neutral mutants have almost the same level of biological activity as compared to wild type candidate polypeptide. The biological activity of the neutral mutants is not statistically different from the wild type candidate polypeptide.

[0078] In one aspect of the invention, the set of mutants comprises at least 2 mutants, wherein one mutant is a loss of function mutant and another mutant is an increase of function mutant. Use of one loss of function mutant and one increase of function mutant provides a gradient in the biological activities that form the functional profile.

[0079] In another aspect of the invention, the set of mutants comprises an R_f mutant, an I_f mutant, a L_f mutant, and a N_f mutant.

[0080] Also, the mutants can be characterized by the interaction of the mutants relative to the wild type candidate polypeptide. As described above, the interaction of the mutants and candidate polypeptide to a particular target polypeptide can be determined by using interaction values obtained from interaction assays. Based on the interaction values to a target polypeptide, the mutants may be categorized as loss of interaction (L or L_i), reduction of interaction (R or R_I), increase of interaction(I or I_I), or neutral (N or N_i) mutants. This categorization is based on the comparison of the interaction values of the mutant to the interaction value of the candidate polypeptide. The loss of interaction mutants are characterized by almost no interaction to the target polypeptide compared to the wild type candidate polypeptide. The interaction value of the loss of interaction mutants is not statistically different from a known inactive polypeptide. The reduction of interaction mutants are characterized by a decrease in interaction to the target polypeptide compared to the wild type candidate polypeptide. The interaction value of the reduction of interaction mutants is statistically lower than the wild type candidate polypeptide and is also statistically greater than the loss of interaction mutants. Also, the R_i mutant has statistically detectable interaction value. The increase of interaction mutants are characterized by an increase in interaction to the target polypeptide compared to the wild type candidate polypeptide. The interaction value of the increase of interaction mutants is statistically greater than the wild type candidate polypeptide. The neutral mutants have almost the same level of interaction as compared to a wild type candidate polypeptide. The interaction value of the neutral mutants is not statistically different from the wild type candidate polypeptide.

[0081] In one embodiment of the invention, dual alanine mutants of the wild type candidate polypeptide are created using the scanning alanine mutagenesis technique. The scanning alanine mutagenesis technique involves the systemic substitution of specific amino acids with alanine residues using standard site-directed mutagenesis techniques. The alanine mutants can be single Ala mutants or dual Ala mutants. For dual Ala mutants, two amino acid residues of the wild type candidate polypeptide are substituted with two alanine residues. The two substituted amino acid residues may be adjacent to each other or separated from each other by other non-substituted amino acid residues. For single Ala mutants, one amino acid residue of the wild type candidate polypeptide is substituted with one alanine residue. In one aspect of the invention, a series of dual Ala mutants are created as described in **FIG. 3A**. In the first mutant, the first and second amino acid residues of the wild type candidate polypeptide are substituted with alanine residues, in the second mutant the fifth and sixth amino acid residues are substituted with alanine residues, and so forth. In this manner, the entire wild type candidate polypeptide chain is scanned with alanine substitutions such that 50% of the wild type sequence is covered. If desired, a second series of dual Ala mutants can be created to cover the remaining 50% of the wild type sequence.

[0082] The inventors of the present invention have observed that alanine mutations at the C and N terminals of a polypeptide are more likely to produce increase of function mutants, whereas alanine mutants in the inner portions of the polypeptide are more likely to be loss of function mutants. See **FIG. 3B**. Mutations at the terminals with amino acids other than alanine, for example arginine, have been observed to produce loss or reduction of function mutants.

[0083] In addition to the L, R, I, and N mutants described above, in one embodiment of the invention, the set of mutants also contains scrambled sequence and reversed peptide sequence mutants of the candidate polypeptide. Scrambled sequence mutants have the same amino acid residues as the wild type polypeptide but these amino acid residues are in a random sequence compared to the wild type polypeptide. Reversed peptide sequence mutants have a reversed amino acid sequence compared to the wild type polypeptide. That is, the C-terminal amino acid residue of the wild type polypeptide is the N-terminal amino acid residue of the mutant, the second amino acid from the C-terminal of the wild type polypeptide is the second amino acid residue from the N-terminal, and so on till the entire wild type amino acid sequence is reversed. As will be described in further detail below, these mutants are used to verify that the identified target polypeptides are biologically relevant in the cellular environment.

[0084] 5.7 Comparison of Interaction Profiles and Functional Profiles and Rank Ordering

[0085] In the present invention, the potential targets are identified as a target or rank ordered based on the degree of correspondence between the interaction profiles and functional profiles. Some methods for comparing interaction and functional profiles are described herein. Based on the disclosure herein, other methods would be apparent to one of skill in the art.

[0086] The term "rank ordering" as used herein means the arranging of things, for example potential target polypep-

tides, in relation to one another based on one or more characteristics. Typically, in the present invention, the characteristic used for rank ordering is the degree of correspondence between the functional and interaction profiles. The terms "degree of correspondence,""correspond,""correspond closely," and their grammatical equivalents are used herein to describe the relationship between functional and interaction profiles. These terms describe the agreement, either mathematical or non-mathematical, between the functional and interaction profiles. The term "degree of correspondence" is used herein to mean the amount or level of agreement between functional and interaction profiles. The term "correspond" as used herein means that the functional and interaction profiles are in agreement, either mathematical or non-mathematical, with each other. Mathematical agreement could include a statistical agreement between the profiles, i.e. the functional and interaction profiles are not statistically different from each other. However, to correspond with each other, it is not necessary that the functional and interaction profiles be in statistical agreement. One manner of determining non-mathematical agreement is with the visual comparison technique described below. "Correspond closely" means that the functional and interaction profiles are in absolute agreement, either mathematical or non-mathematical, with each other. Absolute mathematical agreement includes a statistical agreement between the profiles. The description provided below will further clarify the meaning of these terms.

