US 20050100934A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0100934 A1**

Lee et al. (43) **Pub. Date: May 12, 2005**

(54) **METHOD FOR ASSAYING PROTEIN-PROTEIN INTERACTION**

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(21) Appl. No.: **10/888,313**

(22) Filed: **Jul. 9, 2004**

Related U.S. Application Data

(60) Provisional application No. 60/566,113, filed on Apr. 27, 2004. Provisional application No. 60/511,918, filed on Oct. 15, 2003. Provisional application No. 60/485,968, filed on Jul. 9, 2003.

Publication Classification

(51) **Int. Cl.**[7] **C12Q 1/68**; C12N 15/85

(52) **U.S. Cl.** **435/6**; 435/455

(57) **ABSTRACT**

The invention relates to a method for determining if a test compound, or a mix of compounds, modulates the interaction between two proteins of interest. The determination is made possible via the use of two recombinant molecules, one of which contains the first protein a cleavage site for a proteolytic molecules, and an activator of a gene. The second recombinant molecule includes the second protein and the proteolytic molecule. If the test compound binds to the first protein, a reaction is initiated whereby the activator is cleaved, and activates a reporter gene.

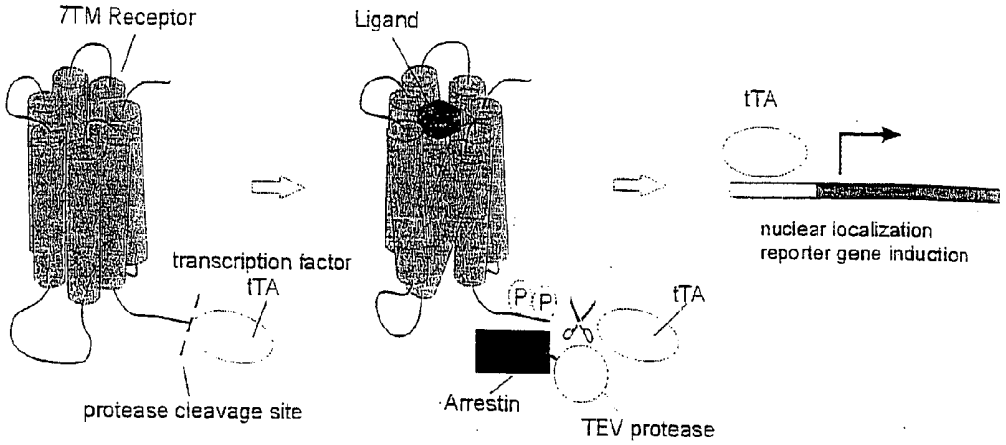
7TM Receptor
Ligand
tTA
transcription factor
tTA
nuclear localization
reporter gene induction
P
P
tTA
protease cleavage site
Arrestin
TEV protease

Fig. 1

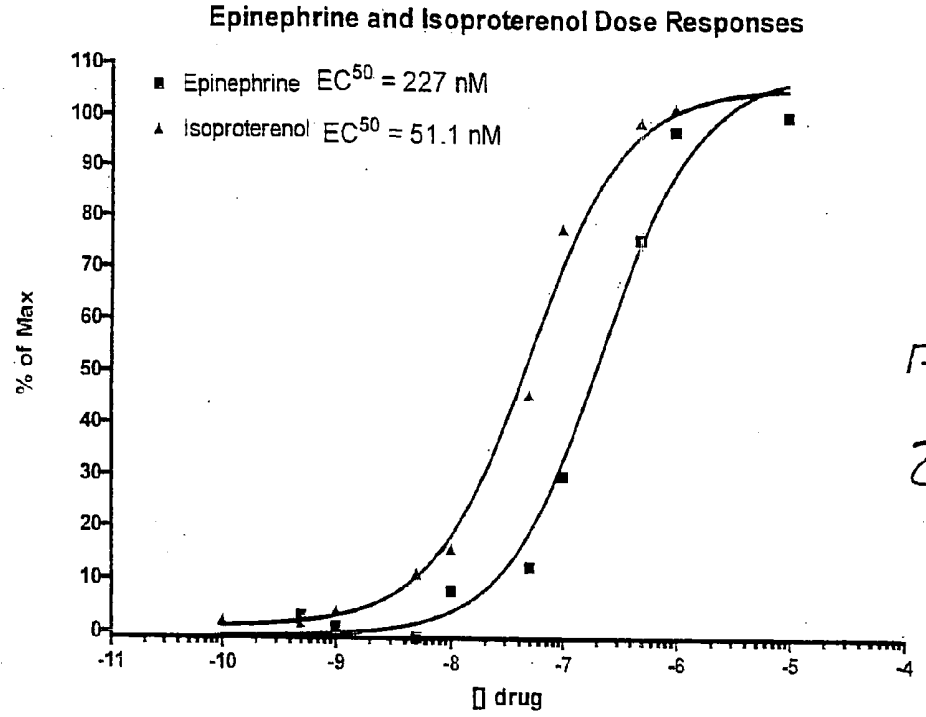
Epinephrine and Isoproterenol Dose Responses
Epinephrine EC50 = 227 nM
Isoproterenol EC50 = 51.1 nM
% of Max
[] drug

Fig 2a

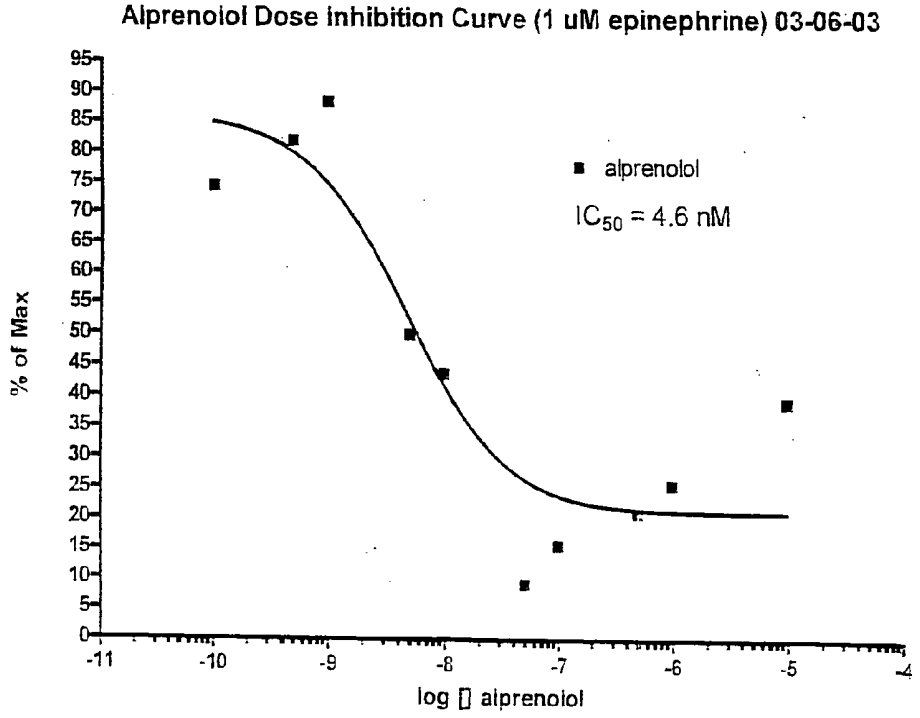
Alprenolol Dose Inhibition Curve (1 uM epinephrine) 03-06-03
alprenolol
IC50 = 4.6 nM
% of Max
log [] alprenolol

Fig 2b

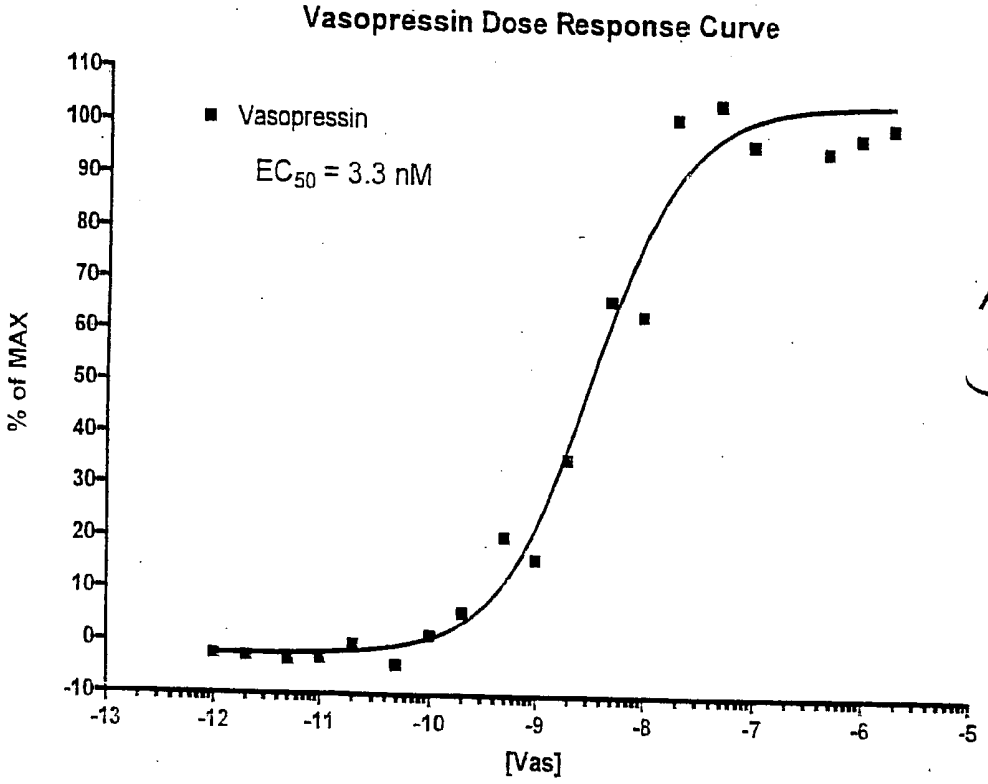
Vasopressin Dose Response Curve
Vasopressin
EC50 = 3.3 nM
% of MAX
110
100
90
80
70
60
50
40
30
20
10
0
-10
-13
-12
-11
-10
-9
-8
-7
-6
-5
[Vas]

Fig 3

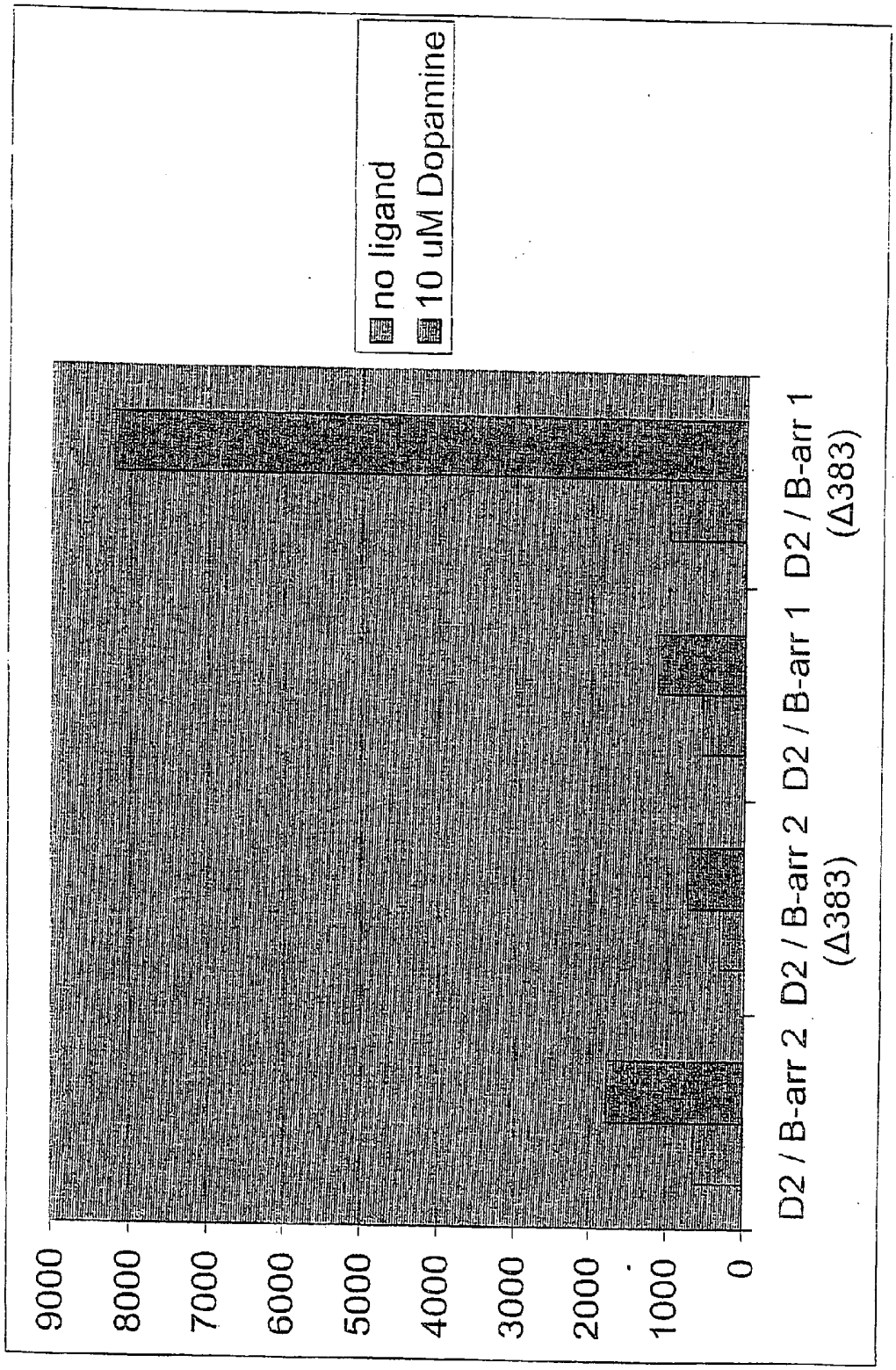
no ligand
10 uM Dopamine
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
D2 / B-arr 2 (Δ383)
D2 / B-arr 2
D2 / B-arr 1
D2 / B-arr 1 (Δ383)

Figure 4

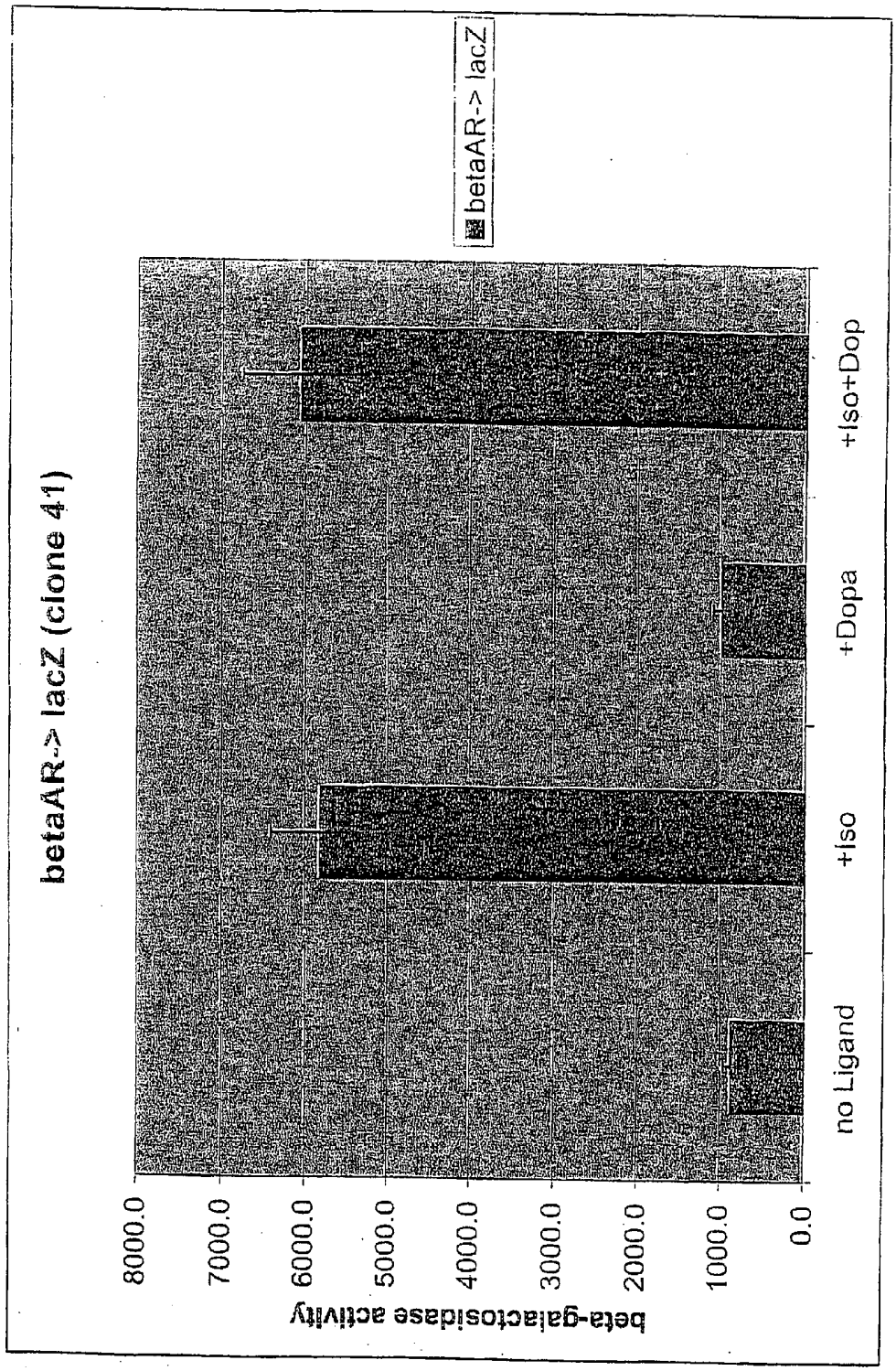
betaAR-> lacZ (clone 41)
betaAR-> lacZ
beta-galactosidase activity
8000.0
7000.0
6000.0
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
no Ligand
+Iso
+Dopa
+Iso+Dop

Figure 5A

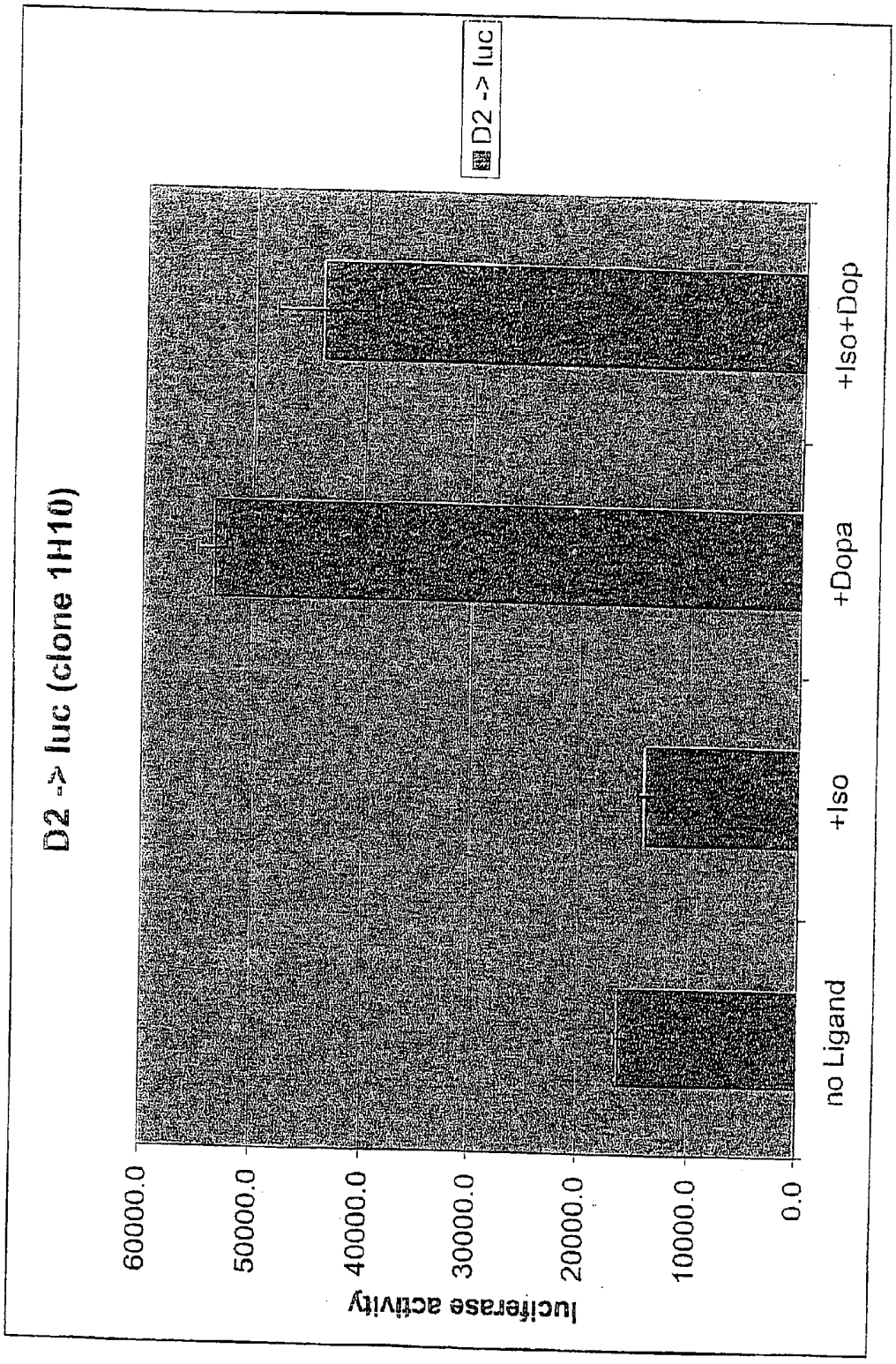
D2 -> luc (clone 1H10)
D2 -> luc
luciferase activity
60000.0
50000.0
40000.0
30000.0
20000.0
10000.0
0.0
no Ligand
+Iso
+Dopa
+Iso+Dop

Figure 5B

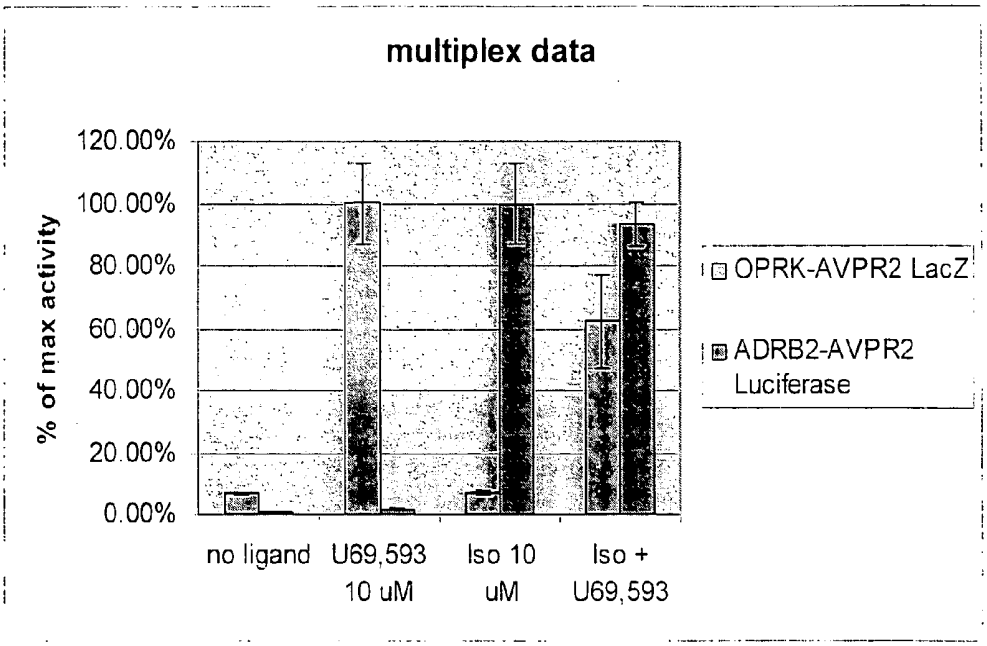
multiplex data
% of max activity
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
no ligand
U69,593 10 uM
Iso 10 uM
Iso + U69,593
OPRK-AVPR2 LacZ
ADRB2-AVPR2 Luciferase

Fig. 6

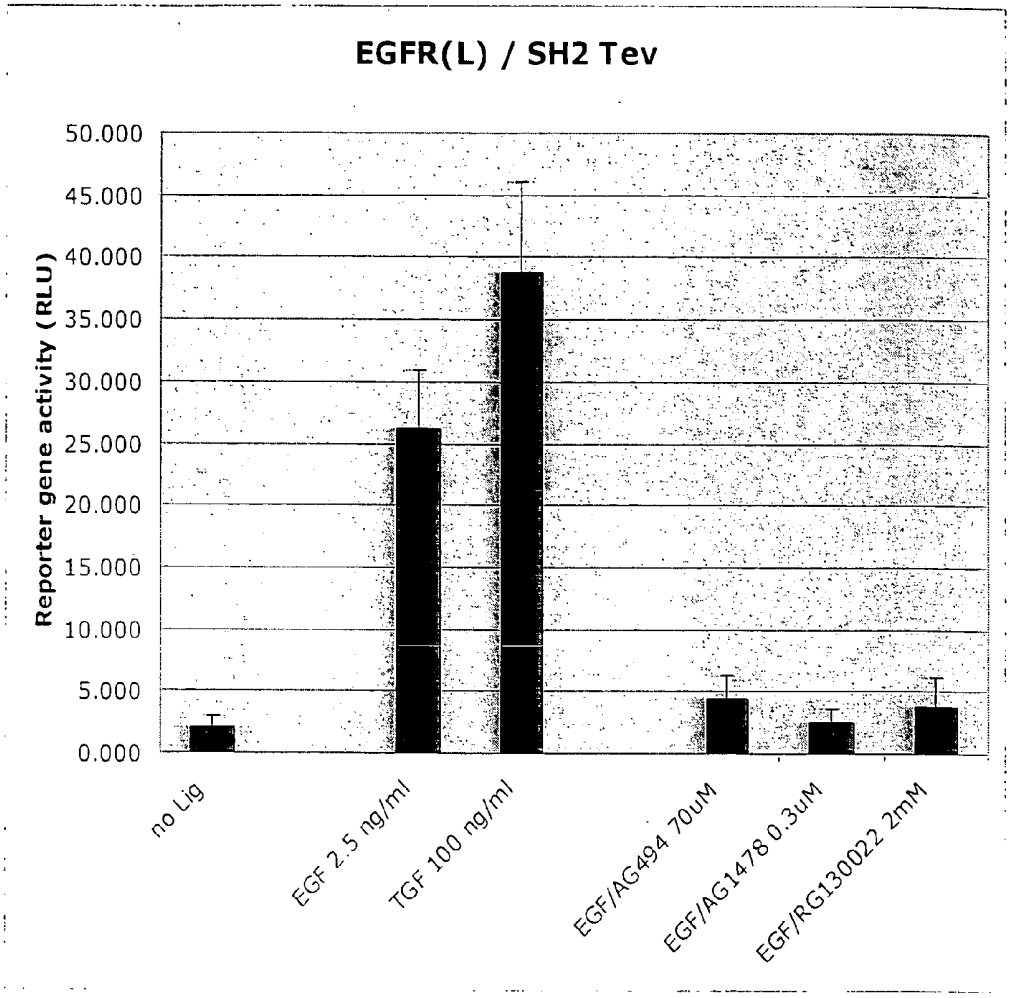
EGFR(L) / SH2 Tev
Reporter gene activity (RLU)
50.000
45.000
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
no Lig
EGF 2.5 ng/ml
TGF 100 ng/ml
EGF/AG494 70uM
EGF/AG1478 0.3uM
EGF/RG130022 2mM

Fig. 7

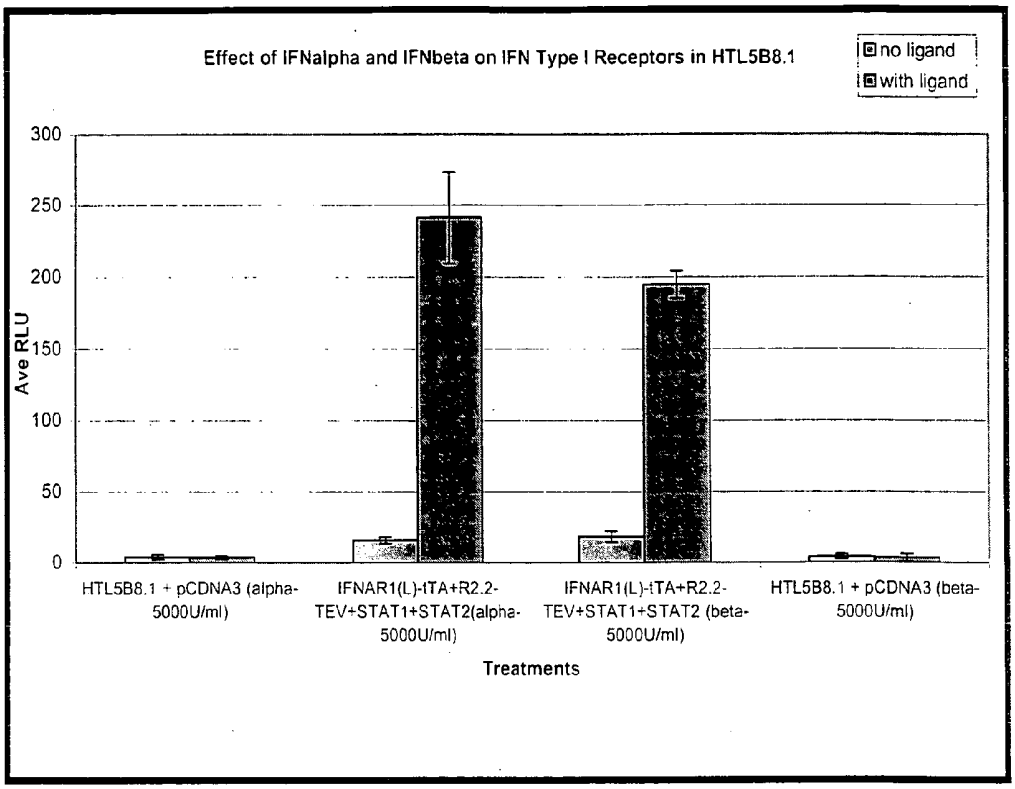
Effect of IFNalpha and IFNbeta on IFN Type I Receptors in HTL5B8.1
no ligand
with ligand
Ave RLU
300
250
200
150
100
50
0
HTL5B8.1 + pCDNA3 (alpha-5000U/ml)
IFNAR1(L)-tTA+R2.2-TEV+STAT1+STAT2(alpha-5000U/ml)
IFNAR1(L)-tTA+R2.2-TEV+STAT1+STAT2 (beta-5000U/ml)
HTL5B8.1 + pCDNA3 (beta-5000U/ml)
Treatments

Fig. 8

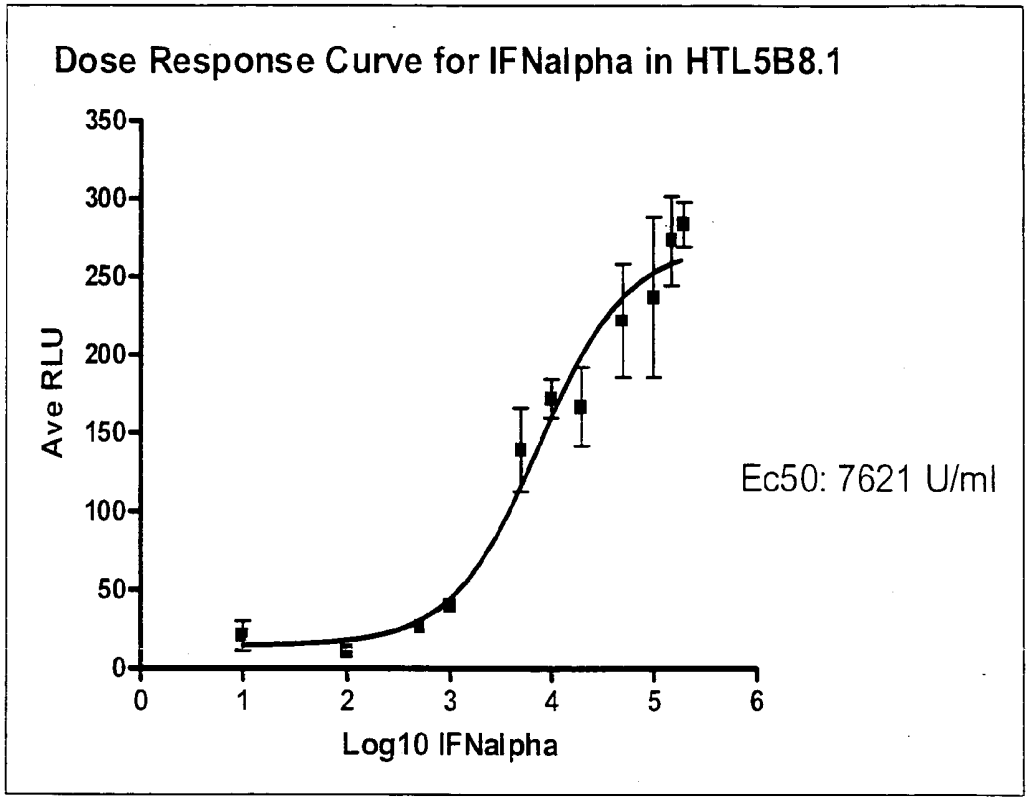
Dose Response Curve for IFNalpha in HTL5B8.1
Ave RLU
350
300
250
200
150
100
50
0
0
1
2
3
4
5
6
Log10 IFNalpha
Ec50: 7621 U/ml

Fig. 9

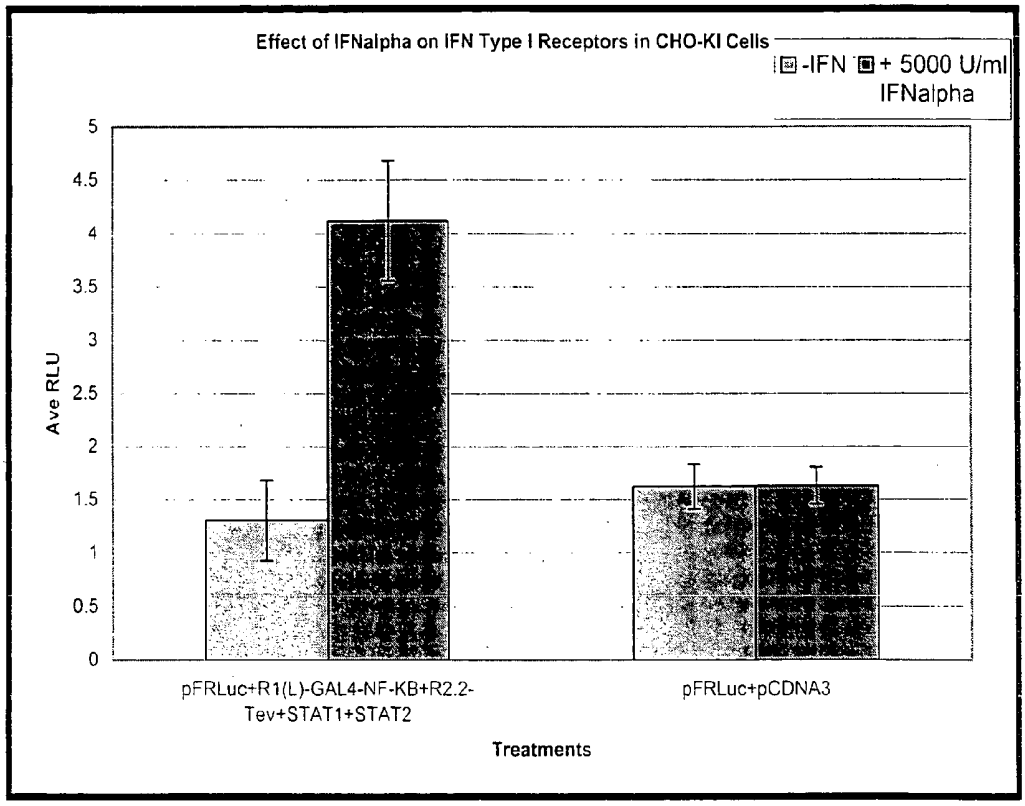
Effect of IFNalpha on IFN Type I Receptors in CHO-KI Cells
-IFN
+ 5000 U/ml IFNalpha
Ave RLU
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
pFRLuc+R1(L)-GAL4-NF-KB+R2.2-Tev+STAT1+STAT2
pFRLuc+pCDNA3
Treatments

Fig. 10

METHOD FOR ASSAYING PROTEIN-PROTEIN INTERACTION

RELATED APPLICATIONS

[0001] This is a continuation-in-part of Application No. 60/566,113 filed Apr. 27, 2004, which is a continuation-in-part of Application No. 60/511,918, filed Oct. 15, 2003, which is a continuation-in-part of Application No. 60/485,968 filed Jul. 9, 2003, all of which are incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to methods for determining interaction between molecules of interest. More particularly, it relates to determining if a particular substance referred to as the test compound modulates the interaction of two or more specific proteins of interest, via determining activation of a reporter gene in a cell, where the activation, or lack thereof, results from the modulation or its absence. The determination occurs using transformed or transfected cells, which are also a feature of the invention, as are the agents used to transform or transfect them.

