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(54) Title: PEPTIDES OF CAV2.2 THAT INHIBIT PAIN

(57) Abstract: The present invention relates to peptides of CaV2.2 and their use in the treatment of pain. The sequence of the peptides is derived from the C-terminus of CaV2.2 and is believed to inhibit the interaction of CaV2.2 with Mint1-PDZ1. The invention is related to use of this peptide to treat pain and to use of this peptide in binding reaction with int-PDZ to screen for small molecules that can inhibit pain.



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DESCRIPTION

PEPTIDES OF CAV2.2 THAT INHIBIT PAIN

BACKGROUND OF THE INVENTION

This application claims benefit of priority to U.S. Provisional Application Serial No. 60/558,383, filed April 1, 2004, the entire contents of which are hereby incorporated by reference.

The government owns rights in the present invention pursuant to grant number NS039552 from the NINDS.

1. Field of the Invention

The present invention relates to fields of molecular biology and pathology. More particularly, the present invention relates to peptides of CaV2.2 and methods for their use in the treatment of pain.

2. Description of Related Art

There are six distinguishable types of voltage-dependent calcium channels (VDCC) presently described, designated L-type, N-type, P/Q-type, L-type, R-type, and T-type, which are expressed throughout the nervous system (Tsien *et al.*, 1991). Presynaptic voltage-gated Ca^{2+} channels mediate rapid Ca^{2+} influx into the synaptic terminal that triggers synaptic vesicle exocytosis and neurotransmitter release (Llinas *et al.*, 1981). N-type Ca^{2+} channels, encoded by CaV2.2 pore-forming subunit (Williams *et al.*, 1992; Ertel *et al.*, 2000) and P/Q-type Ca^{2+} channels, encoded by the CaV2.1 pore-forming subunit (Mori *et al.*, 1991; Ertel *et al.*, 2000), play a predominant role in supporting chemical neurotransmission in central synapses (Takahashi and Momiyama, 1993; Wheeler *et al.*, 1994; Dunlap *et al.*, 1995; Reuter, 1995). Sensation of pain is mediated by nociceptive neurons in the dorsal root ganglia (DRG) (McCleskey and Gold, 1999; Yaksh, 1999). N-type voltage-gated Ca^{2+} channels (Ca_v2.2) are abundantly expressed in DRG neurons (Kerr *et al.*, 1988; Gohil *et al.*, 1994; Westenbroek *et al.*, 1998) and play a predominant role in control of glutamate release from DRG neurons in the spinal cord. Thus, inhibition of N-type Ca^{2+} channels is expected to have anti-nociceptive effect. Indeed, studies have suggested that N-type calcium channel antagonists are mainly effective in reducing pain associated with inflammation and

tissue/nerve injury, although some effect has been shown in acute models of pain. Anti-nociceptive effects of L- and P/Q-type VDCC antagonists have also been reported; however, these effects appear to be moderate at best.

Pain can be essentially divided into 2 broad categories: physiological pain and pathological pain. Physiological pain is good for the organism in that it is protective. To prevent damage to tissue, physiological pain pathways are activated by noxious stimulation. Physiological pain must only be controlled under specific clinical situations, such as during surgery, medical procedures, or following trauma. Drugs that chronically disable pathways that transmit physiological pain are undesirable as they cause the organism to lose the protective function of pain. Pathological pain, on the other hand, is not the result of a noxious stimulation or healing tissue. Pathological pain originates from abnormal function of the nervous system due to nerve lesion or compression, neuropathy, tumor growth, or tissue inflammation. Current therapeutics that are used for the treatment of pathological pain are typically limited by serious side effects and the development of tolerance.

Pain researchers developed three classes of pain animal models: acute (physiological) pain model (hot plate, tail flick, paw pressure), inflammatory models (carrageenan and formalin), and nerve injury (sciatic nerve ligation, focal spinal injury) (Yaksh, 1999). A biphasic behavioural response is observed in the formalin model. The phase I of the response (1-10 min after injection) corresponds to acute afferent input resulting from the activation of primary afferent neurons. The phase II of the response (10 – 60 min) results from sensitization of spinal responses and considered to be an appropriate model for persistent pain (Yaksh, 1999).

Consistent with the role of N-type Ca^{2+} channels in pain pathway, pharmacological block of N-type Ca^{2+} channels by single injection or continuous infusion of synthetic ω -conopeptide SNX-111 inhibited phase II formalin response in rat animal model (Malmberg and Yaksh, 1994, 1995). The role of N-type Ca^{2+} channels in pain pathway was further supported by analysis of $\text{Ca}_v2.2$ knockout mice (Hatakeyama *et al.*, 2001; Kim *et al.*, 2001; Saegusa *et al.*, 2001). All 3 groups observed suppression of phase II formalin response in $\text{Ca}_v2.2$ -/- mice when compared to wild type mice.

These results pointed to N-type Ca^{2+} channels as potential drug target for a treatment of persistent pain. Based on this idea, Elan Pharmaceuticals (initially Neurex) developed a drug Ziconotide (SNX-111, a synthetic version of ω -conotoxin MVIIA). Very promising results were obtained with Ziconotide in clinical trials and currently FDA is considering

Ziconotide for approval. However, although Ziconotide is highly effective for treatment of chronic pain, there is also a number of problems associated with its use. Ziconotide (SNX-111) is a polypeptide with a complex chemical structure and very difficult to synthesize. Ziconotide does not pass blood-brain-barrier and has to be delivered by pump infusion directly into a spinal cord, greatly limiting scope of its applications. In addition, a number of severe side-effects were reported in some patients in response to Ziconotide (Penn and Paice, 2000).

It has been demonstrated that neuron-to-neuron contact is required for N-type Ca^{2+} channel clustering during synapse formation in rat hippocampal neuronal culture (Bahls *et al.*, 1998). More recently, synaptic targeting of an auxiliary P/Q-type Ca^{2+} channel subunit $\beta 4$ was investigated (Wittmann *et al.*, 2000). The present inventors have previously investigated targeting of recombinant N-type Ca^{2+} channels to synaptic locations in rat hippocampal neuronal cultures. It was found that in immature and in mature low-density hippocampal cultures, recombinant N-type Ca^{2+} channels are uniformly distributed in both axonal and somatodendritic compartments. In contrast, in mature high-density cultures, the recombinant N-type Ca^{2+} channels are clustered in presynaptic sites and primarily excluded from the somatodendritic domain. Synaptic clustering of recombinant N-type channels depended critically on the most C-terminal region of the “long” splice variant of the N-type Ca^{2+} channel pore-forming subunit $\text{CaV}2.2\text{a}$ (Williams *et al.*, 1992; Ertel *et al.*, 2000).

In another earlier study, the inventors identified postsynaptic density-95 (PSD-95)/discs large/zona occludens-1 (PDZ) and Src homology 3 (SH3) domain-binding motifs in the same region of the $\text{CaV}2.2$ subunit (Maximov *et al.*, 1999). The association of $\text{CaV}2.2\text{-NC1}$ C termini with the Mint1/CASK/veli-neurexin/neurologin complex (Maximov *et al.*, 1999) provides a possible molecular mechanism for N-type Ca^{2+} channel synaptic targeting during synaptogenesis, and the association of $\text{CaV}2.2\text{a-NC1}$ C terminal with Mint1-PDZ1 and CASK-SH3 domains (Maximov *et al.*, 1999) links synaptic N-type channels to neurexin–neurologin neuronal adhesion complex (Irie *et al.*, 1997; Nguyen and Sudhof, 1997; Butz *et al.*, 1998; Song *et al.*, 1999) and synaptic clustering of the channels and synaptic organization (Fanning and Anderson, 1996; Kornau *et al.*, 1997; Craven and Bredt, 1998). The importance of N-type channel association with Mint1 and neurexins is consistent with impaired presynaptic function in neurons from Mint1 knockout (Ho *et al.*, 2003) and α -neurexins (Missler *et al.*, 2003) knockout mice.

More recently, the inventors have shown that CaV2.2 C termini also bind to INADL-5, PAR6, and MUPP1-9 PDZ domains (Bezprozvanny and Maximov, 2001) and that the proline-rich region of the CaV2.2 C-terminus has been implicated recently in interactions with the SH3 domain of RBP (Hibino *et al.*, 2002). Subsequently, the inventors also demonstrated that these motifs act as synergistic synaptic targeting signals for N-type channels in rat hippocampal neurons (Maximov and Bezprozvanny, 2002). The inventors also demonstrated that introduction of CaV2.2 carboxy-terminal sequence into hippocampal neurons by transfection impairs their presynaptic function (Maximov & Bezprozvanny, 2002). However, there have yet to be reported attempts to specifically block these interactions and determine the ensuing biological consequences, particular with regard to pain.

SUMMARY OF THE INVENTION

Thus, in accordance with the present invention, there is provided an isolated and purified peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1), or conservative variants thereof. The peptide may comprises the sequence QDHWC (SEQ ID NO:2), DQDHWC (SEQ ID NO:3), PDQDHWC (SEQ ID NO:4), HPDQDHWC (SEQ ID NO:5), HHPDQDHWC (SEQ ID NO:6), YHHPDQDHWC (SEQ ID NO:7), SYHHPDQDHWC (SEQ ID NO:8) or HSYHHPDQDHWC (SEQ ID NO:9). The peptide may further comprise a permeant protein delivery motif, such as a TAT sequence or an R9 sequence. The peptide may be 40 residues in length, 30 residues in length, 20 residues in length, 15 residues in length, 12 residues in length, 10 residues in length, 8 residues in length, 7 residues in length, 6 residues in length, 5 residues in length, or 4 residues in length. The peptide may be further comprised within a pharmaceutically acceptable buffer, diluent or excipient, or within a lipid vehicle, such as a liposome.

In another embodiment, there is provided a nucleic acid encoding peptide of 4 to about 50 residues comprising the sequence DHWC or conservative variants thereof operably linked to a promoter. The promoter may be tissue specific or constitutive. Constitutive promoters include CMV IE, RSV, and SV40 large T. The nucleic acid may further comprise a polyadenylation signal. The nucleic acid may be located in a viral vector, such as a retrovirus, adenovirus, adeno-associated virus, vaccinia virus, herpesvirus or polyoma virus. The nucleic acid may also be comprised in a non-viral vector, such as a non-viral vector

comprised in a lipid vehicle, *e.g.*, a liposome. The nucleic acid may further encode a permeant protein delivery motif fused to SEQ ID NO:1.

In yet another embodiment, there is provided a method of inhibiting pain in an animal comprising administering to the animal a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1) or conservative variants thereof, the peptide dispersed in a pharmaceutically acceptable buffer, diluent or excipient. The peptide may be 40 residues in length, 20 residues in length, 12 residues in length, 8 residues in length or 4 residues in length. The peptide may further be comprised within a lipid vehicle, such as a liposome. The pain to be treated may be selected from the group consisting of neuropathic pain, inflammatory pain and pain secondary to cancer. The method may further comprise administering a second anti-pain agent to the animal, such as a steroid, an NTHE, or an opioid. The animal may be a human, a dog, a cat, a rat, a mouse, a horse, a cow or a rabbit.

In still yet another embodiment, there is provided a method of inhibiting pain in an animal comprising administering to the animal a nucleic acid encoding peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1) or conservative variants thereof operably linked to a promoter, the nucleic acid dispersed in a pharmaceutically acceptable buffer, diluent or excipient. The nucleic acid may be located in a viral vector, such as a retrovirus, adenovirus, adeno-associated virus, vaccinia virus, herpesvirus and polyoma virus. The nucleic acid may be comprised in a non-viral vector, such as in a lipid vehicle, *e.g.*, a liposome. The pain to be treated may be neuropathic pain, inflammatory pain or pain secondary to cancer. The method may further comprise administering a second anti-pain agent to the animal, such as a steroid, an NTHE, or an opioid.

In further embodiments, there are provided methods of screening for an anti-pain agents comprising:

- (a) providing a peptide of 4 to about 50 residues comprising the a peptide with one or more conservative variants of DHWC (SEQ ID NO:1);
- (b) contacting the peptide with an animal model of pain; and
- (c) determining the ability of the peptide to inhibit pain in the animal model,

or:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting the peptide and Mint1, Mint1-PDZ1 domain, Mint2, or Mint2-PDZ1 domain in the presence of a candidate substance; and

- (c) determining the ability of candidate substance to inhibit the peptide binding Mint1, Mint1-PDZ1 domain, Mint2, or Mint2-PDZ1 domain,

or:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting said peptide and Mint1-PDZ1/2 domains in the presence of a candidate substance; and
- (c) determining the ability of candidate substance to inhibit the peptide binding Mint1-PDZ1/2 domains,

or:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting said peptide and Mint2 in the presence of a candidate substance; and
- (c) determining the ability of candidate substance to inhibit the peptide binding Mint2,

or:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting said peptide and Mint2-PDZ1 domain in the presence of a candidate substance; and
- (c) determining the ability of candidate substance to inhibit the peptide binding Mint2-PDZ1 domain,

or:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting said peptide and Mint2-PDZ1/2 domains in the presence of a candidate substance; and
- (c) determining the ability of candidate substance to inhibit the peptide binding Mint2-PDZ1/2 domains.

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.”

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 DRAWINGS

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.

FIGS. 1A & 1B – Suppression of phase II, but not phase I formalin response in mouse injected with R9-NC peptide. FIG. 1A. Sequence of R9-NC peptide. FIG. 1B. Results of formalin assay in control mouse (n = 8) and mouse injected with R9-NC 60 min prior to formalin injection (n = 8). Phase I response (1-10 min from formalin injection) and phase II response (11-60 min from formalin injection) are shown.

FIG. 2 – Suppression of phase II, but not phase I formalin response in rats injected with R9-QDHC peptide. The effect of i.v. administration of PBS, R9 (1 mM) or R9-QDHC (1 mM) on phase I and phase II of the formalin assay. Phase I represents the period from 0-10 min following formalin injection and phase II represents the period from 11-60 min following formalin injection. N= 4-7 rats per group. *** indicates a significant difference when compared to PBS injection (p<0.01).

FIGS. 3A-D – Effects of R9-QDHC peptides on synaptic function. Double-staining protocol is shown on the top. Results of first and second destaining are shown for representative puncta for control neurons (FIG. 3A), for neurons loaded with R9

peptide (FIG. 3B) and for neurons loaded with R9-QDHC peptide (FIG. 3C). An average d2/d1 ratios are compared for all 3 groups of neurons (FIG. 3D).

FIG. 4 - Principle of HTRF-based HTS screen for blockers of bio-NC peptide association with GST-Mint1-PDZ1 domain.

FIG. 5 - HTRF data using bio-NC and GST-Mint1-PDZ1. Biotin is a control.

FIG. 6 - Principle of ALPHA-based HTS screen for blockers of bio-NC peptide association with GST-Mint1-PDZ1 domain.

FIG. 7 - ALPHA data using bio-NC and GST-Mint1-PDZ1. Biotin is a control.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

As stated above, the inventors reported previously (Maximov *et al.*, 1999) that there was specific association of the CaV2.2 C-terminal region with the first PDZ domain in the neuronal adaptor protein Mint1 and with the SH3 domain of the adaptor protein CASK. More recently, they have shown that CaV2.2 C termini also bind to INADL-5, PAR6, and MUPP1–9 PDZ domains (Bezprozvanny and Maximov, 2001). The proline-rich region of the CaV2.2 C terminal also has been implicated recently in interactions with the SH3 domain of RBP (Hibino *et al.*, 2002). Thus, a number of adaptor proteins appear to play a role in N-type Ca²⁺ channel synaptic targeting, with Mint1 and CASK being the best candidates for an important interacting role with N-type Ca²⁺ channel synaptic targeting.

In previous reports, the inventors speculated that the association of CaV2.2-NC1 C termini with the Mint1/CASK/veli-neurexin/neurologin complex (Maximov *et al.*, 1999) provided a possible molecular mechanism for N-type Ca²⁺ channel synaptic targeting during synaptogenesis (Maximov and Bezprozvanny, 2002). Here, the inventors now provide evidence that a discrete peptide, derived from the C-terminal region of CaV2.2, can in fact inhibit pain responses *in vivo*. The synthesis and use of such peptides in the treatment of pain is described in greater detail below.

1. CaV2.2 Peptides or Polypeptides

The present invention relates to the use of C-terminal peptides of CaV2.2. Accession nos. for human and rat CaV2.2 proteins are NM000718 (SEQ ID NO:11) and NM147141 (SEQ ID NO:13), respectively. CaV2.2 is the α 1B subunit for an N-type Ca²⁺ channel. It has been localized to the piriform cortex, hippocampus, hypothalamus, locus coeruleus, dorsal raphe, thalamic nuclei, and granular layer of the cortex. The human protein is 2339

residues, and the rat protein is 2333 residues. Each polypeptide concludes with an identical 12 residue sequence of HSYHHPDQDHC (SEQ ID NO:9), which is a subject of the present invention.

Documents relating to CaV2.2 include U.S. Patents 5,429,921, 5,792,846, 5,846,757, 5,851,824, 6,096,514, 6,140,485, 6,229,000, 6,353,091, 6,528,630 and 6,653,097, each of which are hereby incorporated by reference.

A. Structural Features

CaV2.2 peptides will comprise molecules of 4 to about 50 residues in length having the sequence DHWC. A particular preferred length may be less than 35 residues, less than 30 residues, less than 25 residues, less than 20 residues, less than 15 residues, or less than 13, including 4, 5, 6, 7, 8, 9, 10, 11 or 12 residues. The peptides may be generated synthetically or by recombinant techniques, and are purified according to known methods, such as precipitation (*e.g.*, ammonium sulfate), HPLC, ion exchange chromatography, affinity chromatography (including immunoaffinity chromatography) or various size separations (sedimentation, gel electrophoresis, gel filtration).

The peptides may be labeled using various molecules, such as fluorescent, chromogenic or colorimetric agents. The peptides may also be linked to other molecules, including other anti-pain agents. The links may be direct or through distinct linker molecules. The linker molecules in turn may be subject, *in vivo*, to cleavage, thereby releasing the agent from the peptide. Peptides may also be rendered multimeric by linking to larger, and possibly inert, carrier molecules.