[0087] The degree of correspondence between a functional profile and an interaction profile indicates the likelihood of interaction between a potential target that corresponds to the interaction profile being compared and the candidate polypeptide. Close correspondence between a functional profile and an interaction profile indicates that the potential target that corresponds to the interaction profile being compared is a target of the candidate polypeptide.

[0088] In one embodiment of the invention, the correspondence between the functional and interaction profiles is determined by performing a visual comparison. The term "visual comparison" and its grammatical equivalents are used herein to mean an examination by sight to identify similarities or differences in certain features of the functional and interaction profiles. Typically, the features examined include the functional and interaction values of the functional and interaction profiles. The visual comparison is performed to look for similarities or differences between the functional profile for the candidate polypeptide and its mutants and the interaction profile of the candidate polypeptide and its mutants with a particular target polypeptide.

[0089] **FIG. 1** depicts four interaction profiles and one functional profile which can be compared visually to determine the degree of correspondence. The top four panels (I-IV) depict four interaction profiles for four different target polypeptides. The fifth panel (V) depicts the functional profile. The interaction profiles and functional profiles depict the interaction values and functional values, respectively, for the wild type candidate polypeptide and the three mutants. From a simple visual comparison of the interaction profiles to the functional profile of **FIG. 1**, it can be concluded that the fourth interaction profile (panel IV) corresponds closely with the functional profile.

[0090] In still another aspect of the invention, the degree of correspondence is evaluated by using difference values. In the functional profile, the difference in functional values between the candidate polypeptide and each mutant is calculated and converted into a functional difference value. Thus, for each mutant there is a number which indicates the increase or decrease in function compared to the candidate polypeptide. Similarly, in the interaction profile for each potential target, the difference in interaction values between the candidate polypeptide and each mutant is calculated and this difference is converted into an interaction difference value. For each target, the functional and interaction difference values of each mutant are compared. Each target gets a rank depending on the mathematical difference between the difference values of each mutant. The mathematical difference is used to rank order or determine the degree of correspondence. A target gets the highest rank if all the mutants have no mathematical difference between the percentage difference values. The statistical difference between the functional and interaction difference values of each mutant may also be used to rank order or determine the degree of correspondence.

[0091] In another aspect of the invention, the comparison can be performed by plotting the functional profile and the interaction profile on one graph. For a particular target, the interaction values for the candidate polypeptide and its mutants are plotted on the Y-axis. On the X-axis, the functional values from the functional profile are plotted. The ability to draw a straight line through the points on the graph for the candidate polypeptide and mutants derived therefrom, the line having a positive slope between successive points, indicates a higher degree of correspondence between the functional and interaction profiles. This graphical profile technique can be combined with a weighted categorization method. In the weighted categorization method, the mutants are categorized as L, R, N, and I based on the functional and interaction values. The range of functional and interaction values used for each category depends on the assays used. One example of the weighted categorization method is depicted in **FIG. 15**. The weighted categorization method depicted in **FIG. 15** is described below. Particularly, for a high degree of correspondence, each mutant not only falls in the same category based on both the functional and interaction values, but also the line through the points on the graph has a positive slope.

[0092] **FIGS. 2 and 4** depict a categorization method for determining the degree of correspondence between the interaction profiles and functional profiles. In this method, the interaction values of the candidate polypeptide and mutants for a particular target polypeptide are obtained. The interaction values of each mutant are compared to the interaction value for the candidate polypeptide and based on this comparison the mutants are categorized as L (L_i), R (R_i), N (N_i), or I (I_i). Similarly, the functional values of the candidate polypeptide and mutants are obtained using a functional assay. The functional values of each mutant are compared to the functional value for the candidate polypeptide and based on this comparison the mutants are categorized as L (L_f), R (R_f), N (N_f), I (I_f). The categorized mutants are then plotted as depicted in **FIG. 2**. From these plots, the degree of correspondence is determined.

[0093] In one embodiment, as depicted in **FIG. 4, a** mutant is considered as a "failure," if a two, or three step shift is observed along the linear progression from L to R to N to I. A mutant is considered as a "pass," if no shift or a one

step shift is observed along the linear progression from L to R to N to I. In **FIG. 4**, the mutants with no shift are depicted with shaded boxes that fall along the diagonal, and the mutants with one shift are depicted in the lightly shaded boxes. If every mutant in a set is categorized as a pass, the highest degree of correspondence is observed. For example, see panel II of **FIG. 2**.

[**0094**] In another embodiment, the rank ordering of the target polypeptides is performed using the following exceptions: (i) a target polypeptide is considered to have a high degree of correspondence even if a neutral mutant is a failure, as long as two of R_f, L_f, or I_f pass (see panel I of **FIG. 2**); and (ii) a target polypeptide is considered to have a high degree of correspondence even if a I_f is a failure, as long as two of R_f and L_f pass and no R_f and L_f, are failures. Also, while rank ordering the target polypeptides the following restrictions can be considered: (i) a target polypeptide is considered to have no degree of correspondence if a L_f is a failure, unless there are two other L_f that pass; (ii) a target polypeptide is considered to have no degree of correspondence if a R_f is a failure, unless two of R_f, L_f, or I_f pass; and (iii) a target polypeptide is considered to have no degree of correspondence if both R_f and L_f are failures. Thus, one method of rank ordering can be done by using the restrictions and exceptions described above. These restrictions and exceptions are depicted on **FIG. 5**.