BACKGROUND AND RELATED ART

[0003] The study of protein/protein interaction, as exemplified, e.g., by the identification of ligands for receptors, is an area of great interest. Even when a ligand or ligands for a given receptor are known, there is interest in identifying more effective or more selective ligands. GPCRs will be discussed herein as a non-exclusive example of a class of proteins which can be studied in this way.

[0004] The G-protein coupled receptors, or "GPCRs" hereafter, are the largest class of cell surface receptors known for humans. Among the ligands recognized by GPCRs are hormones, neurotransmitters, peptides, glycoproteins, lipids, nucleotides, and ions. They also act as receptors for light, odors, pheromones, and taste. Given these various roles, it is perhaps not surprising that they are the subject of intense research, seeking to identify drugs useful in various conditions. The success rate has been phenomenal. Indeed, Howard, et al. *Trends Pharmacol. Sci.,* 22:132-140 (2001) estimate that over 50% of marketed drugs act on such receptors. "GPCRs" as used herein, refers to any member of the GPCR superfamily of receptors characterized by a seven-transmembrane domain (7TM) structure. Examples of these receptors include, but are not limited to, the class A or "rhodopsin-like" receptors; the class B or "secretin-like" receptors; the class C or "metabotropic glutamate-like" receptors; the Frizzled and Smoothened-related receptors; the adhesion receptor family or EGF-7TM/LNB-7TM receptors; adiponectin receptors and related receptors; and chemosensory receptors including odorant, taste, vomeronasal and pheromone receptors. As examples, the GPCR superfamily in humans includes but is not limited to those receptor molecules described by Vassilatis, et al., *Proc. Natl. Acad. Sci. USA,* 100:4903-4908 (2003); Takeda, et al., FEBS Letters, 520:97-101 (2002); Fredricksson, et al., *Mol. Pharmacol.,* 63:1256-1272 (2003); Glusman, et al., Genome Res., 11:685-702 (2001); and Zozulya, et al., *Genome Biol.,* 2:0018.1-0018.12 (2001), all of which are incorporated by reference.

[0005] The mechanisms of action by which GPCRs function has been explicated to some degree. In brief, when a GPCR binds a ligand, a conformational change results, stimulating a cascade of reactions leading to a change in cell physiology. It is thought that GPCRs transduce signals by modulating the activity of intracellular, heterotrimeric guanine nucleotide binding proteins, or "G proteins". The complex of ligand and receptor stimulates guanine nucleotide exchange and dissociation of the G protein heterotrimer into α and $\beta\gamma$ subunits.

[0006] Both the GTP-bound a subunit and the $\beta\gamma$ dimer can act to regulate various cellular effector proteins, including adenylyl cyclase and phospholipase C (PLC). In conventional cell based assays for GPCRs, receptor activity is monitored by measuring the output of a G-protein regulated effector pathway, such as the accumulation of cAMP that is produced by adenylyl cyclase, or the release of intracellular calcium, which is stimulated by PLC activity.

[0007] Conventional G-protein based, signal transduction assays have been difficult to develop for some targets, as a result of two major issues.

[0008] First, different GPCRs are coupled to different G protein regulated signal transduction pathways, and G-protein based assays are dependent on knowing the G-protein specificity of the target receptor, or require engineering of the cellular system, to force coupling of the target receptor to a particular effect or pathway. Second, all cells express a large number of endogenous GPCRs, as well as other signaling factors. As a result, the effector pathways that are measured may be modulated by other endogenous molecules in addition to the target GPCR, potentially leading to false results.

[0009] Regulation of G-protein activity is not the only result of ligand/GPCR binding. Luttrell, et al., *J. Cell Sci.,* 115:455-465 (2002), and Ferguson, *Pharmacol. Rev.,* 53:1-24 (2001), both of which are incorporated by reference, review other activities which lead to termination of the GPCR signal. These termination processes prevent excessive cell stimulation, and enforce temporal linkage between extracellular signal and corresponding intracellular pathway.

[0010] In the case of binding of an agonist to GPCR, serine and threonine residues at the C terminus of the GPCR molecule are phosphorylated. This phosphorylation is caused by the GPCR kinase, or "GRK," family. Agonist complexed, C-terminal phosphorylated GPCRs interact with arrestin family members, which "arrest" receptor signaling. This binding inhibits coupling of the receptor to G proteins, thereby targeting the receptor for internalization, followed by degradation and/or recycling. Hence, the binding of a ligand to a GPCR can be said to "modulate" the interaction between the GPCR and arrestin protein, since the binding of ligand to GPCR causes the arrestin to bind to the GPCR, thereby modulating its activity. Hereafter, when "modulates" or any form thereof is used, it refers simply to some change in the way the two proteins of the invention interact, when the test compound is present, as compared to how these two proteins interact, in its absence. For example, the presence of the test compound may strengthen or enhance the interaction of the two proteins, weaken it, inhibit it, or lessen it in some way, manner or form which can then be detected.

[0011] This background information has led to alternate methods for assaying activation and inhibition of GPCRs.

These methods involve monitoring interaction with arrestins. A major advantage of this approach is that no knowledge of G-protein pathways is necessary.

[0012] Oakley, et al., *Assay Drug Dev. Technol.,* 1:21-30 (2002) and U.S. Pat. Nos. 5,891,646 and 6,110,693, incorporated by reference, describe assays where the redistribution of fluorescently labelled arrestin molecules in the cytoplasm to activated receptors on the cell surface is measured. These methods rely on high resolution imaging of cells, in order to measure arrestin relocalization and receptor activation. It will be recognized by the skilled artisan that this is a complex, involved procedure.

[0013] Various other U.S. patents and patent applications dealing with these points have issued and been filed. For example, U.S. Pat. No. 6,528,271 to Bohn, et al., deals with assays for screening for pain controlling medications, where the inhibitor of β-arrestin binding is measured. Published U.S. patent applications, such as 2004/0002119, 2003/0157553, 2003/0143626, and 2002/0132327, all describe different forms of assays involving GPCRs. Published application 2002/0106379 describes a construct which is used in an example which follows; however, it does not teach or suggest the invention described herein.

[0014] It is an object of the invention to develop a simpler assay for monitoring and/or determining modulation of specific protein/protein interactions, where the proteins include but are not limited to, membrane bound proteins, such as receptors, GPCRs in particular. How this is accomplished will be seen in the examples which follow.

SUMMARY OF THE INVENTION

[0015] Thus, in accordance with the present invention, there is provided a method for determining if a test compound modulates a specific protein/protein interaction of interest comprising contacting said compound to a cell which has been transformed or transfected with (a) a nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes said first test protein, (ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and (iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and (b) a nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound is to be measured, and (ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site, and determining activity of said reporter gene as a determination of whether said compound modulates said protein/protein interaction.

[0016] The first test protein may be a membrane bound protein, such as a transmembrane receptor, and in particular a GPCR. Particular transmembrane receptors include β-adrenergic receptor (ADRB2), arginine vasopressin receptor 2 (AVPR2). serotonin receptor 1a (HTR1 A), m2 muscarinic acetylcholine receptor (CHRM2), chemokine (C-C motif) receptor 5 (CCR5), dopamine D2 receptor (DRD2), kappa opioid receptor (OPRK), or α1a-adregenic receptor (ADRA1A) although it is to be understood that in all cases the invention is not limited to these specific embodiments. For example, molecules such as the insulin growth factor-1 receptor (IGF-1R), which is a tyrosine kinase, and proteins which are not normally membrane bound, like estrogen receptor 1 (ESR1) and estrogen receptors 2 (ESR2). The protease or portion of a protease may be a tobacco etch virus nuclear inclusion A protease. The protein which activates said reporter gene may be a transcription factor, such as tTA or GAL4. The second protein may be an inhibitory protein, such as an arrestin. The cell may be a eukaryote or a prokaryote. The reporter gene may be an exogenous gene, such as β-galactosidase or luciferase.

[0017] The nucleotide sequence encoding said first test protein may be modified to increase interaction with said second test protein. Such modifications include but are not limited to replacing all or part of the nucleotide sequence of the C-terminal region of said first test protein with a nucleotide sequence which encodes an amino acid sequence which has higher affinity for said second test protein than the original sequence. For example, the C-terminal region may be replaced by a nucleotide sequence encoding the C-terminal region of AVPR2, AGTRLI, GRPR, F2RL1, CXCR2/IL-8b, CCR4, or GRPR.

[0018] The method may comprise contacting more than one test compound to a plurality of samples of cells, each of said samples being contacted by one or more of said test compounds, wherein each of said cell samples have been transformed or transfected with the aforementioned nucleic acid molecules, and determining activity of reporter genes in said plurality of said samples to determine if any of said test compounds modulate a specific, protein/protein interaction. The method may comprise contacting each of said samples with one test compound, each of which differs from all others, or comprise contacting each of said samples with a mixture of said test compounds.

[0019] In another embodiment, there is provided a method for determining if a test compound modulates one or more of a plurality of protein interactions of interest, comprising contacting said test compound to a plurality of samples of cells, each of which has been transformed or transfected with (a) a first nucleic acid molecule comprising, (i) a nucleotide sequence which encodes a first test protein, a nucleotide sequence encoding a cleavage site for a protease, and (ii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, (b) a second nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound of interest is to be measured, (ii) a nucleotide sequence which encodes a protease or a protease which is specific for said cleavage site, wherein said first test protein differs from other first test proteins in each of said plurality of samples, and determining activity of said reporter gene in at one or more of said plurality of samples as a determination of modulation of one or more protein interactions of interest

[0020] The second test protein may be different in each sample or the same in each sample. All of said samples may be combined in a common receptacle, and each sample comprises a different pair of first and second test proteins. Alternatively, each sample may be tested in a different receptacle. The reporter gene in a given sample may differ from the reporter gene in other samples. The mixture of test compounds may comprise or be present in a biological sample, such as cerebrospinal fluid, urine, blood, serum, pus, ascites, synovial fluid, a tissue extract, or an exudate.

[0021] In yet another embodiment, there is provided a recombinant cell, transformed or transfected with (a) a

nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes said first test protein, (ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and (iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and (b) a nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound is to be measured, and (ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site.

[0022] One or both of said nucleic acid molecules may be stably incorporated into the genome of said cell. The cell also may have been transformed or transfected with said reporter gene. The first test protein may be a membrane bound protein, such as a transmembrane receptor, and in particular a GPCR. Particular transmembrane receptors include ADRB2, AVPR2, HTR1A, CHRM2, CCR5, DRD2, OPRK, or ADRA1A.

[0023] The protease or portion of a protease may be a tobacco etch virus nuclear inclusion A protease. The protein which activates said reporter gene may be a transcription factor, such as tTA or GAL4. The second protein may be an inhibitory protein. The cell may be a eukaryote or a prokaryote. The reporter gene may be an exogenous gene, such as β-galactosidase or luciferase. The nucleotide sequence encoding said first test protein may be modified to increase interaction with said second test protein, such as by replacing all or part of the nucleotide sequence of the C-terminal region of said first test protein with a nucleotide sequence which encodes an amino acid sequence which has higher affinity for said second test protein than the original sequence. The C-terminal region may be replaced by a nucleotide sequence encoding the C-terminal region of AVPR2, AGTRLI, GRPR, F2RL1, CXCR2/IL-8B, CCR4, or GRPR.

[0024] In still yet another embodiment, there is provided an isolated nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a test protein (ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and (iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell. The test protein may be a membrane bound protein, such as is a transmembrane receptor. A particular type of transmembrane protein is a GPCR. Particular transmembrane receptors include ADRB2, AVPR2, HTR1A, CHRM2, CCR5, DRD2, OPRK, or ADRA1A. The protease or portion of a protease may be a tobacco etch virus nuclear inclusion A protease. The protein which activates said reporter gene may be a transcription factor, such as tTA or GAL4. As above, the invention is not to be viewed as limited to these specific embodiments.

[0025] In still a further embodiment, there is provided an expression vector comprising an isolated nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a test protein (ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and (iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and further being operably linked to a promoter.

[0026] In still yet a further embodiment, there is provided an isolated nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a test protein whose interaction with another test protein in the presence of a test compound is to be measured, and (ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site. The test protein may be an inhibitory protein, such as an arrestin.

[0027] Also provided is an expression vector comprising an isolated nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a test protein whose interaction with another test protein in the presence of a test compound is to be measured, and (ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site, said nucleic acid further being operably linked to a promoter.

[0028] An additional embodiment comprises a fusion protein produced by expression of:

[0029] an isolated nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a test protein (ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and (iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and further being operably linked to a promoter; or

[0030] an isolated nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a test protein whose interaction with another test protein in the presence of a test compound is to be measured, and (ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site

[0031] In yet another embodiment, there is provided a test kit useful for determining if a test compound modulates a specific protein/protein interaction of interest comprising a separate portion of each of (a) a nucleic acid molecule which comprises, a nucleotide sequence which encodes said first test protein (i) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, (ii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and (b) a nucleic acid molecule which comprises, (i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound is to be measured, (ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site, and container means for holding each of (a) and (b) separately from each other.

[0032] The first test protein may be a membrane bound protein, such as a transmembrane receptor. A particular type of transmembrane receptor is a GPCR. A particular transmembrane protein is a GPCR. Particular transmembrane receptors include ADRB2, AVPR2, HTR1A, CHRM2, CCR5, DRD2, OPRK, or ADRA1A. The protease or portion of a protease may be tobacco etch virus nuclear inclusion A protease. The protein which activates said reporter gene may be a transcription factor, such as tTA or GAL4. The second protein may be an inhibitory protein, such as an arrestin. The kit may further comprise a separate portion of an isolated nucleic acid molecule which encodes a reporter gene. The reporter gene may encode P-galactosidase or luciferase. The nucleotide sequence encoding said first test protein may be modified to increase interaction with said second test protein, such as by replacing all or part of the nucleotide sequence of the C-terminal region of said first test protein

with a nucleotide sequence which encodes an amino acid sequence which has higher affinity for said second test protein than the original sequence. The nucleotide sequence of said C-terminal region may be replaced by a nucleotide sequence encoding the C-terminal region of AVPR2, AGTRLI, GRPR, F2RL1, CXCR2/IL-8B, CCR4, or GRPR.

[0033] It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more,""at least one," and "one or more than one."

[0034] These, and other, embodiments of the invention will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following description, while indicating various embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many substitutions, modifications, additions and/or rearrangements may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such substitutions, modifications, additions and/or rearrangements.

BRIEF DESCRIPTION OF THE FIGURES

[0035] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0036] **FIG. 1** shows the conceptual underpinnings of the invention, pictorially, using ligand-receptor binding as an example.

[0037] **FIGS. 2*a*** and **2*b*** show that the response of targets in assays in accordance with the invention is dose dependent, both for agonists and antagonists.

[0038] **FIG. 3** shows that a dose response curve results with a different target and a different agonist as well.

[0039] **FIG. 4** depicts results obtained in accordance with the invention, using the D2 dopamine receptor.

[0040] **FIGS. 5*a*** and **5*b*** illustrate results of an assay which shows that two molecules can be studied simultaneously.

[0041] **FIG. 6** sets forth the result of another "multiplex" assay, i.e., one where two molecules are studied simultaneously.

[0042] **FIG. 7** presents data obtained from assays measuring EGFR activity.

[0043] **FIG. 8** presents data obtained from assays in accordance with the invention, designed to measure the activity of human type I interferon receptor.

[0044] **FIG. 9** elaborates on the results in **FIG. 7**, showing a dose response curve for IFN-α in the cells used to generate **FIG. 7**.

[0045] **FIG. 10** shows the results of additional experiments where a different transcription factor, and a different cell line, were used.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0046] The present invention relates to methods for determining if a substance of interest modulates interaction of a first test protein, such as a membrane bound protein, like a receptor, e.g., a transmembrane receptor, with a second test protein, like a member of the arrestin family. The methodology involves cotransforming or cotransfecting a cell, which may be prokaryotic or eukaryotic, with two constructs. The first construct includes, a sequence encoding (i) the first test protein, such as a transmembrane receptor, (ii) a cleavage site for a protease, and (iii) a sequence encoding a protein which activates a reporter gene. The second construct includes, (i) a sequence which encodes a second test protein whose interaction with the first test protein is measured and/or determined, and (ii) a nucleotide sequence which encodes a protease or a portion of a protease sufficient to act on the cleavage site that is part of the first construct. In especially preferred embodiments, these constructs become stably integrated into the cells.

[0047] The features of an embodiment of the invention are shown, pictorially, in **FIG. 1**. In brief, first, standard techniques are employed to fuse DNA encoding a transcription factor to DNA encoding a first test protein, such as a transmembrane receptor molecule, being studied. This fusion is accompanied by the inclusion of a recognition and cleavage site for a protease not expressed endogenously by the host cell being used in the experiments.

[0048] DNA encoding this first fusion protein is introduced into and is expressed by a cell which also contains a reporter gene sequence, under the control of a promoter element which is dependent upon the transcription factor fused to the first test protein, e.g., the receptor. If the exogenous protease is not present, the transcription factor remains tethered to the first test protein and is unable to enter the nucleus to stimulate expression of the reporter gene.

[0049] Recombinant techniques can also be used to produce a second fusion protein. In the depicted embodiment, DNA encoding a member of the arrestin family is fused to a DNA molecule encoding the exogenous protease, resulting in a second fusion protein containing the second test protein, i.e., the arrestin family member.

[0050] An assay is then carried out wherein the second fusion protein is expressed, together with the first fusion protein, and a test compound is contacted to the cells, preferably for a specific length of time. If the test compound modulates interaction of the two test proteins, e.g., by stimulating, promoting or enhancing the association of the first and second test proteins, this leads to release of the transcription factor, which in turn moves to the nucleus, and provokes expression of the reporter gene. The activity of the reporter gene is measured.

[0051] In an alternative system, the two test proteins may interact in the absence of the test compound, and the test compound may cause the two test proteins to dissociate, lessen or inhibit their interaction. In such a case, the level of free, functionally active transcription factor in the cell decreases in the presence of the test compound, leading to a decrease in proteolysis, and a measurable decrease in the activity of the reporter gene.

[0052] In the depicted embodiment, the arrestin protein, which is the second test protein, binds to the receptor in the

presence of an agonist; however, it is to be understood that since receptors are but one type of protein, the assay is not dependent upon the use of receptor molecules, nor is agonist binding the only interaction capable of being involved. Any protein will suffice, although the interest in transmembrane proteins is clear. Further, agonist binding to a receptor is not the only type of binding which can be assayed. One can determine antagonists, per se and also determine the relative strengths of different antagonists and/or agonists in accordance with the invention.

[0053] Other details of the invention, include specific methods and technology for making and using the subject matter thereof, are described below.

[0054] I. Expression Constructs and Transformation

[0055] The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (see, for example, Maniatis, et al., Molecular Cloning, A Laboratory Manual (Cold Spring Harbor, 1990) and Ausubel, et al., 1994, Current Protocols In Molecular Biology (John Wiley & Sons, 1996), both incorporated herein by reference).

[0056] The term "expression vector" refers to any type of genetic construct comprising a nucleic acid coding for a RNA capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host cell. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleotide sequences that serve other finctions as well and are described infra.

[0057] In certain embodiments, a plasmid vector is contemplated for use ie in cloning and gene transfer. In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. In a non-limiting example, *E. coli* is often transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, for example, promoters which can be used by the microbial organism for expression of its own proteins.

[0058] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, the phage lambda GEM™-11 may be utilized in making a recombinant phage vector which can be used to transform host cells, such as, for example, *E. coli* LE392.

[0059] Bacterial host cells, for example, *E. coli,* comprising the expression vector, are grown in any of a number of suitable media, for example, LB. The expression of the recombinant protein in certain vectors may be induced, as would be understood by those of skill in the art, by contacting a host cell with an agent specific for certain promoters, e.g., by adding IPTG to the media or by switching incubation to a higher temperature. After culturing the bacteria for a further period, generally of between 2 and 24 h, the cells are collected by centrifugation and washed to remove residual media.

[0060] Many prokaryotic vectors can also be used to transform eukaryotic host cells. However, it may be desirable to select vectors that have been modified for the specific purpose of expressing proteins in eukaryotic host cells. Expression systems have been designed for regulated and/or high level expression in such cells. For example, the insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Pat. Nos. 5,871,986 and 4,879,236, both herein incorporated by reference, and which can be bought, for example, under the name MAXBAC® 2.0 from INVITROGEN® and BACPAcK™ BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH®.

[0061] Other examples of expression systems include STRATAGENEO'S COMPLETE CONTROL™ Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an *E.coli* expression system. Another example of an inducible expression system is available from INVITROGEN, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGENO also provides a yeast expression system called the Pichia methanolica Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast *Pichia methanolica.* One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

[0062] Regulatory Signals

[0063] The construct may contain additional 5' and/or 3' elements, such as promoters, poly A sequences, and so forth. The elements may be derived from the host cell, i.e., homologous to the host, or they may be derived from distinct source, i.e., heterologous.

[0064] "promoter" is a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind, such as RNA polymerase and other transcription factors, to initiate the specific transcription a nucleic acid sequence. The phrases "operatively positioned,""operatively linked,""under control," and "under transcriptional control" mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence.

[0065] A promoter generally comprises a sequence that functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a TATA box, such as, for example, the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 late genes, a discrete element overlying the start site itself helps to fix the place of initiation. Additional promoter elements regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. To bring a coding sequence "under the control of" a promoter, one positions the 5∝ end of the transcription initiation site of the transcriptional reading frame "downstream" of (i.e., 3' of) the chosen promoter. The "upstream" promoter stimulates transcription of the DNA and promotes expression of the encoded RNA.

[0066] The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the tk promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription. A promoter may or may not be used in conjunction with an "enhancer," which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[0067] A promoter may be one naturally associated with a nucleic acid molecule, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a nucleic acid molecule, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid molecule in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid molecule in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other virus, or prokaryotic or eukaryotic cell, and promoters or enhancers not "naturally occurring," i.e., containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. For example, promoters that are most commonly used in recombinant DNA construction include the β-lactamase (penicillinase), lactose and tryptophan (trp) promoter systems. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR™, in connection with the compositions disclosed herein (see U.S. Pat. Nos. 4,683,202 and 5,928,906, each incorporated herein by reference). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

[0068] Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the organelle, cell type, tissue, organ, or organism chosen for expression. Those of skill in the art of molecular biology generally know the use of promoters, enhancers, and cell type combinations for protein expression, (see, for example Sambrook, et al., 1989, incorporated herein by reference). The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

[0069] Additionally any promoter/enhancer combination (as per, for example, the Eukaryotic Promoter Data Base EPDB, www.epd.isb-sib.ch/) could also be used to drive expression. Use of a T3, T7 or SP6 cytoplasmic expression system is another possible embodiment. Eukaryotic cells can support cytoplasmic transcription from certain bacterial promoters if the appropriate bacterial polymerase is provided, either as part of the delivery complex or as an additional genetic expression construct.

[0070] A specific initiation signal also may be required for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be "in-frame" with the reading frame of the desired coding sequence to ensure translation of the entire insert. The exogenous translational control signals and initiation codons can be either natural or synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements.

[0071] In certain embodiments of the invention, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5' methylated Cap dependent translation and begin translation at internal sites (Pelletier and Sonenberg, Nature, 334:320-325 (1988)). IRES elements from two members of the picornavirus family (polio and encephalomyocarditis) have been described (Pelletier and Sonenberg, supra), as well an IRES from a mammalian message (Macejak and Sarnow, *Nature,* 353:90-94 (1991))1991). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message (see U.S. Pat. Nos. 5,925,565 and 5,935,819, each herein incorporated by reference).

[0072] Other Vector Sequence Elements p Vectors can include a multiple cloning site (MCS), which is a nucleic acid region that contains multiple restriction enzyme sites, any of which can be used in conjunction with standard recombinant technology to digest the vector (see, for example, Carbonelli, et al., *FEMS Microbiol. Lett.,* 172(1):75-82 (1999), Levenson, et al., Hum. Gene Ther. 9(8):1233-1236 (1998), and Cocea, *Biotechniques,*

23(5):814-816 (1997)), incorporated herein by reference.) "Restriction enzyme digestion" refers to catalytic cleavage of a nucleic acid molecule with an enzyme that ftunctions only at specific locations in a nucleic acid molecule. Many of these restriction enzymes are commercially available. Use of such enzymes is widely understood by those of skill in the art. Frequently, a vector is linearized or fragmented using a restriction enzyme that cuts within the MCS to enable exogenous sequences to be ligated to the vector. "Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments, which may or may not be contiguous with each other. Techniques involving restriction enzymes and ligation reactions are well known to those of skill in the art of recombinant technology.

[0073] Most transcribed eukaryotic RNA molecules will undergo RNA splicing to remove introns from the primary transcripts. Vectors containing genomic eukaryotic sequences may require donor and/or acceptor splicing sites to ensure proper processing of the transcript for protein expression (see, for example, Chandler, et al., 1997, herein incorporated by reference).

[0074] The vectors or constructs of the present invention will generally comprise at least one termination signal. A "termination signal" or "terminator" comprises a DNA sequence involved in specific termination of an RNA transcript by an RNA polymerase. Thus, in certain embodiments a termination signal that ends the production of an RNA transcript is contemplated. A terminator may be necessary in vivo to achieve desirable message levels.

[0075] In eukaryotic systems, the terminator region may also comprise specific DNA sequences that permit site-specific cleavage of the new transcript so as to expose a polyadenylation site. This signals a specialized endogenous polymerase to add a stretch of about 200 adenosine residues (polyA) to the 3' end of the transcript. RNA molecules modified with this polyA tail appear to more stable and are translated more efficiently. Thus, in other embodiments involving eukaryotes, it is preferred that that terminator comprises a signal for the cleavage of the RNA, and it is more preferred that the terminator signal promotes polyadenylation of the message. The terminator and/or polyadenylation site elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

[0076] Terminators contemplated for use in the invention include any known terminator of transcription described herein or known to one of ordinary skill in the art, including but not being limited to, for example, the termination sequences of genes, such as the bovine growth hormone terminator, viral termination sequences, such as the SV40 terminator. In certain embodiments, the termination signal may be a lack of transcribable or translatable sequence, such as an untranslatable/untranscribable sequence due to a sequence truncation.

[0077] In expression, particularly eukaryotic expression, one will typically include a polyadenylation signal to effect proper polyadenylation of the transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and any such sequence may be employed. Preferred embodiments include the SV40 polyadenylation signal or the bovine growth hormone polyadenylation signal, both of which are convenient, readily available, and known to function well in various target cells. Polyadenylation may increase the stability of the transcript or may facilitate cytoplasmic transport.

[0078] In order to propagate a vector in a host cell, it may contain one or more origins of replication (often termed "ori"), sites, which are specific nucleotide sequences at which replication is initiated. Alternatively, an autonomously replicating sequence (ARS) can be employed if the host cell is yeast.

[0079] Transformation Methodology

[0080] Suitable methods for nucleic acid delivery for use with the current invention are believed to include virtually any method by which a nucleic acid molecule (e.g., DNA) can be introduced into a cell as described herein or as would be known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of DNA such as by ex vivo transfection (Wilson, et al., *Science,* 244:1344-1346 (1989), Nabel et al, *Science,* 244:1342-1344 (1989), by injection (U.S. Pat. Nos. 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection (Harlan and Weintraub, *J. Cell Biol.,* 101(3):1094-1099 (1985); U.S. Pat. No. 5,789,215, incorporated herein by reference); by electroporation (U.S. Pat. No. 5,384,253, incorporated herein by reference; Tur-Kaspa, et al., *Mol. Cell Biol.,* 6:716-718 (1986); Potter, et al., *Proc. Natl. Acad. Sci. USA,* 81:7161-7165 (1984); by calcium phosphate precipitation (Graham and Van Der Eb, *Virology,* 52:456-467 (1973); Chen and Okayama, *Mol. Cell Biol.,* 7(8):2745-2752 (1987); Rippe, et al., *Mol. Cell Biol.,* 10:689-695 (1990); by using DEAE-dextran followed by polyethylene glycol (Gopal, *Mol. Cell Biol.,* 5:1188-190 (1985); by direct sonic loading (Fechheimer, et al, *Proc. Natl. Acad. Sci. USA,* 89(17):8463-8467 (1987); by liposome mediated transfection (Nicolau and Sene, Biochem. & Biophys. Acta., 721:185-190 (1982); Fraley, et al, *Proc. Natl. Acad. Sci. USA,* 76:3348-3352 (1979); Nicolau, et al., *Meth. Enzym.,* 149:157-176 (1987); Wong, et al., *Gene,* 10:879-894 (1980); Kaneda, et al., *Science,* 243:375-378 (1989); Kato, et al., *J. Biol. Chem.,* 266:3361-3364 (1991) and receptor-mediated transfection (Wu and Wu, *J. Biol. Chem.,* 262:4429-4432 (1987); Wu and Wu, 1988); by PEG-mediated transformation of protoplasts (Omirulleh, et al., *Plant Mol. Biol.,* 21(3):415-428 (1987); U.S. Pat. Nos. 4,684,611 and 4,952,500, each incorporated herein by reference); by desiccation/inhibition-mediated DNA uptake (Potrykus, et al. *Mol. Gen. Genet.,* 199(2):169-177 (1985), and any combination of such methods.

[0081] II. Components of the Assay System

[0082] As with the method described herein, the products which are features of the invention have preferred embodiments. For example, in the "three part construct," i.e., that contain sequences encoding a test protein, the cleavage site, and the activator protein, the test protein is preferably a membrane bound protein, such as a transmembrane receptor, e.g., a member of the GPCR family. These sequences can be modified so that the C terminus of the proteins they encode have better and stronger interactions with the second protein. The modifications can include, e.g., replacing a C-terminal encoding sequence of the test protein, such as a GPCR, with the C terminal coding region for AVPR2, AGTRLI, GRPR, F2PLI, CCR4, CXCR2/IL-8, CCR4, or GRPR, all of which are defined supra.

[0083] The protein which activates the reporter gene may be a protein which acts within the nucleus, like a transcription factor (e.g., tTA, GAL4, etc.), or it may be a molecule that sets a cascade of reactions in motion, leading to an intranuclear reaction by another protein. The skilled artisan will be well versed in such cascades.

[0084] The second construct, as described supra, includes a region which encodes a protein that interacts with the first protein, leading to some measurable phenomenon. The protein may be an activator, an inhibitor, or, more, generically, a "modulator" of the first protein. Members of the arrestin family are preferred, especially when the first protein is a GPCR, but other protein encoding sequences may be used, especially when the first protein is not a GPCR. The second part of these two part constructs encodes the protease, or portion of a protease, which acts to remove the activating molecule from the fusion protein encoded by the first construct.

[0085] However, these preferred embodiments do not limit the invention, as discussed in the following additional embodiments.

[0086] Host Cells

[0087] As used herein, the terms "cell,""cell line," and "cell culture" may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. The host cells generally will have been engineered to express a screenable or selectable marker which is activated by the transcription factor that is part of a fusion protein, along with the first test protein.

[0088] In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell that is capable of replicating a vector and/or expressing a heterologous gene encoded by a vector. When host cells are "transfected" or "transformed" with nucleic acid molecules, they are referred to as "engineered" or "recombinant" cells or host cells, e.g., a cell into which an exogenous nucleic acid sequence, such as, for example, a vector, has been introduced. Therefore, recombinant cells are distinguishable from naturally-occurring cells which do not contain a recombinantly introduced nucleic acid.