B. Variants or Analogs of CaV2.2

i) Substitutional Variants

It also is contemplated in the present invention that variants or analogs of CaV2.2 peptides may also inhibit pain. Polypeptide sequence variants of CaV2.2, primarily making conservative amino acid substitutions to SEQ ID NO:1, may provide improved compositions. Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Substitutions of this kind preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in

the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

The following is a discussion based upon changing of the amino acids of a peptide to create an equivalent, or even an improved, second-generation molecule. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a peptide that defines that peptide's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and its underlying DNA coding sequence, and nevertheless obtain a peptide with like properties. It is thus contemplated by the inventors that various changes may be made in the DNA sequences coding the peptide without appreciable loss of their biological utility or activity, as discussed below.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant peptide, which in turn defines the interaction of the peptide with other molecules.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a peptide with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those

which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 $\pm$ 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent and immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Another embodiment for the preparation of polypeptides according to the invention is the use of peptide mimetics. Mimetics are peptide containing molecules that mimic elements of protein secondary structure (Johnson *et al*, 1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimetic is expected to permit molecular interactions similar to the natural molecule. These principles may be used, in conjunction with the principles outline above, to engineer second generation molecules having many of the natural properties of MBPs, but with altered and even improved characteristics.

ii) Altered Amino Acids

The present invention may employ peptides that comprise modified, non-natural and/or unusual amino acids. A table of exemplary, but not limiting, modified, non-natural and/or unusual amino acids is provided herein below. Chemical synthesis may be employed to incorporate such amino acids into the peptides of interest.

Table 1 – Modified, Non-Natural and Unusual Amino Acids

<u>Abbr.</u>	<u>Amino Acid</u>	<u>Abbr.</u>	<u>Amino Acid</u>
Aad	2-Aminoadipic acid	EtAsn	N-Ethylasparagine
BAad	3- Aminoadipic acid	Hyl	Hydroxylysine
BAla	beta-alanine, beta-Amino-propionic acid	AHyl	allo-Hydroxylysine
Abu	2-Aminobutyric acid	3Hyp	3-Hydroxyproline
4Abu	4- Aminobutyric acid, piperidinic acid	4Hyp	4-Hydroxyproline
Acp	6-Aminocaproic acid	Ide	Isodesmosine
Ahe	2-Aminoheptanoic acid	Aile	allo-Isoleucine
Aib	2-Aminoisobutyric acid	MeGly	N-Methylglycine, sarcosine
BAib	3-Aminoisobutyric acid	MeIle	N-Methylisoleucine
Apm	2-Aminopimelic acid	MeLys	6-N-Methyllysine
Dbu	2,4-Diaminobutyric acid	MeVal	N-Methylvaline
Des	Desmosine	Nva	Norvaline
Dpm	2,2'-Diaminopimelic acid	Nle	Norleucine
Dpr	2,3-Diaminopropionic acid	Orn	Ornithine
EtGly	N-Ethylglycine		

iii) Mimetics

In addition to the variants discussed above, the present inventors also contemplate that structurally similar compounds may be formulated to mimic the key portions of peptide or polypeptides of the present invention. Such compounds, which may be termed

peptidomimetics, may be used in the same manner as the peptides of the invention and, hence, also are functional equivalents.

Certain mimetics that mimic elements of protein secondary and tertiary structure are described in Johnson *et al.* (1993). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and/or antigen. A peptide mimetic is thus designed to permit molecular interactions similar to the natural molecule.

Some successful applications of the peptide mimetic concept have focused on mimetics of β -turns within proteins, which are known to be highly antigenic. Likely β -turn structure within a polypeptide can be predicted by computer-based algorithms, as discussed herein. Once the component amino acids of the turn are determined, mimetics can be constructed to achieve a similar spatial orientation of the essential elements of the amino acid side chains.

Other approaches have focused on the use of small, multidisulfide-containing proteins as attractive structural templates for producing biologically active conformations that mimic the binding sites of large proteins (Vita *et al.*, 1998). A structural motif that appears to be evolutionarily conserved in certain toxins is small (30-40 amino acids), stable, and high permissive for mutation. This motif is composed of a beta sheet and an alpha helix bridged in the interior core by three disulfides.

Beta II turns have been mimicked successfully using cyclic L-pentapeptides and those with D-amino acids (Weisshoff *et al.*, 1999). Also, Johannesson *et al.* (1999) report on bicyclic tripeptides with reverse turn inducing properties.

Methods for generating specific structures have been disclosed in the art. For example, alpha-helix mimetics are disclosed in U.S. Patents 5,446,128; 5,710,245; 5,840,833; and 5,859,184. These structures render the peptide or protein more thermally stable, also increase resistance to proteolytic degradation. Six, seven, eleven, twelve, thirteen and fourteen membered ring structures are disclosed.

Methods for generating conformationally restricted beta turns and beta bulges are described, for example, in U.S. Patents 5,440,013; 5,618,914; and 5,670,155. Beta-turns permit changed side substituents without having changes in corresponding backbone conformation, and have appropriate termini for incorporation into peptides by standard synthesis procedures. Other types of mimetic turns include reverse and gamma turns.

Reverse turn mimetics are disclosed in U.S. Patents 5,475,085 and 5,929,237, and gamma turn mimetics are described in U.S. Patents 5,672,681 and 5,674,976.

C. Fusion Proteins

Another variant is a fusion protein. This molecule generally has all or a substantial portion of the original molecule, in this case a peptide comprising the sequence DHWC (SEQ ID NO:1), linked at the N- or C-terminus, to all or a portion of a second peptide or polypeptide. For example, fusions may employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion includes the addition of an immunologically active domain, such as an antibody epitope, to facilitate purification of the fusion protein. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other useful fusions include linking of functional domains, such as active sites from enzymes, glycosylation domains, cellular targeting signals or transmembrane regions. Of particular interest are peptide permeant motifs that improve peptides transfer through membranes. Such motifs include those from TAT and R9:

TAT = RKKRRQRRR (Schwarze *et al.*, 2000; Becker-Hapak *et al.*, 2001; Denicourt and Dowdy, 2003)

R9 = RRRRRRRRRR (Wender *et al.*, 2000)

There also may be instances where a greater degree of intracellular specificity is desired. For example, with targeting nuclear proteins, RNA, DNA or cellular proteins or nucleic acids that are subsequently processed. Thus, one preferably uses localization sequences for such targets.

Localization sequences have been divided into routing signals, sorting signals, retention or salvage signals and membrane topology-stop transfer signals (Yellon *et al.*, 1992). For example, there are signals to target the endoplasmic reticulum (Munro, *et al.*, 1987), the nucleus (Lanford *et al.*, 1986; Stanton *et al.*, 1986; Harlow *et al.*, 1985), the nucleolar region (Kubota *et al.*, 1989; and Siomi *et al.*, 1988), the endosomal compartment (Bakke *et al.*, 1990), mitochondria (Yellon *et al.*, 1992) and liposomes (Letourneur *et al.*, 1992). One preferred nuclear targeting sequence may be the SV40 nuclear localization signal.

D. Purification of Proteins

It may be desirable to purify MBPs, variants, peptide-mimics or analogs thereof. Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. A particularly efficient method of purifying peptides is fast protein liquid chromatography or even HPLC.

Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded protein or peptide. The term "purified protein or peptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally-obtainable state. A purified protein or peptide therefore also refers to a protein or peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity, herein assessed by a "-fold purification number." The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

Various techniques suitable for use in protein purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

There is no general requirement that the protein or peptide always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater “-fold” purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

High Performance Liquid Chromatography (HPLC) is characterized by a very rapid separation with extraordinary resolution of peaks. This is achieved by the use of very fine particles and high pressure to maintain an adequate flow rate. Separation can be accomplished in a matter of minutes, or at most an hour. Moreover, only a very small volume of the sample is needed because the particles are so small and close-packed that the void volume is a very small fraction of the bed volume. Also, the concentration of the sample need not be very great because the bands are so narrow that there is very little dilution of the sample.

Gel chromatography, or molecular sieve chromatography, is a special type of partition chromatography that is based on molecular size. The theory behind gel chromatography is that the column, which is prepared with tiny particles of an inert substance that contain small pores, separates larger molecules from smaller molecules as they pass through or around the pores, depending on their size. As long as the material of which the particles are made does

not adsorb the molecules, the sole factor determining rate of flow is the size. Hence, molecules are eluted from the column in decreasing size, so long as the shape is relatively constant. Gel chromatography is unsurpassed for separating molecules of different size because separation is independent of all other factors such as pH, ionic strength, temperature, *etc.* There also is virtually no adsorption, less zone spreading and the elution volume is related in a simple matter to molecular weight.

Affinity Chromatography is a chromatographic procedure that relies on the specific affinity between a substance to be isolated and a molecule that it can specifically bind to. This is a receptor-ligand type interaction. The column material is synthesized by covalently coupling one of the binding partners to an insoluble matrix. The column material is then able to specifically adsorb the substance from the solution. Elution occurs by changing the conditions to those in which binding will not occur (alter pH, ionic strength, temperature, *etc.*).

A particular type of affinity chromatography useful in the purification of carbohydrate containing compounds is lectin affinity chromatography. Lectins are a class of substances that bind to a variety of polysaccharides and glycoproteins. Lectins are usually coupled to agarose by cyanogen bromide. Concanavalin A coupled to Sepharose was the first material of this sort to be used and has been widely used in the isolation of polysaccharides and glycoproteins other lectins that have been include lentil lectin, wheat germ agglutinin which has been useful in the purification of N-acetyl glucosaminyl residues and *Helix pomatia* lectin. Lectins themselves are purified using affinity chromatography with carbohydrate ligands. Lactose has been used to purify lectins from castor bean and peanuts; maltose has been useful in extracting lectins from lentils and jack bean; N-acetyl-D galactosamine is used for purifying lectins from soybean; N-acetyl glucosaminyl binds to lectins from wheat germ; D-galactosamine has been used in obtaining lectins from clams and L-fucose will bind to lectins from lotus.

The matrix should be a substance that itself does not adsorb molecules to any significant extent and that has a broad range of chemical, physical and thermal stability. The ligand should be coupled in such a way as to not affect its binding properties. The ligand should also provide relatively tight binding. And it should be possible to elute the substance without destroying the sample or the ligand. One of the most common forms of affinity chromatography is immunoaffinity chromatography. The generation of antibodies that would be suitable for use in accord with the present invention is discussed below.

E. Peptide Synthesis

CaV2.2-related peptides may be generated synthetically for use in various embodiments of the present invention. Because of their relatively small size, the peptides of the invention can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart & Young, (1984); Tam *et al.*, (1983); Merrifield, (1986); Barany and Merrifield (1979), each incorporated herein by reference. Short peptide sequences, or libraries of overlapping peptides, usually from about 6 up to about 35 to 50 amino acids, which correspond to the selected regions described herein, can be readily synthesized and then screened in screening assays designed to identify reactive peptides. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression.

2. CaV2.2 Nucleic Acids

Important aspects of the present invention concern isolated DNA segments and recombinant vectors encoding CaV2.2 and peptides thereof, the creation and use of recombinant host cells through the application of DNA technology, that express CaV2.2 or peptides thereof, and biologically functional equivalents thereof. Accession nos. for human and rat CaV2.2 DNA sequences are GI4502522 (SEQ ID NO:10) and GI25453409 (SEQ ID NO:12), respectively.

The present invention concerns DNA segments, isolatable from mammalian cells, such as mouse, rat or human cells, that are free from total genomic DNA and that encode a CaV2.2 polypeptide or peptide. As used herein, the term "DNA segment" refers to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding CaV2.2 refers to a DNA segment that contains wild-type, polymorphic or mutant CaV2.2 coding sequences yet is isolated away from, or purified free from, total mammalian genomic DNA. Included within the term "DNA segment" are DNA segments and also recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like.

A. CaV2.2 Splice Forms

Human (Williams *et al.*, 1992), chicken (Lu and Dunlap, 1999), and rat (Maximov and Bezprozvanny, 2002) CaV2.2 subunits undergo alternative splicing in the C-terminal region. The results suggest that in mature high density cultures, the long CaV2.2a-NC1 splice variant (CaV2.2a) is the axonal/presynaptic isoform, and the short CaV2.2a-NC2 splice variant (CaV2.2b) is the somatodendritic isoform (Maximov and Bezprozvanny, 2002). Similar to the CaV2.2 subunit, the P/Q-type channel pore-forming subunit CaV2.1 is alternatively spliced at the C termini (Zhuchenko *et al.*, 1997).

The long C-terminal splice variant of CaV2.1a, but not the short splice variants, contains a similar PDZ domain-binding motif (Maximov *et al.*, 1999). The inventors have previously suggested that the N-type and the P/Q-type Ca²⁺ channels are targeted to synapses via interactions with a similar or identical set of adaptor proteins (Maximov and Bezprozvanny, 2002). They also have suggested that an alternative splicing of the CaV2.2 and CaV2.1 subunit C termini provides a potential regulatory mechanism of N-type and P/Q-type Ca²⁺ channel sorting (Maximov and Bezprozvanny, 2002). In the case of P/Q-type Ca²⁺ channels, association of CaV2.1 C terminal with an auxiliary $\beta 4$ subunit (Walker *et al.*, 1998) may play an additional role in synaptic targeting (Wittmann *et al.*, 2000). It is also possible that truncation of SH3, PDZ, and $\beta 4$ binding motifs in the CaV2.1 subunit (Fletcher *et al.*, 1996) may lead to mistargeting of P/Q-type Ca²⁺ channels in *leaner* mice, resulting in severe neurological phenotype.

A recent report suggested the importance of alternative splicing in the CaV2.1 subunit II/III loop region for P/Q-type Ca²⁺ channel sorting between axonal and somatodendritic compartments of GABAergic cortical neurons (Timmermann *et al.*, 2002). Novel II/III splice variants of human CaV2.2 subunit that lack the soluble SNARE-binding synprint site were identified recently (Kaneko *et al.*, 2002). At the moment it is not clear whether alternative splicing of the CaV2.2 and CaV2.1 II/III loop and C-terminal regions are independent or correlated events, and future studies will be needed to answer this question. However, these data suggest that the alternative splicing-dependent sorting of Ca²⁺ channels in neurons may be a general phenomenon.

B. Variants

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode a CaV2.2, a peptide, peptide-

mimic or a biologically functional equivalent of a CaV2.2. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein. Accordingly, sequences that have about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%, and any range derivable therein, such as, for example, about 70% to about 80%, and more preferably about 81% and about 90%; or even more preferably, between about 91% and about 99%; of amino acids that are identical or functionally equivalent to the amino acids of SEQ ID NO: 1 or any analog or variant thereof provided the biological activity of the protein is maintained. In particular embodiments, the biological activity of a CaV2.2 C-terminal peptide, or a biologically functional equivalent, comprises binding to Mint1.

It will also be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein, polypeptide or peptide activity where an amino acid sequence expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, *i.e.*, introns, which are known to occur within genes.

3. Screening Assays

The present invention also contemplates the screening of compounds, *e.g.*, peptides, peptide-mimics, variants, analogs or small molecules, for various abilities to interact with Mint1 and/or affect pain signaling in an animal model of pain. Particularly preferred compounds will be those that mimic the function of the CaV2.2 C-terminal peptide disclosed herein. In the screening assays of the present invention, the candidate substance may first be screened for basic biochemical activity -- *e.g.*, binding to a target molecule (*e.g.*, Mint1) -- and then tested for its ability to inhibit pain at the whole animal level.

A. Modulators

The present invention provides methods of screening for agents that bind Mint1. In an embodiment, the present invention is directed to a method of:

- (a) providing a Mint1, Mint1-PDZ1, Mint2, or Mint2-PDZ1 polypeptide;
- (b) contacting the Mint1, Mint1-PDZ1, Mint2, or Mint2-PDZ1 polypeptide with a candidate substance; and
- (c) determining the binding of the candidate substance to the Mint1, Mint1-PDZ1, Mint2, or Mint2-PDZ1 polypeptide,

wherein binding to Mint1 identifies the compound as a putative anti-pain agent.

Measuring binding to Mint1 may be direct, by identifying a Mint1-candidate complex, by identifying labeled candidate associated with Mint1, or by assessing the inhibition of binding of a peptide comprising SEQ ID NO:1 to Mint1 by the candidate. In still yet other embodiments, one would look at the effect of a candidate on pain in an appropriate model.

As used herein, the term "candidate substance" refers to any molecule that may potentially modulate bind to Mint1 in a manner analogous to a peptide having the sequence of SEQ ID NO:1. The candidate substance may be a peptide, or a small molecule inhibitor, or even a nucleic acid molecule. It may prove to be the case that the most useful pharmacological compounds will be compounds that are structurally related to compounds which interact naturally with Mint1. Creating and examining the action of such molecules is known as "rational drug design," and include making predictions relating to the structure of target molecules.

The goal of rational drug design is to produce structural analogs of biologically active polypeptides or target compounds. By creating such analogs, it is possible to fashion drugs which are more active or stable than the natural molecules, which have different susceptibility to alteration or which may affect the function of various other molecules. In one approach, one would generate a three-dimensional structure for a molecule like Mint1, and then design a molecule for its ability to interact with Mint1. This could be accomplished by x-ray crystallography, computer modeling or by a combination of both approaches.

It also is possible to use antibodies to ascertain the structure of a target compound or inhibitor. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. An example of such an approach is to use a peptide of SEQ ID NO:1 as a model, and then make modifications that would improve the ability of the molecule to bind Mint1.

On the other hand, one may simply acquire, from various commercial sources, small molecule libraries that are believed to meet the basic criteria for useful drugs in an effort to "brute force" the identification of useful compounds. Screening of such libraries, including combinatorially generated libraries (e.g., peptide libraries), is a rapid and efficient way to screen large number of related (and unrelated) compounds for activity. Combinatorial approaches also lend themselves to rapid evolution of potential drugs by the creation of second, third and fourth generation compounds modeled of active, but otherwise undesirable compounds.

Candidate compounds may include fragments or parts of naturally-occurring compounds or may be found as active combinations of known compounds which are otherwise inactive. It is proposed that compounds isolated from natural sources, such as animals, bacteria, fungi, plant sources, including leaves and bark, and marine samples may be assayed as candidates for the presence of potentially useful pharmaceutical agents. It will be understood that the pharmaceutical agents to be screened could also be derived or synthesized from chemical compositions or man-made compounds. Thus, it is understood that the candidate substance identified by the present invention may be a polypeptide, polynucleotide, small molecule inhibitor or any other compounds that may be designed through rational drug design starting from known inhibitors of hypertrophic response.

It will, of course, be understood that all the screening methods of the present invention are useful in themselves notwithstanding the fact that effective candidates may not be found. The invention provides methods for screening for such candidates, not solely methods of finding them.