[**0095**] As mentioned above, in addition to the L, N, R, and I mutants, scrambled peptide sequence and reversed peptide sequence mutants of the candidate polypeptide can be used in the methods of the present invention. Use of these mutants allows the confirmation that a particular target-candidate polypeptide interaction is associated with a biologically relevant function of the candidate polypeptide. If the target polypeptide binds only to the candidate polypeptide and not to the scrambled peptide sequence or reversed peptide sequence mutants, then it can be inferred that the interaction of the target polypeptide and candidate polypeptide is associated with a biologically relevant function. However, if the target polypeptide binds to both the candidate polypeptide and the scrambled peptide sequence or reversed peptide sequence mutants, then it cannot be inferred that the interaction of the target polypeptide and candidate polypeptide is associated with a biologically relevant function.

[**0096**] In one embodiment of the invention, the correspondence between the functional and interaction profiles is determined using the apparatus depicted in **FIG. 6**. **FIG. 6** illustrates an apparatus for implementing selected operations associated with the process of **FIG. 7**. The apparatus **600** includes a central processing unit **602** connected to a set of input/output devices **604** via a system bus **606**. The input/output devices **604** may include a keyboard, mouse, scanner, data port, video monitor, liquid crystal display, printer, and the like. A memory **608** in the form of primary and/or secondary memory is also connected to the system bus **606**. The components of **FIG. 6** discussed up to this point characterize a standard computer. This standard computer is programmed in accordance with the invention. In particular, the apparatus **600** is programmed to perform processing operations **704**, **706**, **708**, and **710** of **FIG. 7**.

[**0097**] As shown in **FIG. 6**, the memory **608** stores functional profile data **610**. The functional profile data is obtained according to the techniques described in connection with block **704** of **FIG. 7**. The functional profile data may be loaded into the apparatus **600** using any number of known techniques.

[**0098**] Interaction profile data **612** is also stored in memory **608**. The interaction profile data is obtained according to the techniques described in connection with block **706** of **FIG. 7**. This data may also be loaded using any number of known techniques.

[**0099**] The memory **608** also stores a comparison module **614**. The comparison module **614** includes a set of executable instructions that operate in connection with the central processing unit **602** to compare the functional profile data **610** with the interaction profile data **612**. In other words, the comparison module **614** performs the operation associated with block **708** of **FIG. 7**. The executable code of the comparison module **614** may utilize any number of numerical techniques to compare the functional profile data **610** and the interaction profile data **612**.

[**0100**] The memory **608** also stores a target selection module **616**. The target selection module **616** includes a set of executable instructions to process comparison data created by the comparison module **614**. The executable code of the target selection module **616** may be incorporated into the executable code of the comparison module **614**, but these modules are shown as being separate for the purpose of illustration. In one embodiment, the target selection module **616** includes executable instructions to provide a rank ordering of the comparison data created by the comparison module **614**. Based upon the rank ordering, a target polypeptide is selected. That is, the target selection module **616** identifies a selected target polypeptide based on the closest correspondence between functional and interaction profiles. This operation corresponds to block **710** of **FIG. 7**.

[**0101**] Following identification of a target or rank-ordering of potential targets, genomics and proteomics techniques may be employed to further confirm the interaction between the target or potential target and the candidate polypeptide. For example, the target can be sequenced and based on the sequence educated guesses can be made if the target could in fact interact with the candidate polypeptide in the biological pathway being studied. The sequence of the target can also be compared with other sequences in the art to determine whether a polypeptide known to be connected to the biological pathway being studied has been identified. Other similar techniques may be used in combination with the methods of the present invention.

[**0102**] 5.8 An Embodiment of the Invention

[**0103**] In one embodiment, a method of the invention involves identifying a candidate polypeptide and identifying the target polypeptide of the candidate polypeptide based on the degree of correspondence between the functional and interaction profiles. This embodiment is depicted in **FIG. 7**.

[**0104**] The candidate polypeptide to be evaluated is first identified with an interleukin-4 (IL-4) activity assay. In this assay, cell lines that express the IL-4 receptor and that have been transformed by a recombinant vector comprising a reporter gene operatively linked to an IL-4 inducible element are used. When IL-4 binds to the IL-4 receptor the IL-4 signaling cascade is activated, which in turn leads to the activation of the IL-4 inducible element which leads to the expression of the reporter gene. The level of reporter gene

product expressed is proportional to the activation of the IL-4 signaling cascade. The cells are infected with a retroviral library such that different populations of cells are produced that express the individual peptides in the retroviral library. The random peptides in the peptide library are tested for their potential involvement in the IL-4 signaling cascade. A peptide from the retroviral library that inhibits IL-4 induced activation of the IL-4 inducible element causes a decrease in the level of reporter gene product expressed. A peptide from the retroviral library that increases the IL-4 induced activation of the IL-4 inducible element causes an increase in the level of reporter gene product expressed. Thus, a peptide from the retroviral library may be identified as an activator or inhibitor of the IL-4 signaling pathway by measuring the level of reporter gene product expressed. Based on the needs of the study being conducted, one of the peptides of the retroviral library is selected as the candidate polypeptide (C) (block **700**).

[**0105**] Next, the candidate polypeptide undergoes mutagenesis to create a set of mutants (block **702**). In one embodiment, the dual alanine mutagenesis technique is first used to develop a series of mutants. The mutants developed in this manner are then tested using the IL-4 activity assay to determine the biological activity of the mutants in the assay. Each of the mutants are tested in the IL-4 assay and the level of reporter gene product expressed in response to the activation of the IL-4 signaling cascade is determined in the presence of each mutant. From the series of mutants three mutants are selected for the set of mutants. One of the mutants causes a significant increase in the level of reporter gene product expressed in response to activation of the IL-4 signaling cascade, compared to the candidate polypeptide. The other two mutants cause a significant decrease in the level of reporter gene product expressed, compared to the candidate polypeptide. Thus, the set comprises of one IOF mutant and two LOF mutants.