[0089] Numerous cell lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC), which is an organization that serves as an archive for living cultures and genetic materials (www.atcc.org). An appropriate host can be determined by one of skill in the art based on the vector backbone and the desired result. A plasmid or cosmid, for example, can be introduced into a prokaryote host cell for replication of many vectors. Cell types available for vector replication and/or expressioninclude, but are not limited to, bacteria, such as *E. coil* (e.g., *E. coli* strain RR1, *E. coil* LE392, *E. coil* B, *E. coli* X 1776 (ATCC No. 31537) as well as *E. coli* W3110 (F-, lambda-, prototrophic, ATCC No. 273325), DH5α, JM1O9, and KC8, bacilli such as *Bacillus subtilis;* and other enterobacteriaceae such as *Salmonella typhimurium, Serratia marcescens,* various *Pseudomonas* specie, as well as a number of commercially available bacterial hosts such as SURE® Competent Cells and SOLOPACK™ Gold Cells (STRATAGENE®, La Jolla). In certain embodiments, bacterial cells such as *E. coli* LE392 are particularly contemplated as host cells for phage viruses.

[0090] Examples of eukaryotic host cells for replication and/or expression of a vector include, but are not limited to, HeLa, NIH3T3, Jurkat, 293, COS, CHO, Saos, and PC12. Many host cells from various cell types and organisms are available and would be known to one of skill in the art. Similarly, a viral vector may be used in conjunction with either a eukaryotic or prokaryotic host cell, particularly one that is permissive for replication or expression of the vector.

[0091] Test Proteins

[0092] The present invention contemplates the use of any two proteins for which a physical interaction is known or suspected. The proteins will exist as fusions proteins, a first test protein fused to a transcription factor, and the second test protein fused to a protease that recognizes a cleavage site in the first fusion protein, cleavage of which releases the transcription factor. The only requirements for the test proteins/fusions are (a) that the first test protein cannot localize to the nucleus prior to cleavage, and (b) that the protease must remain active following both fusion to the second test protein and binding of the first test protein to the second test protein.

[0093] With respect to the first construct, the first test protein may be, e.g., a naturally membrane bound protein, or one which has been engineered to become membrane bound, via standard techniques. The first test protein may be, e.g., a transmembrane receptor such as any of the GPCRs, or any other transmembrane receptor of interest, including, but not being limited to, receptor tyrosine kinases, receptor serine threonine kinases, cytokine receptors, and so forth. Further, as it is well known that portions of proteins, will function in the same manner as the full length first test protein, such active portions of a first test protein are encompassed by the definition of protein herein.

[0094] As will be evident to the skilled artisan, the present invention may be used to assay for interaction with any protein, and is not limited in its scope to assaying membrane bound receptor, like the GPCRs. For example, the activity of other classes of transmembrane receptors, including but not limited to: receptor tyrosine kinases (RTKs), such as IGF1R, such as the epidermal growth factor receptor (EGFR), ErbB2/HER2/Neu or related RTKs; receptor serine/threonine kinases, such as Transforming Growth Factor-beta (TGFβ), activin, or Bone Morphogenetic Protein (BMP) receptors; cytokine receptors, such as receptors for the interferon family for interleukin, erythropoietin, G-CSF, GM-CSF, tumor necrosis factor (TNF) and leptin receptors; and other receptors, which are not necessarily normally membrane bound, such as estrogen receptor 1 (ESR1), and estrogen receptor 2 (ESR2). In each case, the method involves transfecting a cell with a modified receptor construct that directs the expression of a chimeric protein containing the receptor of interest, to which is appended, a protease cleavage site followed by a nucleic acid molecule encoding a transcription factor. The cell is co-transfected with a second construct that directs the expression of a chimeric protein consisting of an interacting protein fused, to the protease that recognizes and cleaves the site described supra. In the case of RTKs, such as the EGFR, this interacting protein may consist of a SH2 (Src homology domain 2) containing protein or portion thereof, such as phospho-

lipase C (PLC) or Src homology 2 domain containing transforming protein 1 (SHC1). In the case of receptor serine/threonine kinases, such as TGFβ, activin, BMP receptors, this interacting protein may be a Smad protein or portion thereof. In the case of cytokine receptors, such as interferon-α/β or interferon-γ gamma receptors, this interacting protein may be a signal transducer and activator of transcription (STAT) protein such as, but not being limited to, Stat1, Stat2; Janus kinase (JAK) proteins Jak1, Jak2, or Tyk2; or portions thereof. In each case, the transfected cell contains a reporter gene that is regulated by the transcription factor fused to the receptor. An assay is then performed in which the transfected cells are treated with a test compound for a specific period and the reporter gene activity is measured at the end of the test period. If the test compound activates the receptor of interest, interactions between the receptor of interest and the interacting protein are stimulated, leading to cleavage of the protease site and release of the fused transcription factor, which is in turn measurable as an increase in reporter gene activity.

[0095] Other possible test protein pairs include antibody-ligands, enzyme-substrates, dimerizing proteins, components of signal transduction cascades, and other protein pairs well known to the art.

[0096] Reporters

[0097] The protein which activates a reporter gene may be any protein having an impact on a gene, expression or lack thereof which leads to a detectable signal. Typical protein reporters include enzymes such as chloramphenicol acetyl transferase (CAT), β-glucuronidase (GUS) or β-galactosidase. Also contemplated are fluorescent and chemilluminescent proteins such as green fluorescent protein, red fluorescent protein, cyan fluorescent protein luciferase, beta lactamase, and alkaline phosphatase.

[0098] Transcriptions Factors and Repressors

[0099] In accordance with the present invention, transcription factors are used to activate expression of a reporter gene in an engineered host cell. Transcription factors are typically classified according to the structure of their DNA-binding domain, which are generally (a) zinc fingers, (b) helix-turn-helix, (c) leucine zipper, (d) helix-loop-helix, or (e) high mobility groups. The activator domains of transcription factors interact with the components of the transcriptional apparatus (RNA polymerase) and with other regulatory proteins, thereby affecting the efficiency of DNA binding.

[0100] The Rel/Nuclear Factor kB (NF-kB) and Activating Protein-1 (AP-1) are among the most studied transcription factor families. They have been identified as important components of signal transduction pathways leading to pathological outcomes such as inflammation and tumorogenesis. Other transcription factor families include the heat shock/E2F family, POU family and the ATF family. Particular transcription factors, such as tTA and GAL4, are contemplated for use in accordance with the present invention.

[0101] Though transcription factors are one class of molecules that can be used, the assays may be modified to accept the use of transcriptional repressor molecules, where the measurable signal is downregulation of a signal generator, or even cell death.

[0102] Proteases and Cleavage Sites

[0103] Proteases are well characterized enzymes that cleave other proteins at a particular site. One family, the Ser/Thr proteases, cleave at serine and threonine residues. Other proteases include cysteine or thiol proteases, aspartic proteases, metalloproteinases, aminopeptidases, di & tripeptidases, carboxypeptidases, and peptidyl peptidases. The choice of these is left to the skilled artisan and certainly need not be limited to the molecules described herein. It is well known that enzymes have catalytic domains and these can be used in place of full length proteases. Such are encompassed by the invention as well. A specific embodiment is the tobacco etch virus nuclear inclusion A protease, or an active portion thereof. Other specific cleavage sites for proteases may also be used, as will be clear to the skilled artisan.

[0104] Modification of Test Proteins

[0105] The first test protein may be modified to enhance its binding to the interacting protein in this assay. For example, it is known that certain GPCRs bind arrestins more stably or with greater affinity upon ligand stimulation and this enhanced interaction is mediated by discrete domains, e.g., clusters of serine and threonine residues in the C-terminal tail (Oakley, et al, *J. Biol. Chem.,* 274:32248-32257, 1999 and Oakley, et al., *J. Biol. Chem.,* 276:19452-19460, 2001). Using this as an example, it is clear that the receptor encoding sequence itself may be modified, so as to increase the affinity of the membrane bound protein, such as the receptor, with the protein to which it binds. Exemplary of such modifications are modifications of the C-terminal region of the membrane bound protein, e.g., receptor, such as those described supra, which involve replacing a portion of it with a corresponding region of another receptor, which has higher affinity for the binding protein, but does not impact the receptor function. Examples 16 and 20, supra, show embodiments of this feature of the invention.

[0106] In addition, the second test protein may be modified to enhance its interaction with the first test protein. For example, the assay may incorporate point mutants, truncations or other variants of the second test protein, e.g., arrestin that are known to bind agonist-occupied GPCRs more stably or in a phosphorylation-independent manner (Kovoor, et al., *J. Biol. Chem.,* 274:6831-6834, 1999).

[0107] III. Assay Formats

[0108] As discussed above, the present invention, in one embodiment, offers a straightforward way to assess the interaction of two test proteins when expressed in the same cell. A first construct, as described supra, comprises a sequence encoding a first protein, concatenated to a sequence encoding a cleavage site for a protease or protease portion, which is itself concatenated to a sequence encoding a reporter gene activator. By "concatenated" is meant that the sequences described are fused to produce a single, intact open reading frame, which may be translated into a single polypeptide which contains all the elements. These may, but need not be, separated by additional nucleotide sequences which may or may not encode additional proteins or peptides. A second construct inserted into the recombinant cells is also as described supra, i.e., it contains both a sequence encoding a second protein. and the protease or protease portion. Together, these elements constitute the basic assay

format when combined with a candidate agent whose effect on target protein interaction is sought.

[0109] However, the invention may also be used to assay more than one membrane bound protein, such as a receptor, simultaneously by employing different reporter genes, each of which is stimulated by the activation of a protein, such as the classes of proteins described herein. For example, this may be accomplished by mixing cells transfected with different receptor constructs and different reporter genes, or by fusing different transcription factors to each test receptor, and measuring the activity of each reporter gene upon treatment with the test compound. For example, it may be desirable to determine if a molecule of interest activates a first receptor and also determine if side effects should be expected as a result of interaction with a second receptor. In such a case one may, e.g., involve a first cell line encoding a first receptor and a first reporter, such as lacZ, and a second cell line encoding a second receptor and a second reporter, such as GFP. Preferred embodiments of such a system are seen in Examples 17 and 18. One would mix the two cell lines, add the compound of interest, and look for a positive effect on one, with no effect on the other.

[0110] It is contemplated that the invention relates both to assays where a single pair of interacting test proteins is examined, but more preferably, what will be referred to herein as "multiplex" assays are used. Such assays may be carried out in various ways, but in all cases, more than one pair of test proteins is tested simultaneously. This may be accomplished, e.g., by providing more than one sample of cells, each of which has been transformed or transfected, to test each interacting pair of proteins. The different transformed cells may be combined, and tested simultaneously, in one receptacle, or each different type of transformant may be placed in a different well, and then tested.

[0111] The cells used for the multiplex assays described herein may be, but need not be, the same. Similarly, the reporter system used may, but need not be, the same in each sample. After the sample or samples are placed in receptacles, such as wells of a microarray, one or more compounds may be screened against the plurality of interacting protein pairs set out in the receptacles.

[0112] The fusion proteins expressed by the constructs are also a feature of the invention. Other aspects of the invention which will be clear to the artisan, are antibodies which can identify the fusion proteins as well as various protein based assays for determining the presence of the protein, as well as hybridization assays, such as assays based on PCR, which determine expression of the gene.

[0113] IV. Kits

[0114] Any of the compositions described herein may be comprised in a kit. The kits will thus comprise, in suitable container means for the vectors or cells of the present invention, and any additional agents that can be used in accordance with the present invention.

[0115] The kits may comprise a suitably aliquoted compositions of the present invention. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[0116] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

V. EXAMPLES

[0117] Specific embodiments describing the invention will be seen in the examples which follow, but the invention should not be deemed as limited thereto.

Example 1

[0118] A fusion construct was created, using DNA encoding human β2 adrenergic receptor, referred to hereafter as "ADRB2", in accordance with standard nomenclature. Its nucleotide sequence can be found at GenBank, under Accession Number NM_000024 (SEQ ID NO: 1). The tetracycline controlled transactivator tTA, described by Gossen, et al., *Proc. Natl. Acad. Sci. USA,* 87:5547-5551 (1992), incorporated by reference, was also used. A sequence encoding the recognition and cleavage site for tobacco etch virus nuclear inclusion A protease, described by Parks, et al., *Anal. Biochem.,* 216:413-417 (1994), incorporated by reference, is inserted between these sequences in the fusion coding gene. The CMV promoter region was placed upstream of the ADRB2 coding region, and a poly A sequence was placed downstream of the tTA region.

[0119] A fusion construct was prepared by first generating a form of ADRB2 which lacked internal BamHI and BglII restriction sites. Further, the endogenous stop codon was replaced with a unique BantHI site.

[0120] Overlapping PCR was used to do this. To elaborate, a 5' portion of the coding region was amplified with:

```
gattgaagat ctgccttctt gctggc,     (SEQ ID NO: 2)
and

gcagaacttg gaagacctgc ggagtcc,    (SEQ ID NO: 3)
```

[0121] while a 3' portion of the coding region was amplified with:

```
ggactccgca ggtcttccaa gttctgc,    (SEQ ID NO: 4)
and

ttcggatcct agcagtgagt catttgt.    (SEQ ID NO: 5)
```

[0122] The resulting PCR products have 27 nucleotides of overlapping sequence and were purified via standard agarose

gel electrophoresis. These were mixed together, and amplified with SEQ ID NO: 2, and SEQ ID NO: 5.

[0123] PCR was also used to modify the coding region of tTA so that the endogenous start codon was replaced with a TEV NIa-Pro cleavage site. The cleavage site, defined by the seven amino acid sequence ENLYFQS (SEQ ID NO: 6), is taught by Parks, et al., *Anal. Biochem.,* 216:413-417 (1994), incorporated by reference. The seventh amino acid is known as P1' position, and replacing it with other amino acids is known to reduce the efficiency of cleavage by TEV NIa-Pro. See Kapust, et al., *Biochem. Biophys. Res. Commun.,* 294:949-955 (2002).

[0124] Variants where the seventh amino acid was changed to Tyr, and where it was changed to Leu, were produced. These resulted in intermediate and low efficiency cleavage sites, as compared to the natural high efficiency site.

[0125] A DNA sequence encoding the natural high efficiency site was added to the tTA coding region in two steps. Briefly, BamHII and XbaI restriction sites were added to the 5' end and a XhoI restriction site was added to the 3' end of the tTA coding region by PCR with

```
ccggatcctc tagattagat aaaagtaaag tg  (SEQ ID NO: 7)
and

gactcgagct agcagtatcc tcgcgccccc     (SEQ ID NO: 8)

taccc,
```

[0126] and the TEV NIa-Pro cleavage site was added to the 5' end by ligating an oligonucleotide with the sequence

```
gagaacctgt acttccag        (SEQ ID NO: 9)
```

[0127] between the BamHI and XbaI sites.

[0128] This DNA sequence was modified to encode the intermediate and low efficiency cleavage sites by PCR using:

```
ggatccgaga acctgtactt ccagtacaga   (SEQ ID NO: 10)

tta,
and

ctcgagagat cctcgcgccc cctacccacc.  (SEQ ID NO: 11)

for ENLYFQY,                       (SEQ ID NO: 12)
and

ggatccgaga acctgtactt ccagctaaga   (SEQ ID NO: 13)

tta,
and

ctcgagagat cctcgcgccc cctacccacc   (SEQ ID NO: 11)

for ENLYFQL.                       (SEQ ID NO: 14)
```

[0129] These PCR steps also introduced a BaniHI restriction site 5' to the sequence encoding each cleavage site, and an XhoI restriction site 3' to tTA stop codon.

[0130] The thus modified ADRB2 coding region was digested with PstI, which cuts at nucleotide position 260 in the coding region, and BamHI. This 3' fragment was ligated with the three variants of tTA modified with the TEV NIa-Pro cleavage sites, that had been digested with BamHI and XhoI, and the resulting complexes were cloned into pBlueScript II, which had been digested with PstI and XhoI.

[0131] A NotI restriction site was introduced 5' to the start codon of the ADRB2 coding region, again via PCR, using

```
gcggccgcca ccatgaacgg taccgaaggc   (SEQ ID NO: 15)

cca,
and

ctggtgggtg gcccggtacc a.           (SEQ ID NO: 16)
```

[0132] The 5' fragment of modified ADRB2 coding region was isolated, via digestion with NotI and PstI and was ligated into each of the constructs of the 3' fragment of ADRB2-TEV-NIa-Pro-cleavage site tTA fusions that had been digested previously, to produce three, full length constructs encoding fuision proteins.

[0133] Each construct was digested with NotI and XhoI, and was then inserted into the commercially available expression vector pcDNA 3, digested with NotI and XhoI.

Example 2

[0134] A second construct was also made, whereby the coding sequence for "βarrestin 2 or ARRB2" hereafter (GenBank, NM_004313) (SEQ ID NO: 17), was ligated to the catalytic domain of the TEV NIa protease (i.e., amino acids 189-424 of mature NIa protease, residues 2040-2279) in the TEV protein. To do this, a DNA sequence encoding ARRB2 was modified, so as to add a BaniHI restriction site to its 5' end. Further, the sequence was modified to replace the endogenous stop codon with a BamHI site. The oligonucleotides

```
caggatcctc tggaatgggg gagaaacccg   (SEQ ID NO: 18)

ggacc,
and

ggatccgcag agttgatcat catagtcgtc   (SEQ ID NO: 19)
```

[0135] were used. The resulting PCR product was cloned into the commercially available vector pGEM-T EASY (Promega). The multiple cloning site of the pGEM-T EASY vector includes an EcoRI site 5' to the start codon of ARRB2.

[0136] The TEV NIa-Pro coding region was then modified to replace the endogenous start codon with a BglII site, and to insert at the 3' end a sequence which encodes influenza hemagluttinin epitope YPYDVPDYA (SEQ ID NO: 20) in accordance with Kolodziej, et al., *Meth. Enzymol.,* 194:508-519 (1991), followed by a stop codon, and a NotI restriction site. This was accomplished via PCR, using

```
agatctagct tgtttaaggg accacgtg,     (SEQ ID NO: 21)
and

gcggccgctc aagcgtaatc tggaacatca   (SEQ ID NO: 22)

tatgggtacg agtacaccaa ttcattcatg

ag.
```

[0137] The resulting, modified ARRB2 coding region was digested with EcoRI and BamHI, while the modified TEV coding region was cleaved with BglII and NotI. Both fragments were ligated into a commercially available pcDNA3 expression vector, digested with EcoRI and NotI.

Example 3

[0138] Plasmids encoding ADRB2-TEV-NIa-Pro cleavage site-tTA and the ARRB2-TEV-NIa protease fuision proteins were transfected into HEK-293T cells, and into "clone 41," which is a derivative of HEK-293T, that has a stably integrated β-galactosidase gene under control of a tTA dependent promoter. About 5×10^4 cells were plated in each well of a 24 well plate, in DMEM medium supplemented with 10% fetal bovine serum, 2mM L- glutamine, 100 units/ml penicillin, 100 μg/ml G418, and 5 μg/ml purimycin. Cells were grown to reach 50% confluency the next day, and were then transfected, using 0.4 μg plasmid DNA, and 2 μl Fugene (a proprietary transfection reagent containing lipids and other material). The mix was combined in 100 μl of DMEM medium, and incubated for 15 minutes at room temperature prior to adding cells. Transfected cells were incubated for 8-20 hours before testing by adding drugs which are known agonists for the receptor, and then 16-24 hours after drug addition.

Example 4

[0139] The levels of 0-galactosidase activity in the cells were first measured by staining the cells with a chromogenic substance, i.e., "X-gal," as taught by MacGregor, et al., *Somat. Cell Mol. Genet.,* 13:253-265 (1987), incorporated by reference. Following culture, cells were washed, twice, in D-PBS with calcium and magnesium, fixed for 5 minutes in 4% paraformaldehyde, and then washed two additional times with D-PBS, calcium and magnesium, for 10 minutes each time. Fixed cells were incubated with 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM $MgCl_2$, 0.1% X-Gal, that had been prepared from a 1:40 dilution of 4% X-Gal stock in dimethylformamide, in D-PBS with calcium and magnesium.

[0140] The reaction was incubated in the dark at room temperature for from 3-4 hours, to overnight. Substrate solution was removed, and cells were mounted under glass coverslips with mowiol mounting medium (10% mowiol, 0.1% 1.4-diazabicyclo [2.2.2]octane, 24% glycerol).

[0141] The results indicated that cells transfected with either the ADRB2-TEV-NIa-Pro cleavage site-tTA plasmid alone or the ARRB2-TEV-NIa protease plasmid alone did not express β-galactosidase. A small fraction of cells transfected with both plasmids did express β-galactosidase, probably due to basal levels of interaction between unstimulated ADRB2 and ARRB2. About 3-5 fold more cells expressed the reporter gene after treatment with either 10 μM isoproterenol, or 10 μM epinephrine, both of which are ADRB2 agonists.

[0142] When the cells were pretreated for 5 minutes with the ADRB2 antagonist alprenolol (10 μM), the agonist induced increase in β-galactosidase expressing cells was blocked, and treatment with alprenolol alone had no apparent effect.

[0143] These results show that one can link agonist binding and GPCR stimulation to transcriptional activation of a reporter gene.

Example 5

[0144] A set of experiments were carried out in order to quantify the level of reporter gene activity in the cells more precisely and to maximize the signal-to-background ratio of the assay. This was accomplished by measuring the level of reporter gene induction using a commercially available chemiluminescence assay for β-galactosidase activity. Clone 41 cells were transfected with the ADRB2-tTA fusion constructs, containing either the high, medium or low efficiency cleavage sites, and the ARRB2-TEV-NIa protease expression plasmid described supra. Cells were either untreated or treated with 1 μM isoproterenol 20 hours after the transfection, and the luminescence assay was carried out 24 hours after the drug addition. In brief, following cell culture, the medium was removed, and 50 μl of lysis buffer (100 mM potassium phosphate, pH7.8, 0.2% Triton X-100) was added to each well. The cells were lysed via incubation for 5 minutes, at room temperature, with mild agitation. Lysates were collected and analyzed via commercially available products.

[0145] In all cases, treatment with agonist increased levels of β-galactosidase activity. However, the background level of reporter gene activity in untreated cells was lowest with the low efficiency cleavage site, relative to the medium and high efficiency sites. Further, agonist treatment resulted in a 4.8-fold stimulation of reporter gene activity in cells transfected with the low efficiency cleavage site, compared to 2.8-fold for the medium efficiency cleavage site and 1.2-fold for the high efficiency cleavage site. Thus, the highest signal-to-background ratio is obtained by using the low efficiency protease cleavage site.

Example 6

[0146] These experiments were designed to verify that the agonist stimulated increase in reporter gene expression is dependent on binding and activation of the receptor by the agonist.

[0147] To do this, variants of the ADRB2-tTA fusion constructs were generated following the protocols supra, except each contained a mutant form of the receptor with a single amino acid change from D to S at position 113, which results in a greatly reduced affinity for the agonist isoproterenol. See Strader, et al., *J. Biol. Chem.,* 266:5-8 (1991). Three forms of the mutant receptor-tTA fusion construct with each of the different cleavage sites were formed.

[0148] The levels of β-galactosidase activity were measured in clone 41 cells co-transfected with the ADRB2-tTA fusion constructs containing the D113S point mutation and the ARRB2-TEV-NIa protease expression plasmid described previously. The activity tests were carried out exactly as described, supra. The results indicated that the agonist isoproterenol did not stimulate reporter gene expression in cells expressing the mutant ADRB2-tTA fusion contructs.

Example 7

[0149] These experiments were designed to examine whether the agonist stimulated increase in reporter gene expression is dependent on fusion of TEV NIa-Pro to ARRB2.

[0150] To do this, the levels of β-galactosidase activity were measured in clone 41 cells co-transfected with the

ADRB2-tTA fusion construct containing the low efficiency cleavage site and either the ARRB2-TEV-NIa protease expression plasmid described supra, or a control TEV-NIa protease fusion to the SH2 domain of phospholipase C. The activity tests were carried out exactly as described, supra. The results indicated that agonist-stimulated increase in reporter gene expression was detected only when the TEV protease was fused to ARRB2 and not when fused to an unrelated polypeptide.

Example 8

[0151] These experiments were designed to determine if gene expression is induced selectively by agonists of the target receptor, or if it can be stimulated by other molecules.

[0152] ATP is an agonist for G protein coupled receptors P2Y1 and P2Y2, which are expressed endogenously by HEK-293T cells.

[0153] Experiments were carried out using clone 41 cells which were cotransfected with the ADRB2-tTA fusion construct containing the low efficiency cleavage site and the arrestin-TEV-NIa protease fusion as described supra, which were treated with isoproterenol, ATP, or untreated. The assays were carried out as described, supra.

[0154] The results indicated that induction of reporter gene activity was specific to activation of target receptor. Stimulation of another GPCR pathway was irrelevant.

Example 9

[0155] A set of experiments were carried out using clone 41 cells which were cotransfected with the ADRB2-tTA fusion construct containing the low efficiency cleavage site and the ARRB2-TEV-NIa protease fusion as described supra, which were treated with varying amounts of one of the adrenergic receptor agonists isoproterenol and epinephrine. The assays were carried out as described, supra. The results presented in **FIG. 2***a* show a dose-response curve for the stimulation of reporter gene expression by these two ligands. Each point represents the mean value obtained from three experiments.

[0156] A set of experiments were carried out as described supra, in which the co-transfected clone 41 cells were pretreated with varying concentrations of the adrenergic receptor antagonist alprenolol for 15 minutes, followed by treatment with 1 μM epinephrine. The results shown in **FIG. 2***b* indicate a dose-inhibition curve for this antagonist.

Example 10

[0157] A similar set of constructs were made to establish an assay for the G protein coupled arginine vasopressin receptor 2 (AVPR2). The AVPR2 coding region (Genbank Accession Number: NM_000054) (SEQ ID NO: 23) was modified to place an EcoRI site at the 5' end and replace the stop codon with a BamHI site using PCR with the primers

```
gaattcatgc tcatggcgtc caccac      (SEQ ID NO: 24)
and

ggatcccgat gaagtgtcct tggccag.    (SEQ ID NO: 25)
```

[0158] The modified AVPR2 coding region was ligated into the three ADRB2- tTA constructs described supra, which had been cut with EcoRI and BamHI. This replaced the entire coding sequence of the ADRB2 with the coding sequence of AVPR2.

[0159] Clone 41 cells were co-transfected with the AVPR2-tTA fusion construct containing the low efficiency cleavage site and the ARRB2-TEV-NIa protease fusion described supra, and assays were carried out using varying concentrations (1 pM to 2 μM) of [Arg8] vasopressin, an agonist for AVPR2. The data, presented in **FIG. 3**, shows a dose- response curve for this agonist, with an EC50 of 3.3 nM, which agrees with previously published data (Oakley, R., et. al., *Assay and Drug Development Technologies,* 1:21-30, (2002)). The maximal response resulted in an approximately 40-fold induction of reporter gene expression over the background level.

Example 11

[0160] A similar set of constructs were made to establish an assay for the G protein coupled serotonin receptor 1a (HTRIA). The HTR1A coding region,(Genbank Accession Number: NM_000524) (SEQ ID NO: 26) was modified to place an EcoRI site at the 5' end and replace the stop codon with a BamHI site using PCR with the primers

```
gaattcatgg atgtgctcag ccctgg     (SEQ ID NO: 27)
and

ggatccctgg cggcagaact tacac.     (SEQ ID NO: 28)
```

[0161] The modified HTR1A coding region was ligated into the AVPR2-tTA constructs described supra, which had been cut with EcoRI and BamHI. This replaced the entire coding sequence of AVPR2 with the coding sequence of HTR1A. The resulting construct will be referred to as "HTR1A-tTA" hereafter.

[0162] Clone 41 cells were co-transfected with the HTR1A-tTA fusion construct containing the low efficiency cleavage site and the ARRB2-TEV-NIa protease fusion construct described supra, and assays were carried out using 10 μM 8-hydroxy-DPAT HBr (OH-DPAT), an agonist for the HTR1A, as well as with 10 μM serotonin, a natural agonist for HTR1A. The assays were carried out as described, supra. The maximal response to OH-DPAT resulted in a 6.3-fold induction of reporter gene expression over background level and the maximal response to serotonin resulted in a 4.6-fold induction of reporter gene expression over background level.

Example 12

[0163] Similar constructs were made to establish an assay for the G protein coupled m2 muscarinic acetylcholine receptor (CHRM2). The CHRM2 coding region (Genbank Accession Number: NM_000739) (SEQ ID NO: 29) was modified to place an EcoRI site at the 5' end and replace the stop codon with a BglII site using PCR with the primers

```
gaattcatga ataactcaac aaactcc     (SEQ ID NO: 30)
and

agatctcctt gtagcgccta tgttc.      (SEQ ID NO: 31)
```

[0164] The modified CHRM2 coding region was ligated into the AVPR2-tTA constructs described supra, which had been cut with EcoRI and BamHI. This replaced the entire coding sequence of AVPR2 with the coding sequence of CHRM2.

[0165] Clone 41 cells were co-transfected with the CHRM2-tTA fusion construct containing the high efficiency cleavage site and the ARRB2-TEV-NIa protease fusion described supra, where the ARRB2-protease fusion protein was expressed under the control of the Herpes Simplex Virus thymidine kinase (HSV-TK) promoter, and assays were carried out using 10 μM carbamylcholine Cl (carbochol), an agonist for CHRM2, as described supra. The maximal response to carbochol resulted in a 7.2-fold induction of reporter gene expression over background.

Example 13

[0166] α Constructs were also made to establish an assay for the G protein coupled chemokine (C-C motif) receptor 5 (CCR5). The CCR5 coding region (Genbank Accession Number: NM_000579) (SEQ ID NO: 32) was modified to place Not I site at the 5' end and replace the stop codon with a BamHIl site using PCR with the primers

```
gcggccgcat ggattatcaa gtgtcaagtc c (SEQ ID NO: 33)
and

ggatccctgg cggcagaact tacac.       (SEQ ID NO: 34)
```

[0167] The CCR5 coding region was also modified to place a BsaI site at the 5' end which, when cut, leaves a nucleotide overhang which is compatible with EcoRI cut DNA using the primers

```
ggtctccaat tcatggatta tcaagtgtca   (SEQ ID NO: 35)

agt
and

gacgacagcc aggtacctat c.           (SEQ ID NO: 36)
```

[0168] The first modified coding region was cut with ClaI and BamHI and the second was cut with Bsal and Clal. Both fragments were ligated into the AVPR2-tTA constructs described supra, which had been cut with EcoRI and BamHI. This replaced the entire coding sequence of AVPR2 with the coding sequence of CCR5.

[0169] The CCR5-tTA fusion construct containing the low efficiency cleavage site was transfected into "clone 34" cells, which are a derivative of the HEK cell line "clone 41" described supra, but which contain a stably integrated ARRB2-TEV-NIa protease fusion gene under the control of the CMV promoter. Assays were carried out using 1 μg/ml "Regulated on Activation, Normal T-Cell Expressed and Secreted" (RANTES), a known agonist for CCR5. The maximal response to RANTES, measured as described supra resulted in an approximately 40-fold induction of reporter gene expression over the background.

Example 14

[0170] Next, a set of constructs were made to establish an assay for the G protein coupled dopamine 2 receptor (DRD2). The DRD2 coding region (Genbank Accession Number: NM_000795) (SEQ ID NO: 37) was modified to place an EcoRI site at the 5' end and replace the stop codon with a BglII site using PCR with the primers

```
gaattcatgg atccactgaa tctgtcc   (SEQ ID NO: 38)
and

agatctgcag tggaggatct tcagg.    (SEQ ID NO: 39)
```

[0171] The modified DRD2 coding region was ligated into the AVPR2-tTA constructs described supra, cut with EcoRI and BamHI. This replaced the entire coding sequence of AVPR,2 with the coding sequence of DRD2.

[0172] Clone 41 cells were co-transfected with the DRD2-tTA fusion construct containing the medium efficiency cleavage site and the ARRB2-TEV-NIa protease fusion described supra, and assays were carried out using 10 μM dopamine HCl (dopamine), an agonist for DRD2. Results were measured as in the assays described supra. The maximal response to dopamine resulted in a 2.7-fold induction of reporter gene expression over the background.

Example 15

[0173] These experiments were designed to demonstrate enhancements of the assay using arrestin variants that bind agonist-occupied GPCRs more stably. First, a fusion of the TEV NIa protease to β-arrestin-1 (ARRB1) was constructed. The coding region of ARRB1 (Genbank Accession Number: NM_004041) (SEQ ID NO: 40) was modified to place an Asp718 site at the 5' end and replace the stop codon with a BamHI site using PCR with the primers

```
ggtaccatgg gcgacaaagg gacgcgagtg   (SEQ ID NO: 41)
and

ggatcctctg ttgttgagct gtggagagcc   (SEQ ID NO: 42)

tgtaccatcc tcctcttc.
```

[0174] The resulting modified ARRB1 coding region was cut with Asp718 and EcoRI and with EcoRI and BamHI, while the modified TEV NIa-Pro coding region described supra was cut with BglII and NotI. All three fragments were ligated into a commercially available pcDNA3 expression vector, which had digested with Asp718 and NotI.

[0175] Clone 41 cells were co-transfected with the DRD2-tTA fusion construct containing the medium efficiency cleavage site and the ARRB1-TEV-NIa protease fusion, and assays were carried out using 10 μM dopamine HCl (dopamine), an agonist for the D2 receptor, as described supra. The maximal response to dopamine resulted in a 2.1-fold induction of reporter gene expression over the background.

[0176] Truncation of ARRBlfollowing amino acid 382 has been reported to result in enhanced affinity for agonist-bound GPCRs, independent of GRK-mediated phosphorylation (Kovoor A., et. al., *J. Biol. Chem.*, 274(11):6831-6834 (1999)). To demonstrate the use of such a "constitutively active" arrestin in the present assay, the coding region of β-arrestin-1 was modified to place an Asp718 site at the 5' end and a BamHI site after amino acid 382 using PCR with SEQ ID NO: 41, spra and

[0177] ggatccattt gtgtcaagtt ctatgag (SEQ ID NO: 43).

[0178] This results in a an ARRb1 coding region which is 36 amino acids shorter than the fill-length coding region. The resulting modified ARRB1 coding region, termed "ARRB1 (Δ383)", was cut with Asp718 and EcoRI and with EcoRI and BamHI, while the modified TEV NIa-Pro coding region described supra was cut with BglII and NotI. All three fragments were ligated into a commercially available pcDNA3 expression vector, digested with Asp718 and NotI.