B. *In vitro* Assays

A quick, inexpensive and easy assay to run is a Mint1 binding assay. Binding of a molecule to Mint1 may, in and of itself, be inhibitory, due to steric, allosteric or charge-charge interactions. This can be performed in solution or on a solid phase and can be utilized as a first round screen to rapidly eliminate certain compounds before moving into more sophisticated screening assays.

The target (e.g., Mint1) may be either free in solution, fixed to a support, expressed in or on the surface of a cell. Either the target or the compound may be labeled, thereby permitting determination of binding. Competitive binding assays can be performed in which a peptide comprising SEQ ID NO:1 is used. The peptide may be labeled, or the candidate

may be labeled. One may measure the amount of free label versus bound label to determine binding or inhibition of binding.

A technique for high throughput screening of compounds is described in WO 84/03564. Large numbers of small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with, for example, Mint1 and washed. Bound polypeptide is detected by various methods.

To search for small molecule inhibitors of association between CaV2.2 carboxy-terminal and Mint1-PDZ1 domain, the inventors will utilize a variety of different approaches. First, they will synthesize biotinylated CaV2.2a N-type Ca^{2+} channels carboxy-terminal peptide and express Mint1-PDZ1 domain in bacteria as GST-fusion protein. Next, two methods are contemplated for use in high throughput screens – a scintillation-proximity assay (SPA) and a homogeneous time-resolved fluorescence resonance energy transfer assay (HTRF) (see Whitfield *et al.*, 2003). These methods will be used to screen an 8,000 compound subset selected from a compound library available at UT Southwestern HTS facility. SPA and HTRF pilot screens will be optimized to yield Z score (Zhang *et al.*, 1999) of at least 0.5 with the test library of 8,000 compounds. Identified compounds (hits) will be validated in formalin-induced pain model (Malmberg and Yaksh, 1995).

i) Z Score Determination

Pilot studies will provide the inventors with initial information if SPA and/or HTRF assays can be used to analyze association of bio-NC peptide and GST-Mint1-PDZ1 protein. Recently developed simple statistical parameter (Z score) can be used for evaluation of high throughput screens (Zhang *et al.*, 1999). According to Zhang *et al.* (1999), in order to validate a quality of proposed high throughput screens, one has to calculate a ratio of separation band to the signal dynamic range of the assay. Obtained parameter (Z factor) defines the capability of hit identification for each given high throughput screens at the defined screening conditions.

As a first step of high throughput screen optimization, Zhang *et al.* (1999) recommend to determine Z' factor that is calculated similar to Z factor using only control data. The Z' factor is a characteristic parameter for the quality of assay itself, without intervention of any test compounds. In order to determine Z' score, the inventors will measure SPA signal or HTRF signal for each well of 3 384 well plates with bio-NC peptide and GST-Mint1-PDZ1 protein. These measurements will be analyzed to yield μ_{C+} (mean positive signal) and σ_{C+}

(variability of positive signal). They will also measure SPA signal or HTRF signal for each well of 3 384 well plates with bio-NC-W2338A peptide and GST-Mint1-PDZ1 protein. As NC-W2338A mutant does not bind to Mint1-PDZ1, these measurements will provide us with μ_{C-} (mean negative signal) and σ_{C-} (variability of negative signal).

Once these values are obtained, the inventors will calculate Z' score using formula from (Zhang *et al.*, 1999):

$$Z' = 1 - (3\sigma_{C+} + 3\sigma_{C-})/|\mu_{C+} - \mu_{C-}| \quad (\text{eq 1})$$

If the inventors obtain $Z' > 0.5$ for SPA and/or HTRF assay, they will proceed with pilot screen using 8,000 test compound library. If $Z' < 0.5$ for either assay, the screen will be optimized to result in $Z' > 0.5$. The optimization steps for SPA assay include optimization of SPA beads concentration, replacement of PVT beads with Ysi beads, optimization of ^{35}S -Streptavidin concentration, optimization of bio-NC peptide and GST-Mint1-PDZ1 protein amounts, dilution of samples prior to SPA counting, changes in incubation time and temperature, change in agitation, *etc.* The optimization steps for HTRF assay include changes in EuK-anti-GST-mAb and Streptavidin-XL concentrations, changes in number of flashes per well and in HTRF integration time, optimization of bio-NC peptide and GST-Mint1-PDZ1 protein amounts, dilution of samples prior to HTRF counting, changes in incubation time and temperature, change in agitation, *etc.*

Once $Z' > 0.5$ condition is met, the inventors will fix the assay conditions and perform a pilot screen with the test library of 8,000 compounds (each at 5 μM concentration) and measure SPA signal and/or HTRF signal for each well containing different test compounds mixed with bio-NC peptide and GST-Mint1-PDZ1 protein. The test library of 8,000 compounds is available at UT Southwestern HTS facility. These measurements will be analyzed to yield μ_s (mean sample signal) and σ_s (variability of sample signal). As most of test compounds are not expected to disrupt bio-NC peptide association with GST-Mint1-PDZ1 domain, Z-score will be calculated using negative control (bio-NC-W2338A) data as follows (Zhang *et al.*, 1999):

$$Z = 1 - (3\sigma_s + 3\sigma_{C-})/|\mu_s - \mu_{C-}| \quad (\text{eq 2}).$$

Previous HTS screens at UT Southwestern HTS facility with the test library of 8,000 compounds (at 5 μ M concentration) resulted in a sample mean within 4% from DMSO control and it is most likely that the HTS screen with $Z' > 0.5$ will yield $Z > 0.5$. If $Z > 0.5$, the inventors can proceed with the complete screen. If $Z < 0.5$, the inventors will need to optimize the concentration of test compounds to yield $Z > 0.5$ but still a reasonable "hit rate" (Zhang *et al.*, 1999). The biological activity of the "hits" from the full screen will be tested in formalin-induced pain model (Malmberg and Yaksh, 1995).

ii) Scintillation Proximity Assay

In scintillation-proximity assay (SPA) studies, biotinylated NC peptide (bio-NC) and GST-Mint1-PDZ1 protein will be mixed in SPA buffer (0.2% BSA in PBS) in the presence of 5 μ M concentration of tested small molecule compounds in a white 384 well Optiplates (Packard) in a final volume of 25 μ l for 1 hour. SPA readout will be initiated by addition of Glutathione-PVT SPA beads (RPNQ0030 from Amersham) and ^{35} S-Streptavidin (Amersham SJ436). The plates will be sealed and incubated for 10 min with shaking. Following incubation, the plates will be spun for 5 min at 1,000 g to float PVT SPA beads and the luminescence signal from each well will be determined by CLIPR luminescence plate reader. Association of bio-NC peptide with GST-Mint1-PDZ1 will bring radiolabeled Streptavidin (bio-NC binding partner) in proximity of Glutathione-PVT SPA beads (GST-binding partner), resulting in strong luminescence signal. Control studies will be performed with bio-NC-W2338A peptide instead of bio-NC peptide. NC-W2338A does not bind Mint1-PDZ1 domain and bio-NC-W2338A/GST-Mint1-PDZ1 pair should not result in significant SPA luminescence signal. Results obtained with bio-NC/GST-Mint1-PDZ1 and bio-NC-W2338A/GST-Mint1-PDZ1 pairs will be used to optimize Z' score (Zhang *et al.*, 1999) of the screen. Following optimization of Z' score, pilot HTS screen with a library of 8,000 test compounds will be performed and Z score (Zhang *et al.*, 1999) will be optimized. Compounds that reduce luminescence signal by more than 50% will be selected for further evaluation.

iii) Homogenous Time-Resolved Fluorescence Resonance Energy Transfer

The homogeneous time-resolved fluorescence resonance energy transfer (HTRF) studies are based on the FRET energy transfer between caged donor fluorophore with a

delayed emission and a neighboring acceptor fluorophore. Excitation of a donor fluorophore by a Xenon flash lamp results in prompt fluorescence. Photons emitted during delayed emission stage are absorbed by an acceptor and result in HTRF signal. HTRF signal is collected during integration time that can be adjusted to yield maximal signal to noise ratio. To further improve signal to noise ratio, multiple flashes can be used to stimulate the same sample.

Bio-NC peptide and GST-Mint1-PDZ1 proteins will be mixed in HTRF buffer (0.2% BSA in PBS, 100 mM KF) in the presence of 5 μ M concentration of tested small molecule compounds in a black 384 well plates (Costar) in a final volume of 25 μ l for 1 hour. For donor labeling, Eu³⁺ cryptate (EuK) conjugated anti-GST monoclonal antibodies (CIS Bio International, 61GSTKLA) will be added. For acceptor labeling, Streptavidin-XL (Streptavidin conjugated to XL665, 610SAXLA from CIS Bio International) will be added to yield a final volume of 50 μ l. The plates will be sealed and incubated for 2 h with shaking in the dark.

Following incubation, HTRF measurements will be taken using LJJ Analyst. In these studies, brief Xenon lamp flash will be used for EuK excitation. Prompt EuK fluorescence at 620 nm will be measured for each well on the plate, and then delayed HTRF fluorescence of XL665 at 665 nm will be measured. Time-delayed fluorescence of XL665 will be measured with 50 μ s delay after the excitation and 400 μ s integration time. Due to 50 μ s delay, only the long-lived FRET signal is detected, drastically reducing fluorescence background. The HTRF ratio of A/B counts (665 nm/620 nm), determined for each well, will indicate a degree of bio-NC peptide association with GST-Mint1-PDZ1 protein.

Control studies will be performed with bio-NC-W2338A peptide instead of bio-NC peptide. Results obtained with bio-NC/GST-Mint1-PDZ1 and bio-NC-W2338A/GST-Mint1-PDZ1 pairs will be used to optimize Z' score (Zhang *et al.*, 1999) of the screen. Following optimization of Z' score, pilot HTS screen with a library of 8,000 test compounds will be performed and Z score (Zhang *et al.*, 1999) will be optimized. Compounds that reduce A/B HTRF ratio by more than 50% will be selected for further evaluation.

iv) ALPHASCREEN Assay

The AlphaScreen signal amplification strategy (Perkin Elmers) involves, as a first step, is the conversion of ambient oxygen to the singlet state by a photosensitizer in the Donor bead upon illumination at 680 nm (FIG. 6). The Acceptor beads contain a thioxene

derivative that reacts with the singlet oxygen to generate chemiluminescence at 370 nm. Energy transfer to fluorescent acceptors in the same beads shifts the emission wavelength to 520-620 nm. The half-life of the decay reaction is 0.3 sec, which makes the AlphaScreen fluorescence signal very long lived and allows the technology to operate in time-resolved mode. The short lifetime of singlet oxygen in aqueous solution ($\sim 4 \mu\text{sec}$) allows diffusion over a distance up to $\sim 200 \text{ nm}$ (FIG. 6). The ALPHA measurements can be taken using Perkin Elmers Envision

In one example, bio-NC peptide (63, 189, 1000 nM) and GST-Mint1-PDZ1/2 protein (63, 189, 1000 nM) were mixed in ALPHA buffer (Hepes 25mM pH 7.2; NaCl 100mM; BSA 0.1%) and incubated for 10 h. Anti-GST acceptor beads (Perkin Elmer) and Streptavidin donor beads (Perkin Elmer) were added. The reaction mixture was incubated for 60 more minutes. As a negative control (63, 189, 1000 nM) of Biotin was used in reaction instead of Bio-NC peptide. The reaction mixtures were aliquoted 50 μl per well in black 384 well plates (Costar) for ALPHA measurements. Measurement parameters include excitation at 680 nm, detection - 570 nm; excitation time - 180 ms; total measurement time - 550 ms.

C. *In cyto* Assays

Various cell that express Mint1, Mint1-PDZ1, Mint2, or Mint2-PDZ1 can be utilized for screening of candidate substances. Exemplary cells include, but are not limited to yeast cells, bacterial cells, COS cells, HEK293 cells. Depending on the assay, culture may be required. Labeled candidate substances or competitive inhibitors (a peptide of SEQ ID NO:1) is contacted with the cell and binding assessed. Various readouts for binding of candidate substances to cells may be utilized, including fluorescent microscopy and FACS.

D. *In vivo* Assays

The present invention particularly contemplates the use of various animal models. For example, various animal models of pain may be used to determine if the binding of candidate substances to Mint1 (Mint1-PDZ1, Mint2, Mint2-PDZ1) affects the ability of the animal to perceive pain in animal models of pain. Testing of acute (physiological) pain: (hot plate, tail flick, paw pressure), inflammatory models: (carrageenan, formalin), and nerve injury: (sciatic nerve ligation, focal spinal injury) and other models including muscle inflammation and cancer evoked bone pain.

Treatment of these animals with test compounds will involve the administration of the compound, in an appropriate form, to the animal. Administration will be by any route the could be utilized for clinical or non-clinical purposes, including but not limited to oral, nasal, buccal, or even topical. Alternatively, administration may be by oral, sublingual, intratracheal instillation, bronchial instillation, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Specifically contemplated are oral administration and systemic intravenous injection.

4. Engineering Expression Constructs

In certain embodiments, the present invention involves either the production of CaV2.2 peptides or the administration of a CaV2.2 nucleic acid to an animal. Such methods both rely upon expression constructs containing a CaV2.2 coding region and the means for its expression, plus elements that permit replication of the constructs. A variety of elements and vector types are discussed below.

A. Selectable Markers

In certain embodiments of the invention, expression constructs of the present invention contain nucleic acid constructs whose expression may be identified *in vitro* or *in vivo* by including a marker in the expression construct. Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression construct. Usually the inclusion of a drug selection marker aids in cloning and in the selection of transformants. For example, genes that confer resistance to neomycin, puromycin, hygromycin, DHFR, GPT, zeocin and histidinol are useful selectable markers. Alternatively, enzymes such as herpes simplex virus thymidine kinase (*tk*) may be employed. Immunologic markers also can be employed. The selectable marker employed is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid encoding a gene product. Further examples of selectable markers are well known to one of skill in the art and include reporters such as EGFP, β -gal or chloramphenicol acetyltransferase (CAT).

B. Polyadenylation Signals

One will typically desire to 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 such as human or bovine growth hormone and SV40 polyadenylation signals. Also contemplated as an element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

C. Control Regions

Throughout this application, the term "expression construct" is meant to include any type of genetic construct containing a nucleic acid coding for the peptide of interest. The nucleic acid encoding the peptide is under transcriptional control of a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrase "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation.

For the purpose of recombinant production, prokaryotic (bacteria) and lower eukaryotic organisms (yeast) are preferred. Commercial vectors and expression systems, including appropriate host cells and methods for transformation and culture, are well known to those of skill in the art.

In other embodiments, promoters refer to a group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II. Much of the thinking about how promoters are organized derives from analyses of several viral promoters, including those for the HSV thymidine kinase (*tk*) and SV40 early transcription units. These studies, augmented by more recent work, have shown that promoters are composed of discrete functional modules, each consisting of approximately 7-20 bp of DNA, and containing one or more recognition sites for transcriptional activator or repressor proteins.

At least one module in each promoter 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 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 recently been shown to contain functional elements downstream of the start site as well. 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.

The particular promoter employed to control the expression of a nucleic acid sequence of interest is not believed to be important, so long as it is capable of directing the expression of the nucleic acid in the targeted cell. Thus, where a human cell is targeted, it is preferable to position the nucleic acid coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally speaking, such a promoter might include either a human or viral promoter.

In various embodiments, the human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, the Rous sarcoma virus long terminal repeat, β -actin, rat insulin promoter and glyceraldehyde-3-phosphate dehydrogenase can be used to obtain high-level expression of the coding sequence of interest. The use of other viral or mammalian cellular or bacterial phage promoters which are well-known in the art to achieve expression of a coding sequence of interest is contemplated as well, provided that the levels of expression are sufficient for a given purpose. By employing a promoter with well-known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized.

Selection of a promoter that is regulated in response to specific physiologic or synthetic signals can permit inducible expression of the product. For example in the case where expression of a transgene, or transgenes when a multicistronic vector is utilized, is toxic to the cells in which the vector is produced in, it may be desirable to prohibit or reduce expression of one or more of the transgenes. Examples of transgenes that may be toxic to the producer cell line are pro-apoptotic and cytokine genes. Several inducible promoter systems are available for production of viral vectors where the transgene product may be toxic.

In some circumstances, it may be desirable to regulate expression of a transgene in a gene therapy vector. For example, different viral promoters with varying strengths of activity may be utilized depending on the level of expression desired. In mammalian cells, the CMV immediate early promoter is often used to provide strong transcriptional activation. Modified versions of the CMV promoter that are less potent have also been used when reduced levels of expression of the transgene are desired. When expression of a transgene in hematopoietic cells is desired, retroviral promoters such as the LTRs from MLV or MMTV are often used. Other viral promoters that may be used depending on the desired effect include SV40, RSV

LTR, HIV-1 and HIV-2 LTR, adenovirus promoters such as from the E1A, E2A, or MLP region, AAV LTR, cauliflower mosaic virus, HSV-TK, and avian sarcoma virus.

Similarly tissue specific promoters may be used to effect transcription in specific tissues or cells so as to reduce potential toxicity or undesirable effects to non-targeted tissues. For example, promoters that are selectively active in neuronal tissues, such as dorsal root ganglion (DRG) neurons, nociceptive neurons may find particular utility in accordance with the present invention.

It is envisioned that any of the above promoters alone or in combination with another may be useful according to the present invention depending on the action desired. In addition, this list of promoters should not be construed to be exhaustive or limiting, those of skill in the art will know of other promoters that may be used in conjunction with the promoters and methods disclosed herein.

Enhancers are genetic elements that increase transcription from a promoter located at a distant position on the same molecule of DNA. Enhancers are organized much like promoters. That is, they are composed of many individual elements, each of which binds to one or more transcriptional proteins. The basic distinction between enhancers and promoters is operational. An enhancer region as a whole must be able to stimulate transcription at a distance; this need not be true of a promoter region or its component elements. On the other hand, a promoter must have one or more elements that direct initiation of RNA synthesis at a particular site and in a particular orientation, whereas enhancers lack these specificities. Promoters and enhancers are often overlapping and contiguous, often seeming to have a very similar modular organization.

Below is a list of promoters additional to the tissue specific promoters listed above, cellular promoters/enhancers and inducible promoters/enhancers that could be used in combination with the nucleic acid encoding a gene of interest in an expression construct (Table 2 and Table 3). Additionally, any promoter/enhancer combination (as per the Eukaryotic Promoter Data Base EPDB) could also be used to drive expression of the gene. 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.