[**0106**] A functional profile, as depicted in panel V of **FIG. 1**, is prepared for the candidate polypeptide and the set of mutants (block **704**). The functional profile may be obtained by using the information obtained from the IL-4 activity assay. The functional values are depicted on the Y-axis, which in this case is the level of reporter gene product expressed in response to an IL-4 stimulus.

[**0107**] Next, a screening is performed to identify potential target polypeptides of the candidate polypeptide (block **706**). This may be accomplished by using the yeast-two hybrid assay. By using the yeast-two hybrid assay it is determined that four target polypeptides potentially interact with the candidate polypeptide. For each target polypeptide, using the same yeast-two hybrid assay, the interaction values, i.e., level of transcriptionally competent complex formed as a result of an interaction, of candidate polypeptide and the set of mutants for the target polypeptide is determined. The interaction values of the candidate polypeptide and the set of mutants for each target polypeptide are then used to develop interaction profiles as shown in **FIG. 1**. The interaction values are depicted on the Y-axis.

[**0108**] Next, to identify a target polypeptide, the degree of correspondence between the interaction profiles and the functional profile is determined (block **708**). Based on this correspondence value, a target polypeptide is identified (block **710**). In one embodiment, this is performed by a visual comparison of each interaction profile to the functional profile. As can be seen from **FIG. 1**, the interaction profile in panel IV has the highest degree of correspondence with the functional profile in panel V. That is, for all three the mutants the functional values closely matches the interaction value to the target polypeptide in the interaction assay. Hence, the polypeptide depicted in panel IV of **FIG. 1** is identified as the target polypeptide.

[**0109**] Although this invention has been described for a candidate polypeptide, it will be apparent to one of skill in the art that the methods of the present invention can be used for candidate nucleotides and candidate inorganic molecules. Also, the methods of this invention can be used for target nucleotides and target inorganic molecules.

6. EXAMPLE

[**0110**] 6.1 Identification of Peptide CL06wt as the Candidate Polypeptide from a Random Library of Peptide 20-mers

[**0111**] Peptide CL06wt peptide was identified by screening a library of random peptide 20-mers for the ability to inhibit IL-4 induced germline ϵ promoter transcription using the HBEGF2a/diphtheria dual reporter phenotypic screening system described in WO 01/31232. To construct the random library, A5T4 reporter cells (described in more detail below) were infected with an infectious retroviral library of random peptide 20-mers (prepared as described in WO 97/27213; see also WO 01/34806 at page 39, line 36 through page 40, line 19). The vector used includes a reporter gene encoding blue fluorescent protein (BFP) fused upstream of the region encoding the random peptide via a linker region encoding an α-helical peptide linker (expression fusion product is referred to as "BFP-peptide"). Expression of the BFP-peptide product is controlled by a promoter sensitive to the tetracyline-regulated transactivator such that expression of the BFP-peptide is regulated by tetracycline (Tet) or doxycycline (Dox). See U.S. Patent Application entitled "Methods and Compositions for Screening for Altered Cellular Phenotypes", Attorney Docket No. A-711587/RMS/AXG, filed on Mar. 8, 2002. The BFP reporter gene provides a rapid phenotypic assay to determine whether cells were infected: infected cells express BFP-peptide and fluoresce blue (phenotype BFP^+), uninfected cells do not express BFP-peptide, and do not fluoresce blue (phenotype BFP^-). To reduce the number of stop codons, the region of the vector encoding the random peptide was of the sequence $(NNK)_{20}$, where each N independently represents A, T, C or G and K represents T or G. The library was also biased to account for degeneracy in the genetic code.

[**0112**] The A5T4 reporter cell was engineered from BJAB B-cells (Menezes et al., 1975, *Biomedicine*, 22(4):276-284; Source: Yoshinobu Matsuo, PhD., Fujisaki Cell Center, Hayashibara Biochemical Labs, Inc., 675-1 Fujisaki, Okayama 702-8006, Japan) and includes a reporter gene encoding the HBEGF2a/GFP dual function reporter positioned downstream of an engineered 600 bp IL-4 responsive fragment of the germline ϵ promoter (WO 99/58663) such that the dual function reporter is driven by the germline ϵ promoter. When expressed, the dual function reporter cleaves into two pieces: a heparin-binding epidermal growth factor-like growth factor (HBEGF) and a green fluorescent protein (GFP), via the self-cleaving 2a sequence (WO

99/58663). In this reporter system, cells ectopically expressing HBEGF are capable of translocating diphtheria toxin (DT) into their cytoplasm, leading to rapid, acute cytotoxicity. Cells that do not express HBEGF are spared this fate and continue to survive even in the presence of high concentrations of DT. The A5T4 reporter cell line was further engineered to express the tetracylcine-regulated transactivator (tTA), allowing for regulation of peptide library expression with Tet or Dox. See U.S. Patent Application entitled "Methods and Compositions for Screening for Altered Cellular Phenotypes", Attorney Docket No. A-711587/RMS/AXG, filed on Mar. 8, 2002. Thus, according to this dual phenotypic reporter system, unstimulated control cells express a random peptide fluoresce blue (BFP^+). Following stimulation with IL-4, BFP^+ cells expressing a non-inhibitory peptide fluoresce green and, in addition are sensitive to DT. Stimulated BFP^+ cells expressing an inhibitory peptide do not fluoresce green and are not DT sensitive. The toxin-conditional selection and Tet or Dox-controlled peptide expression features of the A5T4 screening line are illustrated in **FIGS. 8A & 8B**, respectively.