[0179] Clone 41 cells were co-transfected with the DRD2-tTA fusion construct containing the medium efficiency cleavage site and the ARRBl (A383)-TEV-NIa protease fusion, and assays were carried out using 10 μM dopamine HCl (dopamine), an agonist for the DRD2 receptor, as described supra. The maximal response to dopamine resulted in an 8.3-fold induction of reporter gene expression over the background.

[0180] To examine the effect of a comparable truncation of the ARRB2 coding region the coding region of ARRB2 was modified to place an Asp718 site at the 5' end and replaced 81 nucleotides at the 3' end with a BamHI site using PCR with the primers

```
ggtaccatgg gggagaaacc cgggacc   (SEQ ID NO: 44)
and

ggatcctgtg gcatagttgg tatc.     (SEQ ID NO: 45)
```

[0181] This results in a ARRB2 coding region which is 27 amino acids shorter than the full-length coding region. The resulting modified ARRB2 coding region was cut with Asp718 and BamHI, while the modified TEV NIa-Pro coding region described supra was cut with BglII and NotI. Both fragments were ligated into a commercially available pcDNA3 expression vector, digested with Asp718 and NotI.

[0182] Clone 41 cells were co-transfected with the DRD2-tTA fusion construct containing the medium efficiency cleavage site and the ARRB2 (A383)-TEV-NIa protease fusion, and assays were carried out using 10 μM dopamine HCl (doparnine), an agonist for the DRD2 receptor, as described supra. The maximal response to dopamine resulted in a 2.1 -fold induction of reporter gene expression over the background.

[0183] These results, presented in **FIG. 4**, demonstrate that DRD2 dopamine receptor assay shows the highest signal-to-background ratio using the arrestin variant ARRBl (A383).

Example 16

[0184] This set of experiments was carried out to demonstrate enhancements of the assay using receptor modifications that are designed to increase affinity for the interacting protein. In this example, the C-terminal tail domain of a test receptor was replaced with the corresponding tail domain from AVPR2, a receptor known to bind arrestins with high affinity. In these examples the fusion junction was made 15-18 amino acids after the conserved NPXXY motif at the end of the seventh transmembrane helix, which typically corresponds to a position immediately after a putative palmitoylation site in the receptor C-terminus.

[0185] First, PCR was used to produce a DNA fragment encoding the C-terminal 29 amino acids from AVPR2, followed by the low efficiency TEV cleavage site and tTA transcription factor. The fragment was also designed such that the first two amino acids (Ala, A and Arg, R) are encoded by the BssHII restriction site GCGCGC. This was accomplished by amplifying the AVPR2-tTA construct with the low efficiency cleavage site described supra with the primers

```
tgtgcgcgcg gacgcacccc acccagcctg   (SEQ ID NO: 46)

ggt
and

ctcgagagat cctcgcgccc cctacccacc.  (SEQ ID NO: 11)
```

[0186] Next, the coding region of the DRD2 was modified to place an EcoRI site at the 5' end and to insert a BssHII site after the last amino acid in the coding region (Cys-443). This was done using PCR with the primers

```
gaattcatgg atccactgaa tctgtcc      (SEQ ID NO: 47)
and

tgtgcgcgcg cagtggagga tcttcaggaa   (SEQ ID NO: 48)

ggc.
```

[0187] The resulting modified D2 coding region was cut with EcoRI and BssHII and the resulting AVPR2 C-terminal tail-low efficiency cleavage site-tTA fragment was cut with BssHII and BamHI. Both fragments were ligated into the AVPR2-low efficiency cleavage site-tTA construct described supra, cut with EcoRI and BamHI.

[0188] Clone 41 cells were co-transfected with the DRD2-AVPR2 Tail-tTA fusion construct containing the low efficiency TEV cleavage site and the ARRB2-TEV-NIa protease fusion described supra, and assays were carried out using 10 μM dopamine HCl (dopamine), an agonist for the DRD2 receptor. The maximal response to dopamine resulted in an approximately 60-fold induction of reporter gene expression over the background.

[0189] A construct was made which modified the ADRB2 receptor coding region by inserting an Asp718 site at the 5' end and by placing a BssHII site after Cys-341. This was done using PCR with the primers

```
gcggccgcca ccatgaacgg taccgaaggc   (SEQ ID NO: 49)

cca
and

tgtgcgcgcg cacagaagct cctggaaggc.  (SEQ ID NO: 50)
```

[0190] The modified ADRB2 receptor coding region was cut with EcoRI and BssHII and the AVPR2 C-terminal tail-low efficiency cleavage site-tTA fragment was cut with BssHII and BamHI. Both fragments were ligated into the AVPR2-low efficiency cleavage site-tTA construct described supra cut, with EcoRI and BamHI. The resulting construct is "ADRB2-AVPR2 Tail-tTA." (Also see published application U.S. 2002/0106379, supra, SEQ ID NO: 3 in particular.)

[0191] Clone 41 cells were co-transfected with the ADRB2-AVPR2 Tail-tTA fusion construct containing the

low efficiency TEV cleavage site and the ARRB2-TEV-NIa protease fusion described supra, and assays were carried out using 10 μM isoproterenol, an agonist for the ADRB2 receptor. The maximal response to isoproterenol resulted in an approximately 10-fold induction of reporter gene expression over the background.

[0192] A construct was made which modified the kappa opioid receptor (OPRK; Genbank Accession Number: NM_000912) (SEQ ID NO: 51) coding region by placing a BssHII site after Cys-345. This was done using PCR with the primers

```
ggtctacttg atgaattcct ggcc      (SEQ ID NO: 52)
and

gcgcgcacag aagtcccgga aacaccg   (SEQ ID NO: 53)
```

[0193] The modified OPRK receptor coding region was cut with EcoRI and BssHII and AVPR2 C-terminal tail-low efficiency cleavage site-tTA fragment was cut with BssHII and XhoI. Both fragments were ligated into a plasmid containing the modified OPRK receptor sequence, cloned into pcDNA3.1+at Asp718 (5') and XhoI (3'), which had been digested with EcoRI and XhoI.

[0194] Clone 41 cells were co-transfected with the OPRK-AVPR2 Tail-tTA fusion construct containing the low efficiency cleavage site and the ARRB2-TEV-NIa protease fusion described supra, and assays were carried out using 10 μM U-69593, an agonist for the OPRK. The maximal response to U-69593 resulted in an approximately 12-fold induction of reporter gene expression over the background.

Example 17

[0195] This experiment was designed to demonstrate the use of the assay to measure the activity of two test receptors simultaneously using a multiplex format.

[0196] Clone 41 cells and "clone 1H10" cells, which are cells of an HEK-293T cell line containing a stable integration of the luciferase gene under the control of a tTA-dependent promoter, were each plated on 24-well culture dishes and were transiently transfected with the chimeric ADRB2-AVPR2 Tail-tTA or the DRD2-AVPR2 Tail-tTA fusion constructs described supra, respectively. Transient transfections were performed using 100 μlof media, 0.4 μg of DNA and 2 μl of FuGene reagent per well. After 24 hr of incubation, Clone 41 cells expressing ADRB2-AVPR2 Tail-tTA and clone 1H10 cells expressing DRD2-AVPR2 Tail-tTA were trypsinized, mixed in equal amounts, and replated in 12 wells of a 96-well plate. Triplicate wells were incubated without drug addition or were immediately treated with 1 μM isoproterenol, 1 μM dopamine, or a mixture of both agonists at 1 μM. Cells were assayed for reporter gene activity approximately 24 hours after ligand addition. Medium was discarded, cells were lysed in 40 μl lysis buffer [100 mM potassium phosphate pH 7.8, 0.2% Triton X-100] and the cell lysate was assayed for beta-galactosidase and for luciferase activity using commercially available luminescent detection reagents.

[0197] The results are presented in **FIG. 5A and 5B**. Treatment with isoproterenol resulted in an approximately seven-fold induction of beta-galactosidase reporter gene activity, whereas luciferase activity remained unchanged. Treatment with dopamine resulted in a 3.5-fold induction of luciferase activity, while beta-galactosidase activity remained unchanged. Treatment with both isoproterenol and dopamine resulted in seven-fold and three-fold induction of beta-galactosidase and luciferase activity, respectively.

Example 18

[0198] This experiment was designed to demonstrate the use of the assay to measure the activity of two test receptors simultaneously using a multiplex format.

[0199] "Clone 34.9" cells, which are a derivative of clone 41 cells and containing a stably integrated ARRB2-TEV NIa protease fusion protein gene, were transiently transfected with the chimeric OPRK-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA fusion construct described supra. In parallel, "clone HTL 5B8.1" cells, which are an HEK-293T cell line containing a stable integrated luciferase gene under the control of a tTA-dependent promoter, were transiently transfected with the ADRB-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA fusion construct described supra. In each case 5×10^5 cells were plated in each well of a 6-well dish, and cultured for 24 hours in DMEM supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 units/ml penicillin, 500 μg/ml G418, and 3 μg/ml puromycin. Cells were transiently transfected with 100 μl of DMEM, 0.5 μg of OPRK-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA DNA, and 2.5 μl Fugene ("clone 34.9 cells") or with 100 μl of DMEM, 0.5 μg of ADRB2-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA DNA, 0.5 μg of ARRB2-TEV NIa Protease DNA and 5 μl Fugene ("clone HTL 5B8.1 cells"). Transiently transfected cells were cultured for about 24 hours, and were then trypsinized, mixed in equal amounts and replated in wells of a 96 well plate. Cell were incubated for 24 hours before treatment with 10 μM U-69593, 10 μM isoproterenol or a mixture of both agonists at 10 μM. Sixteen wells were assayed for each experimental condition. After 24 hours, cells were lysed and the activity of both beta-galactosidase and luciferase reporter genes were assayed as described supra. The results are presented in **FIG. 6**. Treatment with U-69593 resulted in an approximately 15-fold induction of beta-galactosidase reporter gene activity, whereas luciferase activity remained unchanged. Treatment with isoproterenol resulted in a 145-fold induction of luciferase activity. while beta-galactosidase activity remained unchanged. Treatment with both U-69593 and isoproterenol resulted in nine-fold and 136-fold induction of beta-galactosidase and luciferase activity, respectively.

Example 19

[0200] This experiment was carried out to demonstrate the use of a different transcription factor and promoter in the assay of the invention.

[0201] A fusion construct was created, comprising DNA encoding AVPR2, fused in frame to a DNA sequence encoding the amino acid linker GSENLYFQLR (SEQ ID NO: 54) which included the low efficiency cleavage site for TEV Ni a-Pro described supra, fused in frame to a DNA sequence encoding amino acids 2-147 of the yeast GAL4 protein (GenBank Accession Number P04386) (SEQ ID NO: 55) followed by a linker, i.e., of the sequence PELGSASAELT-MVF (SEQ ID NO: 56), followed by amino acids 368-549

of the murine nuclear factor kappa-B chain p65 protein (GenBank Accession Number A37932) (SEQ ID NO: 57). The CMV promoter was placed upstream of the AVPR2 coding region and a polyA sequence was placed downstream of the GAL4-NFkB region. This construct was designated AVPR2-TEV-NIa-Pro cleavage (Leu)-GAL4.

[0202] HUL 5C1.1 is a derivative of HEK-293T cells, which contain a stably integrated luciferase reporter gene under the control of a GAL4 upstream activating sequence (UAS), commercially available pFR-LUC.

[0203] This AVPR2-TEV-NIa-Pro cleavage (Leu)-GAL4 plasmid was co-transfected along with the β-arrestin2-TEV Nla Protease described supra into HUL 5C1.1 cells. About 2.5×10^4 cells were plated into each well of a 96 well-plate, in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 units/ml penicillin, 500 μg/ml G418, and 3 μg/ml puromycin. Cells were grown to reach 50% confluency the next day and were transfected with 10 μl per well of a mixture consisting of 85 μl of DMEM, 0.1 μg of AVPR2-TEV-Nia-Pro cleavage (Leu)-GAL4 DNA, 0.1 μof ARRB2-TEV Nla Protease DNA, and 1 μl Fugene, which had been incubated for 15 minutes at room temperature prior to addition to the cells. Transfected cells were cultured for about 16 hours before treatment with 10 μM vasopressin. After six hours, cells were lysed and luciferase activity was assayed as described supra. Under these conditions, treatment with vasopressin resulted in a 180-fold increase in reporter gene activity.

Example 20

[0204] This set of experiments were carried out to demonstrate enhancements of the assay using further receptor modifications that are designed to increase the affinity for the interacting protein. In this example, the C-terminal tail domain of the test receptor is replaced with the corresponding tail domain of one of the following receptors: apelin J receptor—AGTRL1 (accession number: NM_005161) (SEQ ID NO: 58), gastrin-releasing peptide receptor—GRPR (accession number: NM_005314) (SEQ ID NO: 59), proteinase-activated receptor 2 —F2RL1 (accession number: NM_005242) (SEQ ID NO: 60), CCR4 (accession number: NM_005508) (SEQ ID NO: 61), chemokine (C-X-C motif) receptor 4 - CXCR4 (accession number: NM_003467) (SEQ ID NO: 62), and interleukin 8 receptor, beta-CXCR2/IL8b (accession number: NM_001557) (SEQ ID NO: 63).

[0205] First PCR was used to produce a DNA fragment encoding the C-terminal tail of the above receptors. These fragments were designed such that the first two amino acids (Ala, A and Arg, R) are encoded by the BssHII restriction site.

[0206] The AGTRL1 C-terminal fragment was amplified with the primers

```
tgtgcgcgcg gccagagcag gtgcgca   (SEQ ID NO: 64)
and

gaggatccgt caaccacaag ggtctc.   (SEQ ID NO: 65)
```

[0207] The GRPR C-terminal fragment was amplified with the primers

```
tgtgcgcgcg gcctgatcat ccggtct   (SEQ ID NO: 66)
and

gaggatccga cataccgctc gtgaca.   (SEQ ID NO: 67)
```

[0208] The F2RL1 C-terminal fragment was amplified with the primers

```
tgtgcgcgca gtgtccgcac tgtaaagc   (SEQ ID NO: 68)
and

gaggatccat aggaggtctt aacagt.    (SEQ ID NO: 69)
```

[0209] The CCR4 C-terminal fragment was amplified with the primers

```
tgtgcgcgcg gccttttgt gctctgc   (SEQ ID NO: 70)
and

gaggatccca gagcatcatg aagatc.   (SEQ ID NO: 71)
```

[0210] The CXCR2/IL8b C-terminal fragment was amplified with the primers

```
tgtgcgcgcg gcttgatcag caagggac   (SEQ ID NO: 72)
and

gaggatccga gagtagtgga agtgtg.    (SEQ ID NO: 73)
```

[0211] The CXCR4 C-terminal fragment was amplified with the primers

```
tgtgcgcgcg ggtccagcct caagatc   (SEQ ID NO: 74)
and

gaggatccgc tggagtgaaa acttga.   (SEQ ID NO: 75)
```

[0212] The resulting DNA fragments encoding the modified C-terminal tail domains of these receptors were cut with BssHII and BamHI and the fragments were ligated in frame to the OPRK receptor coding region, replacing the AVPR2-C-terminal tail fragment, in the OPRK-AVPR2 Tail- TEV-NIa-Pro cleavage (Leu)-tTA expression construct described supra.

[0213] HTL 5B8.1 cells described supra were co-transfected with each of the above modified OPRK coding region—TEV-NIa-Pro cleavage (Leu)—tTA constructs and the β-arrestin 2—TEV NIa protease fusion described supra. About 2.5×10^4 cells per well were plated onto a 96 well-plate, in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 units/ml penicillin, 500 μg/ml G418, and 3 μg/ml puromycin. Cells were grown to reach 50% confluency the next day and were transfected with 10 μl per well of a mixture consisting of 85 μl of DMEM, 0.25 μg of AVPR2-TEV-NIa-Pro cleavage (Leu)-GAL4 DNA, 0.25 μg of ARRB2-TEV NIa protease DNA, and 2.5 μl Fugene (a proprietary transfection reagent containing lipids and other material), which had been incubated for 15 minutes at room temperature prior to addition to the

cells. Transfected cells were cultured for about 16 hours before treatment 10 μM U-69593. After six hours, cells were lysed and luciferase activity was assayed as described supra. Under these conditions, treatment with U-69593 resulted in the following relative increases in reporter gene activity for each of the modified OPRK receptors: OPRK-AGTRL1 C-terminal tail—30 fold; OPRK-GRPR C-terminal tail—312 fold; OPRK-F2RL1 C-terminal tail—69.5 fold; OPRK-CCR4 C-terminal tail—3.5 fold; OPRK-CXCR4 C-terminal tail—9.3 fold; OPRK-IL8b C-terminal tail—113 fold.

Example 21

[0214] This experiment was designed to produce a cell line that stably expressed the ARRB2-TEV NIa protease fusion protein described supra.

[0215] A plasmid was made which expressed the ARRB2-TEV NIa protease fusion protein under the control of the EF1α promoter and also expressed the hygromycin resistance gene under the control of the thyrnidine kinase (TK) promoter.

[0216] This plasmid was transfected into HTL 5B8.1, and clones containing a stable genomic integration of the plasmid were selected by culturing in the presence of 100 μg/ml hygromycin. Resistant clones were isolated and expanded and were screened by transfection of the ADRB2-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA plasmid described supra. Three cell lines that were selected using this procedure were designated "HTLA 4C2.10", "HTLA 2C11.6" and "HTLA 5D4". About 2.5×10^4 cells per well were plated onto a 96 well-plate, in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 units/ml penicillin, 500 μg/ml G418, 3 μg/ml puromycin, and 100 μg/ml hygromycin. Cells were grown to reach 50% confluency the next day and were transfected with 10 μl per well of a mixture consisting of 85 μl of DMEM, 0.25 μg of ADRB2-AVPR2-TEV-NIa-Pro cleavage (Leu)-GAL4 DNA and 0.5 μl Fugene, which had been incubated for 15 minutes at room temperature prior to addition to the cells. Transfected cells were cultured for about 16 hours before treatment 10 μM isoproterenol. After six hours, cells were lysed and luciferase activity was assayed as described supra. Under these conditions, treatment with isoproterenol resulted in a 112-fold ("HTLA 4C2.10"), 56-fold ("HTLA 2C11.6") and 180-fold ("HTLA 5D4") increase in reporter gene activity in the three cell lines, respectively.

Example 22

[0217] This experiment was designed to produce a cell line that stably expressed the ARRB2-TEV NIa protease and the ADRB2-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA fusion proteins described supra.

[0218] The ARRB2-TEV NIa protease plasmid containing the hygromycin resistance gene was transfected together with the ADRB2-AVPR2 Tail-TEV-NIa-Pro cleavage (Leu)-tTA fusion protein plasmid described supra into HTL 5B8.1 cells and clones containing stable genomic integration of the plasmids were selected by culturing in the presence of 100 μg/ml hygromycin. Resistant clones were isolated and expanded, and were screened by treating with 10 μM isoproterenol and measuring the induction of reporter gene activity as described supra. Three cell lines that were selected using this procedure were designated "HTLAR 1E4", "HTLAR 1C10" and "HTLAR 2G2". Treatment with isoproterenol for 6 hours resulted in a 208-fold ("HTLAR 1E4"), 197-fold ("HTLAR 1C10") and 390-fold ("HTLAR 2G2") increase in reporter gene activity in the three cell lines, respectively.

Example 23

[0219] This experiment was designed to demonstrate the use of the assay to measure the activity of the receptor tyrosine kinase epidermal growth factor receptor (EGFR).

[0220] A first fusion construct was created, comprising DNA encoding the human EGFR, which can be found at GenBank under the Accession Number NM_005228 (SEQ ID NO: 76), fused in frame to a DNA sequence encoding amino acids 3-335 of the tetracycline-controlled transactivator tTA, described supra. Inserted between these sequences is a DNA sequence encoding the amino acid sequence GGSGSENLYFQL (SEQ ID NO: 77) which includes the low efficiency cleavage site for TEV NIa-Pro, ENLYFQL (SEQ ID NO: 14), described supra. The CMV promoter was placed upstream of the Epidermal Growth Factor Receptor coding region, and a polyA sequence was placed downstream of the tTA region. This construct is designated EGFR-TEV-NIa-Pro cleavage (Leu)-tTA.

[0221] A second fusion construct was created, comprising DNA encoding the two SH2 domains of human Phospholipase C Gamma 1, corresponding to amino acids 538-759 (GeneBank accession number NP_002651.2) (SEQ ID NO: 78) fused in frame to a DNA sequence encoding the catalytic domain of mature TEV NIa protease, described supra, corresponding to amino acids 2040-2279 (GeneBank accession number AAA47910) (SEQ ID NO: 79). Inserted between these sequences is a linker DNA sequence encoding the amino acids NSSGGNSGS (SEQ ID NO: 80). The CMV promoter was placed upstream of the PLC-Gamma SH2 domain coding sequence and a polyA sequence was placed downstream of the TEV NIa protease sequence. This construct is designated PLC Gammal-TEV.

[0222] The EGFR-TEV-NIa-Pro cleavage (Leu)-tTA and PLC Gammal-TEV fusion constructs were transfected into clone HTL5B8.1 cells described supra. About 2.5×10^4 cells were plated into each well of a 96 well-plate, in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 units/ml penicillin, 500 μg/ml G418, and 3 μg/ml puromycin. Cells were grown to reach 50% confluency the next day and were transfected with 15 μl per well of a mixture consisting of 100 μl of DMEM, 0.4 μg of pcDNA3 DNA ("carrier" vector DNA), 0.04 μg of EGFR-TEV-NIa-Pro cleavage (Leu)-tTA DNA, 0.04 μg of PLC Gammal-TEV DNA, and 2 μl Fugene (a proprietary transfection reagent containing lipids and other material), which had been incubated for 15 minutes at room temperature prior to addition to the cells. Transfected cells were cultured for about 16 hours before treatment with specified receptor agonists and inhibitors. After six hours, cells were lysed and luciferase activity was assayed as described supra. Results are shown in **FIG. 7**.

[0223] The addition of 2.5 ng/ml human Epidermal Growth Factor (corresponding to the EC80 for this ligand) resulted in a 12.3 fold increase of luciferase reporter gene activity, while addition of 100 ng/ml human Transforming Growth Factor—Alpha resulted in an 18.3 fold increase.

Prior treatment with tyrosine kinase inhibitors (70 μM AG-494; 0.3 μM AG-1478; 2 mM RG-130022) before addition of human Epidermal Growth Factor blocked the induction of reporter gene activity.

Example 24

[0224] This experiment was designed to demonstrate the use of the assay to measure the activity of the human Type I Interferon Receptor.

[0225] A fusion construct was created, comprising DNA encoding human Interferon Receptor I (IFNAR1) (557 amino acids), which can be found in Genbank under Accession Number NM_000629 (SEQ ID NO: 81), fused in frame to a DNA sequence encoding amino acids 3-335 of the tetracycline controlled transactivator tTA, described supra. Inserted between these sequences is a DNA sequence encoding the amino acid sequence GSENLYFQL (SEQ ID NO: 82) which includes the low efficiency cleavage site for TEV NIa-Pro, ENLYFQL (SEQ ID NO: 14), described supra. The CMV promoter was placed upstream of the Human Interferon Receptor I (IFNAR1) coding region, and a poly A sequence was placed downstream of the tTA region. This construct is designated IFNAR1-TEV-NIa-Pro cleavage (L)-tTA.

[0226] second fusion construct was created, using DNA encoding Human Interferon Receptor 2, splice variant 2 (IFNAR2.2) (515 amino acids), which can be found at Genbank, under Accession Number L41942 (SEQ ID NO: 83), fused in frame to a DNA sequence encoding the catalytic domain of the TEV NIa protease, described supra corresponding to amino acids 2040-2279 (GenBank accession number AAA47910) (SEQ ID NO: 84). Inserted between these sequences is a DNA sequence encoding the amino acid sequence RS (Arg-Ser). The CMV promoter region was placed upstream of the Human Interferon Receptor 2 (IFNAR2.2) coding region, and a poly A sequence was placed downstream of the TEV region. This construct is designated IFNAR2.2-TEV.

[0227] Expression constructs were also generated in which the genes for Human Signal Transducer and Activator of Transcription 1 (STAT1), found in Genbank, under Accession Number NM_007315 (SEQ ID NO: 85), Human Signal Transducer and Activator of Transcription 2 (STAT2) found in Genbank, under Accession Number NM_005419 (SEQ ID NO: 86), were expressed under the control of the CMV promoter region. These constructs were designated CMV-STAT1 and CMV-STAT2 respectively.

[0228] The IFNAR1-TEV-NIa-Pro cleavage (L)-tTA and IFNAR2.2-TEV fusion constructs, together with CMV-STAT1 and CMV-STAT2 were transiently transfected into HTL5B8.1 cells described supra. About 2.5×10^4 cells were seeded in each well of a 96 well plate and cultured in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml G418, and 5 μg/ml puromycin. After 24 hours of incubation, cells were transfected with 15 ng of each IFNAR1-TEV-NIa-Pro cleavage (L)-tTA, IFNAR2.2-TEV, CMV-STATI and CMV-STAT2 DNA, or with 60 ng control pcDNA plasmid, together with 0.3 μl Fugene per well. Transfected cells were cultured for 8-20 hours before treatment with 5000 U/ml human interferon-alpha or 5000 U/ml human interferon-beta. At the time of interferon addition, medium was aspirated and replaced with 293 SFM II media supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 3 μg/ml puromycin and 500 μg/ml of G418. Interferon-treated cells were cultured for an additional 18-20 hours before they were assayed for luciferase reporter gene activity as described supra. Results are shown in **FIG. 8**. Treatment with 5000 U/ml IFN-α resulted in 15-fold increase in reporter gene activity, while treatment with 5000 U/ml IFN-β resulted in a 10-fold increase. Interferon treatment of HTL5B8.1 cells transfected with the control plasmid pcDNA3 had no effect on reporter gene activity. **FIG. 9** shows a dose-response curve generated for IFN-α in HTL5B8.1 cells transfected with IFNAR1(ENLYFQ(L)-tTa, IFNAR2.2-TEV, STAT1 and STAT2 expression constructs as described supra.

Example 25

[0229] This experiment was designed to demonstrate the use of the assay to measure the activity of the human Type I Interferon Receptor using a different transcription factor and a different cell line.

[0230] A fusion construct was created, using DNA encoding Human Interferon Receptor I (IFNAR1), fused in frame to a DNA sequence encoding the GAL4-NF-κB-fusion, described supra. Inserted between these sequences is a DNA sequence encoding the amino acid sequence GSENLYFQL (SEQ ID NO: 87), which includes the low efficiency cleavage site for TEV NIa-Pro, ENLYFQL (SEQ ID NO: 14), described supra. The CMV promoter was placed upstream of the Human Interferon Receptor I (IFNAR1) coding region, and a poly A sequence was placed downstream of the GAL4-NF-κB region. This construct is designated IFNAR1-TEV- NIa-Pro cleavage (L)-GAL4-NF-κB.

[0231] CHO-K1 cells were then transiently transfected with a mixture of five plasmids: IFNAR1-TEV-NIa-Pro cleavage (L)-GAL4-NF-κB, IFNAR2.2-TEV, CMV-STAT1, CMV-STAT2 and pFR-Luc, a luciferase reporter gene plasmid under the control of a GAL4-dependent promoter. About 1.0×10^4 cells per well were seeded in a 96 well plate 24 hours prior to transfections in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin. Cells were transfected the following day with 10 ng of reporter plasmid (pFR-Luc), plus 20 ng of each of the expression constructs described supra or with 10 ng reporter plasmid plus 80 ng of control pcDNA3 plasmid, together with 0.3 μl Fugene per well. Transfected cells were cultured for 8-20 hours before treatment with 5000 U/ml human interferon-alpha. At the time of interferon addition. medium was aspirated and replaced with DMEM media supplemented with 2 mM L-glutamine, 100 units/ml penicillin. Interferon-treated cells were cultured for an additional 6 hours before they were assayed for luciferase reporter gene activity as described supra. Results are shown in **FIG. 10**. IFN-α treatment of CHO-K1 cells transfected with the reporter, IFNAR and STAT constructs resulted in 3-fold increase in reporter gene activity, while interferon treatment of cells transfected with the reporter and control plasmids had no effect on reporter gene activity.

Example 26

[0232] This set of experiments was carried out to demonstrate additional enhancements of the assay using receptor modifications designed to increase the affinity of the test

receptor for the interacting protein. In these examples, the fusion junction between the test receptor and a C-terminal tail domain of GRPR (Genbank Accession Number: NM_005314) (SEQ ID NO: 59) was made 17-23 amino acids after the conserved NPXXY motif at the end of the seventh transmembrane helix.

[0233] First, PCR was used to produce a DNA fragment encoding the C-terminal 42 amino acids from GRPR beginning 2 amino acids after the putative palmitoylation site (hereafter referred to as GRPR 42aa). The fragment was designed such that the first amino acid of the C-terminal tail is preceded by two amino acids (Ser, S and Arg, R) which are encoded by the XbaI restriction site TCTAGA, and the stop codon is replaced by two amino acids (Gly, G and Ser, S) which are encoded by a BamnHI restriction site GGATCC. This was accomplished by amplifying a plasmid containing the GRPR coding region with primers

```
tctagaggcctgatcatccggtctcac    (SEQ ID NO: 88)
and

gaggatccgacataccgctcgtgaca     (SEQ ID NO: 67)
```

[0234] Next the coding region of OPRK (Genbank Accession Number: NM_000912) (SEQ ID NO: 51) was modified to place insert an XbaI site after Pro-347. This was done using PCR with the primers

```
ggtctacttgatgaattcctggcc        (SEQ ID NO: 52)
and

tctagatggaaaacagaagtcccggaaac   (SEQ ID NO: 89)
```

[0235] In addition, the coding region of ADRAIA (Genbank Accession Number: NM_000680) (SEQ ID NO: 90) was modified to insert an XbaI site after Lys-349. This was done using PCR with the primers

```
ctcggatatctaaacagctgcatcaa      (SEQ ID NO: 91)
and

tctagactttctgcagagacactggattc   (SEQ ID NO: 92)
```

[0236] In addition, the coding region of DRD2 (Genbank Accession Number: NM_000795) (SEQ ID NO: 37) was modified to insert two amino acids (Leu and Arg) and an XbaI site after Cys-343. This was done using PCR with the primers

```
gaattcatggatccactgaatctgtcc      (SEQ ID NO: 38)
and

tctagatcgaaggcagtggaggatcttcagg  (SEQ ID NO: 93)
```

[0237] The modified OPRK receptor coding region was cut with EcoRI and XbaI and the GRPR 42aa C-terminal tail fragment was cut with XbaI and BaniHI. Both fragments were ligated into a plasmid containing the OPRK receptor with the AVPR2 C-terminal tail-low-efficiency cleavage site-tTA described supra which had been digested with EcoRI and BamHI.

[0238] The modified ADRAIA receptor coding region was cut with EcoRV and XbaI and the OPRK-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site was cut with XbaI and XhoI. Both fragments were ligated into a plasmid containing the ADRA1A receptor which had been digested with EcoRV and XhoI.

[0239] The modified DRD2 receptor coding region was cut with EcoRI and XbaI and the OPRK-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site was cut with XbaI and XhoI. Both fragments were ligated into a pcDNA6 plasmid digested with EcoRI and XhoI

[0240] HTLA 2C11.6 cells, described supra, were transfected with OPRK-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site and assays were carried out using 10 μM U-69593, an agonist for OPRK. The maximal response to U-69593 resulted in an approximately 200-fold increase in reporter gene activity.

[0241] HTLA 2C11.6 cells were transfected with ADRA1A-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site and assays were carried out using 10 μM epinephrine, an agonist for ADRA1A. The maximal response to epinephrine resulted in an approximately 14-fold increase in reporter gene activity.

[0242] HTLA 2C11.6 cells were transfected with DRD2-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site and assays were carried out using 10 μM dopamine, an agonist for DRD2. The maximal response to dopamine resulted in an approximately 30-fold increase in reporter gene activity.

Example 27

[0243] This set of experiments were carried out to demonstrate further enhancements of the assay using a different set of test receptor modifications designed to increase the affinity for the interacting protein. In these examples, the C-terminal domain of the test receptor was replaced with a portion of the endogenous C-terminal tail domain of GRPR.

[0244] First, PCR was used to produce a DNA fragment encoding the truncated GRPR tail, specifically a sequence encoding 23 amino acids from Gly-343 to Asn-365. The fragment was designed such that the first amino acid of the C-terminal tail is preceded by two amino acids (Ser, S and Arg, R) which are encoded by the XbaI restriction site TCTAGA. and the Ser-366 is replaced by two amino acids (Gly, G and Ser, S) which are encoded by a BamHI restriction site GGATCC. This was accomplished by amplifying a plasmid containing the GRPR coding region with primers

```
tctagaggcctgatcatccggtctcac    (SEQ ID NO: 94)
and

cggatccgttggtactcttgagg        (SEQ ID NO: 95)
```

[0245] Next the truncated GRPR fragment (hereafter referred to as GRPR 23aa Tail) was cut with XbaI and BamHI and inserted into the OPRK-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site described herein, digested with XbaI and BamHI.

[0246] Similarly, the GRPR 23aa Tail fragment was cut with XbaI and BamHI and inserted into the ADRA1A-GRPR 42aa Tail-tTA fusion construct containing the low efficiency cleavage site described herein, digested with XbaI and BamHI.

[0247] HTLA 2C11.6 cells were transfected with OPRK-GRPR 23aa Tail -tTA fusion construct containing the low efficiency cleavage site and assays were carried out using 10 μM U-69593, an agonist for OPRK. The maximal response to U-69593 resulted in an approximately 115-fold induction of reporter gene expression over the background.

[0248] HTLA 2C11.6 cells were transfected with ADRAIA-GRPR 23aa Tail-tTA fusion construct containing the low efficiency cleavage site and assays were carried out using 10 μM epinephrine, an agonist for ADRA1A. The maximal response to epinephrine resulted in an approximately 102-fold induction of reporter gene expression over the background.

Example 28

[0249] This experiment was designed to demonstrate the use of the assay to measure the activity of the receptor tyrosine kinase Insulin-like Growth Factor-1 Receptor (IGF1R), specifically by monitoring the ligand-induced recruitment of the intracellular signaling protein SHC1 (Src homology 2 domain-containing transforming protein 1).