TABLE 2

PROMOTER

Immunoglobulin Heavy Chain

Immunoglobulin Light Chain

T-Cell Receptor

HLA DQ α and DQ β β -Interferon

Interleukin-2

Interleukin-2 Receptor

MHC Class II 5

MHC Class II HLA-DR α β -Actin

Muscle Creatine Kinase

Prealbumin (Transthyretin)

Elastase *I*

Metallothionein

Collagenase

Albumin Gene

 α -Fetoprotein τ -Globin β -Globin

e-fos

c-HA-ras

Insulin

Neural Cell Adhesion Molecule (NCAM)

 α 1-Antitrypsin

PROMOTER

H2B (TH2B) Histone

Mouse or Type I Collagen

Glucose-Regulated Proteins (GRP94 and GRP78)

Rat Growth Hormone

Human Serum Amyloid A (SAA)

Troponin I (TN I)

Platelet-Derived Growth Factor

Duchenne Muscular Dystrophy

SV40

Polyoma

Retroviruses

Papilloma Virus

Hepatitis B Virus

Human Immunodeficiency Virus

Cytomegalovirus

Gibbon Ape Leukemia Virus

TABLE 3

Element	Inducer
MT II	Phorbol Ester (TPA) Heavy metals
MMTV (mouse mammary tumor virus)	Glucocorticoids
β -Interferon	Poly(rI)X Poly(rc)
Adenovirus 5 E2	Ela
c-jun	Phorbol Ester (TPA), H ₂ O ₂
Collagenase	Phorbol Ester (TPA)
Stromelysin	Phorbol Ester (TPA), IL-1
SV40	Phorbol Ester (TPA)
Murine MX Gene	Interferon, Newcastle Disease Virus
GRP78 Gene	A23187
α -2-Macroglobulin	IL-6
Vimentin	Serum
MHC Class I Gene H-2kB	Interferon
HSP70	Ela, SV40 Large T Antigen
Proliferin	Phorbol Ester-TPA
Tumor Necrosis Factor	FMA
Thyroid Stimulating Hormone α Gene	Thyroid Hormone
Insulin E Box	Glucose

5. Methods of Gene Transfer

In order to effect recombinant express of CaV2.2 peptide, it is necessary to transfer the appropriate expression construct into a host cell of interest. Such transfer may employ viral or non-viral methods of gene transfer. This section provides a discussion of methods and compositions of gene transfer.

A. Viral Vector-Mediated Transfer

In one embodiment of the invention, an expression construct comprises a virus or engineered construct derived from a viral genome. The ability of certain viruses to enter cells

via receptor-mediated endocytosis and to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign genes into mammalian cells (Ridgeway, 1988; Nicolas and Rubenstein, 1988; Baichwal and Sugden, 1986; Temin, 1986). The first viruses used as gene vectors were DNA viruses such as the papovaviruses (simian virus 40, bovine papilloma virus, and polyoma) (Ridgeway, 1988; Baichwal and Sugden, 1986). These have a relatively low capacity for foreign DNA sequences and have a restricted host spectrum. Furthermore, their oncogenic potential and cytopathic effects in permissive cells raise safety concerns. A wide variety of viruses are now used to successfully transfer genetic material to eukaryotic cells.

In certain embodiments, the nucleic acid sequence is incorporated into a viral particle to mediate gene transfer to a cell. Typically, the virus simply will be exposed to the appropriate host cell under physiologic conditions, permitting uptake of the virus. The present methods may be advantageously employed using a variety of viral vectors, as discussed below.

i) Adenovirus

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized DNA genome, ease of manipulation, high titer, wide target-cell range, and high infectivity. The roughly 36 kB viral genome is bounded by 100-200 base pair (bp) inverted terminal repeats (ITR), in which are contained *cis*-acting elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome that contain different transcription units are divided by the onset of viral DNA replication.

The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression, and host cell shut off (Renan, 1990). The products of the late genes (L1, L2, L3, L4 and L5), including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP (located at 16.8 map units) is particularly efficient during the late phase of infection, and all the mRNAs issued from this promoter possess a 5' tripartite leader (TL) sequence which makes them preferred mRNAs for translation.

In order for adenovirus to be optimized for gene therapy, it is necessary to maximize the carrying capacity so that large segments of DNA can be included. It also is very desirable to reduce the toxicity and immunologic reaction associated with certain adenoviral products. The two goals are, to an extent, coterminous in that elimination of adenoviral genes serves both ends. By practice of the present invention, it is possible achieve both these goals while retaining the ability to manipulate the therapeutic constructs with relative ease.

The large displacement of DNA is possible because the *cis* elements required for viral DNA replication all are localized in the inverted terminal repeats (ITR) (100-200 bp) at either end of the linear viral genome. Plasmids containing ITR's can replicate in the presence of a non-defective adenovirus (Hay *et al.*, 1984). Therefore, inclusion of these elements in an adenoviral vector should permit replication.

In addition, the packaging signal for viral encapsidation is localized between 194-385 bp (0.5-1.1 map units) at the left end of the viral genome (Hearing *et al.*, 1987). This signal mimics the protein recognition site in bacteriophage λ DNA where a specific sequence close to the left end, but outside the cohesive end sequence, mediates the binding to proteins that are required for insertion of the DNA into the head structure. E1 substitution vectors of Ad have demonstrated that a 450 bp (0-1.25 map units) fragment at the left end of the viral genome could direct packaging in 293 cells (Levrero *et al.*, 1991).

Previously, it has been shown that certain regions of the adenoviral genome can be incorporated into the genome of mammalian cells and the genes encoded thereby expressed. These cell lines are capable of supporting the replication of an adenoviral vector that is deficient in the adenoviral function encoded by the cell line. There also have been reports of complementation of replication deficient adenoviral vectors by "helping" vectors, *e.g.*, wild-type virus or conditionally defective mutants.

Replication-deficient adenoviral vectors can be complemented, in *trans*, by helper virus. This observation alone does not permit isolation of the replication-deficient vectors, however, since the presence of helper virus, needed to provide replicative functions, would contaminate any preparation. Thus, an additional element was needed that would add specificity to the replication and/or packaging of the replication-deficient vector. That element, as provided for in the present invention, derives from the packaging function of adenovirus.

It has been shown that a packaging signal for adenovirus exists in the left end of the conventional adenovirus map (Tibbetts, 1977). Later studies showed that a mutant with a

deletion in the E1A (194-358 bp) region of the genome grew poorly even in a cell line that complemented the early (E1A) function (Hearing and Shenk, 1983). When a compensating adenoviral DNA (0-353 bp) was recombined into the right end of the mutant, the virus was packaged normally. Further mutational analysis identified a short, repeated, position-dependent element in the left end of the Ad5 genome. One copy of the repeat was found to be sufficient for efficient packaging if present at either end of the genome, but not when moved towards the interior of the Ad5 DNA molecule (Hearing *et al.*, 1987).

By using mutated versions of the packaging signal, it is possible to create helper viruses that are packaged with varying efficiencies. Typically, the mutations are point mutations or deletions. When helper viruses with low efficiency packaging are grown in helper cells, the virus is packaged, albeit at reduced rates compared to wild-type virus, thereby permitting propagation of the helper. When these helper viruses are grown in cells along with virus that contains wild-type packaging signals, however, the wild-type packaging signals are recognized preferentially over the mutated versions. Given a limiting amount of packaging factor, the virus containing the wild-type signals are packaged selectively when compared to the helpers. If the preference is great enough, stocks approaching homogeneity should be achieved.

ii) Retrovirus

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes - gag, pol and env - that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene, termed Ψ , functions as a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5' and 3' ends of the viral genome. These contain strong promoter and enhancer sequences and also are required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding a promoter is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag,

pol and env genes but without the LTR and Ψ components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a human cDNA, together with the retroviral LTR and Ψ sequences is introduced into this cell line (by calcium phosphate precipitation for example), the Ψ sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression of many types of retroviruses require the division of host cells (Paskind *et al.*, 1975).

An approach designed to allow specific targeting of retrovirus vectors recently was developed based on the chemical modification of a retrovirus by the chemical addition of galactose residues to the viral envelope. This modification could permit the specific infection of cells such as hepatocytes *via* asialoglycoprotein receptors, should this be desired.

A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, the infection of a variety of human cells that bore those surface antigens was demonstrated with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

iii) Adeno-associated Virus

AAV utilizes a linear, single-stranded DNA of about 4700 base pairs. Inverted terminal repeats flank the genome. Two genes are present within the genome, giving rise to a number of distinct gene products. The first, the *cap* gene, produces three different virion proteins (VP), designated VP-1, VP-2 and VP-3. The second, the *rep* gene, encodes four non-structural proteins (NS). One or more of these *rep* gene products is responsible for transactivating AAV transcription.

The three promoters in AAV are designated by their location, in map units, in the genome. These are, from left to right, p5, p19 and p40. Transcription gives rise to six transcripts, two initiated at each of three promoters, with one of each pair being spliced. The splice site, derived from map units 42-46, is the same for each transcript. The four non-

structural proteins apparently are derived from the longer of the transcripts, and three virion proteins all arise from the smallest transcript.

AAV is not associated with any pathologic state in humans. Interestingly, for efficient replication, AAV requires "helping" functions from viruses such as herpes simplex virus I and II, cytomegalovirus, pseudorabies virus and, of course, adenovirus. The best characterized of the helpers is adenovirus, and many "early" functions for this virus have been shown to assist with AAV replication. Low level expression of AAV *rep* proteins is believed to hold AAV structural expression in check, and helper virus infection is thought to remove this block.

The terminal repeats of the AAV vector can be obtained by restriction endonuclease digestion of AAV or a plasmid such as p201, which contains a modified AAV genome (Samulski *et al.*, 1987), or by other methods known to the skilled artisan, including but not limited to chemical or enzymatic synthesis of the terminal repeats based upon the published sequence of AAV. The ordinarily skilled artisan can determine, by well-known methods such as deletion analysis, the minimum sequence or part of the AAV ITRs which is required to allow function, *i.e.*, stable and site-specific integration. The ordinarily skilled artisan also can determine which minor modifications of the sequence can be tolerated while maintaining the ability of the terminal repeats to direct stable, site-specific integration.

AAV-based vectors have proven to be safe and effective vehicles for gene delivery *in vitro*, and these vectors are being developed and tested in pre-clinical and clinical stages for a wide range of applications in potential gene therapy, both *ex vivo* and *in vivo* (Carter and Flotte, 1996 ; Chatterjee *et al.*, 1995; Ferrari *et al.*, 1996; Fisher *et al.*, 1996; Flotte *et al.*, 1993; Goodman *et al.*, 1994; Kaplitt *et al.*, 1994; 1996, Kessler *et al.*, 1996; Koeberl *et al.*, 1997; Mizukami *et al.*, 1996).

AAV-mediated efficient gene transfer and expression in the lung has led to clinical trials for the treatment of cystic fibrosis (Flotte and Carter, 1995; Flotte *et al.*, 1993). Similarly, the prospects for treatment of muscular dystrophy by AAV-mediated gene delivery of the dystrophin gene to skeletal muscle, of Parkinson's disease by tyrosine hydroxylase gene delivery to the brain, of hemophilia B by Factor IX gene delivery to the liver, and potentially of myocardial infarction by vascular endothelial growth factor gene to the heart, appear promising since AAV-mediated transgene expression in these organs has recently been shown to be highly efficient (Fisher *et al.*, 1996; Flotte *et al.*, 1993; Kaplitt *et al.*, 1994; 1996; Koeberl *et al.*, 1997; McCown *et al.*, 1996; Ping *et al.*, 1996; Xiao *et al.*, 1996).

iv) Other Viral Vectors

Other viral vectors may be employed as expression constructs in the present invention. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar *et al.*, 1988) canary pox virus, and herpes viruses may be employed. These viruses offer several features for use in gene transfer into various mammalian cells.

B. Non-viral Transfer

Several non-viral methods for the transfer of expression constructs into cells are contemplated by the present invention. These include calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe *et al.*, 1990) DEAE-dextran (Gopal, 1985), electroporation (Tur-Kaspa *et al.*, 1986; Potter *et al.*, 1984), direct microinjection (Harland and Weintraub, 1985), DNA-loaded liposomes (Nicolau and Sene, 1982; Fraley *et al.*, 1979), cell sonication (Fechheimer *et al.*, 1987), gene bombardment using high velocity microprojectiles (Yang *et al.*, 1990), and receptor-mediated transfection (Wu and Wu, 1987; Wu and Wu, 1988).

Once the construct has been delivered into the cell the nucleic acid encoding the therapeutic gene may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the therapeutic gene may be stably integrated into the genome of the cell. This integration may be in the cognate location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In a particular embodiment of the invention, the expression construct may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar Liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved

solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). The addition of DNA to cationic liposomes causes a topological transition from liposomes to optically birefringent liquid-crystalline condensed globules (Radler *et al.*, 1997). These DNA-lipid complexes are potential non-viral vectors for use in gene therapy.

Liposome-mediated nucleic acid delivery and expression of foreign DNA *in vitro* has been very successful. Using the β -lactamase gene, Wong *et al.*, (1980) demonstrated the feasibility of liposome-mediated delivery and expression of foreign DNA in cultured chick embryo, HeLa, and hepatoma cells. Nicolau *et al.*, (1987) accomplished successful liposome-mediated gene transfer in rats after intravenous injection. Also included are various commercial approaches involving "lipofection" technology.

In certain embodiments of the invention, the liposome may be complexed with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda *et al.*, 1989). In other embodiments, the liposome may be complexed or employed in conjunction with nuclear nonhistone chromosomal proteins (HMG-1) (Kato *et al.*, 1991). In yet further embodiments, the liposome may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression constructs have been successfully employed in transfer and expression of nucleic acid *in vitro* and *in vivo*, then they are applicable for the present invention.

Other vector delivery systems which can be employed to deliver a nucleic acid encoding a therapeutic gene into cells are receptor-mediated delivery vehicles. These take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific (Wu and Wu, 1993).

Receptor-mediated gene targeting vehicles generally consist of two components: a cell receptor-specific ligand and a DNA-binding agent. Several ligands have been used for receptor-mediated gene transfer. The most extensively characterized ligands are asialoorosomucoid (ASOR) (Wu and Wu, 1987) and transferrin (Wagner *et al.*, 1990). Recently, a synthetic neoglycoprotein, which recognizes the same receptor as ASOR, has been used as a gene delivery vehicle (Ferkol *et al.*, 1993; Perales *et al.*, 1994) and epidermal growth factor (EGF) has also been used to deliver genes to squamous carcinoma cells (Myers, EPO 0273085).

In other embodiments, the delivery vehicle may comprise a ligand and a liposome. For example, Nicolau *et al.*, (1987) employed lactosyl-ceramide, a galactose-terminal asialganglioside, incorporated into liposomes and observed an increase in the uptake of the insulin gene by hepatocytes. Thus, it is feasible that a nucleic acid encoding a therapeutic gene also may be specifically delivered into a cell type such as prostate, epithelial or tumor cells, by any number of receptor-ligand systems with or without liposomes. For example, the human prostate-specific antigen (Watt *et al.*, 1986) may be used as the receptor for mediated delivery of a nucleic acid in prostate tissue.

In another embodiment of the invention, the expression construct may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is applicable particularly for transfer *in vitro*, however, it may be applied for *in vivo* use as well. Dubensky *et al.*, (1984) successfully injected polyomavirus DNA in the form of CaPO_4 precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Neshif (1986) also demonstrated that direct intraperitoneal injection of CaPO_4 precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a CAM also may be transferred in a similar manner *in vivo* and express CAM.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

6. Methods of Treating Pain

The present invention also contemplates method of inhibiting pain using peptides or mimetics of the carboxy-terminus of CaV2.2. Binding of such agents to Mint1 has now been shown to reduce pain *in vivo*. Thus, it is contemplated that the administration of CaV2.2 or mimetics into subjects will reduce or even prevent pain.

As discussed above, pain can be essentially divided into 2 broad categories: physiological pain and pathological pain. Physiological pain is good for the organism in that

it is protective. To prevent damage to tissue, physiological pain pathways are activated by noxious stimulation. Physiological pain must only be controlled under specific clinical situations, such as during surgery, medical procedures, or following trauma. Drugs that chronically disable pathways that transmit physiological pain are undesirable as they cause the organism to lose the protective function of pain. Pathological pain, on the other hand, is not the result of a noxious stimulation or healing tissue. Pathological pain originates from abnormal function of the nervous system due to nerve lesion or compression, neuropathy, tumor growth, or tissue inflammation. Current therapeutics that are used for the treatment of pathological pain are typically limited by serious side effects and the development of tolerance.

A. Physiological Pain

The sensory experience of physiological (acute) pain caused by a noxious stimulus is mediated by a specialized high-threshold sensory system. This system extends from the periphery through the spinal cord, brain stem and thalamus to the cerebral cortex where the sensation is perceived. A withdrawal response is initiated to prevent tissue damage. Physiological pain is a vital sensation and is associated with survival of the organism.

B. Inflammatory Pain

If tissue damage occurs in spite of the protection rendered by the physiological pain system (*i.e.*, via trauma, surgery, or inflammatory disease), the body shifts from protecting against noxious stimulation to promoting the healing of injured tissue. Inflammatory pain helps to achieve this goal by increasing sensitivity to stimuli that are not normally painful (hyperalgesia). By having a heightened perception of pain, the organism limits movement and enables healing. When the inflammation is reduced, pain dissipates. In the case of chronic inflammation (*e.g.*, rheumatoid arthritis), however, pain states inflammatory pain persists. Cancer pain can sometimes fit into this pain category as some tumors will release inflammatory mediators that serve to sensitive nerves in the surrounding tissue.

C. Neuropathic Pain

Neuropathic pain may result from lesions to the peripheral nervous system, as in patients with diabetes, post-herpetic neuralgias, AIDS, or in patients with spinal cord injuries. Cancer pain can fit into the category of neuropathic pain if tumor growth creates nerve impingements.

D. Genetic Based Therapies

Specifically, the present inventors intend to provide, to a cell, an expression construct that expresses a CaV2.2 peptide or variant thereof. The lengthy discussion of expression vectors and the genetic elements employed therein is incorporated into this section by reference. Particularly preferred expression vectors are viral vectors such as adenovirus, adeno-associated virus, herpesvirus, vaccinia virus and retrovirus. Also preferred is liposomally-encapsulated expression vector.