[**0113**] Following infection, the library was enriched for cells expressing peptides that inhibit IL-4 induced germline ϵ transcription as generally outlined in the top half of **FIG. 9** and sorted by FACS into single cell clones. The clones were then screened as generally illustrated in the lower half of **FIG. 9**. Briefly, for screening, each clone was divided into two populations and one population was treated with Dox (10 ng/ml). After 5 days, both populations were stimulated with IL-4 (final conc. 60 U/mL; PeptroTech, Inc.) and, after 3 more days, both populations were analyzed by FACS to measure BFP and GFP fluorescence. FACS data were converted to reporter ratios, which are defined as the ratio of the geometric mean of GFP fluorescence of the +IL-4/−Dox and +IL-4/+Dox populations. Cells expressing a peptide that inhibits germline ϵ transcription have reporter ratios of ≧1.1. A reporter ratio of ≧1.2 is indicative of strong inhibition.

[**0114**] The sequences of peptides expressed by positive clones (reporter ratios of ≧1.1) were obtained by RT-PCR amplification of the integrated peptide-expressing sequences. In this experiment, of 2.4×10^9 A5T4 cells infected, 218 positive clones were identified, 199 of which were unique. From this same experiment, 155 total clones with a reporter ratio of ≧1.19 were identified, 136 of which were unique. Clone CL06, which encodes peptide CL06wt, was amongst the positive clones identified (clone CL06 had a reporter ration of 1.3).

[**0115**] 6.2 Clone CL06 Transfers its Phenotype Into Naïve Cells

[**0116**] The ability of peptide CL06wt to inhibit germline ϵ promoter transcription was confirmed in naïve cells. Briefly, Phoenix cells were transfected with a retroviral vector encoding a BFP-peptide CL06wt fusion as described in WO 97/27213. Naïve A5T4 cells were infected with the resultant virions and grown for 3 days. The infected cells were stimulated with IL-4 (60 U/mL) and, after 3 days, the cells were assessed by FACS for BFP and GFP. The FACS data are presented in **FIG. 10**. In **FIG. 10**, Panel A provides the BFP fluorescence. As illustrated in **FIG. 10**, there are two populations of cells: cells that express BFP-peptide (BFP^+) and cells that do not (BFP^-). The GFP fluorescence data corresponding to the BFP^+/BFP^- populations are presented in Panel B. The reporter ratio is determined as illustrated in panel C of **FIG. 10** ("geo mean" refers to the geometric mean).

[**0117**] 6.3 Peptide CL06wt Inhibits Trascription of an Endogenous Germline ϵ Promoter

[**0118**] The ability of peptide CL06wt to inhibit transcription of an endogenous germline ϵ promoter was confirmed using a TAQMAN® assay (Roche Molecular, Alameda, Calif.). Briefly, A5T4 cells were infected with virions capable of expressing peptide CL06wt (prepared as described above). Infected cells were divided into two populations. One population was exposed to Dox (10 ng/ml). Both populations were stimulated with IL-4 (60 U/ml). After 3 days, the cells were pelleted and the pellets assayed for ϵ promoter transcription using a TAQMAN assay performed as described in Applied Biosystems Protocol 4310299 (available at http:\\www.appliedbiosystems.com). The primers and probe used were as follows (the probe was labeled at the 5'-end with Fam and at the 3'-end with Tamra):

```
ϵ forward primer:
ATCCACAGGCACCAAATGGA          (SEQ ID NO:7)

ϵ reverse primer:
GGAAGACGGATGGGCTCTG           (SEQ ID NO:8)

ϵ probe:
ACCCGGCGCTTCAGCCTCCA          (SEQ ID NO:9)
```

[**0119**] The measured Taqman ϵ inhibition ratio, defined as the ratio of the relative expression units of +IL-4/−Dox to +IL-4/+Dox cells, was 1.37 (average of 3 values; p=0.002), indicating that peptide CL06wt strongly inhibits the endogenous germline ϵ promoter.

[**0120**] 6.4 Peptide CL06wt is Selective for the Germline ϵ Promoter

[**0121**] To demonstrate selectivity for the germline ϵ promoter, peptide CL06wt was tested for inhibition of germline α transcription. The assay was similar to that described in the immediately preceding section, except that ST486 cells (ATCC # CRL-1647) engineered to express the tetracycline-regulated transactivator were infected and the infected cells were stimulated with TGF-β (Preprotech, 40 ng/ml). The primers and probe used were as follows (the probe was labeled at the 5'-end with Fam and at the 3'-end with Tamra):

```
α forward primer:
CAGCACTGCGGGCCC               (SEQ ID NO:10)

α reverse primer:
TCAGCGGGAAGACCTTGG            (SEQ ID NO:11)

α probe:
CCAGCAGCCTGACCAGCATCCC        (SEQ ID NO:12)
```

[**0122**] The measured Taqman α inhibition ratio was 0.93 (average of 3 values; p=0.6867), indicating that peptide CL06wt (SEQ ID NO:1) does not inhibit transcription of the germline α promoter. These data confirm that peptide CL06wt (SEQ ID NO:1) is a selective inhibitor of germline ϵ transcription.

[0123] 6.5 Peptide CL06wt Mediates its Inhibitory Action by Interacting with Retinoid X Receptor-β

[0124] 6.5.1 Screening for Potential Target Polypeptides for Peptide CL06wt

[0125] The potential target polypeptides for peptide CL06wt were screened for in a β-galactosidase yeast two-hybrid (YTH) assay using peptide CL06wt as bait and a cDNA library constructed from the A5T4 reporter cell line as prey. Interaction was assessed by β-galactosidase quantification using BetaFluor (Novagen) as a substrate. A negative interaction control (no cDNA fused downstream of the GAL4 activation domain sequence). A general outline of the YTH assay is illustrated in **FIG. 11A**. Following clustering, filtering to remove non-specific bait hits (e.g., GFP and BFP), singletons and clusters recognized by 10 or more cDNA baits based upon historical YTH assays, and prioritization, four prey clones were identified as potential targets, designated CL06-nonstim-7, CL06-stim-14, CL06-stim-15 and CL06-stim-2. Sequence searching revealed that the CL06-stim-2 clone encoded the following polypeptide:

[0126] CL06-stim-2: gi|542994|pir||S37781 retinoid X receptor beta [human]

[0127] Potential targets identified in the YTH assay were reconfirmed as generally outlined in **FIG. 12**.