[0250] A first fusion construct was created, comprising DNA encoding the human IGF-1R, which can be found at GenBank under the Accession Number NM_000875 (SEQ ID NO: 96), fused in frame to a DNA sequence encoding amino acids 3-335 of the tetracycline-controlled transactivator tTA, described supra. Inserted between these sequences is a DNA sequence encoding the amino acid sequence GSENLYFQL (SEQ ID NO: 82) which includes the low efficiency cleavage site for TEV NIa-Pro, ENLYFQL (SEQ ID NO: 14), described supra. The CMV promoter was placed upstream of the IGF1R coding region, and a polyA sequence was placed downstream of the tTA region. This construct is designated IGF I R-TEV-NIa-Pro cleavage (Leu)-tTA.

[0251] A second fusion construct was created, comprising DNA encoding the PTB domain of human SHC1, corresponding to amino acids 1-238 (GeneBank accession number BC014158) (SEQ ID NO: 97) fused in frame to a DNA sequence encoding the catalytic domain of mature TEV NIa protease, described supra, corresponding to amino acids 2040-2279 (GeneBank accession number AAA47910) (SEQ ID NO: 79). Inserted between these sequences is a linker DNA sequence encoding the amino acids NSGS (SEQ ID NO: 98). The CMV promoter was placed upstream of the SHC1 PTB domain coding sequence and a polyA sequence was placed downstream of the TEV NIa protease sequence. This construct is designated SHC1-TEV.

[0252] The IGFIR-TEV-NIa-Pro cleavage (Leu)-tTA and SHC1-TEV fusion constructs were transfected into clone HTL5B8.1 cells described supra. About 2.5×10^4 cells were plated into each well of a 96 well-plate, in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 100 units/ml penicillin, 500 μg/ml G418, and 3 μg/ml puromycin. Cells were grown to reach 50% confluency the next day and were transfected with 15 μl per well of a mixture consisting of 100 μl of DMEM, 0.2 μg of IGF1R-TEV-NIa-Pro cleavage (Leu)-tTA DNA, 0.2 μg of SHC1-TEV DNA, and 2 μl Fugene (a proprietary transfection reagent containing lipids and other material), which had been incubated for 15 minutes at room temperature prior to addition to the cells. Transfected cells were cultured for about 16 hours before treatment with a specific receptor agonist. After 24 hours, cells were lysed and luciferase activity was assayed as described supra.

[0253] The addition of 1 μM human Insulin-like Growth Factor 1 resulted in a 90 fold increase of luciferase reporter gene activity.

Example 29

[0254] This experiment was designed to demonstrate the use of the assay to measure the interaction of two test proteins that are not normally membrane bound. In this example, the assay was used to measure the ligand-induced dimerization of the nuclear steroid hormone receptors, ESR1 (estrogen receptor 1 or ER alpha) and ESR2 (estrogen receptor 2 or ER beta). In this example, ESR1 is fused to the transcription factor tTA, where the cleavage site for the TEV NIa-Pro protease is inserted between the ESR1 and tTA sequences. This ESR1-tTA fusion is tethered to the membrane by a fusion to the intracellular, C-terrminal end of the transmembrane protein CD8. CD8 essentially serves as an inert scaffold that tethers ESR1 to the cytoplasmic side of the cell membrane. The transcription factor fused thereto cannot enter the nucleus until interaction with ESR2 and protease. Any transmembrane protein could be used. This CD8-ESR1-TEV NIa Pro cleavage-tTA fusion protein is expressed together with a second fusion protein comprised of ESR2 and the TEV NIa-Pro protease in a cell line containing a tTA-dependent reporter gene. The estrogen-induced dimerization of ESR1 and ESR2 thereby triggers the release of the tTA transcription factor from the membrane bound fusion, which is detected by the subsequent induction in reporter gene activity.

[0255] A fusion construct was created, comprising DNA encoding human CD8 gene (235 amino acids), which can be found in Genbank under Accession Number NM_001768 (SEQ ID NO: 99), fused in frame to a DNA sequence encoding the human ESR1 (596 amino acids), which can be found in Genbank under Accession Number NM_000125 (SEQ ID NO: 100). Inserted between these sequences is a DNA sequence encoding the amino acid sequence GRA (Gly-Arg-Ala). The resulting construct is then fused in frame to a DNA sequence encoding amino acids 3-335 of the tetracycline controlled transactivator tTA, described supra. Inserted between these sequences is a DNA sequence encoding the amino acid sequence GSENLYFQL (SEQ ID NO: 82) which includes the low efficiency cleavage site for TEV NIa-Pro, ENLYFQL (SEQ ID NO: 14), described supra. The CMV promoter was placed upstream of the Human CD8 coding region, and a poly A sequence was placed downstream of the tTA region. This construct is designated CD8-ESR1 -TEV-NIa-Pro cleavage (L)-tTA.

[0256] A second fusion construct was created, using DNA encoding Human Estrogen Receptor beta (ESR2) (530 amino acids), which can be found at Genbank, under Accession Number NM_001437 (SEQ ID NO: 101), fused in frame to a DNA sequence encoding the catalytic domain of the TEV NIa protease, described supra, corresponding to amino acids 2040-2279 (GenBank accession number AAA47910) (SEQ ID NO: 84). Inserted between these sequences is a DNA sequence encoding the amino acid sequence RS (Arg-Ser). The CMV promoter region was placed upstream of the Human Estrogen Receptor beta

(ESR2) coding region, and a poly A sequence was placed downstream of the TEV region. This construct is designated ESR2-TEV.

[0257] The CD8-ESRI-TEV-NIa-Pro cleavage (L)-tTA and ESR2-TEV fusion constructs, together with pCDNA3 were transiently transfected into HTL5B8.1 cells described supra. About 2.0×10^4 cells were seeded in each well of a 96 well plate and cultured in phenol-free DMEM medium supplemented with 10% fetal bovine serum., 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml G418, and 5 μg/ml puromycin. After 24 hours of incubation, cells were transfected with a mixture of 5 ng of ESR1-TEV-Nia-Pro cleavage (L)-tTA, 15 ng of ESR2-TEV and 40 ng of pCDNA3, together with 0.3 μl Fugene per well. 6 hours after transfection, the cells were washed with PBS and incubated in 100 μl of phenol-free DMEM without serum for 24 hours before treatment with 50 nM 17-β Estradiol. Ligand-treated cells were cultured for an additional 18-20 hours before they were assayed for luciferase reporter gene activity as described supra. Treatment with 50 nM 17-β Estradiol resulted in a 16-fold increase in reporter gene activity.

[0258] Other features of the invention will be clear to the skilled artisan and need not be reiterated here.

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<210> SEQ ID NO 26
<211> LENGTH: 1266
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

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ctgctgctgg gcacgctcat cttctgcgcg gtgctgggca atgcgtgcgt ggtggctgcc 180

atcgccttgg agcgctccct gcagaacgtg gccaattatc ttattggctc tttggcggtc 240

accgacctca tggtgtcggt gttggtgctg cccatggccg cgctgtatca ggtgctcaac 300

aagtggacac tgggccaggt aacctgcgac ctgttcatcg ccctcgacgt gctgtgctgc 360

acctcatcca tcttgcacct gtgcgccatc gcgctggaca ggtactgggc catcacggac 420

cccatcgact acgtgaacaa gaggacgccc cggccgcgtg cgctcatctc gctcacttgg 480

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gcgcgcttcc gcatccgcaa gacggtcaaa aaggtggaga agaccggagc ggacacccgc 720

catggagcat ctcccgcccc gcagcccaag aagagtgtga atggagagtc ggggagcagg 780

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aggcaaggtg acgatggcgc cgccctggag gtgatcgagg tgcaccgagt gggcaactcc 900

aaagagcact tgcctctgcc cagcgaggct ggtcctaccc cttgtgcccc cgcctctttc 960

gagaggaaaa atgagcgcaa cgccgaggcg aagcgcaaga tggccctggc ccgagagagg 1020

aagacagtga agacgctggg catcatcatg ggcaccttca tcctctgctg gctgcccttc 1080

ttcatcgtgg ctcttgttct gcccttctgc gagagcagct gccacatgcc caccctgttg 1140

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<210> SEQ ID NO 27
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 27

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<210> SEQ ID NO 28
<211> LENGTH: 25

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<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 28

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<210> SEQ ID NO 29
<211> LENGTH: 1401
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 29

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aacatcctag tcatggtttc cattaaagtc aaccgccacc tccagaccgt caacaattac 180

tttttattca gcttggcctg tgctgacctt atcataggtg ttttctccat gaacttgtac 240

accctctaca ctgtgattgg ttactggcct ttgggacctg tggtgtgtga cctttggcta 300

gccctggact atgtggtcag caatgcctca gttatgaatc tgctcatcat cagctttgac 360

aggtacttct gtgtcacaaa acctctgacc tacccagtca agcggaccac aaaaatggca 420

ggtatgatga ttgcagctgc ctgggtcctc tctttcatcc tctgggctcc agccattctc 480

ttctggcagt tcattgtagg ggtgagaact gtggaggatg gggagtgcta cattcagttt 540

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aagaaggagc ctgttgccaa ccaagacccc gtttctccaa gtctggtaca aggaaggata 720

gtgaagccaa acaataacaa catgcccagc agtgacgatg gcctggagca caacaaaatc 780

cagaatggca aagcccccag ggatcctgtg actgaaaact gtgttcaggg agaggagaag 840

gagagctcca atgactccac ctcagtcagt gctgttgcct ctaatatgag agatgatgaa 900

ataacccagg atgaaaacac agtttccact tccctgggcc attccaaaga tgagaactct 960

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gtagcccgca agattgtgaa gatgactaag cagcctgcaa aaaagaagcc tcctccttcc 1140

cgggaaaaga aagtcaccag gacaatcttg gctattctgt tggctttcat catcacttgg 1200

gccccataca atgtcatggt gctcattaac acctttttgtg caccttgcat ccccaacact 1260

gtgtggacaa ttggttactg gctttgttac atcaacagca ctatcaaccc tgcctgctat 1320

gcactttgca atgccacctt caagaagacc tttaaacacc ttctcatgtg tcattataag 1380

aacataggcg ctacaaggta a 1401

<210> SEQ ID NO 30
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 30

gaattcatga ataactcaac aaactcc 27

<210> SEQ ID NO 31
<211> LENGTH: 25

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<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 31

agatctcctt gtagcgccta tgttc 25

<210> SEQ ID NO 32
<211> LENGTH: 3655
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

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ctgtgtagtg ggatgagcag agaacaaaaa caaaataatc cagtgagaaa agcccgtaaa 120

taaaccttca gaccagagat ctattctcca gcttatttta agctcaactt aaaaagaaga 180

actgttctct gattcttttc gccttcaata cacttaatga tttaactcca ccctccttca 240

aaagaaacag catttcctac ttttatactg tctatatgat tgatttgcac agctcatctg 300

gccagaagag ctgagacatc cgttccccta caagaaactc tccccgggtg gaacaagatg 360

gattatcaag tgtcaagtcc aatctatgac atcaattatt atacatcgga gccctgccaa 420

aaaatcaatg tgaagcaaat cgcagcccgc ctcctgcctc cgctctactc actggtgttc 480

atctttggtt ttgtgggcaa catgctggtc atcctcatcc tgataaactg caaaaggctg 540

aagagcatga ctgacatcta cctgctcaac ctggccatct ctgacctgtt tttccttctt 600

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caactcttga cagggctcta ttttataggc ttcttctctg gaatcttctt catcatcctc 720

ctgacaatcg ataggtacct ggctgtcgtc catgctgtgt ttgctttaaa agccaggacg 780

gtcacctttg gggtggtgac aagtgtgatc acttgggtgg tggctgtgtt tgcgtctctc 840

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tttccataca gtcagtatca attctggaag aatttccaga cattaaagat agtcatcttg 960

gggctggtcc tgccgctgct tgtcatggtc atctgctact cgggaatcct aaaaactctg 1020

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acagagactc ttgggatgac gcactgctgc atcaacccca tcatctatgc ctttgtcggg 1260

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caacttttta cctagtacaa ggcaacatat aggttgtaaa tgtgtttaaa acaggtcttt 1860

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gtgatttccc ctccaaggta tggttaataa gtttcactga cttagaacca ggcgagagac 1980

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accctctggg ccaagtcaaa gacattctga catcttagta tttgcatatt cttatgtatg 3600

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<210> SEQ ID NO 33
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 33

gcggccgcat ggattatcaa gtgtcaagtc c 31

<210> SEQ ID NO 34
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

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<400> SEQUENCE: 34

ggatccctgg cggcagaact tacac 25

<210> SEQ ID NO 35
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 35

ggtctccaat tcatggatta tcaagtgtca agt 33

<210> SEQ ID NO 36
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 36

gacgacagcc aggtacctat c 21

<210> SEQ ID NO 37
<211> LENGTH: 2643
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

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cctatggctt gaagagcctg gccacccagt ggctccaccg ccctgatgga tccactgaat 180

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atgcccaatg gcaaaacccg gacctccctc aagaccatga gccgtaggaa gctctcccag 1260

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<210> SEQ ID NO 38
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 38

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<210> SEQ ID NO 39
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 39

agatctgcag tggaggatct tcagg 25

<210> SEQ ID NO 40
<211> LENGTH: 1301
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 40

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gatgacaagg aggaagagga ggatggtacc ggctctccac agctcaacaa cagatagacg 1260

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<210> SEQ ID NO 41
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 41

ggtaccatgg gcgacaaagg gacgcgagtg 30

<210> SEQ ID NO 42
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 42

ggatcctctg ttgttgagct gtggagagcc tgtaccatcc tcctcttc 48

<210> SEQ ID NO 43
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 43

ggatccattt gtgtcaagtt ctatgag 27

<210> SEQ ID NO 44
<211> LENGTH: 27

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<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 44

ggtaccatgg gggagaaacc cgggacc 27

<210> SEQ ID NO 45
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 45

ggatcctgtg gcatagttgg tatc 24

<210> SEQ ID NO 46
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 46

tgtgcgcgcg gacgcacccc acccagcctg ggt 33

<210> SEQ ID NO 47
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 47

gaattcatgg atccactgaa tctgtcc 27

<210> SEQ ID NO 48
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 48

tgtgcgcgcg cagtggagga tcttcaggaa ggc 33

<210> SEQ ID NO 49
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 49

gcggccgcca ccatgaacgg taccgaaggc cca 33

<210> SEQ ID NO 50
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 50

tgtgcgcgcg cacagaagct cctggaaggc 30

<210> SEQ ID NO 51
<211> LENGTH: 1602
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

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tgcgccccga gcgcctgcct gccccccaac agcagcgcct ggtttcccgg ctgggccgag 480

cccgacagca acggcagcgc cggctcggag gacgcgcagc tggagcccgc gcacatctcc 540

ccggccatcc cggtcatcat cacggcggtc tactccgtag tgttcgtcgt gggcttggtg 600

ggcaactcgc tggtcatgtt cgtgatcatc cgatacacaa agatgaagac agcaaccaac 660

atttacatat ttaacctggc tttggcagat gctttagtta ctacaaccat gccctttcag 720

agtacggtct acttgatgaa ttcctggcct tttggggatg tgctgtgcaa gatagtaatt 780

tccattgatt actacaacat gttcaccagc atcttcacct tgaccatgat gagcgtggac 840

cgctacattg ccgtgtgcca ccccgtgaag gctttggact tccgcacacc cttgaaggca 900

aagatcatca atatctgcat ctggctgctg tcgtcatctg ttggcatctc tgcaatagtc 960

cttggaggca ccaaagtcag ggaagacgtc gatgtcattg agtgctcctt gcagttccca 1020

gatgatgact actcctggtg ggacctcttc atgaagatct gcgtcttcat ctttgccttc 1080

gtgatccctg tcctcatcat catcgtctgc tacaccctga tgatcctgcg tctcaagagc 1140

gtccggctcc tttctggctc ccgagagaaa gatcgcaacc tgcgtaggat caccagactg 1200

gtcctggtgg tggtggcagt cttcgtcgtc tgctggactc ccattcacat attcatcctg 1260

gtggaggctc tggggagcac ctcccacagc acagctgctc tctccagcta ttacttctgc 1320

atcgccttag gctataccaa cagtagcctg aatcccattc tctacgcctt tcttgatgaa 1380

aacttcaagc ggtgtttccg ggacttctgc tttccactga agatgaggat ggagcggcag 1440

agcactagca gagtccgaaa tacagttcag gatcctgctt acctgaggga catcgatggg 1500

atgaataaac cagtatgact agtcgtggag atgtcttcgt acagttcttc gggaagagag 1560

gagttcaatg atctaggttt aactcagatc actactgcag tc 1602

<210> SEQ ID NO 52
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 52

ggtctacttg atgaattcct ggcc 24

<210> SEQ ID NO 53
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 53

gcgcgcacag aagtcccgga aacaccg 27

<210> SEQ ID NO 54
<211> LENGTH: 10

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<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 54

Gly Ser Glu Asn Leu Tyr Phe Gln Leu Arg
5 10

<210> SEQ ID NO 55
<211> LENGTH: 881
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 55

Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
20 25 30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
35 40 45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
130 135 140

Thr Val Ser Ile Asp Ser Ala Ala His His Asp Asn Ser Thr Ile Pro
145 150 155 160

Leu Asp Phe Met Pro Arg Asp Ala Leu His Gly Phe Asp Trp Ser Glu
165 170 175

Glu Asp Asp Met Ser Asp Gly Leu Pro Phe Leu Lys Thr Asp Pro Asn
180 185 190

Asn Asn Gly Phe Phe Gly Asp Gly Ser Leu Leu Cys Ile Leu Arg Ser
195 200 205

Ile Gly Phe Lys Pro Glu Asn Tyr Thr Asn Ser Asn Val Asn Arg Leu
210 215 220

Pro Thr Met Ile Thr Asp Arg Tyr Thr Leu Ala Ser Arg Ser Thr Thr
225 230 235 240

Ser Arg Leu Leu Gln Ser Tyr Leu Asn Asn Phe His Pro Tyr Cys Pro
245 250 255

Ile Val His Ser Pro Thr Leu Met Met Leu Tyr Asn Asn Gln Ile Glu
260 265 270

Ile Ala Ser Lys Asp Gln Trp Gln Ile Leu Phe Asn Cys Ile Leu Ala
275 280 285

Ile Gly Ala Trp Cys Ile Glu Gly Glu Ser Thr Asp Ile Asp Val Phe
290 295 300

Tyr Tyr Gln Asn Ala Lys Ser His Leu Thr Ser Lys Val Phe Glu Ser
305 310 315 320

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Gly Ser Ile Ile Leu Val Thr Ala Leu His Leu Leu Ser Arg Tyr Thr
               325                 330                 335

Gln Trp Arg Gln Lys Thr Asn Thr Ser Tyr Asn Phe His Ser Phe Ser
            340                 345                 350

Ile Arg Met Ala Ile Ser Leu Gly Leu Asn Arg Asp Leu Pro Ser Ser
        355                 360                 365

Phe Ser Asp Ser Ser Ile Leu Glu Gln Arg Arg Arg Ile Trp Trp Ser
    370                 375                 380

Val Tyr Ser Trp Glu Ile Gln Leu Ser Leu Leu Tyr Gly Arg Ser Ile
385                 390                 395                 400

Gln Leu Ser Gln Asn Thr Ile Ser Phe Pro Ser Ser Val Asp Asp Val
               405                 410                 415

Gln Arg Thr Thr Thr Gly Pro Thr Ile Tyr His Gly Ile Ile Glu Thr
            420                 425                 430

Ala Arg Leu Leu Gln Val Phe Thr Lys Ile Tyr Glu Leu Asp Lys Thr
        435                 440                 445

Val Thr Ala Glu Lys Ser Pro Ile Cys Ala Lys Lys Cys Leu Met Ile
    450                 455                 460

Cys Asn Glu Ile Glu Glu Val Ser Arg Gln Ala Pro Lys Phe Leu Gln
465                 470                 475                 480

Met Asp Ile Ser Thr Thr Ala Leu Thr Asn Leu Leu Lys Glu His Pro
               485                 490                 495

Trp Leu Ser Phe Thr Arg Phe Glu Leu Lys Trp Lys Gln Leu Ser Leu
            500                 505                 510

Ile Ile Tyr Val Leu Arg Asp Phe Phe Thr Asn Phe Thr Gln Lys Lys
        515                 520                 525

Ser Gln Leu Glu Gln Asp Gln Asn Asp His Gln Ser Tyr Glu Val Lys
    530                 535                 540

Arg Cys Ser Ile Met Leu Ser Asp Ala Ala Gln Arg Thr Val Met Ser
545                 550                 555                 560

Val Ser Ser Tyr Met Asp Asn His Asn Val Thr Pro Tyr Phe Ala Trp
               565                 570                 575

Asn Cys Ser Tyr Tyr Leu Phe Asn Ala Val Leu Val Pro Ile Lys Thr
            580                 585                 590

Leu Leu Ser Asn Ser Lys Ser Asn Ala Glu Asn Asn Glu Thr Ala Gln
        595                 600                 605

Leu Leu Gln Gln Ile Asn Thr Val Leu Met Leu Leu Lys Lys Leu Ala
    610                 615                 620

Thr Phe Lys Ile Gln Thr Cys Glu Lys Tyr Ile Gln Val Leu Glu Glu
625                 630                 635                 640

Val Cys Ala Pro Phe Leu Leu Ser Gln Cys Ala Ile Pro Leu Pro His
               645                 650                 655

Ile Ser Tyr Asn Asn Ser Asn Gly Ser Ala Ile Lys Asn Ile Val Gly
            660                 665                 670

Ser Ala Thr Ile Ala Gln Tyr Pro Thr Leu Pro Glu Glu Asn Val Asn
        675                 680                 685

Asn Ile Ser Val Lys Tyr Val Ser Pro Gly Ser Val Gly Pro Ser Pro
    690                 695                 700

Val Pro Leu Lys Ser Gly Ala Ser Phe Ser Asp Leu Val Lys Leu Leu
705                 710                 715                 720

Ser Asn Arg Pro Pro Ser Arg Asn Ser Pro Val Thr Ile Pro Arg Ser
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               725                 730                 735

Thr Pro Ser His Arg Ser Val Thr Pro Phe Leu Gly Gln Gln Gln Gln
            740                 745                 750

Leu Gln Ser Leu Val Pro Leu Thr Pro Ser Ala Leu Phe Gly Gly Ala
        755                 760                 765

Asn Phe Asn Gln Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr
    770                 775                 780

Phe Thr Asn Ser Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn
785                 790                 795                 800

Ser Gln Ala Leu Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn
               805                 810                 815

Phe Met Asn Asn Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn
            820                 825                 830

Ser Lys Pro Leu Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala
        835                 840                 845

Phe Gly Ile Thr Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val
    850                 855                 860

Tyr Asn Tyr Leu Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys
865                 870                 875                 880

Glu
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<210> SEQ ID NO 56
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 56

```
Pro Gln Lys Gly Ser Ala Ser Glu Lys Thr Met Val Phe
               5                   10
```