Those of skill in the art are well aware of how to apply gene delivery to *in vivo* and *ex vivo* situations. For viral vectors, one generally will prepare a viral vector stock. Depending on the kind of virus and the titer attainable, one will deliver 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} or 1×10^{12} infectious particles to the patient. Similar figures may be extrapolated for liposomal or other non-viral formulations by comparing relative uptake efficiencies. Formulation as a pharmaceutically acceptable composition is discussed below.

E. Protein Therapy

Another therapy approach is the provision, to a subject, of CaV2.2 peptides, synthetic or recombinant, or variants, mimetics or analogs thereof. Formulations would be selected based on the route of administration and purpose including, but not limited to, parenteral formulations, topical formulations, liposomal formulations and classic pharmaceutical preparations for oral administration.

F. Combined Therapy

In order to increase the effectiveness of CaV2.2 peptides or mimics or analogs thereof, it may be desirable to combine these compositions with another agent effective in the treatment of pain. The terms "contacted" and "exposed," when applied to a cell, tissue or organism, are used herein to describe the process by which a CaV2.2 peptide or mimic or analog and/or another anti-pain agent are delivered to a target cell, tissue or organism or are placed in direct juxtaposition with the target cell, tissue or organism. Anti-pain agents include, but are not limited to, steroids, NSAIDS (COX-2 inhibitors, salicylates, indoleacetic acid derivatives, fenamates, benzothiazine derivatives, pyrrolacetic acids), and analgesics & opioids (lidocaine, morphine, fentanyl, midazolam, propofol, lorazepam, haloperidol, thiopental, pentobarbital, diazepam).

The CaV2.2 peptide or mimic or analog may precede, be co-current with and/or follow the other agent(s) by intervals ranging from minutes to weeks. In embodiments where the CaV2.2 peptide or mimic or analog, and other agent(s) are applied separately to a cell, tissue or organism, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the peptide and agent(s) would still be able to exert an advantageously combined effect on the cell, tissue or organism. For example, in such instances, it is contemplated that one may contact the cell, tissue or organism with two, three, four or more modalities substantially simultaneously (*i.e.*, within less than about a minute) as the CaV2.2 peptide or mimic or analog. In other aspects, one or more agents may be administered within of from substantially simultaneously, about 1 minute, about 5 minutes, about 10 minutes, about 20 minutes about 30 minutes, about 45 minutes, about 60 minutes, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 18 hours, about 24 hours, about 36 hours, about 48 hours, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 21 days, about 4 weeks, about 5 weeks, about 6 weeks, about 7 week or about 8 weeks or more, and any range derivable therein, prior to and/or after administering the CaV2.2 peptide or mimic or analog.

Various combination regimens of the CaV2.2 treatment and one or more other anti-pain agents may be employed. Non-limiting examples of such combinations are shown below, wherein a CaV2.2 composition is "A" and the other anti-pain agent is "B":

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A		
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A		

Administration of the CaV2.2 composition to a cell, tissue or organism may follow general protocols for the administration of pharmaceuticals, taking into account the toxicity, if any. It is expected that the treatment cycles would be repeated as necessary. In particular embodiments, it is contemplated that various additional agents may be applied in any combination with the present invention.

G. Pharmaceutical Formulations

Pharmaceutical formulations of the present invention comprise an effective amount of a CaV2.2 agent dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refer to compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of such pharmaceutical compositions are known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art. Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The pharmaceuticals of the present invention may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present invention can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, , intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, inhalation (*e.g.*, aerosol), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (*e.g.*, liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art.

The actual dosage amount of a composition of the present invention administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent

therapeutic interventions, idiopathy of the patient and on the route of administration. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, *etc.*, can be administered, based on the numbers described above.

In any case, the composition may comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

The pharmaceuticals may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine.

In embodiments where the composition is in a liquid form, a carrier can be a solvent or dispersion medium comprising but not limited to, water, ethanol, polyol (*e.g.*, glycerol,

propylene glycol, liquid polyethylene glycol, *etc.*), lipids (*e.g.*, triglycerides, vegetable oils, liposomes) and combinations thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin; by the maintenance of the required particle size by dispersion in carriers such as, for example liquid polyol or lipids; by the use of surfactants such as, for example hydroxypropylcellulose; or combinations thereof such methods. In many cases, it will be preferable to include isotonic agents, such as, for example, sugars, sodium chloride or combinations thereof.

In certain embodiments, the compositions are prepared for administration by such routes as oral ingestion. In these embodiments, the solid composition may comprise, for example, solutions, suspensions, emulsions, tablets, pills, capsules (*e.g.*, hard or soft shelled gelatin capsules), sustained release formulations, buccal compositions, troches, elixirs, suspensions, syrups, wafers, or combinations thereof. Oral compositions may be incorporated directly with the food of the diet. Preferred carriers for oral administration comprise inert diluents, assimilable edible carriers or combinations thereof. In other aspects of the invention, the oral composition may be prepared as a syrup or elixir. A syrup or elixir, and may comprise, for example, at least one active agent, a sweetening agent, a preservative, a flavoring agent, a dye, a preservative, or combinations thereof.

In certain preferred embodiments an oral composition may comprise one or more binders, excipients, disintegration agents, lubricants, flavoring agents, and combinations thereof. In certain embodiments, a composition may comprise one or more of the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, *etc.*; or combinations thereof the foregoing. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated

above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The preparation of highly concentrated compositions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein.

In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

7. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Materials and Methods

First, animals are injected with a 10 μ M (20 μ l) solution of the test compound (dissolved in PBS) in the dorsal surface of the hindpaw. One hour following the injection of the test compound, animals receive a formalin injection (20 μ l of a 5% solution) into the

dorsal surface of the hindpaw. The contralateral paw is not injected. Following injection, animals are immediately transferred to a plexiglass observation cage. Each animal is observed for a total of 60 minutes following formalin injection.. The period spent biting, scratching, or licking the injected hindpaw is recorded (pain score). Data is presented as cumulative time spent biting, scratching, and licking in phase I (0-10 min) and phase II (11-60 min).

Example 2 – Results

One hour following delivery of either saline (control) or R9-NC into the dorsum of the hindpaw, formalin (20 μ l of a 5% solution) was injected into the same hindpaw. No effect of R9-NC on phase I of the formalin assay was observed. (FIG. 1B) In contrast, a significant reduction of phase II of the formalin assay in the R9-NC treated group was observed when compared to the control group. (FIG. 1B) These data indicate that R9-NC does not alter physiological pain (phase I), but it does block pain that is produced by central sensitization (phase II). Additionally, no untoward side effect of the R9-NC was observed when injected in the hindpaw.

As is shown in FIG. 2, the peptide R9-QDHCW (full sequence RRRRRRRRRQDHCW; SEQ ID NO__) also inhibits phase II but not phase I responses in the rat formalin model. Male Sprague Dawley rats (175-225 gm) received a tail vein injection of either PBS, R9 (1 mM), or R9-QDHCW (1 mM) following which they were placed in a plexiglass observation chamber to allow for acclimation. Thirty minutes following the tail vein injection, the dorsal surface of one hind paw was injected with 50 μ l of a 5% formalin solution. Observers, blind to drug treatment, scored the number of biting, licking, and scratching behaviors of the injected hindlimb and paw. Data were analyzed with SigmaStat Software (SPSS, Inc. v. 2.03) using ANOVA followed by a Tukey post hoc analysis.

No significant differences between any group were observed during phase I of the formalin assay [$F_{(2,16)} = 0.691$ $p > 0.5$]. In contrast, there was a significant reduction in formalin induced phase II behaviors in the R9-QDHCW treated animals when compared to the PBS treated rats [$F_{(2,17)} = 4.815$ $p = 0.007$]. PBS vs R9 for phase II was not significantly different (n.s) and $p = 0.744$. Further, delivery of the peptide to rats had no apparent adverse effects.

An FM1-43 imaging was used to study synaptic effects of R9-QDHWC competitive peptide that corresponds to Mint-PDZ binding site on $\text{Ca}_v2.2$ subunit. In these experiments we adapted double-staining protocol from (Reuter, 1995). First the mature hippocampal neuronal cultures were stained with FM1-43 dye for 90 sec in the presence of 47 mM KCl (FIG. 3, top). Following staining and washout of the dye (10 min) the neurons were stimulated by 90 mM KCl and the initial rate of destaining (d1) was measured at each puncta (FIG. 3, top). After completion of the first destaining protocol, the same neuronal culture was re-stained with FM1-43 for 90 sec in the presence of 47 mM KCl. Following re-staining and washout of the dye (10 min), neurons were incubated with 50 μM of R9-QDHWC or R9 peptides for 15 min. The control group of neurons was incubated for 15 min with addition of PBS. Following loading with R9 peptides, neurons were washed for 10 min and subjected to 90 mM KCl stimulation. The initial rate of destaining (d2) was once again measured at each puncta (FIG. 3, top).

To compare the results obtained during first and second destaining protocols and to minimize puncta-to-puncta variability, the inventors calculated the d2/d1 ratios for each puncta. They found that, for control and R9-loaded neurons, the average d2/d1 ratio was equal to 0.6 ± 0.2 ($n = 30$) and 0.55 ± 0.15 ($n = 28$), respectively (FIGS. 3A, 3B, 3D), but for R9-QDHWC loaded neurons the ratio was reduced to 0.32 ± 0.18 ($n = 35$) (FIGS. 3C, 3D). Thus, loading of hippocampal neurons with R9-QDHWC peptide resulted in specific impairment of synaptic function in our experiments.

The homogenous time-resolved fluorescence resonance energy transfer (HTRF) experiments are based on the FRET energy transfer between caged donor fluorophore with a delayed emission and a neighboring acceptor fluorophore (FIG. 4). Excitation of a donor fluorophore by a Xenon flash lamp results in prompt fluorescence (FIG. 4). Photons emitted during delayed emission stage are absorbed by an acceptor and result in HTRF signal. Bio-NC peptide (1000 nM) and GST-Mint1-PDZ1/2 protein (1000 nM) were mixed in HTRF buffer (0.2% BSA in PBS, 100 mM KF) and incubated for 90 min. For donor labeling, Eu^{3+} cryptate (EuK) conjugated anti-GST monoclonal antibodies (CIS Bio International, 61GSTKLA) were added (10 nM). For acceptor labeling, Streptavidin-XL (Streptavidin conjugated to XL665, 610SAXLA from CIS Bio International) were added (100 nM). The reaction mixture was incubated for 60 more minutes. As a negative control 1000 nM of Biotin was used in reaction instead of Bio-NC peptide. The reaction mixtures were aliquoted 50 μl per well in black 384 well plates (Costar) for HTRF measurements.

The HTRF measurements were taken using Perkin Elmers Envision available at UT Southwestern HTS facility. Parameters: Excitation at 320 nm. Detection - Channel 1: 665nm; Channel 2: 590nm. Delay 50 μ s. Time between flashes: 2000 μ s. Number of flashes: 200/well. The HTRF ratio of 665 nm/620 nm (channel1/channel2).

The inventors determined that HTRF ratio is equal to 0.06 for bio-NC/GST-Mint1-PDZ-1/2 pair (FIG. 5). In control experiments, they determined that HTRF ratio is less than 0.04 for Biotin/GST-Mint1-PDZ-1/2 pair (FIG. 5). Thus, the signal/background ratio in present conditions was 1.7 (FIG. 5). The data are highly reproducible (FIG. 5), but further optimization is required to improve S/B ratio for HTS screen.

The initial step in the AlphaScreen signal amplification strategy (Perkin Elmers) is the conversion of ambient oxygen to the singlet state by a photosensitizer in the Donor bead upon illumination at 680 nm (FIG. 6). The Acceptor beads contain a thioxene derivative that reacts with the singlet oxygen to generate chemiluminescence at 370 nm. Energy transfer to fluorescent acceptors in the same beads shifts the emission wavelength to 520-620 nm. The half-life of the decay reaction is 0.3 sec, which makes the AlphaScreen fluorescence signal very long lived and allows the technology to operate in time-resolved mode. The short lifetime of singlet oxygen in aqueous solution (~ 4 μ sec) allows diffusion over a distance up to ~ 200 nm (FIG. 6).

In the inventors' experiments, bio-NC peptide (63, 189, 1000 nM) and GST-Mint1-PDZ1/2 protein (63, 189, 1000 nM) were mixed in ALPHA buffer (Hepes 25 mM, pH 7.2; NaCl 100 mM; BSA 0.1%) and incubated for 10 h. Anti-GST acceptor beads (Perkin-Elmer) and Streptavidin donor beads (Perkin-Elmer) were added. The reaction mixture was incubated for 60 more minutes. As a negative control (63, 189, 1000 nM) of Biotin was used in reaction instead of Bio-NC peptide. The reaction mixtures were aliquoted 50 μ l per well in black 384 well plates (Costar) for ALPHA measurements.

The ALPHA measurements were taken using Perkin Elmers Envision available at UT Southwestern HTS facility. Parameters: Excitation at 680 nm. Detection - 570 nm. Excitation time: 180 ms. Total measurement time: 550 ms.

The inventors determined that ALPHA signal is equal to 22,000 for bio-NC/GST-Mint1-PDZ-1/2 pair (FIG. 7, 189 nM concentration). In control experiments, they determined that ALPHA signal is less than 1,500 for Biotin/GST-Mint1-PDZ-1/2 pair (FIG. 7). Thus, the signal/background ratio in this conditions is 14 (FIG. 7). The data are highly reproducible (FIG. 7).

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. An isolated and purified peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1), or conservative variants thereof.
2. The peptide of claim 1, wherein said peptide comprises the sequence QDHWC (SEQ ID NO:2).
3. The peptide of claim 1, wherein said peptide comprises the sequence of DQDHWVC (SEQ ID NO:3), PDQDHWC (SEQ ID NO:4), HPDQDHWC (SEQ ID NO:5), HHPDQDHWC (SEQ ID NO:6), YHHPDQDHWC (SEQ ID NO:7), SYHHPDQDHWC (SEQ ID NO:8) or HSYHHPDQDHWC (SEQ ID NO:9).
4. The peptide of claim 1, further comprising a permeant protein delivery motif.
5. The peptide of claim 4, wherein said permeant protein delivery motif is a TAT sequence or an R9 sequence.
6. The peptide of claim 1, wherein said peptide is 40 residues in length.
7. The peptide of claim 1, wherein said peptide is 30 residues in length.
8. The peptide of claim 1, wherein said peptide is 20 residues in length.
9. The peptide of claim 1, wherein said peptide is 15 residues in length.
10. The peptide of claim 1, wherein said peptide is 12 residues in length.
11. The peptide of claim 1, wherein said peptide is 10 residues in length.
12. The peptide of claim 1, wherein said peptide is 8 residues in length.
13. The peptide of claim 1, wherein said peptide is 7 residues in length.

14. The peptide of claim 1, wherein said peptide is 6 residues in length.
15. The peptide of claim 1, wherein said peptide is 5 residues in length.
16. The peptide of claim 1, wherein said peptide is 4 residues in length.
17. The peptide of claim 1, further comprised within a pharmaceutically acceptable buffer, diluent or excipient.
18. The peptide of claim 1, further comprised within a lipid vehicle.
19. The peptide of claim 18, wherein said lipid vehicle is a liposome.
20. A nucleic acid encoding peptide of 4 to about 50 residues comprising the sequence DHWC or conservative variants thereof operably linked to a promoter.
21. The nucleic acid of claim 20, wherein said promoter is tissue specific or constitutive.
22. The nucleic acid of claim 20, wherein said promoter is selected from the group consisting of CMV IE, RSV, and SV40 large T.
23. The nucleic acid of claim 20, wherein said nucleic acid further comprises a polyadenylation signal.
24. The nucleic acid of claim 20, wherein said nucleic acid is located in a viral vector.
25. The nucleic acid of claim 24, wherein said viral vector is selected from the group consisting of retrovirus, adenovirus, adeno-associated virus, vaccinia virus, herpesvirus and polyoma virus.
26. The nucleic acid of claim 20, wherein said nucleic acid is comprised in a non-viral vector.

27. The nucleic acid of claim 26, wherein said non-viral vector is comprised in a lipid vehicle.
28. The nucleic acid of claim 27, wherein said lipid vehicle is a liposome.
29. The nucleic acid of claim 20, wherein said nucleic acid further encodes a permeant protein delivery motif fused to SEQ ID NO:1.
30. A method of inhibiting pain in an animal comprising administering to said animal a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1) or conservative variants thereof, said peptide dispersed in a pharmaceutically acceptable buffer, diluent or excipient.
31. The method of claim 30, wherein said peptide is 40 residues in length.
32. The method of claim 30, wherein said peptide is 20 residues in length.
33. The method of claim 30, wherein said peptide is 12 residues in length.
34. The method of claim 30, wherein said peptide is 8 residues in length.
35. The method of claim 30, wherein said peptide is 4 residues in length.
36. The method of claim 30, wherein said peptide is further comprised within a lipid vehicle.
37. The method of claim 36, wherein said lipid vehicle is a liposome.
38. The method of claim 30, wherein the pain to be treated is selected from the group consisting of neuropathic pain, inflammatory pain and pain secondary to cancer.
39. The method of claim 30, further comprising administering a second anti-pain agent to said animal.

40. The method of claim 30, wherein said second anti-pain agent is a steroid, an NSAID, or an opioid.
41. The method of claim 30, wherein said animal is a human.
42. The method of claim 30, wherein said animal is a dog, a cat, a rat, a mouse, a horse, a cow or a rabbit.
43. A method of inhibiting pain in an animal comprising administering to said animal a nucleic acid encoding peptide of 4 to about 50 residues comprising the sequence DHWC or conservative variants thereof operably linked to a promoter, said nucleic acid dispersed in a pharmaceutically acceptable buffer, diluent or excipient.
44. The method of claim 43, wherein said nucleic acid is located in a viral vector.
45. The method of claim 44, wherein said viral vector is selected from the group consisting of retrovirus, adenovirus, adeno-associated virus, vaccinia virus, herpesvirus and polyoma virus.
46. The method of claim 43, wherein said nucleic acid is comprised in a non-viral vector.
47. The method of claim 43, wherein the pain to be treated is selected from the group consisting of neuropathic pain, inflammatory pain and pain secondary to cancer.
48. The method of claim 43, further comprising administering a second anti-pain agent to said animal.
49. A method of screening for an anti-pain agent comprising:
 - (a) providing a peptide of 4 to about 50 residues comprising the a peptide with one or more conservative variants of DHWC (SEQ ID NO:1);
 - (b) contacting said peptide with an animal model of pain; and
 - (c) determining the ability of the peptide to inhibit pain in said animal model.