[0128] 6.5.2 Confirmation that Peptide CL06wt Mediates its Inhibitory Action by Interacting with Retinoid X Receptor-β

[0129] Confirmation of colony CL06-stim-2 (hRXR-β) as the target polypeptide for peptide CL06wt was confirmed using functional and interaction profiles. The functional profile was obtained using the A5T4 reporter cell line and the interaction profiles were obtained using the YTH assay and compared for correspondence. The main concept underlying this profiling method is that mutants will tend to act the same way in both the functional assay and an interaction assay with the target polypeptide of the wild-type peptide. That is, a mutant that exhibits an increase in function (as compared to the wild-type peptide) in the functional assay will exhibit an increase in interaction (as compared to the wild-type peptide) in a YTH assay with the target polypeptide of the wild-type peptide. Stated another way, the target polypeptide will yield an interaction profile that corresponds closely to the functional profile when compared visually or by other means.

[0130] The functional profiles for a library of mutants derived from CL06wt was obtained by constructing and screening for activity in the A5T4 reporter cell line, in the manner described above. The activity of each mutant at the germline ϵ promoter is reflected in the reporter ratio, described in panel C of **FIG. 10**. The reporter ratios were the functional values that were used to develop the functional profiles.

[0131] TABLE 1 depicts the amino acid sequences (the dual alanine mutations are underlined) of the mutants tested and the measured reporter ratios when screening in the A5T4 reporter line.

TABLE 1

Peptide Name	Peptide Sequence	Reporter Ratio	SEQ ID NO
CL06wt	M S L C S Y A H S Y A A	1.30	(SEQ ID NO:1)
CL06MS	AA L C S Y A H S Y A A	1.56	(SEQ ID NO:2)
CL06LC	M S AA S Y A H S Y A A	1.06	(SEQ ID NO:3)
CL06SY1	M S L C AA A H S Y A A	1.09	(SEQ ID NO:4)
CL06AH	M S L C S Y A A S Y A A		(SEQ ID NO:5)
CL06SY2	M S L C S Y A H AA A A		(SEQ ID NO:6)

[0132] The interaction of CL06wt peptide and its mutants with the clones identified as potential targets for the peptide CL06wt was then quantified in a β-galactosidase YTH assay. The YTH assay was performed in the manner described above. A general outline of this YTH assay is illustrated in **FIG. 11B**. The functional profile was developed by comparing the (β-galactosidase activity of each mutant to the wild type peptide.

[0133] Next, the interaction and functional profiles were compared for correspondence using three different techniques. In one technique, the comparison was performed using graphical representation of the YTH interaction profile as depicted in **FIG. 13**. In **FIG. 13**, the bar graphs illustrate the fluorescence data for CL06wt and its mutants, for each identified potential target. The functional profile is depicted as the listing of functional category (described below) and reporter ratios beneath each peptide. A visual inspection of these profiles indicates that the interaction profile of CL06-stim-2 (hRXR-β) most closely corresponds to the functional profile of CL06 wt and mutants derived therefrom.

[0134] In a second technique, the comparison was performed by categorizing the mutants of CL06wt based on the functional and interaction assays. Based on the reporter ratio, described in panel C of **FIG. 10**, each mutant was categorized into one of four functional categories: (1) reduction of function (ROF); (2) loss of function (LOF); (3) increase of function (IOF) or (4) neutral (N). As mentioned previously, cells expressing a mutant that inhibits germline ϵ transcription have reporter ratios of $\geqq 1.1$. A cell expressing a loss of function mutant has a reporter ratio of <1.11. An increase of function mutant shows a >50% increase in reporter function and a loss of function of mutant shows a >50% decrease in reporter ratio. The criteria for this functional categorization are depicted in panel D of **FIG. 10**. Using this criteria, peptide CL06MS (SEQ ID NO:2) was designated an IOF mutant, and peptides CL06LC (SEQ ID NO:3) and CL06SY1 (SEQ ID NO:4) were designated as LOF mutants.

[0135] The interaction of these IOF and LOF mutants with different polypeptides encoded by the clones CL06-nonstim-7, CL06-stim-14, CL06-stim-15 and CL06-stim-2 were quantified using the YTH assay described above. Based on the YTH assay, the mutants were categorized into the following four interaction categories: (1) reduction of interaction (ROI); (2) loss of interaction (LOI); (3) increase of interaction (IOI); and (4) neutral (N), using the criteria illustrated in **FIG. 5**.

[0136] Based on these functional and interaction categorizations, a graphic profile representation as depicted in **FIG. 14** was obtained. In such a graphic profile represen-

tation, profiles that correspond have shaded boxes that fall along the diagonal (i.e., mutants that are IOF mutants in the functional assays are IOI mutants in the interaction assay). Thus, graphic profiles of the target polypeptide, i.e., true interacting partner, for the wild-type peptide will have shaded boxes falling along the diagonal. Graphic profiles that do not show correspondence will have shaded boxes in the cells marked "fail," as depicted in **FIG. 4**. Graphic profiles that show some degree of correspondence have shaded boxes in the lightly-shaded cells of **FIG. 14**. Criteria for determining correspondence using this graphic representation is presented in **FIG. 5**. Graphic profiles for CL06wt mutants for the four potential targets are depicted in **FIG. 14**. Based on the criteria in **FIG. 5**, it was concluded that the profile of CL06-stim-2(hRXR-β) most closely corresponds to the functional profile of CL06wt and mutants derived therefrom.