<210> SEQ ID NO 57
<211> LENGTH: 549
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

```
Met Asp Asp Leu Phe Pro Leu Ile Phe Pro Ser Glu Pro Ala Gln Ala
               5                   10                  15

Ser Gly Pro Tyr Val Glu Ile Ile Glu Gln Pro Lys Gln Arg Gly Met
            20                  25                  30

Arg Phe Arg Tyr Lys Cys Glu Gly Arg Ser Ala Gly Ser Ile Pro Gly
        35                  40                  45

Glu Arg Ser Thr Asp Thr Thr Lys Thr His Pro Thr Ile Lys Ile Asn
    50                  55                  60

Gly Tyr Thr Gly Pro Gly Thr Val Arg Ile Ser Leu Val Thr Lys Asp
65                  70                  75                  80

Pro Pro His Arg Pro His Pro His Glu Leu Val Gly Lys Asp Cys Arg
               85                  90                  95

Asp Gly Tyr Tyr Glu Ala Asp Leu Cys Pro Asp Arg Ser Ile His Ser
            100                 105                 110

Phe Gln Asn Leu Gly Ile Gln Cys Val Lys Lys Arg Asp Leu Glu Gln
        115                 120                 125

Ala Ile Ser Gln Arg Ile Gln Thr Asn Asn Asn Pro Phe His Val Pro
    130                 135                 140
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Ile Glu Glu Gln Arg Gly Asp Tyr Asp Leu Asn Ala Val Arg Leu Cys
145                 150                 155                 160

Phe Gln Val Thr Val Arg Asp Pro Ala Gly Arg Pro Leu Leu Leu Thr
                165                 170                 175

Pro Val Leu Ser His Pro Ile Phe Asp Asn Arg Ala Pro Asn Thr Ala
            180                 185                 190

Glu Leu Lys Ile Cys Arg Val Asn Arg Asn Ser Gly Ser Cys Leu Gly
        195                 200                 205

Gly Asp Glu Ile Phe Leu Leu Cys Asp Lys Val Gln Lys Glu Asp Ile
    210                 215                 220

Glu Val Tyr Phe Thr Gly Pro Gly Trp Glu Ala Arg Gly Ser Phe Ser
225                 230                 235                 240

Gln Ala Asp Val His Arg Gln Val Ala Ile Val Phe Arg Thr Pro Pro
                245                 250                 255

Tyr Ala Asp Pro Ser Leu Gln Ala Pro Val Arg Val Ser Met Gln Leu
            260                 265                 270

Arg Arg Pro Ser Asp Arg Glu Leu Ser Glu Pro Met Glu Phe Gln Tyr
        275                 280                 285

Leu Pro Asp Thr Asp Asp Arg His Arg Ile Glu Glu Lys Arg Lys Arg
    290                 295                 300

Thr Tyr Glu Thr Phe Lys Ser Ile Met Lys Lys Ser Pro Phe Asn Gly
305                 310                 315                 320

Pro Thr Glu Pro Arg Pro Pro Thr Arg Arg Ile Ala Val Pro Thr Arg
                325                 330                 335

Asn Ser Thr Ser Val Pro Lys Pro Ala Pro Gln Pro Tyr Thr Phe Pro
            340                 345                 350

Ala Ser Leu Ser Thr Ile Asn Phe Asp Glu Phe Ser Pro Met Leu Leu
        355                 360                 365

Pro Ser Gly Gln Ile Ser Asn Gln Ala Leu Ala Leu Ala Pro Ser Ser
    370                 375                 380

Ala Pro Val Leu Ala Gln Thr Met Val Pro Ser Ser Ala Met Val Pro
385                 390                 395                 400

Leu Ala Gln Pro Pro Ala Pro Ala Pro Val Leu Thr Pro Gly Pro Pro
                405                 410                 415

Gln Ser Leu Ser Ala Pro Val Pro Lys Ser Thr Gln Ala Gly Glu Gly
            420                 425                 430

Thr Leu Ser Glu Ala Leu Leu His Leu Gln Phe Asp Ala Asp Glu Asp
        435                 440                 445

Leu Gly Ala Leu Leu Gly Asn Ser Thr Asp Pro Gly Val Phe Thr Asp
    450                 455                 460

Leu Ala Ser Val Asp Asn Ser Glu Phe Gln Gln Leu Leu Asn Gln Gly
465                 470                 475                 480

Val Ser Met Ser His Ser Thr Ala Glu Pro Met Leu Met Glu Tyr Pro
                485                 490                 495

Glu Ala Ile Thr Arg Leu Val Thr Gly Ser Gln Arg Pro Pro Asp Pro
            500                 505                 510

Ala Pro Thr Pro Leu Gly Thr Ser Gly Leu Pro Asn Gly Leu Ser Gly
        515                 520                 525

Asp Glu Asp Phe Ser Ser Ile Ala Asp Met Asp Phe Ser Ala Leu Leu
    530                 535                 540
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Ser Gln Ile Ser Ser
545

<210> SEQ ID NO 58
<211> LENGTH: 1833
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

ggaggtggga ggagggagtg acgagtcaag gaggagacag ggacgcagga gggtgcaagg 60

aagtgtctta actgagacgg gggtaaggca agagagggtg gaggaaattc tgcaggagac 120

aggcttcctc cagggtctgg agaacccaga ggcagctcct cctgagtgct gggaaggact 180

ctgggcatct tcagcccttc ttactctctg aggctcaagc cagaaattca ggctgcttgc 240

agagtgggtg acagagccac ggagctggtg tccctgggac cctctgcccg tcttctctcc 300

actccccagc atggaggaag gtggtgattt tgacaactac tatggggcag acaaccagtc 360

tgagtgtgag tacacagact ggaaatcctc gggggccctc atccctgcca tctacatgtt 420

ggtcttcctc ctgggcacca cgggcaacgg tctggtgctc tggaccgtgt ttcggagcag 480

ccgggagaag aggcgctcag ctgatatctt cattgctagc ctggcggtgg ctgacctgac 540

cttcgtggtg acgctgcccc tgtgggctac ctacacgtac cgggactatg actggccctt 600

tgggaccttc ttctgcaagc tcagcagcta cctcatcttc gtcaacatgt acgccagcgt 660

cttctgcctc accggcctca gcttcgaccg ctacctggcc atcgtgaggc cagtggccaa 720

tgctcggctg aggctgcggg tcagcggggc cgtggccacg gcagttcttt gggtgctggc 780

cgccctcctg gccatgcctg tcatggtgtt acgcaccacc ggggacttgg agaacaccac 840

taaggtgcag tgctacatgg actactccat ggtggccact gtgagctcag agtgggcctg 900

ggaggtgggc cttggggtct cgtccaccac cgtgggcttt gtggtgccct tcaccatcat 960

gctgacctgt tacttcttca tcgcccaaac catcgctggc cacttccgca aggaacgcat 1020

cgagggcctg cggaagcggc gccggctgct cagcatcatc gtggtgctgg tggtgacctt 1080

tgccctgtgc tggatgccct accacctggt gaagacgctg tacatgctgg gcagcctgct 1140

gcactggccc tgtgactttg acctcttcct catgaacatc ttcccctact gcacctgcat 1200

cagctacgtc aacagctgcc tcaacccctt cctctatgcc tttttcgacc cccgcttccg 1260

ccaggcctgc acctccatgc tctgctgtgg ccagagcagg tgcgcaggca cctcccacag 1320

cagcagtggg gagaagtcag ccagctactc ttcggggcac agccaggggc ccggccccaa 1380

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tgtggttgac tagggctggg agcagagaga agcctggcgc cctcggccct ccccggcctt 1500

tgcccttgct ttctgaaaat cagagtcacc tcctctgccc agagctgtcc tcaaagcatc 1560

cagtgaacac tggaagaggc ttctagaagg gaagaaattg tccctctgag gccgccgtgg 1620

gtgacctgca gagacttcct gcctggaact catctgtgaa ctgggacaga agcagaggag 1680

gctgcctgct gtgatacccc cttacctccc ccagtgcctt cttcagaata tctgcactgt 1740

cttctgatcc tgttagtcac tgtggttcat caaataaaac tgtttgtgca actgttgtgt 1800

ccaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 1833

<210> SEQ ID NO 59
<211> LENGTH: 1666

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

aactgcagcc agggagactc agactagaat ggaggtagaa agaactgatg cagagtgggt 60

ttaattctaa gccttttgt ggctaagttt tgttgttgtt aacttattga atttagagtt 120

gtattgcact ggtcatgtga aagccagagc agcaccagtg tcaaaatagt gacagagagt 180

tttgaatacc atagttagta tatatgtact cagagtattt ttattaaaga aggcaaagag 240

cccggcatag atcttatctt catcttcact cggttgcaaa atcaatagtt aagaaatagc 300

atctaaggga acttttaggt gggaaaaaaa atctagagat ggctctaaat gactgtttcc 360

ttctgaactt ggaggtggac catttcatgc actgcaacat ctccagtcac agtgcggatc 420

tccccgtgaa cgatgactgg tcccacccgg ggatcctcta tgtcatccct gcagtttatg 480

gggttatcat tctgataggc ctcattggca acatcacttt gatcaagatc ttctgtacag 540

tcaagtccat gcgaaacgtt ccaaacctgt tcatttccag tctggctttg ggagacctgc 600

tcctcctaat aacgtgtgct ccagtggatg ccagcaggta cctggctgac agatggctat 660

ttggcaggat tggctgcaaa ctgatcccct ttatacagct tacctctgtt ggggtgtctg 720

tcttcacact cacggcgctc tcggcagaca gatacaaagc cattgtccgg ccaatggata 780

tccaggcctc ccatgccctg atgaagatct gcctcaaagc cgcctttatc tggatcatct 840

ccatgctgct ggccattcca gaggccgtgt tttctgacct ccatcccttc catgaggaaa 900

gcaccaacca gaccttcatt agctgtgccc catacccaca ctctaatgag cttcacccca 960

aaatccattc tatggcttcc tttctggtct tctacgtcat cccactgtcg atcatctctg 1020

tttactacta cttcattgct aaaaatctga tccagagtgc ttacaatctt cccgtggaag 1080

ggaatataca tgtcaagaag cagattgaat cccggaagcg acttgccaag acagtgctgg 1140

tgtttgtggg cctgttcgcc ttctgctggc tccccaatca tgtcatctac ctgtaccgct 1200

cctaccacta ctctgaggtg gacacctcca tgctccactt tgtcaccagc atctgtgccc 1260

gcctcctggc cttcaccaac tcctgcgtga acccctttgc cctctacctg ctgagcaaga 1320

gtttcaggaa acagttcaac actcagctgc tctgttgcca gcctggcctg atcatccggt 1380

ctcacagcac tggaaggagt acaacctgca tgacctccct caagagtacc aacccctccg 1440

tggccacctt tagcctcatc aatggaaaca tctgtcacga gcggtatgtc tagattgacc 1500

cttgattttg ccccctgagg gacggttttg ctttatggct agacaggaac ccttgcatcc 1560

attgttgtgt ctgtgccctc caaagagcct tcagaatgct cctgagtggt gtaggtgggg 1620

gtggggaggc ccaaatgatg gatcaccatt atattttgaa agaagc 1666

<210> SEQ ID NO 60
<211> LENGTH: 2876
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 60

tgaaacctaa cccgccctgg ggaggcgcgc agcagaggct ccgattcggg gcaggtgaga 60

ggctgacttt ctctcggtgc gtccagtgga gctctgagtt tcgaatcggc ggcggcggat 120

tccccgcgcg cccggcgtcg gggcttccag gaggatgcgg agccccagcg cggcgtggct 180

gctgggggcc gccatcctgc tagcagcctc tctctcctgc agtggcacca tccaaggaac 240

-continued

caatagatcc tctaaaggaa gaagccttat tggtaaggtt gatggcacat cccacgtcac 300

tggaaaagga gttacagttg aaacagtctt ttctgtggat gagttttctg catctgtcct 360

cactggaaaa ctgaccactg tcttccttcc aattgtctac acaattgtgt ttgtggtggg 420

tttgccaagt aacggcatgg ccctgtgggt ctttcttttc cgaactaaga agaagcaccc 480

tgctgtgatt tacatggcca atctggcctt ggctgacctc ctctctgtca tctggttccc 540

cttgaagatt gcctatcaca tacatggcaa caactggatt tatggggaag ctctttgtaa 600

tgtgcttatt ggctttttct atggcaacat gtactgttcc attctcttca tgacctgcct 660

cagtgtgcag aggtattggg tcatcgtgaa ccccatgggg cactccagga agaaggcaaa 720

cattgccatt ggcatctccc tggcaatatg gctgctgatt ctgctggtca ccatcccttt 780

gtatgtcgtg aagcagacca tcttcattcc tgccctgaac atcacgacct gtcatgatgt 840

tttgcctgag cagctcttgg tgggagacat gttcaattac ttcctctctc tggccattgg 900

ggtctttctg ttcccagcct tcctcacagc ctctgcctat gtgctgatga tcagaatgct 960

gcgatcttct gccatggatg aaaactcaga gaagaaaagg aagagggcca tcaaactcat 1020

tgtcactgtc ctggccatgt acctgatctg cttcactcct agtaaccttc tgcttgtggt 1080

gcattatttt ctgattaaga gccagggcca gagccatgtc tatgccctgt acattgtagc 1140

cctctgcctc tctaccctta acagctgcat cgaccccttt gtctattact ttgtttcaca 1200

tgatttcagg gatcatgcaa agaacgctct cctttgccga agtgtccgca ctgtaaagca 1260

gatgcaagta tccctcacct caaagaaaca ctccaggaaa tccagctctt actcttcaag 1320

ttcaaccact gttaagacct cctattgagt tttccaggtc ctcagatggg aattgcacag 1380

taggatgtgg aacctgttta atgttatgag gacgtgtctg ttatttccta atcaaaaagg 1440

tctcaccaca taccatgtgg atgcagcacc tctcaggatt gctaggagct cccctgtttg 1500

catgagaaaa gtagtccccc aaattaacat cagtgtctgt ttcagaatct ctctactcag 1560

atgaccccag aaactgaacc aacagaagca gacttttcag aagatggtga agacagaaac 1620

ccagtaactt gcaaaaagta gacttggtgt gaagactcac ttctcagctg aaattatata 1680

tatacacata tatatatttt acatctggga tcatgataga cttgttaggg cttcaaggcc 1740

ctcagagatg atcagtccaa ctgaacgacc ttacaaatga ggaaaccaag ataaatgagc 1800

tgccagaatc aggtttccaa tcaacagcag tgagttggga ttggacagta gaatttcaat 1860

gtccagtgag tgaggttctt gtaccacttc atcaaaatca tggatcttgg ctgggtgcgg 1920

tgcctcatgc ctgtaatcct agcactttgg gaggctgagg caggcaatca cttgaggtca 1980

ggagttcgag accagcctgg ccatcatggc gaaacctcat ctctactaaa aatacaaaag 2040

ttaaccaggt gtgtggtgca cgtttgtaat cccagttact caggaggctg aggcacaaga 2100

attgagtatc actttaactc aggaggcaga ggttgcagtg agccgagatt gcaccactgc 2160

actccagctt gggtgataaa ataaaataaa atagtcgtga atcttgttca aaatgcagat 2220

tcctcagatt caataatgag agctcagact gggaacaggg cccaggaatc tgtgtggtac 2280

aaacctgcat ggtgtttatg cacacagaga tttgagaacc attgttctga atgctgcttc 2340

catttgacaa agtgccgtga taatttttga aaagagaagc aaacaatggt gtctctttta 2400

tgttcagctt ataatgaaat ctgtttgttg acttattagg actttgaatt atttctttat 2460

taaccctctg agtttttgta tgtattatta ttaaagaaaa atgcaatcag gattttaaac 2520

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atgtaaatac aaattttgta taacttttga tgacttcagt gaaattttca ggtagtctga 2580

gtaatagatt gttttgccac ttagaatagc atttgccact tagtatttta aaaaataatt 2640

gttggagtat ttattgtcag ttttgttcac ttgttatcta atacaaaatt ataaagcctt 2700

cagagggttt ggaccacatc tctttggaaa atagtttgca acatatttaa gagatacttg 2760

atgccaaaat gactttatac aacgattgta tttgtgactt ttaaaaataa ttattttatt 2820

gtgtaattga tttataaata acaaaatttt ttttacaact taaaaaaaaa aaaaaa 2876

<210> SEQ ID NO 61
<211> LENGTH: 1668
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

gggagataac tcgtgctcac aggaagccac gcacccttga aaggcaccgg gtccttctta 60

gcatcgtgct tcctgagcaa gcctggcatt gcctcacaga ccttcctcag agccgctttc 120

agaaaagcaa gctgcttctg gttgggccca gacctgcctt gaggagcctg tagagttaaa 180

aaatgaaccc cacggatata gcagacacca ccctcgatga aagcatatac agcaattact 240

atctgtatga aagtatcccc aagccttgca ccaaagaagg catcaaggca tttggggagc 300

tcttcctgcc cccactgtat tccttggttt ttgtatttgg tctgcttgga aattctgtgg 360

tggttctggt cctgttcaaa tacaagcggc tcaggtccat gactgatgtg tacctgctca 420

accttgccat ctcggatctg ctcttcgtgt tttccctccc tttttggggc tactatgcag 480

cagaccagtg ggtttttggg ctaggtctgt gcaagatgat ttcctggatg tacttggtgg 540

gcttttacag tggcatattc tttgtcatgc tcatgagcat tgatagatac ctggcaattg 600

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ctgagcgcaa ccatacctac tgcaaaacca agtactctct caactccacg acgtggaagg 780

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cggtgaagat gatctttgcc gtggtggtcc tcttccttgg gttctggaca ccttacaaca 960

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atcccatcat ctacttttt ctgggggaga aatttcgcaa gtacatccta cagctcttca 1140

aaacctgcag gggccttttt gtgctctgcc aatactgtgg gctcctccaa atttactctg 1200

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cctggcaagg gttcacctgg gctgaggcat ccttcctcac accaggcttg cctgcaggca 1500

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ggcaaagact attcccttct aacctgaact gatgggtttc tccagaggga attgcagagt 1620

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<210> SEQ ID NO 62
<211> LENGTH: 1679
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

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caccgcatct ggagaaccag cggttaccat ggaggggatc agtatataca cttcagataa 120

ctacaccgag gaaatgggct caggggacta tgactccatg aaggaaccct gtttccgtga 180

agaaaatgct aatttcaata aaatcttcct gcccaccatc tactccatca tcttcttaac 240

tggcattgtg ggcaatggat tggtcatcct ggtcatgggt taccagaaga aactgagaag 300

catgacggac aagtacaggc tgcacctgtc agtggccgac ctcctctttg tcatcacgct 360

tcccttctgg gcagttgatg ccgtggcaaa ctggtacttt gggaacttcc tatgcaaggc 420

agtccatgtc atctacacag tcaacctcta cagcagtgtc ctcatcctgg ccttcatcag 480

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ctcgtggtag gactgtagaa aagggaactg aacattccag agcgtgtagt gaatcacgta 1440

aagctagaaa tgatccccag ctgtttatgc atagataatc tctccattcc cgtggaacgt 1500

ttttcctgtt cttaagacgt gattttgctg tagaagatgg cacttataac caaagcccaa 1560

agtggtatag aaatgctggt ttttcagttt tcaggagtgg gttgatttca gcacctacag 1620

tgtacagtct tgtattaagt tgttaataaa agtacatgtt aaacttactt agtgttatg 1679

<210> SEQ ID NO 63
<211> LENGTH: 2859
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

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aagccatcag acaggaagat gtgaaaatcc ccagcactca tcccagaatc actaagtggc 120

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acctgtcctg ggccaaagtc ccaggacaga cctcattgtt cctctgtggg aatacctccc 180

caggagggca tcctggattt ccccccttgca acccaggtca gaagtttcat cgtcaaggtt 240

gtttcatctt ttttttcctg tctaacagct ctgactacca cccaaccttg aggcacagtg 300

aagacatcgg tggccactcc aataacagca ggtcacagct gctcttctgg aggtgtccta 360

caggtgaaaa gcccagcgac ccagtcagga tttaagttta cctcaaaaat ggaagatttt 420

aacatggaga gtgacagctt tgaagatttc tggaaaggtg aagatcttag taattacagt 480

tacagctcta ccctgccccc ttttctacta gatgccgccc catgtgaacc agaatccctg 540

gaaatcaaca agtattttgt ggtcattatc tatgccctgg tattcctgct gagcctgctg 600

ggaaactccc tcgtgatgct ggtcatctta tacagcaggg tcggccgctc cgtcactgat 660

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tccactggtt cttcttggtc tcagtgtcaa tgcagccccc attgtggtca caggaagtag 1620

aggaggccac gttcttacta gtttcccttg catggtttag aaagcttgcc ctggtgcctc 1680

acccccttgcc ataattacta tgtcatttgc tggagctctg cccatcctgc ccctgagccc 1740

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cattaggatg gctagtatca aaagaaagaa aatcaggctg gccaacgggg tgaaaccctg 1860

tctctactaa aaatacaaaa aaaaaaaaaa attagccggg cgtggtggtg agtgcctgta 1920

atcacagcta cttgggaggc tgagatggga gaatcacttg aacccgggag gcagaggttg 1980

cagtgagccg agattgtgcc cctgcactcc agcctgagcg acagtgagac tctgtctcag 2040

tccatgaaga tgtagaggag aaactggaac tctcgagcgt tgctgggggg gattgtaaaa 2100

tggtgtgacc actgcagaag acagtatggc agctttcctc aaaacttcag acatagaatt 2160

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ttgaacccat atttgtacac caatattcat agcagcttat tcacaagacc caaaaggcag 2280

aagcaaccca aatgttcatc aatgaatgaa tgaatggcta agcaaaatgt gatatgtacc 2340

taacgaagta tccttcagcc tgaaagagga atgaagtact catacatgtt acaacacgga 2400

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cgaaccttga aaactttatg ctaagtgaaa taagccagac atcaacagat aaatagttta 2460

tgattccacc tacatgaggt actgagagtg aacaaattta cagagacaga aagcagaaca 2520

gtgattacca gggactgagg ggaggggagc atgggaagtg acggtttaat gggcacaggg 2580

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tgtgacttaa tgccactaaa ttgacactta aaaatggttt aaatggtcaa ttttgttatg 2700

tatattttat atcaatttaa aaaaaaacct gagccccaaa aggtatttta atcaccaagg 2760

ctgattaaac caaggctaga accacctgcc tatatttttt gttaaatgat ttcattcaat 2820

atctttttt taataaacca tttttacttg ggtgtttat 2859

<210> SEQ ID NO 64
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 64

tgtgcgcgcg gccagagcag gtgcgca 27

<210> SEQ ID NO 65
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 65

gaggatccgt caaccacaag ggtctc 26

<210> SEQ ID NO 66
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 66

tgtgcgcgcg gcctgatcat ccggtct 27

<210> SEQ ID NO 67
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 67

gaggatccga cataccgctc gtgaca 26

<210> SEQ ID NO 68
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 68

tgtgcgcgca gtgtccgcac tgtaaagc 28

<210> SEQ ID NO 69
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 69

gaggatccat aggaggtctt aacagt 26

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<210> SEQ ID NO 70
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 70

tgtgcgcgcg gccttttgt gctctgc 27

<210> SEQ ID NO 71
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 71

gaggatccca gagcatcatg aagatc 26

<210> SEQ ID NO 72
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 72

tgtgcgcgcg gcttgatcag caagggac 28

<210> SEQ ID NO 73
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 73

gaggatccga gagtagtgga agtgtg 26

<210> SEQ ID NO 74
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 74

tgtgcgcgcg ggtccagcct caagatc 27

<210> SEQ ID NO 75
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 75

gaggatccgc tggagtgaaa acttga 26

<210> SEQ ID NO 76
<211> LENGTH: 5616
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 76

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gccgaggcgg ccggagtccc gagctagccc cggcggccgc cgccgcccag accggacgac 120

aggccacctc gtcggcgtcc gcccgagtcc ccgcctcgcc gccaacgcca caaccaccgc 180

gcacggcccc ctgactccgt ccagtattga tcgggagagc cggagcgagc tcttcgggga 240

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gcagcgatgc gaccctccgg gacggccggg gcagcgctcc tggcgctgct ggctgcgctc 300

tgcccggcga gtcgggctct ggaggaaaag aaagtttgcc aaggcacgag taacaagctc 360

acgcagttgg gcacttttga agatcatttt ctcagcctcc agaggatgtt caataactgt 420

gaggtggtcc ttgggaattt ggaaattacc tatgtgcaga ggaattatga tctttccttc 480

ttaaagacca tccaggaggt ggctggttat gtcctcattg ccctcaacac agtggagcga 540

attcctttgg aaaacctgca gatcatcaga ggaaatatgt actacgaaaa ttcctatgcc 600

ttagcagtct tatctaacta tgatgcaaat aaaaccggac tgaaggagct gcccatgaga 660

aatttacagg aaatcctgca tggcgccgtg cggttcagca acaaccctgc cctgtgcaac 720

gtggagagca tccagtggcg ggacatagtc agcagtgact ttctcagcaa catgtcgatg 780

gacttccaga accacctggg cagctgccaa aagtgtgatc caagctgtcc caatgggagc 840

tgctggggtg caggagagga gaactgccag aaactgacca aaatcatctg tgcccagcag 900

tgctccgggc gctgccgtgg caagtccccc agtgactgct gccacaacca gtgtgctgca 960

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caagagagga tgacacatca aataataact cggattccag cccacattgg attcatcagc 4740

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acccccaaa attagtttgt gttacttatg gaagatagtt ttctcctttt acttcacttc 4980

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ctctggccac aacagggcat tttacaggtg cgaatgacag tagcattatg agtagtgtgg 5160

aattcaggta gtaaatatga aactagggtt tgaaattgat aatgctttca caacatttgc 5220

agatgtttta gaaggaaaaa agttccttcc taaaataatt tctctacaat tggaagattg 5280

gaagattcag ctagttagga gcccaccttt tttcctaatc tgtgtgtgcc ctgtaacctg 5340

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catccaattt atcaaggaag aaatggttca gaaaatattt tcagcctaca gttatgttca 5460

gtcacacaca catacaaaat gttccttttg cttttaaagt aatttttgac tcccagatca 5520

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ctatattcat ttccactcta aaaaaaaaaa aaaaaa 5616

<210> SEQ ID NO 77
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 77

Gly Gly Ser Gly Ser Glu Asn Leu Tyr Phe Gln Leu
5 10

<210> SEQ ID NO 78
<211> LENGTH: 1291
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 78

Met Ala Gly Ala Ala Ser Pro Cys Ala Asn Gly Cys Gly Pro Gly Ala
5 10 15

Pro Ser Asp Ala Glu Val Leu His Leu Cys Arg Ser Leu Glu Val Gly
20 25 30

Thr Val Met Thr Leu Phe Tyr Ser Lys Lys Ser Gln Arg Pro Glu Arg
35 40 45

Lys Thr Phe Gln Val Lys Leu Glu Thr Arg Gln Ile Thr Trp Ser Arg
50 55 60

Gly Ala Asp Lys Ile Glu Gly Ala Ile Asp Ile Arg Glu Ile Lys Glu
65 70 75 80

Ile Arg Pro Gly Lys Thr Ser Arg Asp Phe Asp Arg Tyr Gln Glu Asp
85 90 95

Pro Ala Phe Arg Pro Asp Gln Ser His Cys Phe Val Ile Leu Tyr Gly
100 105 110

Met Glu Phe Arg Leu Lys Thr Leu Ser Leu Gln Ala Thr Ser Glu Asp
115 120 125

Glu Val Asn Met Trp Ile Lys Gly Leu Thr Trp Leu Met Glu Asp Thr
130 135 140

Leu Gln Ala Pro Thr Pro Leu Gln Ile Glu Arg Trp Leu Arg Lys Gln

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145                 150                 155                 160

Phe Tyr Ser Val Asp Arg Asn Arg Glu Asp Arg Ile Ser Ala Lys Asp
                165                 170                 175

Leu Lys Asn Met Leu Ser Gln Val Asn Tyr Arg Val Pro Asn Met Arg
            180                 185                 190

Phe Leu Arg Glu Arg Leu Thr Asp Leu Glu Gln Arg Ser Gly Asp Ile
        195                 200                 205

Thr Tyr Gly Gln Phe Ala Gln Leu Tyr Arg Ser Leu Met Tyr Ser Ala
    210                 215                 220

Gln Lys Thr Met Asp Leu Pro Phe Leu Glu Ala Ser Thr Leu Arg Ala
225                 230                 235                 240

Gly Glu Arg Pro Glu Leu Cys Arg Val Ser Leu Pro Glu Phe Gln Gln
                245                 250                 255

Phe Leu Leu Asp Tyr Gln Gly Glu Leu Trp Ala Val Asp Arg Leu Gln
            260                 265                 270

Val Gln Glu Phe Met Leu Ser Phe Leu Arg Asp Pro Leu Arg Glu Ile
        275                 280                 285

Glu Glu Pro Tyr Phe Phe Leu Asp Glu Phe Val Thr Phe Leu Phe Ser
    290                 295                 300

Lys Glu Asn Ser Val Trp Asn Ser Gln Leu Asp Ala Val Cys Pro Asp
305                 310                 315                 320

Thr Met Asn Asn Pro Leu Ser His Tyr Trp Ile Ser Ser Ser His Asn
                325                 330                 335

Thr Tyr Leu Thr Gly Asp Gln Phe Ser Ser Glu Ser Ser Leu Glu Ala
            340                 345                 350

Tyr Ala Arg Cys Leu Arg Met Gly Cys Arg Cys Ile Glu Leu Asp Cys
        355                 360                 365

Trp Asp Gly Pro Asp Gly Met Pro Val Ile Tyr His Gly His Thr Leu
    370                 375                 380

Thr Thr Lys Ile Lys Phe Ser Asp Val Leu His Thr Ile Lys Glu His
385                 390                 395                 400

Ala Phe Val Ala Ser Glu Tyr Pro Val Ile Leu Ser Ile Glu Asp His
                405                 410                 415

Cys Ser Ile Ala Gln Gln Arg Asn Met Ala Gln Tyr Phe Lys Lys Val
            420                 425                 430

Leu Gly Asp Thr Leu Leu Thr Lys Pro Val Glu Ile Ser Ala Asp Gly
        435                 440                 445

Leu Pro Ser Pro Asn Gln Leu Lys Arg Lys Ile Leu Ile Lys His Lys
    450                 455                 460

Lys Leu Ala Glu Gly Ser Ala Tyr Glu Glu Val Pro Thr Ser Met Met
465                 470                 475                 480

Tyr Ser Glu Asn Asp Ile Ser Asn Ser Ile Lys Asn Gly Ile Leu Tyr
                485                 490                 495

Leu Glu Asp Pro Val Asn His Glu Trp Tyr Pro His Tyr Phe Val Leu
            500                 505                 510

Thr Ser Ser Lys Ile Tyr Tyr Ser Glu Glu Thr Ser Ser Asp Gln Gly
        515                 520                 525

Asn Glu Asp Glu Glu Glu Pro Lys Glu Val Ser Ser Ser Thr Glu Leu
    530                 535                 540

His Ser Asn Glu Lys Trp Phe His Gly Lys Leu Gly Ala Gly Arg Asp
545                 550                 555                 560
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Gly Arg His Ile Ala Glu Arg Leu Leu Thr Glu Tyr Cys Ile Glu Thr
                565                 570                 575

Gly Ala Pro Asp Gly Ser Phe Leu Val Arg Glu Ser Glu Thr Phe Val
            580                 585                 590

Gly Asp Tyr Thr Leu Ser Phe Trp Arg Asn Gly Lys Val Gln His Cys
        595                 600                 605

Arg Ile His Ser Arg Gln Asp Ala Gly Thr Pro Lys Phe Phe Leu Thr
    610                 615                 620

Asp Asn Leu Val Phe Asp Ser Leu Tyr Asp Leu Ile Thr His Tyr Gln
625                 630                 635                 640

Gln Val Pro Leu Arg Cys Asn Glu Phe Glu Met Arg Leu Ser Glu Pro
                645                 650                 655

Val Pro Gln Thr Asn Ala His Glu Ser Lys Glu Trp Tyr His Ala Ser
            660                 665                 670

Leu Thr Arg Ala Gln Ala Glu His Met Leu Met Arg Val Pro Arg Asp
        675                 680                 685

Gly Ala Phe Leu Val Arg Lys Arg Asn Glu Pro Asn Ser Tyr Ala Ile
    690                 695                 700

Ser Phe Arg Ala Glu Gly Lys Ile Lys His Cys Arg Val Gln Gln Glu
705                 710                 715                 720

Gly Gln Thr Val Met Leu Gly Asn Ser Glu Phe Asp Ser Leu Val Asp
                725                 730                 735

Leu Ile Ser Tyr Tyr Glu Lys His Pro Leu Tyr Arg Lys Met Lys Leu
            740                 745                 750

Arg Tyr Pro Ile Asn Glu Glu Ala Leu Glu Lys Ile Gly Thr Ala Glu
        755                 760                 765

Pro Asp Tyr Gly Ala Leu Tyr Glu Gly Arg Asn Pro Gly Phe Tyr Val
    770                 775                 780

Glu Ala Asn Pro Met Pro Thr Phe Lys Cys Ala Val Lys Ala Leu Phe
785                 790                 795                 800

Asp Tyr Lys Ala Gln Arg Glu Asp Glu Leu Thr Phe Ile Lys Ser Ala
                805                 810                 815

Ile Ile Gln Asn Val Glu Lys Gln Glu Gly Gly Trp Trp Arg Gly Asp
            820                 825                 830

Tyr Gly Gly Lys Lys Gln Leu Trp Phe Pro Ser Asn Tyr Val Glu Glu
        835                 840                 845

Met Val Asn Pro Val Ala Leu Glu Pro Glu Arg Glu His Leu Asp Glu
    850                 855                 860