50. A method of screening for an anti-pain agent comprising:
- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
 - (b) contacting said peptide and Mint1 in the presence of a candidate substance; and
 - (c) determining the ability of candidate substance to inhibit the peptide binding Mint1.
51. A method of screening for an anti-pain agent comprising:
- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
 - (b) contacting said peptide and Mint1-PDZ1 domain in the presence of a candidate substance; and
 - (c) determining the ability of candidate substance to inhibit the peptide binding Mint1-PDZ1 domain.
52. A method of screening for an anti-pain agent comprising:
- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
 - (b) contacting said peptide and Mint1-PDZ1/2 domains in the presence of a candidate substance; and
 - (c) determining the ability of candidate substance to inhibit the peptide binding Mint1-PDZ1/2 domains.
53. A method of screening for an anti-pain agent comprising:
- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
 - (b) contacting said peptide and Mint2 in the presence of a candidate substance; and

- (c) determining the ability of candidate substance to inhibit the peptide binding Mint2.

54. A method of screening for an anti-pain agent comprising:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting said peptide and Mint2-PDZ1 domain in the presence of a candidate substance; and
- (c) determining the ability of candidate substance to inhibit the peptide binding Mint2-PDZ1 domain.

55. A method of screening for an anti-pain agent comprising:

- (a) providing a peptide of 4 to about 50 residues comprising the sequence DHWC (SEQ ID NO:1);
- (b) contacting said peptide and Mint2-PDZ1/2 domains in the presence of a candidate substance; and

determining the ability of candidate substance to inhibit the peptide binding Mint2-PDZ1/2 domains.

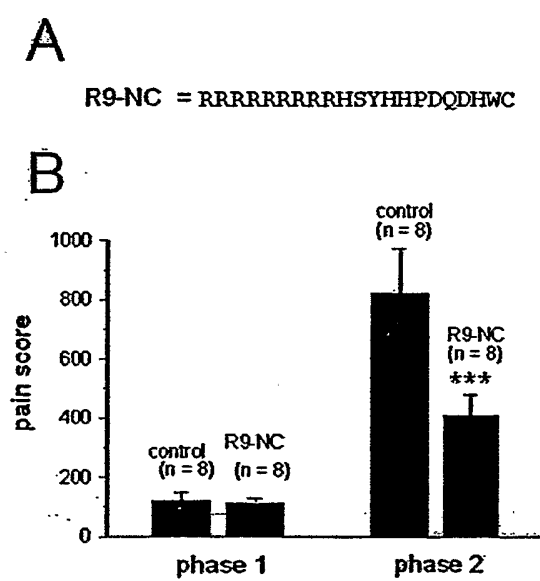
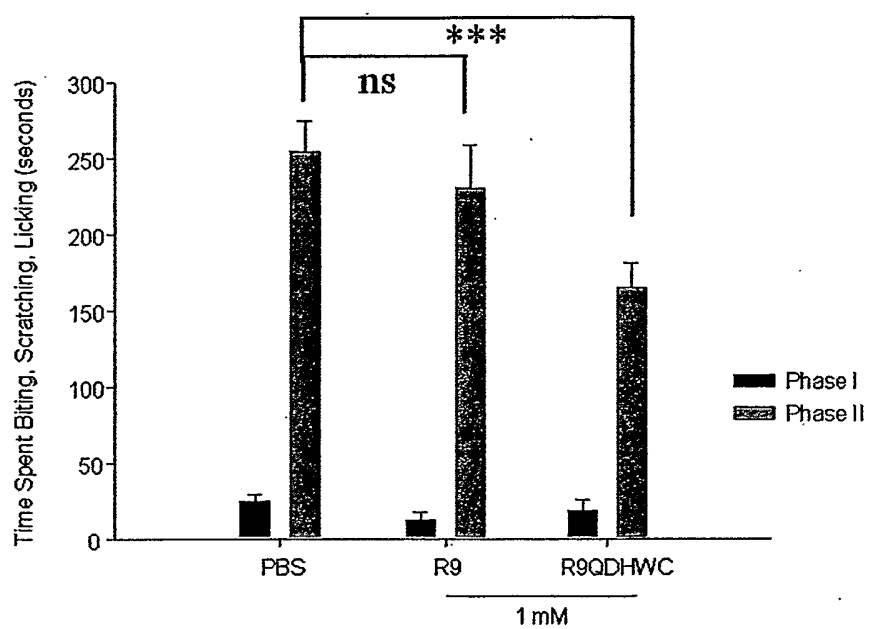
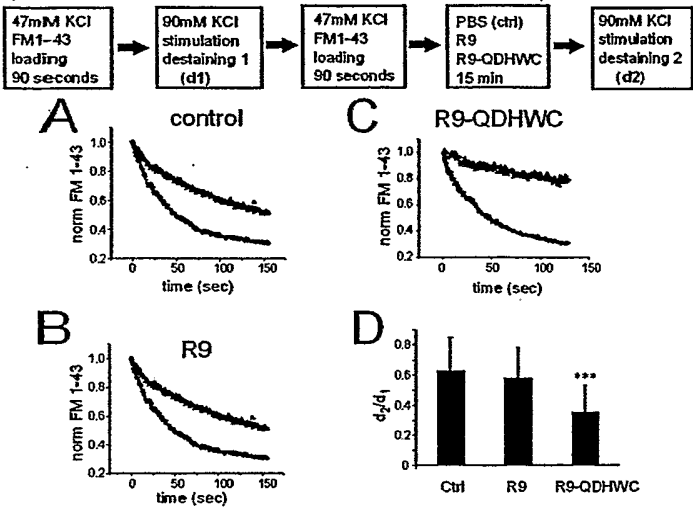


FIG. 1A-B

**FIG. 2**



FIGS. 3A-D

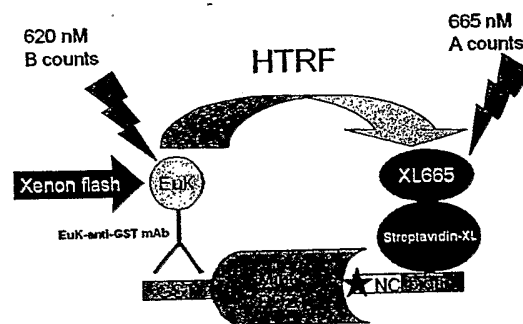


FIG. 4

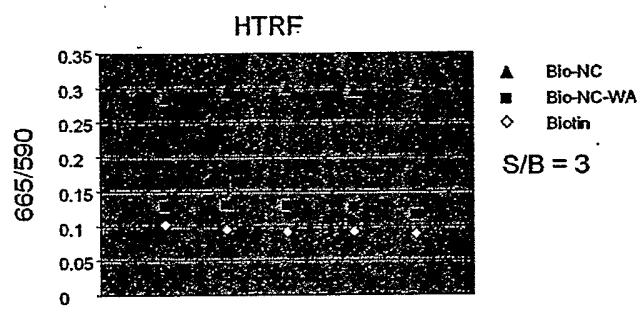


FIG. 5

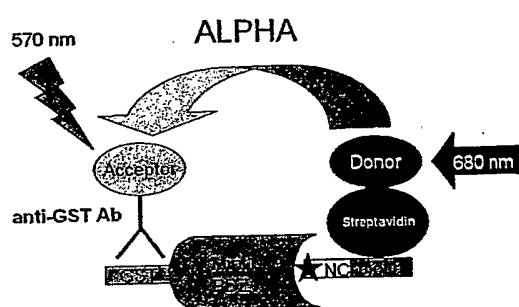
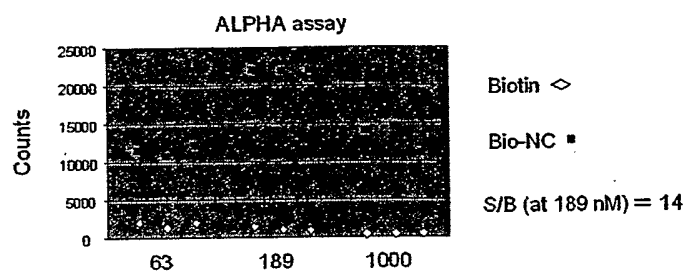


FIG. 6

**FIG. 7**

SEQUENCE LISTING

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BEZPROZVANNY, ILYA

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<130> UTFD:1558WO

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<211> 7364

<212> DNA

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<220>

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gggatgcacg	cggggcccgg	gagcc	atg	gtc	cgc	ttc	ggg	gac	gag	ctg	ggc	172
			Met	Val	Arg	Phe	Gly	Asp	Glu	Leu	Gly	
			1				5					

ggc	cgc	tat	gga	ggc	ccc	ggc	ggc	gga	gag	cgg	gcc	cgg	ggc	ggc	ggg	220
Gly	Arg	Tyr	Gly	Gly	Pro	Gly	Gly	Gly	Glu	Arg	Ala	Arg	Gly	Gly	Gly	
10					15				20						25	

gcc	ggc	ggg	gcg	ggg	ggc	ccg	ggt	ccc	ggg	ggg	ctg	cag	ccc	ggc	cag	268
Ala	Gly	Gly	Ala	Gly	Gly	Pro	Gly	Pro	Gly	Gly	Leu	Gln	Pro	Gly	Gln	
			30					35						40		

cgg	gtc	ctc	tac	aag	caa	tcg	atc	gcg	cag	cgc	gcg	cgg	acc	atg	gcg	316
Arg	Val	Leu	Tyr	Lys	Gln	Ser	Ile	Ala	Gln	Arg	Ala	Arg	Thr	Met	Ala	
			45					50					55			

ctg	tac	aac	ccc	atc	ccg	gtc	aag	cag	aac	tgc	ttc	acc	gtc	aac	cgc	364
Leu	Tyr	Asn	Pro	Ile	Pro	Val	Lys	Gln	Asn	Cys	Phe	Thr	Val	Asn	Arg	
		60					65					70				

tcg	ctc	ttc	gtc	ttc	agc	gag	gac	aac	gtc	gtc	cgc	aaa	tac	gcg	aag	412
Ser	Leu	Phe	Val	Phe	Ser	Glu	Asp	Asn	Val	Val	Arg	Lys	Tyr	Ala	Lys	
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atc gcc aac tgc atc gtg ctg gcc ctg gag cag cac ctg cct gat ggg	508
Ile Ala Asn Cys Ile Val Leu Ala Leu Glu Gln His Leu Pro Asp Gly	
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gac aaa acg ccc atg tcc gag cgg ctg gac gac acg gag ccc tat ttc	556
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125 130 135	
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Glu Cys Arg Glu Tyr Trp Pro Gly Pro Asn Phe Gly Ile Thr Asn Phe	
285 290 295	
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Asp Asn Ile Leu Phe Ala Ile Leu Thr Val Phe Gln Cys Ile Thr Met	
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Glu Gly Trp Thr Asp Ile Leu Tyr Asn Thr Asn Asp Ala Ala Gly Asn	
315 320 325	
acc tgg aac tgg ctc tac ttc atc cct ctc atc atc atc ggc tcc ttc	1180

Thr 330	Trp	Asn	Trp	Leu	Tyr 335	Phe	Ile	Pro	Leu	Ile 340	Ile	Ile	Gly	Ser	Phe 345		
ttc	atg	ctc	aac	ctg	gtg	ctg	ggc	gtg	ctc	tgc	ggg	gag	ttt	gcc	aag	1228	
Phe	Met	Leu	Asn	Leu	Val	Leu	Gly	Val	Leu	Ser	Gly	Glu	Phe	Ala	Lys		
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Glu	Arg	Glu	Arg	Val	Glu	Asn	Arg	Arg	Ala	Phe	Leu	Lys	Leu	Arg	Arg		
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Gln	Gln	Gln	Ile	Glu	Arg	Glu	Leu	Asn	Gly	Tyr	Leu	Glu	Trp	Ile	Phe		
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Lys	Ala	Glu	Glu	Val	Met	Leu	Ala	Glu	Glu	Asp	Arg	Asn	Ala	Glu	Glu		
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410					415					420					425		
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Cys	Ala	Val	Gly	Ser	Pro	Phe	Ala	Arg	Ala	Ser	Leu	Lys	Ser	Gly	Lys		
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Cys	Val	Val	Ala	Leu	Asn	Thr	Leu	Cys	Val	Ala	Met	Val	His	Tyr	Asn		
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Gln	Pro	Arg	Arg	Leu	Thr	Thr	Thr	Leu	Tyr	Phe	Ala	Glu	Phe	Val	Phe		
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Leu	Gly	Leu	Phe	Leu	Thr	Glu	Met	Ser	Leu	Lys	Met	Tyr	Gly	Leu	Gly		
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Ile	Val	Gly	Ser	Val	Phe	Glu	Val	Val	Trp	Ala	Ala	Ile	Lys	Pro	Gly		
	555					560					565						
agc	tcc	ttt	ggg	atc	agt	gtg	ctg	cgg	gcc	ctc	cgc	ctg	ctg	agg	atc	1900	
Ser	Ser	Phe	Gly	Ile	Ser	Val	Leu	Arg	Ala	Leu	Arg	Leu	Leu	Arg	Ile		

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ttc aaa gtc acg aag tac tgg agc tcc ctg cgg aac ctg gtg gtc tcc				1948
Phe Lys Val Thr Lys Tyr Trp Ser Ser Leu Arg Asn Leu Val Val Ser				
	590	595	600	
ctg ctg aac tcc atg aag tcc atc atc agc ctg ctc ttc ttg ctc ttc				1996
Leu Leu Asn Ser Met Lys Ser Ile Ile Ser Leu Leu Phe Leu Leu Phe				
	605	610	615	
ctg ttc att gtg gtc ttc gcc ctg ctg ggg atg cag ctg ttt ggg gga				2044
Leu Phe Ile Val Val Phe Ala Leu Leu Gly Met Gln Leu Phe Gly Gly				
	620	625	630	
cag ttc aac ttc cag gat gag act ccc aca acc aac ttc gac acc ttc				2092
Gln Phe Asn Phe Gln Asp Glu Thr Pro Thr Thr Asn Phe Asp Thr Phe				
	635	640	645	
cct gcc gcc atc ctc act gtc ttc cag atc ctg acg gga gag gac tgg				2140
Pro Ala Ala Ile Leu Thr Val Phe Gln Ile Leu Thr Gly Glu Asp Trp				
	650	655	660	665
aat gca gtg atg tat cac ggg atc gaa tcg caa ggc ggc gtc agc aaa				2188
Asn Ala Val Met Tyr His Gly Ile Glu Ser Gln Gly Gly Val Ser Lys				
	670	675	680	
ggc atg ttc tcg tcc ttt tac ttc att gtc ctg aca ctg ttc gga aac				2236
Gly Met Phe Ser Ser Phe Tyr Phe Ile Val Leu Thr Leu Phe Gly Asn				
	685	690	695	
tac act ctg ctg aat gtc ttt ctg gcc atc gct gtg gac aac ctg gcc				2284
Tyr Thr Leu Leu Asn Val Phe Leu Ala Ile Ala Val Asp Asn Leu Ala				
	700	705	710	
aac gcc caa gag ctg acc aag gat gaa gag gag atg gaa gaa gca gcc				2332
Asn Ala Gln Glu Leu Thr Lys Asp Glu Glu Glu Met Glu Glu Ala Ala				
	715	720	725	
aat cag aag ctt gct ctg caa aag gcc aaa gaa gtg gct gaa gtc agc				2380
Asn Gln Lys Leu Ala Leu Gln Lys Ala Lys Glu Val Ala Glu Val Ser				
	730	735	740	745
ccc atg tct gcc gcg aac atc tcc atc gcc gcc agg cag cag aac tcg				2428
Pro Met Ser Ala Ala Asn Ile Ser Ile Ala Ala Arg Gln Gln Asn Ser				
	750	755	760	
gcc aag gcg cgc tcg gtg tgg gag cag cgg gcc agc cag cta cgg ctg				2476
Ala Lys Ala Arg Ser Val Trp Glu Gln Arg Ala Ser Gln Leu Arg Leu				
	765	770	775	
cag aac ctg cgg gcc agc tgc gag gcg ctg tac agc gag atg gac ccc				2524
Gln Asn Leu Arg Ala Ser Cys Glu Ala Leu Tyr Ser Glu Met Asp Pro				
	780	785	790	
gag gag cgg ctg cgc ttc gcc act acg cgc cac ctg cgg ccc gac atg				2572
Glu Glu Arg Leu Arg Phe Ala Thr Thr Arg His Leu Arg Pro Asp Met				
	795	800	805	
aag acg cac ctg gac cgg ccg ctg gtg gtg gag ctg ggc cgc gac ggc				2620
Lys Thr His Leu Asp Arg Pro Leu Val Val Glu Leu Gly Arg Asp Gly				
	810	815	820	825

gcg cgg ggg ccc gtg gga ggc aaa gcc cga cct gag gct gcg gag gcc	2668
Ala Arg Gly Pro Val Gly Gly Lys Ala Arg Pro Glu Ala Ala Glu Ala	
830 835 840	
ccc gag ggc gtc gac cct ccg cgc agg cac cac cgg cac cgc gac aag	2716
Pro Glu Gly Val Asp Pro Pro Arg Arg His His Arg His Arg Asp Lys	
845 850 855	
gac aag acc ccc gcg gcg ggg gac cag gac cga gca gag gcc ccg aag	2764
Asp Lys Thr Pro Ala Ala Gly Asp Gln Asp Arg Ala Glu Ala Pro Lys	
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Ala Glu Ser Gly Glu Pro Gly Ala Arg Glu Glu Arg Pro Arg Pro His	
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Arg Ser His Ser Lys Glu Ala Ala Gly Pro Pro Glu Ala Arg Ser Glu	
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Arg Gly Arg Gly Pro Gly Pro Glu Gly Gly Arg Arg His His Arg Arg	
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Gly Ser Pro Glu Glu Ala Ala Glu Arg Glu Pro Arg Arg His Arg Ala	
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His Arg His Gln Asp Pro Ser Lys Glu Cys Ala Gly Ala Lys Gly Glu	
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Arg Arg Ala Arg His Arg Gly Gly Pro Arg Ala Gly Pro Arg Glu Ala	
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Glu Ser Gly Glu Glu Pro Ala Arg Arg His Arg Ala Arg His Lys Ala	
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Gln Pro Ala His Glu Ala Val Glu Lys Glu Thr Thr Glu Lys Glu Ala	
990 995 1000	
acg gag aag gag gct gag ata gtg gaa gcc gac aag gaa aag gag ctc	3196
Thr Glu Lys Glu Ala Glu Ile Val Glu Ala Asp Lys Glu Lys Glu Leu	
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cgg aac cac cag ccc cgg gag cca cac tgt gac ctg gag acc agt ggg	3244
Arg Asn His Gln Pro Arg Glu Pro His Cys Asp Leu Glu Thr Ser Gly	
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act gtg act gtg ggt ccc atg cac aca ctg ccc agc acc tgt ctc cag	3292
Thr Val Thr Val Gly Pro Met His Thr Leu Pro Ser Thr Cys Leu Gln	
1035 1040 1045	
aag gtg gag gaa cag cca gag gat gca gac aat cag cgg aac gtc act	3340
Lys Val Glu Glu Gln Pro Glu Asp Ala Asp Asn Gln Arg Asn Val Thr	
1050 1055 1060 1065	