[**0137**] These graphical comparisons may be implemented using the apparatus of **FIG. 6**. In such an embodiment, standard techniques are used to convert shade values to corresponding numerical values that are processed by the comparison module **1614** and the target selection module **1616**. Alternately, the apparatus of **FIG. 6** may be used to produce the shade data that is visually inspected. For example, the output devices of the input/output devices **1604** may be used to display visual shade data.

[**0138**] In a third technique, the comparison was performed using a weighted categorization process as depicted in **FIG. 15**. In **FIG. 15**, along the X-axis is plotted the functional profile and the interaction profile is plotted along the Y-axis. On the X-axis, the reporter ratios of CL06wt and its three mutants are plotted. On the Y-axis, the β-galactosidase signal from the YTH assay is plotted for CL06wt and the three mutants. The X-axis is then categorized into L (loss of function), R (reduction of function), N (neutral), and I (increase of function) based on the reporter ratios as described in panel D of **FIG. 10**. A ratio of <1.11 is categorized as an L and N includes ratios ±50% of CL06wt's ratio. A ratio that is greater than +50% of CL06wt's ratio is I. R includes ratios greater than 1.11 and ratios less than −50% of CL06wt's ratio. Similarly, the Y-axis is categorized based on the criteria in **FIG. 5**. In such a graphic profile, profiles that correspond closely have interaction values (in this case, β-galactosidase signal) and functional values (in this case, reporter ratios) that fall along a line with a positive slope. Also, close correspondence is indicated when the mutants are categorized in the same category using both the reporter ratio and the β-galactosidase signal, i.e., a mutant is categorized as a L based on the reporter ratio and the same mutant is also categorized as a L based on the β-galactosidase signal. In this case, the interaction and functional profiles for S2 (hRXR-β) falls along a line with a positive slope. Also, for S2 (hRXR-β) each of the three mutants fall into the same category based on both reporter ratio and β-galactosidase signal.

[**0139**] The plot in **FIG. 15** may be generated using the apparatus of **FIG. 6**. Alternately, the apparatus of **FIG. 6** may be used to perform computations corresponding to the processing of the information within **FIG. 15**.

[**0140**] Based on the close correspondence observed between the interaction and functional profiles using the three different techniques, hRXR-β was identified as a target for CL06wt.

[**0141**] 6.6 Retinoids Inhibit Germline ϵ Promoter in the A5T4 Reporter Line

[**0142**] IL-4 stimulated A5T4 reporter cells were contacted with varying concentrations of retinoids a-tRA and 13-cRA (0.03 μM, 0.3 μM, 3 μM and 30 μM) and assessed for inhibition of germline ϵ promoter with the dual reporter system described above. DMSO and a compound known to inhibit germline ϵ promoter were tested as controls. Both retinoids and the positive control inhibited germline ϵ promoter in a dose-dependent manner. DMSO did not appreciably inhibit germline ϵ promoter at any of the concentrations tested. These data demonstrate that an agonist of RXR inhibits IL-4 induced germline ϵ promoter.

[**0143**] While the invention has been described by reference to various specific embodiments, skilled artisans will recognize that numerous modifications may be made thereto without departing from the spirit and the scope of the appended claims.

[**0144**] All references cited throughout the disclosure are incorporated herein by reference in their entireties for all purposes.

What is claimed is:

1. A method of rank ordering potential targets of a candidate polypeptide, comprising:

(a) comparing a functional profile for a set of polypeptides, wherein said set of polypeptides comprises the candidate polypeptide and a set of mutants derived therefrom, and a plurality of interaction profiles for the set of polypeptides to identify those interaction profiles that correspond to said functional profile, wherein each interaction profile corresponds to a potential target for the candidate polypeptide; and

(b) creating a rank order of said potential targets based upon the degree of correspondence between each interaction profile and functional profile, wherein the rank order of a potential target identifies the likelihood of interaction between the potential target and candidate polypeptide.

2. The method of claim 1, wherein comparing comprises:

(a) from the functional profile, obtaining a difference between each mutant functional value and candidate polypeptide functional value to obtain a series of functional difference values;

(b) from the interaction profile of each potential target, obtaining a difference between each mutant interaction value and candidate polypeptide interaction value to obtain a series of interaction difference values; and

(c) for each potential target, producing a set of difference values, wherein each difference value is the difference between the functional difference value and corresponding interaction difference value, thereby identifying those interaction profiles that correspond to the functional profile based on the difference values.

3. The method of claim 2, wherein (c) comprises:

determining the statistical difference between the functional difference values and corresponding interaction difference values.

4. The method of claim 1, wherein comparing comprises:

selecting those interaction profiles that correspond to the functional profile based on visual comparison of the functional values in the functional profile with corresponding interaction values in the interaction profile of each potential target.

5. The method of claim 1, further comprising identifying the candidate polypeptide in a functional assay.

6. The method of claim 5 further comprising identifying the candidate polypeptide in an interleukin-4 activity assay.

7. The method of claim 5, wherein the mutants are dual alanine mutants; the interaction profiles are identified in a yeast two hybrid assay; and the functional assay is an interleukin-4 activity assay.

8. The method of claim 1, wherein the mutants comprising the set of mutants are dual alanine mutants.

9. The method of claim 1, wherein the set of mutants comprises at least two mutants selected from the group consisting of loss of function, reduction of function, and increase of function mutants.

10. The method of claim 1, wherein the set of mutants comprises scrambled sequence and reversed peptide sequence mutants.

11. A method of rank ordering potential targets of a candidate polypeptide comprising:

(a) generating a functional profile for a set of polypeptides comprising the candidate polypeptide and a set of mutants derived therefrom;

(b) generating a plurality of interaction profiles for the set of polypeptides, wherein each interaction profile corresponds to a potential target for the candidate polypeptide; and

(c) creating a rank order of the potential targets based upon the degree of correspondence between each interaction profile and functional profile, wherein the rank order of a potential target identifies the likelihood of interaction between the potential target and candidate polypeptide.