Asn Ser Pro Leu Gly Asp Leu Leu Arg Gly Val Leu Asp Val Pro Ala
865                 870                 875                 880

Cys Gln Ile Ala Ile Arg Pro Glu Gly Lys Asn Asn Arg Leu Phe Val
                885                 890                 895

Phe Ser Ile Ser Met Ala Ser Val Ala His Trp Ser Leu Asp Val Ala
            900                 905                 910

Ala Asp Ser Gln Glu Glu Leu Gln Asp Trp Val Lys Lys Ile Arg Glu
        915                 920                 925

Val Ala Gln Thr Ala Asp Ala Arg Leu Thr Glu Gly Lys Ile Met Glu
    930                 935                 940

Arg Arg Lys Lys Ile Ala Leu Glu Leu Ser Glu Leu Val Val Tyr Cys
945                 950                 955                 960
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Arg Pro Val Pro Phe Asp Glu Glu Lys Ile Gly Thr Glu Arg Ala Cys
                965                 970                 975

Tyr Arg Asp Met Ser Ser Phe Pro Glu Thr Lys Ala Glu Lys Tyr Val
            980                 985                 990

Asn Lys Ala Lys Gly Lys Lys Phe Leu Gln Tyr Asn Arg Leu Gln Leu
        995                 1000                1005

Ser Arg Ile Tyr Pro Lys Gly Gln Arg Leu Asp Ser Ser Asn Tyr Asp
    1010                1015                1020

Pro Leu Pro Met Trp Ile Cys Gly Ser Gln Leu Val Ala Leu Asn Phe
1025                1030                1035                1040

Gln Thr Pro Asp Lys Pro Met Gln Met Asn Gln Ala Leu Phe Met Thr
                1045                1050                1055

Gly Arg His Cys Gly Tyr Val Leu Gln Pro Ser Thr Met Arg Asp Glu
            1060                1065                1070

Ala Phe Asp Pro Phe Asp Lys Ser Ser Leu Arg Gly Leu Glu Pro Cys
        1075                1080                1085

Ala Ile Ser Ile Glu Val Leu Gly Ala Arg His Leu Pro Lys Asn Gly
    1090                1095                1100

Arg Gly Ile Val Cys Pro Phe Val Glu Ile Glu Val Ala Gly Ala Glu
1105                1110                1115                1120

Tyr Asp Ser Thr Lys Gln Lys Thr Glu Phe Val Val Asp Asn Gly Leu
                1125                1130                1135

Asn Pro Val Trp Pro Ala Lys Pro Phe His Phe Gln Ile Ser Asn Pro
            1140                1145                1150

Glu Phe Ala Phe Leu Arg Phe Val Val Tyr Glu Glu Asp Met Phe Ser
        1155                1160                1165

Asp Gln Asn Phe Leu Ala Gln Ala Thr Phe Pro Val Lys Gly Leu Lys
    1170                1175                1180

Thr Gly Tyr Arg Ala Val Pro Leu Lys Asn Asn Tyr Ser Glu Asp Leu
1185                1190                1195                1200

Glu Leu Ala Ser Leu Leu Ile Lys Ile Asp Ile Phe Pro Ala Lys Gln
                1205                1210                1215

Glu Asn Gly Asp Leu Ser Pro Phe Ser Gly Thr Ser Leu Arg Glu Arg
            1220                1225                1230

Gly Ser Asp Ala Ser Gly Gln Leu Phe His Gly Arg Ala Arg Glu Gly
        1235                1240                1245

Ser Phe Glu Ser Arg Tyr Gln Gln Pro Phe Glu Asp Phe Arg Ile Ser
    1250                1255                1260

Gln Glu His Leu Ala Asp His Phe Asp Ser Arg Glu Arg Arg Ala Pro
1265                1270                1275                1280

Arg Arg Thr Arg Val Asn Gly Asp Asn Arg Leu
                1285                1290


<210> SEQ ID NO 79
<211> LENGTH: 3054
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 79

Met Ala Leu Ile Phe Gly Thr Val Asn Ala Asn Ile Leu Lys Glu Val
                5                   10                  15

Phe Gly Gly Ala Arg Met Ala Cys Val Thr Ser Ala His Met Ala Gly
            20                  25                  30
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Ala Asn Gly Ser Ile Leu Lys Lys Ala Glu Glu Thr Ser Arg Ala Ile
        35                  40                  45

Met His Lys Pro Val Ile Phe Gly Glu Asp Tyr Ile Thr Glu Ala Asp
    50                  55                  60

Leu Pro Tyr Thr Pro Leu His Leu Glu Val Asp Ala Glu Met Glu Arg
65                  70                  75                  80

Met Tyr Tyr Leu Gly Arg Arg Ala Leu Thr His Gly Lys Arg Arg Lys
                85                  90                  95

Val Ser Val Asn Asn Lys Arg Asn Arg Arg Arg Lys Val Ala Lys Thr
            100                 105                 110

Tyr Val Gly Arg Asp Ser Ile Val Glu Lys Ile Val Val Pro His Thr
        115                 120                 125

Glu Arg Lys Val Asp Thr Thr Ala Ala Val Glu Asp Ile Cys Asn Glu
    130                 135                 140

Ala Thr Thr Gln Leu Val His Asn Ser Met Pro Lys Arg Lys Lys Gln
145                 150                 155                 160

Lys Asn Phe Leu Pro Ala Thr Ser Leu Ser Asn Val Tyr Ala Gln Thr
                165                 170                 175

Trp Ser Ile Val Arg Lys Arg His Met Gln Val Glu Ile Ile Ser Lys
            180                 185                 190

Lys Ser Val Arg Ala Arg Val Lys Arg Phe Glu Gly Ser Val Gln Leu
        195                 200                 205

Phe Ala Ser Val Arg His Met Tyr Gly Glu Arg Lys Arg Val Asp Leu
    210                 215                 220

Arg Ile Asp Asn Trp Gln Gln Glu Thr Leu Leu Asp Leu Ala Lys Arg
225                 230                 235                 240

Phe Lys Asn Glu Arg Val Asp Gln Ser Lys Leu Thr Phe Gly Ser Ser
                245                 250                 255

Gly Leu Val Leu Arg Gln Gly Ser Tyr Gly Pro Ala His Trp Tyr Arg
            260                 265                 270

His Gly Met Phe Ile Val Arg Gly Arg Ser Asp Gly Met Leu Val Asp
        275                 280                 285

Ala Arg Ala Lys Val Thr Phe Ala Val Cys His Ser Met Thr His Tyr
    290                 295                 300

Ser Asp Lys Ser Ile Ser Glu Ala Phe Phe Ile Pro Tyr Ser Lys Lys
305                 310                 315                 320

Phe Leu Glu Leu Arg Pro Asp Gly Ile Ser His Glu Cys Thr Arg Gly
                325                 330                 335

Val Ser Val Glu Arg Cys Gly Glu Val Ala Ala Ile Leu Thr Gln Ala
            340                 345                 350

Leu Ser Pro Cys Gly Lys Ile Thr Cys Lys Arg Cys Met Val Glu Thr
        355                 360                 365

Pro Asp Ile Val Glu Gly Glu Ser Gly Glu Ser Val Thr Asn Gln Gly
    370                 375                 380

Lys Leu Leu Ala Met Leu Lys Glu Gln Tyr Pro Asp Phe Pro Met Ala
385                 390                 395                 400

Glu Lys Leu Leu Thr Arg Phe Leu Gln Gln Lys Ser Leu Val Asn Thr
                405                 410                 415

Asn Leu Thr Ala Cys Val Ser Val Lys Gln Leu Ile Gly Asp Arg Lys
            420                 425                 430
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Gln Ala Pro Phe Thr His Val Leu Ala Val Ser Glu Ile Leu Phe Lys
        435                 440                 445

Gly Asn Lys Leu Thr Gly Ala Asp Leu Glu Glu Ala Ser Thr His Met
    450                 455                 460

Leu Glu Ile Ala Arg Phe Leu Asn Asn Arg Thr Glu Asn Met Arg Ile
465                 470                 475                 480

Gly His Leu Gly Ser Phe Arg Asn Lys Ile Ser Ser Lys Ala His Val
                485                 490                 495

Asn Asn Ala Leu Met Cys Asp Asn Gln Leu Asp Gln Asn Gly Asn Phe
            500                 505                 510

Ile Trp Gly Leu Arg Gly Ala His Ala Lys Arg Phe Leu Lys Gly Phe
        515                 520                 525

Phe Thr Glu Ile Asp Pro Asn Glu Gly Tyr Asp Lys Tyr Val Ile Arg
    530                 535                 540

Lys His Ile Arg Gly Ser Arg Lys Leu Ala Ile Gly Asn Leu Ile Met
545                 550                 555                 560

Ser Thr Asp Phe Gln Thr Leu Arg Gln Gln Ile Gln Gly Glu Thr Ile
                565                 570                 575

Glu Arg Lys Glu Ile Gly Asn His Cys Ile Ser Met Arg Asn Gly Asn
            580                 585                 590

Tyr Val Tyr Pro Cys Cys Cys Val Thr Leu Glu Asp Gly Lys Ala Gln
        595                 600                 605

Tyr Ser Asp Leu Lys His Pro Thr Lys Arg His Leu Val Ile Gly Asn
    610                 615                 620

Ser Gly Asp Ser Lys Tyr Leu Asp Leu Pro Val Leu Asn Glu Glu Lys
625                 630                 635                 640

Met Tyr Ile Ala Asn Glu Gly Tyr Cys Tyr Met Asn Ile Phe Phe Ala
                645                 650                 655

Leu Leu Val Asn Val Lys Glu Glu Asp Ala Lys Asp Phe Thr Lys Phe
            660                 665                 670

Ile Arg Asp Thr Ile Val Pro Lys Leu Gly Ala Trp Pro Thr Met Gln
        675                 680                 685

Asp Val Ala Thr Ala Cys Tyr Leu Leu Ser Ile Leu Tyr Pro Asp Val
    690                 695                 700

Leu Arg Ala Glu Leu Pro Arg Ile Leu Val Asp His Asp Asn Lys Thr
705                 710                 715                 720

Met His Val Leu Asp Ser Tyr Gly Ser Arg Thr Thr Gly Tyr His Met
                725                 730                 735

Leu Lys Met Asn Thr Thr Ser Gln Leu Ile Glu Phe Val His Ser Gly
            740                 745                 750

Leu Glu Ser Glu Met Lys Thr Tyr Asn Val Gly Gly Met Asn Arg Asp
        755                 760                 765

Val Val Thr Gln Gly Ala Ile Glu Met Leu Ile Lys Ser Ile Tyr Lys
    770                 775                 780

Pro His Leu Met Lys Gln Leu Leu Glu Glu Glu Pro Tyr Ile Ile Val
785                 790                 795                 800

Leu Ala Ile Val Ser Pro Ser Ile Leu Ile Ala Met Tyr Asn Ser Gly
                805                 810                 815

Thr Phe Glu Gln Ala Leu Gln Met Trp Leu Pro Asn Thr Met Arg Leu
            820                 825                 830

Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu Ala Gln Lys Leu Thr Leu
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        835                 840                 845

Ala Asp Leu Phe Val Gln Gln Arg Asn Leu Ile Asn Glu Tyr Ala Gln
    850                 855                 860

Val Ile Leu Asp Asn Leu Ile Asp Gly Val Arg Val Asn His Ser Leu
865                 870                 875                 880

Ser Leu Ala Met Glu Ile Val Thr Ile Lys Leu Ala Thr Gln Glu Met
                885                 890                 895

Asp Met Ala Leu Arg Glu Gly Gly Tyr Ala Val Thr Ser Glu Lys Val
            900                 905                 910

His Glu Met Leu Glu Lys Asn Tyr Val Lys Ala Leu Lys Asp Ala Trp
        915                 920                 925

Asp Glu Leu Thr Trp Leu Glu Lys Phe Ser Ala Ile Arg His Ser Arg
    930                 935                 940

Lys Leu Leu Lys Phe Gly Arg Lys Pro Leu Ile Met Lys Asn Thr Val
945                 950                 955                 960

Asp Cys Gly Gly His Ile Asp Leu Ser Val Lys Ser Leu Phe Lys Phe
                965                 970                 975

His Leu Glu Leu Leu Lys Gly Thr Ile Ser Arg Ala Val Asn Gly Gly
            980                 985                 990

Ala Arg Lys Val Arg Val Ala Lys  Asn Ala Met Thr Lys  Gly Val Phe
        995                 1000                1005

Leu Lys  Ile Tyr Ser Met Leu  Pro Asp Val Tyr Lys  Phe Ile Thr Val
    1010                1015                1020

Ser  Ser Val Leu Ser Leu  Leu Leu Thr Phe Leu  Phe Gln Ile Asp Cys
1025                1030                1035                1040

Met Ile Arg Ala His  Arg Glu Ala Lys Val  Ala Ala Gln Leu Gln  Lys
                1045                1050                1055

Glu Ser Glu Trp  Asp Asn Ile Ile Asn  Arg Thr Phe Gln Tyr  Ser Lys
            1060                1065                1070

Leu Glu Asn  Pro Ile Gly Tyr Arg  Ser Thr Ala Glu Glu  Arg Leu Gln
        1075                1080                1085

Ser Glu  His Pro Glu Ala Phe  Glu Tyr Tyr Lys Phe  Cys Ile Gly Lys
    1090                1095                1100

Glu  Asp Leu Val Glu Gln  Ala Lys Gln Pro Glu  Ile Ala Tyr Phe Glu
1105                1110                1115                1120

Lys Ile Ile Ala Phe  Ile Thr Leu Val Leu  Met Ala Phe Asp Ala  Glu
                1125                1130                1135

Arg Ser Asp Gly  Val Phe Lys Ile Leu  Asn Lys Phe Lys Gly  Ile Leu
            1140                1145                1150

Ser Ser Thr  Glu Arg Glu Ile Ile  Tyr Thr Gln Ser Leu  Asp Asp Tyr
        1155                1160                1165

Val Thr  Thr Phe Asp Asp Asn  Met Thr Ile Asn Leu  Glu Leu Asn Met
    1170                1175                1180

Asp  Glu Leu His Lys Thr  Ser Leu Pro Gly Val  Thr Phe Lys Gln Trp
1185                1190                1195                1200

Trp Asn Asn Gln Ile  Ser Arg Gly Asn Val  Lys Pro His Tyr Arg  Thr
                1205                1210                1215

Glu Gly His Phe  Met Glu Phe Thr Arg  Asp Thr Ala Ala Ser  Val Ala
            1220                1225                1230

Ser Glu Ile  Ser His Ser Pro Ala  Arg Asp Phe Leu Val  Arg Gly Ala
        1235                1240                1245
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Val Gly  Ser Gly Lys Ser Thr  Gly Leu Pro Tyr His  Leu Ser Lys Arg
    1250                1255                1260

Gly  Arg Val Leu Met Leu  Glu Pro Thr Arg Pro  Leu Thr Asp Asn Met
1265                1270                1275                1280

His Lys Gln Leu Arg  Ser Glu Pro Phe Asn  Cys Phe Pro Thr Leu  Arg
               1285                1290                1295

Met Arg Gly Lys  Ser Thr Phe Gly Ser  Ser Pro Ile Thr Val  Met Thr
           1300                1305                1310

Ser Gly Phe  Ala Leu His His Phe  Ala Arg Asn Ile Ala  Glu Val Lys
        1315                1320                1325

Thr Tyr  Asp Phe Val Ile Ile  Asp Glu Cys His Val  Asn Asp Ala Ser
    1330                1335                1340

Ala  Ile Ala Phe Arg Asn  Leu Leu Phe Glu His  Glu Phe Glu Gly Lys
1345                1350                1355                1360

Val Leu Lys Val Ser  Ala Thr Pro Pro Gly  Arg Glu Val Glu Phe  Thr
               1365                1370                1375

Thr Gln Phe Pro  Val Lys Leu Lys Ile  Glu Glu Ala Leu Ser  Phe Gln
           1380                1385                1390

Glu Phe Val  Ser Leu Gln Gly Thr  Gly Ala Asn Ala Asp  Val Ile Ser
        1395                1400                1405

Cys Gly  Asp Asn Ile Leu Val  Tyr Val Ala Ser Tyr  Asn Asp Val Asp
    1410                1415                1420

Ser  Leu Gly Lys Leu Leu  Val Gln Lys Gly Tyr  Lys Val Ser Lys Ile
1425                1430                1435                1440

Asp Gly Arg Thr Met  Lys Ser Gly Gly Thr  Glu Ile Ile Thr Glu  Gly
               1445                1450                1455

Thr Ser Val Lys  Lys His Phe Ile Val  Ala Thr Asn Ile Ile  Glu Asn
           1460                1465                1470

Gly Val Thr  Ile Asp Ile Asp Val  Val Val Asp Phe Gly  Thr Lys Val
        1475                1480                1485

Val Pro  Val Leu Asp Val Asp  Asn Arg Ala Val Gln  Tyr Asn Lys Thr
    1490                1495                1500

Val  Val Ser Tyr Gly Glu  Arg Ile Gln Lys Leu  Gly Arg Val Gly Arg
1505                1510                1515                1520

His Lys Glu Gly Val  Ala Leu Arg Ile Gly  Gln Thr Asn Lys Thr  Leu
               1525                1530                1535

Val Glu Ile Pro  Glu Met Val Ala Thr  Glu Ala Ala Phe Leu  Cys Phe
           1540                1545                1550

Met Tyr Asn  Leu Pro Val Thr Thr  Gln Ser Val Ser Thr  Thr Leu Leu
        1555                1560                1565

Glu Asn  Ala Thr Leu Leu Gln  Ala Arg Thr Met Ala  Gln Phe Glu Leu
    1570                1575                1580

Ser  Tyr Phe Tyr Thr Ile  Asn Phe Val Arg Phe  Asp Gly Ser Met His
1585                1590                1595                1600

Pro Val Ile His Asp  Lys Leu Lys Arg Phe  Lys Leu His Thr Cys  Glu
               1605                1610                1615

Thr Phe Leu Asn  Lys Leu Ala Ile Pro  Asn Lys Gly Leu Ser  Ser Trp
           1620                1625                1630

Leu Thr Ser  Gly Glu Tyr Lys Arg  Leu Gly Tyr Ile Ala  Glu Asp Ala
        1635                1640                1645
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Gly Ile  Arg Ile Pro Phe Val  Cys Lys Glu Ile Pro  Asp Ser Leu His
    1650                1655                1660

Glu  Glu Ile Trp His Ile  Val Val Ala His Lys  Gly Asp Ser Gly Ile
1665                1670                1675                1680

Gly Arg Leu Thr Ser  Val Gln Ala Ala Lys  Val Val Tyr Thr Leu  Gln
                1685                1690                1695

Thr Asp Val His  Ser Ile Ala Arg Thr  Leu Ala Cys Ile Asn  Arg Arg
            1700                1705                1710

Ile Ala Asp  Glu Gln Met Lys Gln  Ser His Phe Glu Ala  Ala Thr Gly
        1715                1720                1725

Arg Ala  Phe Ser Phe Thr Asn  Tyr Ser Ile Gln Ser  Ile Phe Asp Thr
    1730                1735                1740

Leu  Lys Ala Asn Tyr Ala  Thr Lys His Thr Lys  Glu Asn Ile Ala Val
1745                1750                1755                1760

Leu Gln Gln Ala Lys  Asp Gln Leu Leu Glu  Phe Ser Asn Leu Ala  Lys
                1765                1770                1775

Asp Gln Asp Val  Thr Gly Ile Ile Gln  Asp Phe Asn His Leu  Glu Thr
            1780                1785                1790

Ile Tyr Leu  Gln Ser Asp Ser Glu  Val Ala Lys His Leu  Lys Leu Lys
        1795                1800                1805

Ser His  Trp Asn Lys Ser Gln  Ile Thr Arg Asp Ile  Ile Ile Ala Leu
    1810                1815                1820

Ser  Val Leu Ile Gly Gly  Gly Trp Met Leu Ala  Thr Tyr Phe Lys Asp
1825                1830                1835                1840

Lys Phe Asn Glu Pro  Val Tyr Phe Gln Gly  Lys Lys Asn Gln Lys  His
                1845                1850                1855

Lys Leu Lys Met  Arg Glu Ala Arg Gly  Ala Arg Gly Gln Tyr  Glu Val
            1860                1865                1870

Ala Ala Glu  Pro Glu Ala Leu Glu  His Tyr Phe Gly Ser  Ala Tyr Asn
        1875                1880                1885

Asn Lys  Gly Lys Arg Lys Gly  Thr Thr Arg Gly Met  Gly Ala Lys Ser
    1890                1895                1900

Arg  Lys Phe Ile Asn Met  Tyr Gly Phe Asp Pro  Thr Asp Phe Ser Tyr
1905                1910                1915                1920

Ile Arg Phe Val Asp  Pro Leu Thr Gly His  Thr Ile Asp Glu Ser  Thr
                1925                1930                1935

Asn Ala Pro Ile  Asp Leu Val Gln His  Glu Phe Gly Lys Val  Arg Thr
            1940                1945                1950

Arg Met Leu  Ile Asp Asp Glu Ile  Glu Pro Gln Ser Leu  Ser Thr His
        1955                1960                1965

Thr Thr  Ile His Ala Tyr Leu  Val Asn Ser Gly Thr  Lys Lys Val Leu
    1970                1975                1980

Lys  Val Asp Leu Thr Pro  His Ser Ser Leu Arg  Ala Ser Glu Lys Ser
1985                1990                1995                2000

Thr Ala Ile Met Gly  Phe Pro Glu Arg Glu  Asn Glu Leu Arg Gln  Thr
                2005                2010                2015

Gly Met Ala Val  Pro Val Ala Tyr Asp  Gln Leu Pro Pro Lys  Asn Glu
            2020                2025                2030

Asp Leu Thr  Phe Glu Gly Glu Ser  Leu Phe Lys Gly Pro  Arg Asp Tyr
        2035                2040                2045

Asn Pro  Ile Ser Ser Thr Ile  Cys His Leu Thr Asn  Glu Ser Asp Gly
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    2050                2055                2060

His  Thr Thr Ser Leu Tyr  Gly Ile Gly Phe Gly  Pro Phe Ile Ile Thr
2065                2070                2075                2080

Asn Lys His Leu Phe  Arg Arg Asn Asn Gly  Thr Leu Leu Val Gln  Ser
               2085                2090                2095

Leu His Gly Val  Phe Lys Val Lys Asn  Thr Thr Thr Leu Gln  Gln His
           2100                2105                2110

Leu Ile Asp  Gly Arg Asp Met Ile  Ile Ile Arg Met Pro  Lys Asp Phe
       2115                2120                2125

Pro Pro  Phe Pro Gln Lys Leu  Lys Phe Arg Glu Pro  Gln Arg Glu Glu
    2130                2135                2140

Arg  Ile Cys Leu Val Thr  Thr Asn Phe Gln Thr  Lys Ser Met Ser Ser
2145                2150                2155                2160

Met Val Ser Asp Thr  Ser Cys Thr Phe Pro  Ser Ser Asp Gly Ile  Phe
               2165                2170                2175

Trp Lys His Trp  Ile Gln Thr Lys Asp  Gly Gln Cys Gly Ser  Pro Leu
           2180                2185                2190

Val Ser Thr  Arg Asp Gly Phe Ile  Val Gly Ile His Ser  Ala Ser Asn
       2195                2200                2205

Phe Thr  Asn Thr Asn Asn Tyr  Phe Thr Ser Val Pro  Lys Asn Phe Met
    2210                2215                2220

Glu  Leu Leu Thr Asn Gln  Glu Ala Gln Gln Trp  Val Ser Gly Trp Arg
2225                2230                2235                2240

Leu Asn Ala Asp Ser  Val Leu Trp Gly Gly  His Lys Val Phe Met  Ser
               2245                2250                2255

Lys Pro Glu Glu  Pro Phe Gln Pro Val  Lys Glu Ala Thr Gln  Leu Met
           2260                2265                2270

Asn Glu Leu  Val Tyr Ser Gln Gly  Glu Lys Arg Lys Trp  Val Val Glu
       2275                2280                2285

Ala Leu  Ser Gly Asn Leu Arg  Pro Val Ala Glu Cys  Pro Ser Gln Leu
    2290                2295                2300

Val  Thr Lys His Val Val  Lys Gly Lys Cys Pro  Leu Phe Glu Leu Tyr
2305                2310                2315                2320

Leu Gln Leu Asn Pro  Glu Lys Glu Ala Tyr  Phe Lys Pro Met Met  Gly
               2325                2330                2335

Ala Tyr Lys Pro  Ser Arg Leu Asn Arg  Glu Ala Phe Leu Lys  Asp Ile
           2340                2345                2350

Leu Lys Tyr  Ala Ser Glu Ile Glu  Ile Gly Asn Val Asp  Cys Asp Leu
       2355                2360                2365

Leu Glu  Leu Ala Ile Ser Met  Leu Val Thr Lys Leu  Lys Ala Leu Gly
    2370                2375                2380

Phe  Pro Thr Val Asn Tyr  Ile Thr Asp Pro Glu  Glu Ile Phe Ser Ala
2385                2390                2395                2400

Leu Asn Met Lys Ala  Ala Met Gly Ala Leu  Tyr Lys Gly Lys Lys  Lys
               2405                2410                2415

Glu Ala Leu Ser  Glu Leu Thr Leu Asp  Glu Gln Glu Ala Met  Leu Lys
           2420                2425                2430

Ala Ser Cys  Leu Arg Leu Tyr Thr  Gly Lys Leu Gly Ile  Trp Asn Gly
       2435                2440                2445

Ser Leu  Lys Ala Glu Leu Arg  Pro Ile Glu Lys Val  Glu Asn Asn Lys
    2450                2455                2460
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Thr  Arg Thr Phe Thr Ala  Ala Pro Ile Asp Thr  Leu Leu Ala Gly Lys
2465                2470                2475                2480

Val Cys Val Asp Asp  Phe Asn Asn Gln Phe  Tyr Asp Leu Asn Ile  Lys
                2485                2490                2495

Ala Pro Trp Thr  Val Gly Met Thr Lys  Phe Tyr Gln Gly Trp  Asn Glu
            2500                2505                2510

Leu Met Glu  Ala Leu Pro Ser Gly  Trp Val Tyr Cys Asp  Ala Asp Gly
        2515                2520                2525

Ser Gln  Phe Asp Ser Ser Leu  Thr Pro Phe Leu Ile  Asn Ala Val Leu
    2530                2535                2540

Lys  Val Arg Leu Ala Phe  Met Glu Glu Trp Asp  Ile Gly Glu Gln Met
2545                2550                2555                2560

Leu Arg Asn Leu Tyr  Thr Glu Ile Val Tyr  Thr Pro Ile Leu Thr  Pro
                2565                2570                2575

Asp Gly Thr Ile  Ile Lys Lys His Lys  Gly Asn Asn Ser Gly  Gln Pro
            2580                2585                2590

Ser Thr Val  Val Asp Asn Thr Leu  Met Val Ile Ile Ala  Met Leu Tyr
        2595                2600                2605

Thr Cys  Glu Lys Cys Gly Ile  Asn Lys Glu Glu Ile  Val Tyr Tyr Val
    2610                2615                2620

Asn  Gly Asp Asp Leu Leu  Ile Ala Ile His Pro  Asp Lys Ala Glu Arg
2625                2630                2635                2640

Leu Ser Arg Phe Lys  Glu Ser Phe Gly Glu  Leu Gly Leu Lys Tyr  Glu
                2645                2650                2655

Phe Asp Cys Thr  Thr Arg Asp Lys Thr  Gln Leu Trp Phe Met  Ser His
            2660                2665                2670

Arg Ala Leu  Glu Arg Asp Gly Met  Tyr Ile Pro Lys Leu  Glu Glu Glu
        2675                2680                2685

Arg Ile  Val Ser Ile Leu Glu  Trp Asp Arg Ser Lys  Glu Pro Ser His
    2690                2695                2700

Arg  Leu Glu Ala Ile Cys  Ala Ser Met Ile Glu  Ala Trp Gly Tyr Asp
2705                2710                2715                2720

Lys Leu Val Glu Glu  Ile Arg Asn Phe Tyr  Ala Trp Val Leu Glu  Gln
                2725                2730                2735

Ala Pro Tyr Ser  Gln Leu Ala Glu Glu  Gly Lys Ala Pro Tyr  Leu Ala
            2740                2745                2750

Glu Thr Ala  Leu Lys Phe Leu Tyr  Thr Ser Gln His Gly  Thr Asn Ser
        2755                2760                2765

Glu Ile  Glu Glu Tyr Leu Lys  Val Leu Tyr Asp Tyr  Asp Ile Pro Thr
    2770                2775                2780

Thr  Glu Asn Leu Tyr Phe  Gln Ser Gly Thr Val  Asp Ala Gly Ala Asp
2785                2790                2795                2800

Ala Gly Lys Lys Lys  Asp Gln Lys Asp Asp  Lys Val Ala Glu Gln  Ala
                2805                2810                2815

Ser Lys Asp Arg  Asp Val Asn Ala Gly  Thr Ser Gly Thr Phe  Ser Val
            2820                2825                2830

Pro Arg Ile  Asn Ala Met Ala Thr  Lys Leu Gln Tyr Pro  Arg Met Arg
        2835                2840                2845

Gly Glu  Val Val Val Asn Leu  Asn His Leu Leu Gly  Tyr Lys Pro Gln
    2850                2855                2860
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Gln  Ile Asp Leu Ser Asn  Ala Arg Ala Thr His  Glu Gln Phe Ala Ala
2865                2870                2875                2880

Trp His Gln Ala Val  Met Thr Ala Tyr Gly  Val Asn Glu Glu Gln  Met
               2885                2890                2895

Lys Ile Leu Leu  Asn Gly Phe Met Val  Trp Cys Ile Glu Asn  Gly Thr
           2900                2905                2910

Ser Pro Asn  Leu Asn Gly Thr Trp  Val Met Met Asp Gly  Glu Asp Gln
        2915                2920                2925

Val Ser  Tyr Pro Leu Lys Pro  Met Val Glu Asn Ala  Gln Pro Thr Leu
    2930                2935                2940

Arg  Gln Ile Met Thr His  Phe Ser Asp Leu Ala  Glu Ala Tyr Ile Glu
2945                2950                2955                2960

Met Arg Asn Arg Glu  Arg Pro Tyr Met Pro  Arg Tyr Gly Leu Gln  Arg
               2965                2970                2975

Asn Ile Thr Asp  Met Ser Leu Ser Arg  Tyr Ala Phe Asp Phe  Tyr Glu
           2980                2985                2990

Leu Thr Ser  Lys Thr Pro Val Arg  Ala Arg Glu Ala His  Met Gln Met
        2995                3000                3005

Lys Ala  Ala Ala Val Arg Asn  Ser Gly Thr Arg Leu  Phe Gly Leu Asp
    3010                3015                3020

Gly  Asn Val Gly Thr Ala  Glu Glu Asp Thr Glu  Arg His Thr Ala His
3025                3030                3035                3040

Asp Val Asn Arg Asn  Met His Thr Leu Leu  Gly Val Arg Gln
               3045                3050
```