cgc atg ggc agt cag ccc cca gac ccg aac act att gta cat atc cca	3388
Arg Met Gly Ser Gln Pro Pro Asp Pro Asn Thr Ile Val His Ile Pro	
1070 1075 1080	
gtg atg ctg acg ggc cct ctt ggg gaa gcc acg gtc gtt ccc agt ggt	3436
Val Met Leu Thr Gly Pro Leu Gly Glu Ala Thr Val Val Pro Ser Gly	
1085 1090 1095	
aac gtg gac ctg gaa agc caa gca gag ggg aag aag gag gtg gaa gcg	3484
Asn Val Asp Leu Glu Ser Gln Ala Glu Gly Lys Lys Glu Val Glu Ala	
1100 1105 1110	
gat gac gtg atg agg agc ggc ccc cgg cct atc gtc cca tac agc tcc	3532
Asp Asp Val Met Arg Ser Gly Pro Arg Pro Ile Val Pro Tyr Ser Ser	
1115 1120 1125	
atg ttc tgt tta agc ccc acc aac ctg ctc cgc cgc ttc tgc cac tac	3580
Met Phe Cys Leu Ser Pro Thr Asn Leu Leu Arg Arg Phe Cys His Tyr	
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Ile Val Thr Met Arg Tyr Phe Glu Val Val Ile Leu Val Val Ile Ala	
1150 1155 1160	
ttg agc agc atc gcc ctg gct gct gag gac cca gtg cgc aca gac tcg	3676
Leu Ser Ser Ile Ala Leu Ala Ala Glu Asp Pro Val Arg Thr Asp Ser	
1165 1170 1175	
ccc agg aac aac gct ctg aaa tac ctg gat tac att ttc act ggt gtc	3724
Pro Arg Asn Asn Ala Leu Lys Tyr Leu Asp Tyr Ile Phe Thr Gly Val	
1180 1185 1190	
ttt acc ttt gag atg gtg ata aag atg atc gac ttg gga ctg ctg ctt	3772
Phe Thr Phe Glu Met Val Ile Lys Met Ile Asp Leu Gly Leu Leu Leu	
1195 1200 1205	
cac cct gga gcc tat ttc cgg gac ttg tgg aac att ctg gac ttc att	3820
His Pro Gly Ala Tyr Phe Arg Asp Leu Trp Asn Ile Leu Asp Phe Ile	
1210 1215 1220 1225	
gtg gtc agt ggc gcc ctg gtg gcg ttt gct ttc tca gga tcc aaa ggg	3868
Val Val Ser Gly Ala Leu Val Ala Phe Ala Phe Ser Gly Ser Lys Gly	
1230 1235 1240	
aaa gac atc aat acc atc aag tct ctg aga gtc ctt cgt gtc ctg cgg	3916
Lys Asp Ile Asn Thr Ile Lys Ser Leu Arg Val Leu Arg Val Leu Arg	
1245 1250 1255	
ccc ctc aag acc atc aaa cgg ctg ccc aag ctc aag gct gtg ttt gac	3964
Pro Leu Lys Thr Ile Lys Arg Leu Pro Lys Leu Lys Ala Val Phe Asp	
1260 1265 1270	
tgt gtg gtg aac tcc ctg aag aat gtc ctc aac atc ttg att gtc tac	4012
Cys Val Val Asn Ser Leu Lys Asn Val Leu Asn Ile Leu Ile Val Tyr	
1275 1280 1285	
atg ctc ttc atg ttc ata ttt gcc gtc att gcg gtg cag ctc ttc aaa	4060
Met Leu Phe Met Phe Ile Phe Ala Val Ile Ala Val Gln Leu Phe Lys	
1290 1295 1300 1305	
ggg aag ttt ttc tac tgc aca gat gaa tcc aag gag ctg gag agg gac	4108

Gly Lys Phe Phe Tyr Cys Thr Asp Glu Ser Lys Glu Leu Glu Arg Asp	
1310 1315 1320	
tgc agg ggt cag tat ttg gat tat gag aag gag gaa gtg gaa gct cag	4156
Cys Arg Gly Gln Tyr Leu Asp Tyr Glu Lys Glu Glu Val Glu Ala Gln	
1325 1330 1335	
ccc agg cag tgg aag aaa tac gac ttt cac tac gac aat gtg ctc tgg	4204
Pro Arg Gln Trp Lys Lys Tyr Asp Phe His Tyr Asp Asn Val Leu Trp	
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gct ctg ctg acg ctg ttc aca gtg tcc acg gga gaa ggc tgg ccc atg	4252
Ala Leu Leu Thr Leu Phe Thr Val Ser Thr Gly Glu Gly Trp Pro Met	
1355 1360 1365	
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Val Leu Lys His Ser Val Asp Ala Thr Tyr Glu Glu Gln Gly Pro Ser	
1370 1375 1380 1385	
cct ggg tac cgc atg gag ctg tcc atc ttc tac gtg gtc tac ttt gtg	4348
Pro Gly Tyr Arg Met Glu Leu Ser Ile Phe Tyr Val Val Tyr Phe Val	
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Val Phe Pro Phe Phe Phe Val Asn Ile Phe Val Ala Leu Ile Ile Ile	
1405 1410 1415	
acc ttc cag gag cag ggg gac aag gtg atg tct gaa tgc agc ctg gag	4444
Thr Phe Gln Glu Gln Gly Asp Lys Val Met Ser Glu Cys Ser Leu Glu	
1420 1425 1430	
aag aac gag agg gct tgc att gac ttc gcc atc agc gcc aaa ccc ctg	4492
Lys Asn Glu Arg Ala Cys Ile Asp Phe Ala Ile Ser Ala Lys Pro Leu	
1435 1440 1445	
aca cgg tac atg ccc caa aac cgg cag tcg ttc cag tat aag acg tgg	4540
Thr Arg Tyr Met Pro Gln Asn Arg Gln Ser Phe Gln Tyr Lys Thr Trp	
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aca ttt gtg gtc tcc ccg ccc ttt gaa tac ttc atc atg gcc atg ata	4588
Thr Phe Val Val Ser Pro Pro Phe Glu Tyr Phe Ile Met Ala Met Ile	
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gcc ctc aac act gtg gtg ctg atg atg aag ttc tat gat gca ccc tat	4636
Ala Leu Asn Thr Val Val Leu Met Met Lys Phe Tyr Asp Ala Pro Tyr	
1485 1490 1495	
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Glu Tyr Glu Leu Met Leu Lys Cys Leu Asn Ile Val Phe Thr Ser Met	
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Phe Ser Met Glu Cys Val Leu Lys Ile Ile Ala Phe Gly Val Leu Asn	
1515 1520 1525	
tat ttc aga gat gcc tgg aat gtc ttt gac ttt gtc act gtg ttg gga	4780
Tyr Phe Arg Asp Ala Trp Asn Val Phe Asp Phe Val Thr Val Leu Gly	
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agt att act gat att tta gta aca gag att gcg gaa acg aac aat ttc	4828
Ser Ile Thr Asp Ile Leu Val Thr Glu Ile Ala Glu Thr Asn Asn Phe	

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Ile Asn Leu Ser Phe Leu Arg Leu Phe Arg Ala Ala Arg Leu Ile Lys			
1565	1570	1575	
ctg ctc cgc cag ggc tac acc atc cgc atc ctg ctg tgg acc ttt gtc			4924
Leu Leu Arg Gln Gly Tyr Thr Ile Arg Ile Leu Leu Trp Thr Phe Val			
1580	1585	1590	
cag tcc ttc aag gcc ctg ccc tac gtg tgt ctg ctc att gcc atg ctg			4972
Gln Ser Phe Lys Ala Leu Pro Tyr Val Cys Leu Leu Ile Ala Met Leu			
1595	1600	1605	
ttc ttc atc tac gcc atc atc ggc atg cag gtg ttt ggg aat att gcc			5020
Phe Phe Ile Tyr Ala Ile Ile Gly Met Gln Val Phe Gly Asn Ile Ala			
1610	1615	1620	1625
ctg gat gat gac acc agc atc aac cgc cac aac aac ttc cgg acg ttt			5068
Leu Asp Asp Asp Thr Ser Ile Asn Arg His Asn Asn Phe Arg Thr Phe			
1630	1635	1640	
ttg caa gcc ctg atg ctg ctg ttc agg agc gcc acg ggg gag gcc tgg			5116
Leu Gln Ala Leu Met Leu Leu Phe Arg Ser Ala Thr Gly Glu Ala Trp			
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cac gag atc atg ctg tcc tgc ctg agc aac cag gcc tgt gat gag cag			5164
His Glu Ile Met Leu Ser Cys Leu Ser Asn Gln Ala Cys Asp Glu Gln			
1660	1665	1670	
gcc aat gcc acc gag tgt gga agt gac ttt gcc tac ttc tac ttc gtc			5212
Ala Asn Ala Thr Glu Cys Gly Ser Asp Phe Ala Tyr Phe Tyr Phe Val			
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tcc ttc atc ttc ctg tgc tcc ttt ctg atg ttg aac ctc ttt gtg gct			5260
Ser Phe Ile Phe Leu Cys Ser Phe Leu Met Leu Asn Leu Phe Val Ala			
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gtg atc atg gac aat ttt gag tac ctc acg cgg gac tct tcc atc cta			5308
Val Ile Met Asp Asn Phe Glu Tyr Leu Thr Arg Asp Ser Ser Ile Leu			
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ggt cct cac cac ttg gat gag ttc atc cgg gtc tgg gct gaa tac gac			5356
Gly Pro His His Leu Asp Glu Phe Ile Arg Val Trp Ala Glu Tyr Asp			
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ccg gct gcg tgt ggg cgc atc agt tac aat gac atg ttt gag atg ctg			5404
Pro Ala Ala Cys Gly Arg Ile Ser Tyr Asn Asp Met Phe Glu Met Leu			
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aaa cac atg tcc ccg cct ctg ggg ctg ggg aag aaa tgc cct gct cga			5452
Lys His Met Ser Pro Leu Gly Leu Gly Lys Lys Cys Pro Ala Arg			
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Val Ala Tyr Lys Arg Leu Val Arg Met Asn Met Pro Ile Ser Asn Glu			
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gac atg act gtt cac ttc acg tcc acg ctg atg gcc ctc atc cgg acg			5548
Asp Met Thr Val His Phe Thr Ser Thr Leu Met Ala Leu Ile Arg Thr			
1790	1795	1800	

gca ctg gag atc aag ctg gcc cca gct ggg aca aag cag cat cag tgt	5596
Ala Leu Glu Ile Lys Leu Ala Pro Ala Gly Thr Lys Gln His Gln Cys	
1805 1810 1815	
gac gcg gag ttg agg aag gag att tcc gtt gtg tgg gcc aat ctg ccc	5644
Asp Ala Glu Leu Arg Lys Glu Ile Ser Val Val Trp Ala Asn Leu Pro	
1820 1825 1830	
cag aag act ttg gac ttg ctg gta cca ccc cat aag cct gat gag atg	5692
Gln Lys Thr Leu Asp Leu Leu Val Pro Pro His Lys Pro Asp Glu Met	
1835 1840 1845	
aca gtg ggg aag gtt tat gca gct ctg atg ata ttt gac ttc tac aag	5740
Thr Val Gly Lys Val Tyr Ala Ala Leu Met Ile Phe Asp Phe Tyr Lys	
1850 1855 1860 1865	
cag aac aaa acc acc aga gac cag atg cag cag gct cct gga ggc ctc	5788
Gln Asn Lys Thr Thr Arg Asp Gln Met Gln Gln Ala Pro Gly Gly Leu	
1870 1875 1880	
tcc cag atg ggt cct gtg tcc ctg ttc cac cct ctg aag gcc acc ctg	5836
Ser Gln Met Gly Pro Val Ser Leu Phe His Pro Leu Lys Ala Thr Leu	
1885 1890 1895	
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Glu Gln Thr Gln Pro Ala Val Leu Arg Gly Ala Arg Val Phe Leu Arg	
1900 1905 1910	
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Gln Lys Ser Ser Thr Ser Leu Ser Asn Gly Gly Ala Ile Gln Asn Gln	
1915 1920 1925	
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Glu Ser Gly Ile Lys Glu Ser Val Ser Trp Gly Thr Gln Arg Thr Gln	
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Asp Ala Pro His Glu Ala Arg Pro Pro Leu Glu Arg Gly His Ser Thr	
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Glu Ile Pro Val Gly Arg Ser Gly Ala Leu Ala Val Asp Val Gln Met	
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Gln Ser Ile Thr Arg Arg Gly Pro Asp Gly Glu Pro Gln Pro Gly Leu	
1980 1985 1990	
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Glu Ser Gln Gly Arg Ala Ala Ser Met Pro Arg Leu Ala Ala Glu Thr	
1995 2000 2005	
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Gln Pro Val Thr Asp Ala Ser Pro Met Lys Arg Ser Ile Ser Thr Leu	
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Ala Gln Arg Pro Arg Gly Thr His Leu Cys Ser Thr Thr Pro Asp Arg	
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Pro Pro Pro Ser Gln Ala Ser Ser His His His His His Arg Cys His	
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Gly Arg Arg Gln Leu Pro Gln Thr Pro Leu Thr Pro Arg Pro Ser Ile	
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acc tac aag acg gcc aac tcc tca ccc atc cac ttc gcc ggg gct cag	6796
Thr Tyr Lys Thr Ala Asn Ser Ser Pro Ile His Phe Ala Gly Ala Gln	
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Glu His Asn Ala Leu Leu Gln Arg Asp Pro Leu Ser Gln Pro Leu Ala	
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Ser Glu Ala Ser Val His Ala Leu Pro Glu Asp Thr Leu Thr Phe Glu	
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 Ile Ala Gln Arg Ala Arg Thr Met Ala Leu Tyr Asn Pro Ile Pro Val
 50 55 60
 Lys Gln Asn Cys Phe Thr Val Asn Arg Ser Leu Phe Val Phe Ser Glu
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 Asp Asn Val Val Arg Lys Tyr Ala Lys Arg Ile Thr Glu Trp Pro Pro
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 Phe Glu Tyr Met Ile Leu Ala Thr Ile Ile Ala Asn Cys Ile Val Leu
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 Ala Leu Glu Gln His Leu Pro Asp Gly Asp Lys Thr Pro Met Ser Glu
 115 120 125
 Arg Leu Asp Asp Thr Glu Pro Tyr Phe Ile Gly Ile Phe Cys Phe Glu
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 Ala Gly Ile Lys Ile Ile Ala Leu Gly Phe Val Phe His Lys Gly Ser
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 Tyr Leu Arg Asn Gly Trp Asn Val Met Asp Phe Val Val Val Leu Thr
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 Gly Ile Leu Ala Thr Ala Gly Thr Asp Phe Asp Leu Arg Thr Leu Arg
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 Ala Val Arg Val Leu Arg Pro Leu Lys Leu Val Ser Gly Ile Pro Ser
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 Leu Gln Val Val Leu Lys Ser Ile Met Lys Ala Met Val Pro Leu Leu
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 Gln Ile Gly Leu Leu Leu Phe Phe Ala Ile Leu Met Phe Ala Ile Ile
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 Gly Leu Glu Phe Tyr Met Gly Lys Phe His Lys Ala Cys Phe Pro Asn
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Pro	Ala	Arg	Leu	Cys	Glu	Gly	Asp	Thr	Glu	Cys	Arg	Glu	Tyr	Trp	Pro
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Gly	Pro	Asn	Phe	Gly	Ile	Thr	Asn	Phe	Asp	Asn	Ile	Leu	Phe	Ala	Ile
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Leu	Thr	Val	Phe	Gln	Cys	Ile	Thr	Met	Glu	Gly	Trp	Thr	Asp	Ile	Leu
305				310						315					320
Tyr	Asn	Thr	Asn	Asp	Ala	Ala	Gly	Asn	Thr	Trp	Asn	Trp	Leu	Tyr	Phe
			325					330						335	
Ile	Pro	Leu	Ile	Ile	Ile	Gly	Ser	Phe	Phe	Met	Leu	Asn	Leu	Val	Leu
			340					345					350		
Gly	Val	Leu	Ser	Gly	Glu	Phe	Ala	Lys	Glu	Arg	Glu	Arg	Val	Glu	Asn
	355					360						365			
Arg	Arg	Ala	Phe	Leu	Lys	Leu	Arg	Arg	Gln	Gln	Gln	Ile	Glu	Arg	Glu
	370				375						380				
Leu	Asn	Gly	Tyr	Leu	Glu	Trp	Ile	Phe	Lys	Ala	Glu	Val	Met	Leu	
385				390					395						400
Ala	Glu	Glu	Asp	Arg	Asn	Ala	Glu	Glu	Lys	Ser	Pro	Leu	Asp	Val	Leu
			405						410					415	
Lys	Arg	Ala	Ala	Thr	Lys	Lys	Ser	Arg	Asn	Asp	Leu	Ile	His	Ala	Glu
			420					425					430		
Glu	Gly	Glu	Asp	Arg	Phe	Ala	Asp	Leu	Cys	Ala	Val	Gly	Ser	Pro	Phe
	435					440						445			
Ala	Arg	Ala	Ser	Leu	Lys	Ser	Gly	Lys	Thr	Glu	Ser	Ser	Ser	Tyr	Phe
	450					455					460				
Arg	Arg	Lys	Glu	Lys	Met	Phe	Arg	Phe	Phe	Ile	Arg	Arg	Met	Val	Lys
465				470					475						480
Ala	Gln	Ser	Phe	Tyr	Trp	Val	Val	Leu	Cys	Val	Val	Ala	Leu	Asn	Thr
			485					490					495		
Leu	Cys	Val	Ala	Met	Val	His	Tyr	Asn	Gln	Pro	Arg	Arg	Leu	Thr	Thr
			500					505					510		
Thr	Leu	Tyr	Phe	Ala	Glu	Phe	Val	Phe	Leu	Gly	Leu	Phe	Leu	Thr	Glu
	515						520					525			
Met	Ser	Leu	Lys	Met	Tyr	Gly	Leu	Gly	Pro	Arg	Ser	Tyr	Phe	Arg	Ser
	530					535					540				
Ser	Phe	Asn	Cys	Phe	Asp	Phe	Gly	Val	Ile	Val	Gly	Ser	Val	Phe	Glu
545				550					555						560
Val	Val	Trp	Ala	Ala	Ile	Lys	Pro	Gly	Ser	Ser	Phe	Gly	Ile	Ser	Val
			565					570					575		
Leu	Arg	Ala	Leu	Arg	Leu	Leu	Arg	Ile	Phe	Lys	Val	Thr	Lys	Tyr	Trp
			580					585					590		
Ser	Ser	Leu	Arg	Asn	Leu	Val	Val	Ser	Leu	Leu	Asn	Ser	Met	Lys	Ser
	595						600					605			
Ile	Ile	Ser	Leu	Leu	Phe	Leu	Leu	Phe	Leu	Phe	Ile	Val	Val	Phe	Ala
	610					615					620				
Leu	Leu	Gly	Met	Gln	Leu	Phe	Gly	Gly	Gln	Phe	Asn	Phe	Gln	Asp	Glu
625				630					635						640
Thr	Pro	Thr	Thr	Asn	Phe	Asp	Thr	Phe	Pro	Ala	Ala	Ile	Leu	Thr	Val
			645					650						655	
Phe	Gln	Ile	Leu	Thr	Gly	Glu	Asp	Trp	Asn	Ala	Val	Met	Tyr	His	Gly
			660					665					670		
Ile	Glu	Ser	Gln	Gly	Gly	Val	Ser	Lys	Gly	Met	Phe	Ser	Ser	Phe	Tyr
	675						680					685			
Phe	Ile	Val	Leu	Thr	Leu	Phe	Gly	Asn	Tyr	Thr	Leu	Leu	Asn	Val	Phe
	690					695					700				
Leu	Ala	Ile	Ala	Val	Asp	Asn	Leu	Ala	Asn	Ala	Gln	Glu	Leu	Thr	Lys
705				710					715						720
Asp	Glu	Glu	Glu	Met	Glu	Glu	Ala	Ala	Asn	Gln	Lys	Leu	Ala	Leu	Gln
			725					730					735		
Lys	Ala	Lys	Glu	Val	Ala	Glu	Val	Ser	Pro	Met	Ser	Ala	Ala	Asn	Ile
			740					745					750		