12. The method of claim 11, further comprising identifying the potential targets in an interaction assay.

13. The method of claim 11, further comprising identifying the candidate polypeptide in a functional assay.

14. The method of claim 13, further comprising identifying the candidate polypeptide in an interleukin-4 activity assay.

15. The method of claim 13, wherein the mutants are dual alanine mutants; the interaction profiles are identified in a yeast two hybrid assay; and the functional assay is an interleukin-4 activity assay.

16. The method of claim 11, wherein the mutants comprising the set of mutants are dual alanine mutants.

17. The method of claim 11, wherein the set of mutants comprises at least two mutants selected from the group consisting of loss of function, reduction of function, and increase of function mutants.

18. The method of claim 11, wherein the set of mutants comprises scrambled sequence and reversed peptide sequence mutants.

19. The method of claim 11, wherein the interaction profiles are identified in a yeast two hybrid assay.

20. A method of rank ordering potential targets of a candidate polypeptide, comprising:

(a) identifying a set of potential targets for the candidate polypeptide in an interaction assay;

(b) generating a functional profile for a set of polypeptides comprising the candidate polypeptide and a set of mutants derived therefrom;

(c) for each potential target identified in (a), generating an interaction profile for the set of polypeptides;

(d) selecting those interaction profiles that correspond to the functional profile; and

(e) creating a rank order of said potential targets based upon the degree of correspondence between each interaction profile and functional profile, wherein the rank order of a potential target identifies the likelihood of interaction between the potential target and candidate polypeptide.

21. The method of claim 20, wherein selecting comprises:

(a) from the functional profile, obtaining a difference between each mutant functional value and candidate polypeptide functional value to obtain a series of functional difference values;

(b) from the interaction profile of each potential target, obtaining a difference between each mutant interaction value and candidate polypeptide interaction value to obtain a series of interaction difference values; and

(c) for each potential target, producing a set of difference values, wherein each difference value is the difference between the functional difference value and corresponding interaction difference value, thereby identifying those interaction profiles that correspond to the functional profile based on the difference values.

22. The method of claim 21, wherein (c) comprises:

determining the statistical difference between the functional difference values and their respective interaction difference values.

23. The method of claim 20, wherein selecting comprises:

selecting those interaction profiles that correspond to the functional profile based on visual comparison of the functional values in the functional profile with corresponding interaction values in the interaction profile of each target.

24. The method of claim 20, further comprising identifying the candidate polypeptide in a functional assay.

25. The method of claim 24, further comprising identifying the candidate polypeptide in an interleukin-4 activity assay.

26. The method of claim 24, wherein the mutants are dual alanine mutants; the interaction profiles are identified in a yeast two hybrid assay; and the functional assay is an interleukin-4 activity assay.

27. The method of claim 20, wherein the mutants comprising the set of mutants are dual alanine mutants.

28. The method of claim 20, wherein the set of mutants comprises at least two mutants selected from the group consisting of loss of function, reduction of function, and increase of function mutants.

29. The method of claim 20, wherein the set of mutants comprises scrambled sequence and reversed peptide sequence mutants.

30. The method of claim 20, wherein the interaction assay is a yeast two hybrid assay.

31. A method of identifying a target of a candidate polypeptide comprising:

(a) identifying a set of potential targets for the candidate polypeptide in an interaction assay;

(b) generating a functional profile for a set of polypeptides comprising the candidate polypeptide and a set of mutants derived therefrom;

(c) for each potential target identified in (a), generating an interaction profile for the set of polypeptides;

(d) identifying an interaction profile that corresponds closely to the functional profile, thereby identifying the potential target that corresponds to the identified interaction profile as being a target of the candidate polypeptide.

32. The method of claim 31, further comprising identifying the candidate polypeptide in a functional assay.

33. The method of claim 32, further comprising identifying the candidate polypeptide in an interleukin-4 activity assay.

34. The method of claim 32, wherein the mutants are dual alanine mutants; the interaction profiles are identified in a yeast two hybrid assay; and the functional assay is an interleukin-4 activity assay.

35. The method of claim 31, wherein the mutants comprising the set of mutants are dual alanine mutants.

36. The method of claim 31, wherein the set of mutants comprises at least two mutants selected from the group consisting of loss of function, reduction of function, and increase of function mutants.

37. The method of claim 31, wherein the set of mutants comprises scrambled sequence and reversed peptide sequence mutants.

38. The method of claim 31, wherein the interaction assay is a yeast two hybrid assay.

39. A computer implemented method of rank ordering potential targets of a candidate polypeptide, comprising:

(a) comparing a functional profile for a set of polypeptides, wherein said set of polypeptides comprises the candidate polypeptide and a set of mutants derived therefrom, and a plurality of interaction profiles for the set of polypeptides to identify those interaction profiles that correspond to said functional profile, wherein each interaction profile corresponds to a potential target for the candidate polypeptide; and

(b) creating a rank order of said potential targets based upon the degree of correspondence between each interaction profile and functional profile, wherein the rank order of a potential target identifies the likelihood of interaction between the potential target and candidate polypeptide.

40. The method of claim 39, wherein comparing comprises:

(a) from the functional profile, obtaining a difference between each mutant functional value and candidate polypeptide functional value to obtain a series of functional difference values;

(b) from the interaction profile of each potential target, obtaining a difference between each mutant interaction value and candidate polypeptide interaction value to obtain a series of interaction difference values; and

(c) for each potential target, producing a set of difference values, wherein each difference value is the difference between the functional difference value and corresponding interaction difference value, thereby identifying those interaction profiles that correspond to the functional profile based on the difference values.

41. The method of claim 40, wherein (c) comprises:

determining the statistical difference between the functional difference values and corresponding interaction difference values.

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