<210> SEQ ID NO 80
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 80

```
Asn Ser Ser Gly Gly Asn Ser Gly Ser
               5
```

<210> SEQ ID NO 81
<211> LENGTH: 2755
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 81

```
ttaggacggg gcgatggcgg ctgagaggag ctgcgcgtgc gcgaacatgt aactggtggg     60

atctgcggcg gctcccagat gatggtcgtc ctcctgggcg cgacgaccct agtgctcgtc    120

gccgtgggcc catgggtgtt gtccgcagcc gcaggtggaa aaaatctaaa atctcctcaa    180

aaagtagagg tcgacatcat agatgacaac tttatcctga ggtggaacag gagcgatgag    240

tctgtcggga atgtgacttt ttcattcgat tatcaaaaaa ctgggatgga taattggata    300

aaattgtctg ggtgtcagaa tattactagt accaaatgca acttttcttc actcaagctg    360

aatgtttatg aagaaattaa attgcgtata agagcagaaa aagaaaacac ttcttcatgg    420

tatgaggttg actcatttac accatttcgc aaagctcaga ttggtcctcc agaagtacat    480

ttagaagctg aagataaggc aatagtgata cacatctctc ctggaacaaa agatagtgtt    540

atgtgggctt tggatggttt aagctttaca tatagcttac ttatctggaa aaactcttca    600

ggtgtagaag aaaggattga aaatatttat tccagacata aaatttataa actctcacca    660
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gagactactt attgtctaaa agttaaagca gcactactta cgtcatggaa aattggtgtc 720

tatagtccag tacattgtat aaagaccaca gttgaaaatg aactacctcc accagaaaat 780

atagaagtca gtgtccaaaa tcagaactat gttcttaaat gggattatac atatgcaaac 840

atgacctttc aagttcagtg gctccacgcc tttttaaaaa ggaatcctgg aaaccatttg 900

tataaatgga aacaaatacc tgactgtgaa aatgtcaaaa ctacccagtg tgtctttcct 960

caaaacgttt tccaaaaagg aatttacctt ctccgcgtac aagcatctga tggaaataac 1020

acatcttttt ggtctgaaga gataaagttt gatactgaaa tacaagcttt cctacttcct 1080

ccagtcttta acattagatc ccttagtgat tcattccata tctatatcgg tgctccaaaa 1140

cagtctggaa acacgcctgt gatccaggat tatccactga tttatgaaat tatttttgg 1200

gaaaacactt caaatgctga gagaaaaatt atcgagaaaa aaactgatgt tacagttcct 1260

aatttgaaac cactgactgt atattgtgtg aaagccagag cacacaccat ggatgaaaag 1320

ctgaataaaa gcagtgtttt tagtgacgct gtatgtgaga aaacaaaacc aggaaatacc 1380

tctaaaattt ggcttatagt tggaatttgt attgcattat ttgctctccc gtttgtcatt 1440

tatgctgcga aagtcttctt gagatgcatc aattatgtct tctttccatc acttaaacct 1500

tcttccagta tagatgagta tttctctgaa cagccattga agaatcttct gctttcaact 1560

tctgaggaac aaatcgaaaa atgtttcata attgaaaata taagcacaat tgctacagta 1620

gaagaaacta atcaaactga tgaagatcat aaaaaatata gttcccaaac tagccaagat 1680

tcaggaaatt attctaatga agatgaaagc gaaagtaaaa caagtgaaga actacagcag 1740

gactttgtat gaccagaaat gaactgtgtc aagtataagg tttttcagca ggagttacac 1800

tgggagcctg aggtcctcac cttcctctca gtaactacag agaggacgtt tcctgtttag 1860

ggaaagaaaa aacatcttca gatcataggt cctaaaaata cgggcaagct cttaactatt 1920

taaaaatgaa attacaggcc cgggcacggt ggctcacacc tgtaatccca gcactttggg 1980

aggctgaggc aggcagatca tgaggtcaag agatcgagac cagcctggcc aacgtggtga 2040

aaccccatct ctactaaaaa tacaaaaatt agccgggtag taggtaggcg cgcgcctgtt 2100

gtcttagcta ctcaggaggc tgaggcagga gaatcgcttg aaaacaggag gtggaggttg 2160

cagtgagccg agatcacgcc actgcactcc agcctggtga cagcgtgaga ctctttaaaa 2220

aaagaaatta aaagagttga gacaaacgtt tcctacattc ttttccatgt gtaaaatcat 2280

gaaaaagcct gtcaccggac ttgcattgga tgagatgagt cagaccaaaa cagtggccac 2340

ccgtcttcct cctgtgagcc taagtgcagc cgtgctagct gcgcaccgtg gctaaggatg 2400

acgtctgtgt tcctgtccat cactgatgct gctggctact gcatgtgcca cacctgtctg 2460

ttcgccattc ctaacattct gtttcattct tcctcgggag atatttcaaa catttggtct 2520

tttcttttaa cactgagggt aggcccttag gaaatttatt taggaaagtc tgaacacgtt 2580

atcacttggt tttctggaaa gtagcttacc ctagaaaaca gctgcaaatg ccagaaagat 2640

gatccctaaa aatgttgagg gacttctgtt cattcatccc gagaacattg gcttccacat 2700

cacagtatct acccttacat ggtttaggat taaagccagg caatctttta ctatg 2755

<210> SEQ ID NO 82
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

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<400> SEQUENCE: 82

Gly Ser Glu Asn Leu Tyr Phe Gln Leu
5

<210> SEQ ID NO 83
<211> LENGTH: 2897
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 83

cccgcactaa agacgcttct tcccggcggg taggaatccc gccggcgagc cgaacagttc 60

cccgagcgca gcccgcggac caccacccgg ccgcacgggc cgcttttgtc ccccgcccgc 120

cgcttctgtc cgagaggccg cccgcgaggc gcatcctgac cgcgagcgtc gggtcccaga 180

gccgggcgcg gctggggccc gaggctagca tctctcggga gccgcaaggc gagagctgca 240

aagtttaatt agacacttca gaattttgat cacctaatgt tgatttcaga tgtaaaagtc 300

aagagaagac tctaaaaata gcaaagatgc ttttgagcca gaatgccttc atcttcagat 360

cacttaattt ggttctcatg gtgtatatca gcctcgtgtt tggtatttca tatgattcgc 420

ctgattacac agatgaatct tgcactttca agatatcatt gcgaaatttc cggtccatct 480

tatcatggga attaaaaaac cactccattg taccaactca ctatacattg ctgtatacaa 540

tcatgagtaa accagaagat ttgaaggtgg ttaagaactg tgcaaatacc acaagatcat 600

tttgtgacct cacagatgag tggagaagca cacacgaggc ctatgtcacc gtcctagaag 660

gattcagcgg gaacacaacg ttgttcagtt gctcacacaa tttctggctg gccatagaca 720

tgtcttttga accaccagag tttgagattg ttggttttac caaccacatt aatgtgatgg 780

tgaaatttcc atctattgtt gaggaagaat tacagtttga tttatctctc gtcattgaag 840

aacagtcaga gggaattgtt aagaagcata aacccgaaat aaaaggaaac atgagtggaa 900

atttcaccta tatcattgac aagttaattc caaacacgaa ctactgtgta tctgtttatt 960

tagagcacag tgatgagcaa gcagtaataa agtctccctt aaaatgcacc ctccttccac 1020

ctggccagga atcagaatca gcagaatctg ccaaaatagg aggaataatt actgtgtttt 1080

tgatagcatt ggtcttgaca agcaccatag tgacactgaa atggattggt tatatatgct 1140

taagaaatag cctccccaaa gtcttgaatt ttcataactt tttagcctgg ccatttccta 1200

acctgccacc gttggaagcc atggatatgg tggaggtcat ttacatcaac agaaagaaga 1260

aagtgtggga ttataattat gatgatgaaa gtgatagcga tactgaggca gcgcccagga 1320

caagtggcgg tggctatacc atgcatggac tgactgtcag gcctctgggt caggcctctg 1380

ccacctctac agaatcccag ttgatagacc cggagtccga ggaggagcct gacctgcctg 1440

aggttgatgt ggagctcccc acgatgccaa aggacagccc tcagcagttg gaactcttga 1500

gtgggccctg tgagaggaga aagagtccac tccaggaccc ttttcccgaa gaggactaca 1560

gctccacgga ggggtctggg ggcagaatta ccttcaatgt ggacttaaac tctgtgtttt 1620

tgagagttct tgatgacgag gacagtgacg acttagaagc ccctctgatg ctatcgtctc 1680

atctggaaga gatggttgac ccagaggatc ctgataatgt gcaatcaaac catttgctgg 1740

ccagcgggga agggacacag ccaacctttc ccagcccctc ttcagagggc ctgtggtccg 1800

aagatgctcc atctgatcaa agtgacactt ctgagtcaga tgttgacctt ggggatggtt 1860

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atataatgag atgactccaa aactattgaa tgaacttgga cagacaagca cctacagggt 1920

tctttgtctc tgcatcctaa cttgctgcct tatcgtctgc aagtgttctc caagggaagg 1980

aggaggaaac tgtggtgttc ctttcttcca ggtgacatca cctatgcaca ttcccagtat 2040

ggggaccata gtatcattca gtgcattgtt tacatattca aagtggtgca ctttgaagga 2100

agcacatgtg cacctttcct ttacactaat gcacttagga tgtttctgca tcatgtctac 2160

cagggagcag ggttccccac agtttcagag gtggtccagg accctatgat atttctcttc 2220

tttcgttctt ttttttttt ttttgagaca gagtctcgtt ctgtcgccca agctggagcg 2280

caatggtgtg atcttggctc actgcaacat ccgcctcccg ggttcaggtg attctcctgc 2340

ctcagcctcc ctcgcaagta gctgggatta caggcgcctg ccaccatgcc tagcaaattt 2400

ttgtattttt agtggagaca ggattttacc atgttggcca ggctggtctc gaactcctga 2460

cctcaagtga tctgccctcc tcagcctcgt aaagtgctgg gattacaggg gtgagccgct 2520

gtgcctggct ggccctgtga tatttctgtg aaataaattg ggccagggtg ggagcaggga 2580

aagaaaagga aaatagtagc aagagctgca aagcaggcag gaagggagga ggagagccag 2640

gtgagcagtg gagagaaggg gggccctgca caaggaaaca gggaagagcc atcgaagttt 2700

cagtcggtga gccttgggca cctcacccat gtcacatcct gtctcctgca attggaattc 2760

caccttgtcc agccctcccc agttaaagtg gggaagacag actttaggat cacgtgtgtg 2820

actaatacag aaaggaaaca tggcgtcggg gagagggata aaacctgaat gccatatttt 2880

aagttaaaaa aaaaaaa 2897

<210> SEQ ID NO 84
<211> LENGTH: 3054
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 84

Met Ala Leu Ile Phe Gly Thr Val Asn Ala Asn Ile Leu Lys Glu Val
1 5 10 15

Phe Gly Gly Ala Arg Met Ala Cys Val Thr Ser Ala His Met Ala Gly
20 25 30

Ala Asn Gly Ser Ile Leu Lys Lys Ala Glu Glu Thr Ser Arg Ala Ile
35 40 45

Met His Lys Pro Val Ile Phe Gly Glu Asp Tyr Ile Thr Glu Ala Asp
50 55 60

Leu Pro Tyr Thr Pro Leu His Leu Glu Val Asp Ala Glu Met Glu Arg
65 70 75 80

Met Tyr Tyr Leu Gly Arg Arg Ala Leu Thr His Gly Lys Arg Arg Lys
85 90 95

Val Ser Val Asn Asn Lys Arg Asn Arg Arg Arg Lys Val Ala Lys Thr
100 105 110

Tyr Val Gly Arg Asp Ser Ile Val Glu Lys Ile Val Val Pro His Thr
115 120 125

Glu Arg Lys Val Asp Thr Thr Ala Ala Val Glu Asp Ile Cys Asn Glu
130 135 140

Ala Thr Thr Gln Leu Val His Asn Ser Met Pro Lys Arg Lys Lys Gln
145 150 155 160

Lys Asn Phe Leu Pro Ala Thr Ser Leu Ser Asn Val Tyr Ala Gln Thr
165 170 175

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Trp Ser Ile Val Arg Lys Arg His Met Gln Val Glu Ile Ile Ser Lys
            180                 185                 190

Lys Ser Val Arg Ala Arg Val Lys Arg Phe Glu Gly Ser Val Gln Leu
        195                 200                 205

Phe Ala Ser Val Arg His Met Tyr Gly Glu Arg Lys Arg Val Asp Leu
    210                 215                 220

Arg Ile Asp Asn Trp Gln Gln Glu Thr Leu Leu Asp Leu Ala Lys Arg
225                 230                 235                 240

Phe Lys Asn Glu Arg Val Asp Gln Ser Lys Leu Thr Phe Gly Ser Ser
                245                 250                 255

Gly Leu Val Leu Arg Gln Gly Ser Tyr Gly Pro Ala His Trp Tyr Arg
            260                 265                 270

His Gly Met Phe Ile Val Arg Gly Arg Ser Asp Gly Met Leu Val Asp
        275                 280                 285

Ala Arg Ala Lys Val Thr Phe Ala Val Cys His Ser Met Thr His Tyr
    290                 295                 300

Ser Asp Lys Ser Ile Ser Glu Ala Phe Phe Ile Pro Tyr Ser Lys Lys
305                 310                 315                 320

Phe Leu Glu Leu Arg Pro Asp Gly Ile Ser His Glu Cys Thr Arg Gly
                325                 330                 335

Val Ser Val Glu Arg Cys Gly Glu Val Ala Ala Ile Leu Thr Gln Ala
            340                 345                 350

Leu Ser Pro Cys Gly Lys Ile Thr Cys Lys Arg Cys Met Val Glu Thr
        355                 360                 365

Pro Asp Ile Val Glu Gly Glu Ser Gly Glu Ser Val Thr Asn Gln Gly
    370                 375                 380

Lys Leu Leu Ala Met Leu Lys Glu Gln Tyr Pro Asp Phe Pro Met Ala
385                 390                 395                 400

Glu Lys Leu Leu Thr Arg Phe Leu Gln Gln Lys Ser Leu Val Asn Thr
                405                 410                 415

Asn Leu Thr Ala Cys Val Ser Val Lys Gln Leu Ile Gly Asp Arg Lys
            420                 425                 430

Gln Ala Pro Phe Thr His Val Leu Ala Val Ser Glu Ile Leu Phe Lys
        435                 440                 445

Gly Asn Lys Leu Thr Gly Ala Asp Leu Glu Glu Ala Ser Thr His Met
    450                 455                 460

Leu Glu Ile Ala Arg Phe Leu Asn Asn Arg Thr Glu Asn Met Arg Ile
465                 470                 475                 480

Gly His Leu Gly Ser Phe Arg Asn Lys Ile Ser Ser Lys Ala His Val
                485                 490                 495

Asn Asn Ala Leu Met Cys Asp Asn Gln Leu Asp Gln Asn Gly Asn Phe
            500                 505                 510

Ile Trp Gly Leu Arg Gly Ala His Ala Lys Arg Phe Leu Lys Gly Phe
        515                 520                 525

Phe Thr Glu Ile Asp Pro Asn Glu Gly Tyr Asp Lys Tyr Val Ile Arg
    530                 535                 540

Lys His Ile Arg Gly Ser Arg Lys Leu Ala Ile Gly Asn Leu Ile Met
545                 550                 555                 560

Ser Thr Asp Phe Gln Thr Leu Arg Gln Gln Ile Gln Gly Glu Thr Ile
                565                 570                 575
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Glu Arg Lys Glu Ile Gly Asn His Cys Ile Ser Met Arg Asn Gly Asn
            580                 585                 590

Tyr Val Tyr Pro Cys Cys Cys Val Thr Leu Glu Asp Gly Lys Ala Gln
        595                 600                 605

Tyr Ser Asp Leu Lys His Pro Thr Lys Arg His Leu Val Ile Gly Asn
    610                 615                 620

Ser Gly Asp Ser Lys Tyr Leu Asp Leu Pro Val Leu Asn Glu Glu Lys
625                 630                 635                 640

Met Tyr Ile Ala Asn Glu Gly Tyr Cys Tyr Met Asn Ile Phe Phe Ala
                645                 650                 655

Leu Leu Val Asn Val Lys Glu Glu Asp Ala Lys Asp Phe Thr Lys Phe
            660                 665                 670

Ile Arg Asp Thr Ile Val Pro Lys Leu Gly Ala Trp Pro Thr Met Gln
        675                 680                 685

Asp Val Ala Thr Ala Cys Tyr Leu Leu Ser Ile Leu Tyr Pro Asp Val
    690                 695                 700

Leu Arg Ala Glu Leu Pro Arg Ile Leu Val Asp His Asp Asn Lys Thr
705                 710                 715                 720

Met His Val Leu Asp Ser Tyr Gly Ser Arg Thr Thr Gly Tyr His Met
                725                 730                 735

Leu Lys Met Asn Thr Thr Ser Gln Leu Ile Glu Phe Val His Ser Gly
            740                 745                 750

Leu Glu Ser Glu Met Lys Thr Tyr Asn Val Gly Gly Met Asn Arg Asp
        755                 760                 765

Val Val Thr Gln Gly Ala Ile Glu Met Leu Ile Lys Ser Ile Tyr Lys
    770                 775                 780

Pro His Leu Met Lys Gln Leu Leu Glu Glu Glu Pro Tyr Ile Ile Val
785                 790                 795                 800

Leu Ala Ile Val Ser Pro Ser Ile Leu Ile Ala Met Tyr Asn Ser Gly
                805                 810                 815

Thr Phe Glu Gln Ala Leu Gln Met Trp Leu Pro Asn Thr Met Arg Leu
            820                 825                 830

Ala Asn Leu Ala Ala Ile Leu Ser Ala Leu Ala Gln Lys Leu Thr Leu
        835                 840                 845

Ala Asp Leu Phe Val Gln Gln Arg Asn Leu Ile Asn Glu Tyr Ala Gln
    850                 855                 860

Val Ile Leu Asp Asn Leu Ile Asp Gly Val Arg Val Asn His Ser Leu
865                 870                 875                 880

Ser Leu Ala Met Glu Ile Val Thr Ile Lys Leu Ala Thr Gln Glu Met
                885                 890                 895

Asp Met Ala Leu Arg Glu Gly Gly Tyr Ala Val Thr Ser Glu Lys Val
            900                 905                 910

His Glu Met Leu Glu Lys Asn Tyr Val Lys Ala Leu Lys Asp Ala Trp
        915                 920                 925

Asp Glu Leu Thr Trp Leu Glu Lys Phe Ser Ala Ile Arg His Ser Arg
    930                 935                 940

Lys Leu Leu Lys Phe Gly Arg Lys Pro Leu Ile Met Lys Asn Thr Val
945                 950                 955                 960

Asp Cys Gly Gly His Ile Asp Leu Ser Val Lys Ser Leu Phe Lys Phe
                965                 970                 975

His Leu Glu Leu Leu Lys Gly Thr Ile Ser Arg Ala Val Asn Gly Gly
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           980                 985                 990

Ala Arg Lys Val Arg Val Ala Lys  Asn Ala Met Thr Lys  Gly Val Phe
        995                 1000                1005

Leu Lys  Ile Tyr Ser Met Leu  Pro Asp Val Tyr Lys  Phe Ile Thr
    1010                1015                1020

Val Ser  Ser Val Leu Ser Leu  Leu Leu Thr Phe Leu  Phe Gln Ile
    1025                1030                1035

Asp Cys  Met Ile Arg Ala His  Arg Glu Ala Lys Val  Ala Ala Gln
    1040                1045                1050

Leu Gln  Lys Glu Ser Glu Trp  Asp Asn Ile Ile Asn  Arg Thr Phe
    1055                1060                1065

Gln Tyr  Ser Lys Leu Glu Asn  Pro Ile Gly Tyr Arg  Ser Thr Ala
    1070                1075                1080

Glu Glu  Arg Leu Gln Ser Glu  His Pro Glu Ala Phe  Glu Tyr Tyr
    1085                1090                1095

Lys Phe  Cys Ile Gly Lys Glu  Asp Leu Val Glu Gln  Ala Lys Gln
    1100                1105                1110

Pro Glu  Ile Ala Tyr Phe Glu  Lys Ile Ile Ala Phe  Ile Thr Leu
    1115                1120                1125

Val Leu  Met Ala Phe Asp Ala  Glu Arg Ser Asp Gly  Val Phe Lys
    1130                1135                1140

Ile Leu  Asn Lys Phe Lys Gly  Ile Leu Ser Ser Thr  Glu Arg Glu
    1145                1150                1155

Ile Ile  Tyr Thr Gln Ser Leu  Asp Asp Tyr Val Thr  Thr Phe Asp
    1160                1165                1170

Asp Asn  Met Thr Ile Asn Leu  Glu Leu Asn Met Asp  Glu Leu His
    1175                1180                1185

Lys Thr  Ser Leu Pro Gly Val  Thr Phe Lys Gln Trp  Trp Asn Asn
    1190                1195                1200

Gln Ile  Ser Arg Gly Asn Val  Lys Pro His Tyr Arg  Thr Glu Gly
    1205                1210                1215

His Phe  Met Glu Phe Thr Arg  Asp Thr Ala Ala Ser  Val Ala Ser
    1220                1225                1230

Glu Ile  Ser His Ser Pro Ala  Arg Asp Phe Leu Val  Arg Gly Ala
    1235                1240                1245

Val Gly  Ser Gly Lys Ser Thr  Gly Leu Pro Tyr His  Leu Ser Lys
    1250                1255                1260

Arg Gly  Arg Val Leu Met Leu  Glu Pro Thr Arg Pro  Leu Thr Asp
    1265                1270                1275

Asn Met  His Lys Gln Leu Arg  Ser Glu Pro Phe Asn  Cys Phe Pro
    1280                1285                1290

Thr Leu  Arg Met Arg Gly Lys  Ser Thr Phe Gly Ser  Ser Pro Ile
    1295                1300                1305

Thr Val  Met Thr Ser Gly Phe  Ala Leu His His Phe  Ala Arg Asn
    1310                1315                1320

Ile Ala  Glu Val Lys Thr Tyr  Asp Phe Val Ile Ile  Asp Glu Cys
    1325                1330                1335

His Val  Asn Asp Ala Ser Ala  Ile Ala Phe Arg Asn  Leu Leu Phe
    1340                1345                1350

Glu His  Glu Phe Glu Gly Lys  Val Leu Lys Val Ser  Ala Thr Pro
    1355                1360                1365
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Pro Gly  Arg Glu Val Glu Phe  Thr Thr Gln Phe Pro  Val Lys Leu
    1370                1375                1380

Lys Ile  Glu Glu Ala Leu Ser  Phe Gln Glu Phe Val  Ser Leu Gln
    1385                1390                1395

Gly Thr  Gly Ala Asn Ala Asp  Val Ile Ser Cys Gly  Asp Asn Ile
    1400                1405                1410

Leu Val  Tyr Val Ala Ser Tyr  Asn Asp Val Asp Ser  Leu Gly Lys
    1415                1420                1425

Leu Leu  Val Gln Lys Gly Tyr  Lys Val Ser Lys Ile  Asp Gly Arg
    1430                1435                1440

Thr Met  Lys Ser Gly Gly Thr  Glu Ile Ile Thr Glu  Gly Thr Ser
    1445                1450                1455

Val Lys  Lys His Phe Ile Val  Ala Thr Asn Ile Ile  Glu Asn Gly
    1460                1465                1470

Val Thr  Ile Asp Ile Asp Val  Val Val Asp Phe Gly  Thr Lys Val
    1475                1480                1485

Val Pro  Val Leu Asp Val Asp  Asn Arg Ala Val Gln  Tyr Asn Lys
    1490                1495                1500

Thr Val  Val Ser Tyr Gly Glu  Arg Ile Gln Lys Leu  Gly Arg Val
    1505                1510                1515

Gly Arg  His Lys Glu Gly Val  Ala Leu Arg Ile Gly  Gln Thr Asn
    1520                1525                1530

Lys Thr  Leu Val Glu Ile Pro  Glu Met Val Ala Thr  Glu Ala Ala
    1535                1540                1545

Phe Leu  Cys Phe Met Tyr Asn  Leu Pro Val Thr Thr  Gln Ser Val
    1550                1555                1560

Ser Thr  Thr Leu Leu Glu Asn  Ala Thr Leu Leu Gln  Ala Arg Thr
    1565                1570                1575

Met Ala  Gln Phe Glu Leu Ser  Tyr Phe Tyr Thr Ile  Asn Phe Val
    1580                1585                1590

Arg Phe  Asp Gly Ser Met His  Pro Val Ile His Asp  Lys Leu Lys
    1595                1600                1605

Arg Phe  Lys Leu His Thr Cys  Glu Thr Phe Leu Asn  Lys Leu Ala
    1610                1615                1620

Ile Pro  Asn Lys Gly Leu Ser  Ser Trp Leu Thr Ser  Gly Glu Tyr
    1625                1630                1635

Lys Arg  Leu Gly Tyr Ile Ala  Glu Asp Ala Gly Ile  Arg Ile Pro
    1640                1645                1650

Phe Val  Cys Lys Glu Ile Pro  Asp Ser Leu His Glu  Glu Ile Trp
    1655                1660                1665

His Ile  Val Val Ala His Lys  Gly Asp Ser Gly Ile  Gly Arg Leu
    1670                1675                1680

Thr Ser  Val Gln Ala Ala Lys  Val Val Tyr Thr Leu  Gln Thr Asp
    1685                1690                1695

Val His  Ser Ile Ala Arg Thr  Leu Ala Cys Ile Asn  Arg Arg Ile
    1700                1705                1710

Ala Asp  Glu Gln Met Lys Gln  Ser His Phe Glu Ala  Ala Thr Gly
    1715                1720                1725

Arg Ala  Phe Ser Phe Thr Asn  Tyr Ser Ile Gln Ser  Ile Phe Asp
    1730                1735                1740
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Thr Leu  Lys Ala Asn Tyr Ala  Thr Lys His Thr Lys  Glu Asn Ile
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Ala Val  Leu Gln Gln Ala Lys  Asp Gln Leu Leu Glu  Phe Ser Asn
    1760                1765                1770

Leu Ala  Lys Asp Gln Asp Val  Thr Gly Ile Ile Gln  Asp Phe Asn
    1775                1780                1785

His Leu  Glu Thr Ile Tyr Leu  Gln Ser Asp Ser Glu  Val Ala Lys
    1790                1795                1800

His Leu  Lys Leu Lys Ser His  Trp Asn Lys Ser Gln  Ile Thr Arg
    1805                1810                1815

Asp Ile  Ile Ile Ala Leu Ser  Val Leu Ile Gly Gly  Gly Trp Met
    1820                1825                1830

Leu Ala  Thr Tyr Phe Lys Asp  Lys Phe Asn Glu Pro  Val Tyr Phe
    1835                1840                1845

Gln Gly  Lys Lys Asn Gln Lys  His Lys Leu Lys Met  Arg Glu Ala
    1850                1855                1860

Arg Gly  Ala Arg Gly Gln Tyr  Glu Val Ala Ala Glu  Pro Glu Ala
    1865                1870                1875

Leu Glu  His Tyr Phe Gly Ser  Ala Tyr Asn Asn Lys  Gly Lys Arg
    1880                1885                1890

Lys Gly  Thr Thr Arg Gly Met  Gly Ala Lys Ser Arg  Lys Phe Ile
    1895                1900                1905

Asn Met  Tyr Gly Phe Asp Pro  Thr Asp Phe Ser Tyr  Ile Arg Phe
    1910                1915                1920

Val Asp  Pro Leu Thr Gly His  Thr Ile Asp Glu Ser  Thr Asn Ala
    1925                1930                1935

Pro Ile  Asp Leu Val Gln His  Glu Phe Gly Lys Val  Arg Thr Arg
    1940                1945                1950

Met Leu  Ile Asp Asp Glu Ile  Glu Pro Gln Ser Leu  Ser Thr His
    1955                1960                1965

Thr Thr  Ile His Ala Tyr Leu  Val Asn Ser Gly Thr  Lys Lys Val
    1970                1975                1980

Leu Lys  Val Asp Leu Thr Pro  His Ser Ser Leu Arg  Ala Ser Glu
    1985                1990                1995

Lys Ser  Thr Ala Ile Met Gly  Phe Pro Glu Arg Glu  Asn Glu Leu
    2000                2005                2010

Arg Gln  Thr Gly Met Ala Val  Pro Val Ala Tyr Asp  Gln Leu Pro
    2015                2020                2025

Pro Lys  Asn Glu Asp Leu Thr  Phe Glu Gly Glu Ser  Leu Phe Lys
    2030                2035                2040

Gly Pro  Arg Asp Tyr Asn Pro  Ile Ser Ser Thr Ile  Cys His Leu
    2045                2050                2055

Thr Asn  Glu Ser Asp Gly His  Thr Thr Ser Leu Tyr  Gly Ile Gly
    2060                2065                2070

Phe Gly  Pro Phe Ile Ile Thr  Asn Lys His Leu Phe  Arg Arg Asn
    2075                2080                2085

Asn Gly  Thr Leu Leu Val Gln  Ser Leu His Gly Val  Phe Lys Val
    2090                2095                2100

Lys Asn  Thr Thr Thr Leu Gln  Gln His Leu Ile Asp  Gly Arg Asp
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Met Ile  Ile Ile Arg Met Pro  Lys Asp Phe Pro Pro  Phe Pro Gln
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Lys Leu  Lys Phe Arg Glu Pro  Gln Arg Glu Glu Arg  Ile Cys Leu
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Val Thr  Thr Asn Phe Gln Thr  Lys Ser Met Ser Ser  Met Val Ser
    2150                2155                2160

Asp Thr  Ser Cys Thr Phe Pro  Ser Ser Asp Gly Ile  Phe Trp Lys
    2165                2170                2175

His Trp  Ile Gln Thr Lys Asp  Gly Gln Cys Gly Ser  Pro Leu Val
    2180                2185                2190

Ser Thr  Arg Asp Gly Phe Ile  Val Gly Ile His Ser  Ala Ser Asn
    2195                2200                2205

Phe Thr  Asn Thr Asn Asn Tyr  Phe Thr Ser Val Pro  Lys Asn Phe
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Met Glu  Leu Leu Thr Asn Gln  Glu Ala Gln Gln Trp  Val Ser Gly
    2225                2230                2235

Trp Arg  Leu Asn Ala Asp Ser  Val Leu Trp Gly Gly  His Lys Val
    2240                2245                2250

Phe Met  Ser Lys Pro Glu Glu  Pro Phe Gln Pro Val  Lys Glu Ala
    2255                2260                2265

Thr Gln  Leu Met Asn Glu Leu  Val Tyr Ser Gln Gly  Glu Lys Arg
    2270                2275                2280

Lys Trp  Val Val Glu Ala Leu  Ser Gly Asn Leu Arg  Pro Val Ala
    2285                2290                2295

Glu Cys  Pro Ser Gln Leu Val  Thr Lys His Val Val  Lys Gly Lys
    2300                2305                2310

Cys Pro  Leu Phe Glu Leu Tyr  Leu Gln Leu Asn Pro  Glu Lys Glu
    2315                2320                2325

Ala Tyr  Phe Lys Pro Met Met  Gly Ala Tyr Lys Pro  Ser Arg Leu
    2330                2335                2340

Asn Arg  Glu Ala Phe Leu Lys  Asp Ile Leu Lys Tyr  Ala Ser Glu
    2345                2350                2355

Ile Glu  Ile Gly Asn Val Asp  Cys Asp Leu Leu Glu  Leu Ala Ile
    2360                2365                2370

Ser Met  Leu Val Thr Lys Leu  Lys Ala Leu Gly Phe  Pro Thr Val
    2375                2380                2385

Asn Tyr  Ile Thr Asp Pro Glu  Glu Ile Phe Ser Ala  Leu Asn Met
    2390                2395                2400

Lys Ala  Ala Met Gly Ala Leu  Tyr Lys Gly Lys Lys  Lys Glu Ala
    2405                2410                2415

Leu Ser  Glu Leu Thr Leu Asp  Glu Gln Glu Ala Met  Leu Lys Ala
    2420                2425                2430

Ser Cys  Leu Arg Leu Tyr Thr  Gly Lys Leu Gly Ile  Trp Asn Gly
    2435                2440                2445

Ser Leu  Lys Ala Glu Leu Arg  Pro Ile Glu Lys Val  Glu Asn Asn
    2450                2455                2460

Lys Thr  Arg Thr Phe Thr Ala  Ala Pro Ile Asp Thr  Leu Leu Ala
    2465                2470                2475

Gly Lys  Val Cys Val Asp Asp  Phe Asn Asn Gln Phe  Tyr Asp Leu
    2480                2485                2490

Asn Ile  Lys Ala Pro Trp Thr  Val Gly Met Thr Lys  Phe Tyr Gln
    2495                2500                2505
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Gly Trp  Asn Glu Leu Met Glu  Ala Leu Pro Ser Gly  Trp Val Tyr
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Cys Asp  Ala Asp Gly Ser Gln  Phe Asp Ser Ser Leu  Thr Pro Phe
    2525                2530                2535

Leu Ile  Asn Ala Val Leu Lys  Val Arg Leu Ala Phe  Met Glu Glu
    2540                2545                2550

Trp Asp  Ile Gly Glu Gln Met  Leu Arg Asn Leu Tyr  Thr Glu Ile
    2555                2560                2565

Val Tyr  Thr Pro Ile Leu Thr  Pro Asp Gly Thr Ile  Ile Lys Lys
    2570                2575                2580

His Lys  Gly Asn Asn Ser Gly  Gln Pro Ser Thr Val  Val Asp Asn
    2585                2590                2595

Thr Leu  Met Val Ile Ile Ala  Met Leu Tyr Thr Cys  Glu Lys Cys
    2600                2605                2610

Gly Ile  Asn Lys Glu Glu Ile  Val Tyr Tyr Val Asn  Gly Asp Asp
    2615                2620                2625

Leu Leu  Ile Ala Ile His Pro  Asp Lys Ala Glu Arg  Leu Ser Arg
    2630                2635                2640

Phe Lys  Glu Ser Phe Gly Glu  Leu Gly Leu Lys Tyr  Glu Phe Asp
    2645                2650                2655

Cys Thr  Thr Arg Asp Lys Thr  Gln Leu Trp Phe Met  Ser His Arg
    2660                2665                2670

Ala Leu  Glu Arg Asp Gly Met  Tyr Ile Pro Lys Leu  Glu Glu Glu
    2675                2680                2685

Arg Ile  Val Ser Ile Leu Glu  Trp Asp Arg Ser Lys  Glu Pro Ser
    2690                2695                2700

His Arg  Leu Glu Ala Ile Cys  Ala Ser Met Ile Glu  Ala Trp Gly
    2705                2710                2715

Tyr Asp  Lys Leu Val Glu Glu  Ile Arg Asn Phe Tyr  Ala Trp Val
    2720                2725                2730

Leu Glu  Gln Ala Pro Tyr Ser  Gln Leu Ala Glu Glu  Gly Lys Ala
    2735                2740                2745

Pro Tyr  Leu Ala Glu Thr Ala  Leu Lys Phe Leu Tyr  Thr Ser Gln
    2750                2755                2760

His Gly  Thr Asn Ser Glu Ile  Glu Glu Tyr Leu Lys  Val Leu Tyr
    2765                2770                2775

Asp Tyr  Asp Ile Pro Thr Thr  Glu Asn Leu Tyr Phe  Gln Ser Gly
    2780                2785                2790

Thr Val  Asp Ala Gly Ala Asp  Ala Gly Lys Lys Lys  Asp Gln Lys
    2795                2800                2805

Asp Asp  Lys Val Ala Glu Gln  Ala Ser Lys Asp Arg  Asp Val Asn
    2810                2815                2820

Ala Gly  Thr Ser Gly Thr Phe  Ser Val Pro Arg Ile  Asn Ala Met
    2825                2830                2835

Ala Thr  Lys Leu Gln Tyr Pro  Arg Met Arg Gly Glu  Val Val Val
    2840                2845                2850

Asn Leu  Asn His Leu Leu Gly  Tyr Lys Pro Gln Gln  Ile Asp Leu
    2855                2860                2865

Ser Asn  Ala Arg Ala Thr His  Glu Gln Phe Ala Ala  Trp His Gln
    2870                2875                2880
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Ala Val  Met Thr Ala Tyr Gly  Val Asn Glu Glu Gln  Met Lys Ile
    2885                2890                2895

Leu Leu  Asn Gly Phe Met Val  Trp Cys Ile Glu Asn  Gly Thr Ser
    2900                2905                2910

Pro Asn  Leu Asn Gly Thr Trp  Val Met Met Asp Gly  Glu Asp Gln
    2915                2920                2925

Val Ser  Tyr Pro Leu Lys Pro  Met Val Glu Asn Ala  Gln Pro Thr
    2930                2935                2940

Leu Arg  Gln Ile Met Thr His  Phe Ser Asp Leu Ala  Glu Ala Tyr
    2945                2950                2955

Ile Glu  Met Arg Asn Arg Glu  Arg Pro Tyr Met Pro  Arg Tyr Gly
    2960                2965                2970

Leu Gln  Arg Asn Ile Thr Asp  Met Ser Leu Ser Arg  Tyr Ala Phe
    2975                2980                2985

Asp Phe  Tyr Glu Leu Thr Ser  Lys Thr Pro Val Arg  Ala Arg Glu
    2990                2995                3000

Ala His  Met Gln Met Lys Ala  Ala Ala Val Arg Asn  Ser Gly Thr
    3005                3010                3015

Arg Leu  Phe Gly Leu Asp Gly  Asn Val Gly Thr Ala  Glu Glu Asp
    3020                3025                3030

Thr Glu  Arg His Thr Ala His  Asp Val Asn Arg Asn  Met His Thr
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Leu Leu  Gly Val Arg Gln
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<400> SEQUENCE: 92

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We claim:

1. A method for determining if a test compound modulates a specific protein/protein interaction of interest comprising contacting said compound to a cell which has been transformed or transfected with

(a) a nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes said first test protein,

(ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and

(iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and

(b) a nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound is to be measured, and

(ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site, and determining activity of said reporter gene as a determination of whether said compound modulates said protein/protein interaction.

2. The method of claim 1, wherein said first test protein is a membrane bound protein.

3. The method of claim 2, wherein said membrane bound protein is a transmembrane receptor.

4. The method of claim 3, wherein said transmembrane receptor is a GPCR.

5. The method of claim 1, wherein said protease or portion of a protease is tobacco etch virus nuclear inclusion A protease.

6. The method of claim 1, wherein said protein which activates said reporter gene is a transcription factor.

7. The method of claim 6, wherein said transcription factor is tTA or GAL4.

8. The method of claim 1, wherein said second protein is an inhibitory protein.

9. The method of claim 8, wherein said inhibitory protein is an arrestin, and said first protein is a transmembrane receptor.

10. The method of claim 1, wherein said cell is a eukaryote.

11. The method of claim 1, wherein said reporter gene is an exogenous gene.

12. The method of claim 11, wherein said exogenous gene encodes 0-galactosidase or luciferase.

13. The method of claim 1, wherein the nucleotide sequence encoding said first test protein is modified to increase interaction with said second test protein.

14. The method of claim 13, wherein said modification comprises replacing all or part of the nucleotide sequence of the C-terminal region of said first test protein with a nucleotide sequence which encodes an amino acid sequence which has higher affinity for said second test protein than the original sequence.

15. The method of claim 14, wherein the nucleotide sequence of said C-terminal region is replaced by a nucleotide sequence encoding all or a part of the C-terminal region of AVPR2, AGTRLI, GRPR, F2PL1, CXCR2/IL-8B, CCR4, or GRPR.

16. The method of claim 1, comprising contacting more than one compound to a plurality of samples of cells, each of said samples being contacted by one or more of said compounds, wherein each of said cell samples have been transformed or transfected with (a) and (b), and determining activity of reporter genes in said plurality of said samples to determine if any of said compounds modulates said specific, protein/protein interaction.

17. The method of claim 2, wherein said membrane bound protein is β2-adrenergic receptor (ADRB2), arginine vasopressin receptor 2 (AVPR2), serotonin receptor 1a (HTR1A), m2 muscarinic acetylcholine receptor (CHRM2), chemokine (C-C motif) receptor 5 (CCR5), dopamine D2 receptor (DRD2), kappa opioid receptor (OPRK), or ADRA1A.

18. The method of claim 16, comprising contacting each of said samples with one compound, each of which differs from all others.

19. The method of claim 16, comprising contacting each of said samples with a mixture of said compounds.

20. A method for determining if a test compound modulates one or more of a plurality of protein interactions of interest, comprising contacting said test compound to a plurality of samples of cells, each of which has been transformed or transfected with

(a) a first nucleic acid molecule comprising:

(i) a nucleotide sequence which encodes a first test protein,

(ii) a nucleotide sequence encoding a cleavage site for a protease, and

(iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell,

(b) a second nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound of interest is to be measured,

(ii) a nucleotide sequence which encodes a protease or a protease which is specific for said cleavage site, wherein said first test protein differs from other first test proteins in each of said plurality of samples, and determining activity of said reporter gene in at one or more of said plurality of samples as a determination of modulation of one or more protein interactions of interest

21. The method of claim 20, wherein said second test protein is different in each sample.

22. The method of claim 20, wherein said second test protein is the same in each sample.

23. The method of claim 20, wherein all of said samples are combined in a common receptacle, and each samples comprises a different pair of first and second test proteins.

24. The method of claim 20, wherein each sample is tested in a different receptacle.

25. The method of claim 20, wherein the reporter gene in a given sample differs from the reporter gene in other samples.

26. The method of claim 19, wherein said mixture of compounds comprises a biological sample.

27. The method of claim 26, wherein said biological sample is cerebrospinal fluid, urine, blood, serum, pus, ascites, synovial fluid, a tissue extract, or an exudate.

28. Recombinant cell, transformed or transfected with:

(a) a nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes said first test protein,

(ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and

(iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and

(b) a nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound is to be measured, and

(ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site.

29. The recombinant cell of claim 28, wherein one or both of said nucleic acid molecules are stably incorporated into the genome of said cell.

30. The recombinant cell of claim 28, wherein said cell has been transformed or transfected with said reporter gene.

31. The recombinant cell of claim 28, wherein said first test protein is a membrane bound protein.

32. The recombinant cell of claim 31, wherein said membrane bound protein is a transmembrane receptor.

33. The recombinant cell of claim 32, wherein said transmembrane receptor is a GPCR.

34. The recombinant cell of claim 28, wherein said protease or portion of a protease is tobacco etch virus nuclear inclusion A protease.

35. The recombinant cell of claim 28, wherein said protein which activates said reporter gene is a transcription factor.

36. The recombinant cell of claim 31, wherein said membrane bound protein is ADBR2, AVPR2, HTR1A, CHRM2, CCR5, DRD2, or OPRK.

37. The recombinant cell of claim 28, wherein said transcription factor is tTA or GAL4.

38. The recombinant cell of claim 28, wherein said second protein is an inhibitory protein.

39. The recombinant cell of claim 38, wherein said inhibitory protein is an arrestin, and said first protein is a transmembrane receptor.

40. The recombinant cell of claim 28, wherein said cell is a eukaryote.

41. The recombinant cell of claim 28, wherein said cell is a prokaryote.

42. The recombinant cell of claim 28, wherein said reporter gene is an exogenous gene.

43. The recombinant cell of claim 42, wherein said exogenous gene encodes β-galactosidase or luciferase.

44. The recombinant cell of claim 28, wherein the nucleotide sequence encoding said first test protein is modified to increase interaction with said second test protein.

45. The recombinant cell of claim 44, wherein said modification comprises replacing all or part of the nucleotide sequence of the C-terminal region of said first test protein with a nucleotide sequence which encodes an amino acid sequence which has higher affinity for said second test protein than the original sequence.

46. The recombinant cell of claim 44, wherein the nucleotide sequence of said C-terminal region is replaced by a nucleotide sequence encoding the C-terminal region of AVPR2, AGTRLI, GRPR, F2PL1, CXCR2/IL-8B, or CCR4.

47. An isolated nucleic acid molecule which comprises, in 5' to 3' order,

(i) a nucleotide sequence which encodes a test protein,

(ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease, and

(iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell.

48. The isolated nucleic acid molecule of claim 47, wherein said test protein is a membrane bound protein.

49. The isolated nucleic acid molecule of claim 48, wherein said membrane bound protein is a transmembrane receptor.

50. The isolated nucleic acid molecule of claim 49, wherein said transmembrane receptor is a GPCR.

51. The isolated nucleic acid molecule of claim 47, wherein said protease or portion of a protease is tobacco etch virus nuclear inclusion A protease.

52. The isolated nucleic acid molecule of claim 47, wherein said protein which activates said reporter gene is a transcription factor.

53. The isolated nucleic acid molecule of claim 52, wherein said transcription factor is tTA or GAL4.

54. The isolated nucleic acid molecule of claim 48, wherein said membrane bound protein is ADBR2, AVPR2, HTR1A, CHRM2, CCR5, DRD2, or OPRK.

55. Expression vector comprising the isolated nucleic acid molecule of claim 47, operably linked to a promoter.

56. An isolated nucleic acid molecule which comprises:

(i) nucleotide sequence which encodes a test protein whose interaction with another test protein in the presence of a test compound is to be measured, and

(ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site.

57. The isolated nucleic acid molecule of claim 56, wherein said test protein is an inhibitory protein.

58. The isolated nucleic acid molecule of claim 57, wherein said inhibitory protein is an arrestin.

59. Expression vector comprising the isolated nucleic acid molecule of claim 56, operably linked to a promoter.

60. A fusion protein produced by expression of the isolated nucleic acid molecule of claim 47.

61. A fusion protein produced by expression of the isolated nucleic acid molecule of claim 56.

62. A test kit useful for detennining if a test compound modulates a specific protein/protein interaction of interest comprising a separate portion of each of:

(a) a nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes said first test protein,

(ii) a nucleotide sequence encoding a cleavage site for a protease or a portion of a protease,

(iii) a nucleotide sequence which encodes a protein which activates a reporter gene in said cell, and

(b) a nucleic acid molecule which comprises:

(i) a nucleotide sequence which encodes a second test protein whose interaction with said first test protein in the presence of said test compound is to be measured,

(ii) a nucleotide sequence which encodes a protease or a portion of a protease which is specific for said cleavage site, and

(c) container means for holding each of (a) and (b) separately from each other.

63. The test kit of claim 62, wherein said first test protein is a membrane bound protein.

64. The test kit of claim 63, wherein said membrane bound protein is a transmembrane receptor.

65. The test kit of claim 64, wherein said transmembrane receptor is a GPCR.

66. The test kit of claim 62, wherein said protease or portion of a protease is tobacco etch virus nuclear inclusion A protease.

67. The test kit of claim 62, wherein said protein which activates said reporter gene is a transcription factor.

68. The test kit of claim 67, wherein said transcription factor is tTA or GAL4.

69. The test kit of claim 62, wherein said second protein is an inhibitory protein.

70. The test kit of claim 69, wherein said inhibitory protein is an arrestin, and said first protein is a transmembrane receptor.

71. The test kit of claim 61, further comprising a separate portion of an isolated nucleic acid molecule which encodes a reporter gene.

72. The test kit of claim 71, wherein said reporter gene encodes 0-galactosidase or luciferase.

73. The test kit of claim 62, wherein the nucleotide sequence encoding said first test protein is modified to increase interaction with said second test protein.

74. The test kit of claim 73, wherein said modification comprises replacing all or part of the nucleotide sequence of the C-terminal region of said first test protein with a nucleotide sequence which encodes an amino acid sequence which has higher affinity for said second test protein than the original sequence.

75. The test kit of claim 74, wherein said nucleotide sequence of said C-terminal region is replaced by a nucleotide sequence encoding the C-terminal region of AVPR2, AGTRLI, GRPR, F2PL1, CXCR2/IL-8B or CCR4.

76. The test kit of claim 63, wherein said membrane bound protein is ADRB2, AVPR2, HTR1A, CHRM2, CCR5, DRD2, or OPRK.

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专利名称(译)	测定蛋白质 - 蛋白质相互作用的方法		
公开(公告)号	US20050100934A1	公开(公告)日	2005-05-12
申请号	US10/888313	申请日	2004-07-09
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IPC分类号	C12N C12N15/85 C12Q1/68 G01N33/53 G01N33/68 G01N33/94		
CPC分类号	C12N15/1055 G01N33/6845 G01N2500/10 G01N2333/726 G01N33/9406		
优先权	60/566113 2004-04-27 US 60/485968 2003-07-09 US 60/511918 2003-10-15 US		
其他公开文献	US7049076		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及用于确定测试化合物或化合物的混合物是否调节两种目的蛋白质之间的相互作用的方法。通过使用两种重组分子可以进行测定，其中一种重组分子含有第一种蛋白质，一种蛋白水解分子的切割位点，和一种基因的激活因子。第二重组分子包括第二蛋白质和蛋白水解分子。如果测试化合物与第一种蛋白质结合，则启动反应，从而切割活化剂，并激活报告基因。

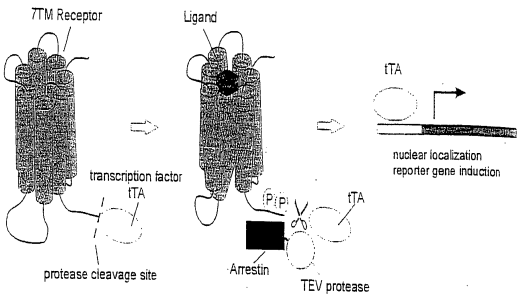
7TM Receptor
Ligand
tTA
nuclear localization
reporter gene induction
transcription factor
tTA
protease cleavage site
P
P
tTA
Arrestin
TEV protease

Fig. 1