Ser Ile Ala Ala Arg Gln Gln Asn Ser Ala Lys Ala Arg Ser Val Trp
 755 760 765
 Glu Gln Arg Ala Ser Gln Leu Arg Leu Gln Asn Leu Arg Ala Ser Cys
 770 775 780
 Glu Ala Leu Tyr Ser Glu Met Asp Pro Glu Glu Arg Leu Arg Phe Ala
 785 790 795 800
 Thr Thr Arg His Leu Arg Pro Asp Met Lys Thr His Leu Asp Arg Pro
 805 810 815
 Leu Val Val Glu Leu Gly Arg Asp Gly Ala Arg Gly Pro Val Gly Gly
 820 825 830
 Lys Ala Arg Pro Glu Ala Ala Glu Ala Pro Glu Gly Val Asp Pro Pro
 835 840 845
 Arg Arg His His Arg His Arg Asp Lys Asp Lys Thr Pro Ala Ala Gly
 850 855 860
 Asp Gln Asp Arg Ala Glu Ala Pro Lys Ala Glu Ser Gly Glu Pro Gly
 865 870 875 880
 Ala Arg Glu Glu Arg Pro Arg Pro His Arg Ser His Ser Lys Glu Ala
 885 890 895
 Ala Gly Pro Pro Glu Ala Arg Ser Glu Arg Gly Arg Gly Pro Gly Pro
 900 905 910
 Glu Gly Gly Arg Arg His His Arg Arg Gly Ser Pro Glu Glu Ala Ala
 915 920 925
 Glu Arg Glu Pro Arg Arg His Arg Ala His Arg His Gln Asp Pro Ser
 930 935 940
 Lys Glu Cys Ala Gly Ala Lys Gly Glu Arg Arg Ala Arg His Arg Gly
 945 950 955 960
 Gly Pro Arg Ala Gly Pro Arg Glu Ala Glu Ser Gly Glu Glu Pro Ala
 965 970 975
 Arg Arg His Arg Ala Arg His Lys Ala Gln Pro Ala His Glu Ala Val
 980 985 990
 Glu Lys Glu Thr Thr Glu Lys Glu Ala Thr Glu Lys Glu Ala Glu Ile
 995 1000 1005
 Val Glu Ala Asp Lys Glu Lys Glu Leu Arg Asn His Gln Pro Arg Glu
 1010 1015 1020
 Pro His Cys Asp Leu Glu Thr Ser Gly Thr Val Thr Val Gly Pro Met
 1025 1030 1035 1040
 His Thr Leu Pro Ser Thr Cys Leu Gln Lys Val Glu Glu Gln Pro Glu
 1045 1050 1055
 Asp Ala Asp Asn Gln Arg Asn Val Thr Arg Met Gly Ser Gln Pro Pro
 1060 1065 1070
 Asp Pro Asn Thr Ile Val His Ile Pro Val Met Leu Thr Gly Pro Leu
 1075 1080 1085
 Gly Glu Ala Thr Val Val Pro Ser Gly Asn Val Asp Leu Glu Ser Gln
 1090 1095 1100
 Ala Glu Gly Lys Lys Glu Val Glu Ala Asp Asp Val Met Arg Ser Gly
 1105 1110 1115 1120
 Pro Arg Pro Ile Val Pro Tyr Ser Ser Met Phe Cys Leu Ser Pro Thr
 1125 1130 1135
 Asn Leu Leu Arg Arg Phe Cys His Tyr Ile Val Thr Met Arg Tyr Phe
 1140 1145 1150
 Glu Val Val Ile Leu Val Val Ile Ala Leu Ser Ser Ile Ala Leu Ala
 1155 1160 1165
 Ala Glu Asp Pro Val Arg Thr Asp Ser Pro Arg Asn Asn Ala Leu Lys
 1170 1175 1180
 Tyr Leu Asp Tyr Ile Phe Thr Gly Val Phe Thr Phe Glu Met Val Ile
 1185 1190 1195 1200
 Lys Met Ile Asp Leu Gly Leu Leu Leu His Pro Gly Ala Tyr Phe Arg
 1205 1210 1215
 Asp Leu Trp Asn Ile Leu Asp Phe Ile Val Val Ser Gly Ala Leu Val
 1220 1225 1230
 Ala Phe Ala Phe Ser Gly Ser Lys Gly Lys Asp Ile Asn Thr Ile Lys

1235	1240	1245
Ser Leu Arg Val Leu Arg Val Leu Arg Pro Leu Lys Thr Ile Lys Arg		
1250	1255	1260
Leu Pro Lys Leu Lys Ala Val Phe Asp Cys Val Val Asn Ser Leu Lys		
1265	1270	1275
Asn Val Leu Asn Ile Leu Ile Val Tyr Met Leu Phe Met Phe Ile Phe		
1285	1290	1295
Ala Val Ile Ala Val Gln Leu Phe Lys Gly Lys Phe Phe Tyr Cys Thr		
1300	1305	1310
Asp Glu Ser Lys Glu Leu Glu Arg Asp Cys Arg Gly Gln Tyr Leu Asp		
1315	1320	1325
Tyr Glu Lys Glu Glu Val Glu Ala Gln Pro Arg Gln Trp Lys Lys Tyr		
1330	1335	1340
Asp Phe His Tyr Asp Asn Val Leu Trp Ala Leu Leu Thr Leu Phe Thr		
1345	1350	1355
Val Ser Thr Gly Glu Gly Trp Pro Met Val Leu Lys His Ser Val Asp		
1365	1370	1375
Ala Thr Tyr Glu Glu Gln Gly Pro Ser Pro Gly Tyr Arg Met Glu Leu		
1380	1385	1390
Ser Ile Phe Tyr Val Val Tyr Phe Val Val Phe Pro Phe Phe Phe Val		
1395	1400	1405
Asn Ile Phe Val Ala Leu Ile Ile Ile Thr Phe Gln Glu Gln Gly Asp		
1410	1415	1420
Lys Val Met Ser Glu Cys Ser Leu Glu Lys Asn Glu Arg Ala Cys Ile		
1425	1430	1435
Asp Phe Ala Ile Ser Ala Lys Pro Leu Thr Arg Tyr Met Pro Gln Asn		
1445	1450	1455
Arg Gln Ser Phe Gln Tyr Lys Thr Trp Thr Phe Val Val Ser Pro Pro		
1460	1465	1470
Phe Glu Tyr Phe Ile Met Ala Met Ile Ala Leu Asn Thr Val Val Leu		
1475	1480	1485
Met Met Lys Phe Tyr Asp Ala Pro Tyr Glu Tyr Glu Leu Met Leu Lys		
1490	1495	1500
Cys Leu Asn Ile Val Phe Thr Ser Met Phe Ser Met Glu Cys Val Leu		
1505	1510	1515
Lys Ile Ile Ala Phe Gly Val Leu Asn Tyr Phe Arg Asp Ala Trp Asn		
1525	1530	1535
Val Phe Asp Phe Val Thr Val Leu Gly Ser Ile Thr Asp Ile Leu Val		
1540	1545	1550
Thr Glu Ile Ala Glu Thr Asn Asn Phe Ile Asn Leu Ser Phe Leu Arg		
1555	1560	1565
Leu Phe Arg Ala Ala Arg Leu Ile Lys Leu Leu Arg Gln Gly Tyr Thr		
1570	1575	1580
Ile Arg Ile Leu Leu Trp Thr Phe Val Gln Ser Phe Lys Ala Leu Pro		
1585	1590	1595
Tyr Val Cys Leu Leu Ile Ala Met Leu Phe Phe Ile Tyr Ala Ile Ile		
1605	1610	1615
Gly Met Gln Val Phe Gly Asn Ile Ala Leu Asp Asp Asp Thr Ser Ile		
1620	1625	1630
Asn Arg His Asn Asn Phe Arg Thr Phe Leu Gln Ala Leu Met Leu Leu		
1635	1640	1645
Phe Arg Ser Ala Thr Gly Glu Ala Trp His Glu Ile Met Leu Ser Cys		
1650	1655	1660
Leu Ser Asn Gln Ala Cys Asp Glu Gln Ala Asn Ala Thr Glu Cys Gly		
1665	1670	1675
Ser Asp Phe Ala Tyr Phe Tyr Phe Val Ser Phe Ile Phe Leu Cys Ser		
1685	1690	1695
Phe Leu Met Leu Asn Leu Phe Val Ala Val Ile Met Asp Asn Phe Glu		
1700	1705	1710
Tyr Leu Thr Arg Asp Ser Ser Ile Leu Gly Pro His His Leu Asp Glu		
1715	1720	1725

Phe Ile Arg Val Trp Ala Glu Tyr Asp Pro Ala Ala Cys Gly Arg Ile
 1730 1735 1740
 Ser Tyr Asn Asp Met Phe Glu Met Leu Lys His Met Ser Pro Pro Leu
 1745 1750 1755 1760
 Gly Leu Gly Lys Lys Cys Pro Ala Arg Val Ala Tyr Lys Arg Leu Val
 1765 1770 1775
 Arg Met Asn Met Pro Ile Ser Asn Glu Asp Met Thr Val His Phe Thr
 1780 1785 1790
 Ser Thr Leu Met Ala Leu Ile Arg Thr Ala Leu Glu Ile Lys Leu Ala
 1795 1800 1805
 Pro Ala Gly Thr Lys Gln His Gln Cys Asp Ala Glu Leu Arg Lys Glu
 1810 1815 1820
 Ile Ser Val Val Trp Ala Asn Leu Pro Gln Lys Thr Leu Asp Leu Leu
 1825 1830 1835 1840
 Val Pro Pro His Lys Pro Asp Glu Met Thr Val Gly Lys Val Tyr Ala
 1845 1850 1855
 Ala Leu Met Ile Phe Asp Phe Tyr Lys Gln Asn Lys Thr Thr Arg Asp
 1860 1865 1870
 Gln Met Gln Gln Ala Pro Gly Gly Leu Ser Gln Met Gly Pro Val Ser
 1875 1880 1885
 Leu Phe His Pro Leu Lys Ala Thr Leu Glu Gln Thr Gln Pro Ala Val
 1890 1895 1900
 Leu Arg Gly Ala Arg Val Phe Leu Arg Gln Lys Ser Ser Thr Ser Leu
 1905 1910 1915 1920
 Ser Asn Gly Gly Ala Ile Gln Asn Gln Glu Ser Gly Ile Lys Glu Ser
 1925 1930 1935
 Val Ser Trp Gly Thr Gln Arg Thr Gln Asp Ala Pro His Glu Ala Arg
 1940 1945 1950
 Pro Pro Leu Glu Arg Gly His Ser Thr Glu Ile Pro Val Gly Arg Ser
 1955 1960 1965
 Gly Ala Leu Ala Val Asp Val Gln Met Gln Ser Ile Thr Arg Arg Gly
 1970 1975 1980
 Pro Asp Gly Glu Pro Gln Pro Gly Leu Glu Ser Gln Gly Arg Ala Ala
 1985 1990 1995 2000
 Ser Met Pro Arg Leu Ala Ala Glu Thr Gln Pro Val Thr Asp Ala Ser
 2005 2010 2015
 Pro Met Lys Arg Ser Ile Ser Thr Leu Ala Gln Arg Pro Arg Gly Thr
 2020 2025 2030
 His Leu Cys Ser Thr Thr Pro Asp Arg Pro Pro Pro Ser Gln Ala Ser
 2035 2040 2045
 Ser His His His His Arg Cys His Arg Arg Arg Asp Arg Lys Gln
 2050 2055 2060
 Arg Ser Leu Glu Lys Gly Pro Ser Leu Ser Ala Asp Met Asp Gly Ala
 2065 2070 2075 2080
 Pro Ser Ser Ala Val Gly Pro Gly Leu Pro Pro Gly Glu Gly Pro Thr
 2085 2090 2095
 Gly Cys Arg Arg Glu Arg Glu Arg Arg Gln Glu Arg Gly Arg Ser Gln
 2100 2105 2110
 Glu Arg Arg Gln Pro Ser Ser Ser Ser Ser Glu Lys Gln Arg Phe Tyr
 2115 2120 2125
 Ser Cys Asp Arg Phe Gly Gly Arg Glu Pro Pro Lys Pro Lys Pro Ser
 2130 2135 2140
 Leu Ser Ser His Pro Thr Ser Pro Thr Ala Gly Gln Glu Pro Gly Pro
 2145 2150 2155 2160
 His Pro Gln Gly Ser Gly Ser Val Asn Gly Ser Pro Leu Leu Ser Thr
 2165 2170 2175
 Ser Gly Ala Ser Thr Pro Gly Arg Gly Gly Arg Arg Gln Leu Pro Gln
 2180 2185 2190
 Thr Pro Leu Thr Pro Arg Pro Ser Ile Thr Tyr Lys Thr Ala Asn Ser
 2195 2200 2205
 Ser Pro Ile His Phe Ala Gly Ala Gln Thr Ser Leu Pro Ala Phe Ser

105	110	115	
gat ggg gac aag act ccc atg tct gaa cga ctg gat gac acg gaa cct Asp Gly Asp Lys Thr Pro Met Ser Glu Arg Leu Asp Asp Thr Glu Pro 120 125 130 135			496
tac ttc atc ggc atc ttt tgc ttc gag gcg ggc atc aag atc ata gct Tyr Phe Ile Gly Ile Phe Cys Phe Glu Ala Gly Ile Lys Ile Ile Ala 140 145 150			544
ctg ggc ttc gtg ttc cac aaa ggc tcc tac ctc cgg aat ggc tgg aac Leu Gly Phe Val Phe His Lys Gly Ser Tyr Leu Arg Asn Gly Trp Asn 155 160 165			592
gtc atg gac ttc gtg gtg gtc ctc aca ggg att ctt gcc aca gct gga Val Met Asp Phe Val Val Val Leu Thr Gly Ile Leu Ala Thr Ala Gly 170 175 180			640
act gac ttt gat ctg cgc acc ctg agg gct gtg cgt gtg ctt agg ccc Thr Asp Phe Asp Leu Arg Thr Leu Arg Ala Val Arg Val Leu Arg Pro 185 190 195			688
ctg aag ttg gtg tct gga att cca agc ttg cag gtg gtg ctc aag tcc Leu Lys Leu Val Ser Gly Ile Pro Ser Leu Gln Val Val Leu Lys Ser 200 205 210 215			736
atc atg aag gcc atg gtc ccg ctg ctg cag atc ggg ctg ctg ctc ttc Ile Met Lys Ala Met Val Pro Leu Leu Gln Ile Gly Leu Leu Leu Phe 220 225 230			784
ttc gcc atc ctc atg ttc gct atc atc ggc ctc gag ttc tat atg ggc Phe Ala Ile Leu Met Phe Ala Ile Ile Gly Leu Glu Phe Tyr Met Gly 235 240 245			832
aaa ttc cat aag gcc tgc ttc ccc aac agc aca gat gca gag cct gtg Lys Phe His Lys Ala Cys Phe Pro Asn Ser Thr Asp Ala Glu Pro Val 250 255 260			880
ggt gac ttt cct tgt ggc aag gag gcc cct gct cgt ctg tgt gac agt Gly Asp Phe Pro Cys Gly Lys Glu Ala Pro Ala Arg Leu Cys Asp Ser 265 270 275			928
gac acc gaa tgc cgg gag tac tgg cca gga ccc aac ttt ggc atc acc Asp Thr Glu Cys Arg Glu Tyr Trp Pro Gly Pro Asn Phe Gly Ile Thr 280 285 290 295			976
aat ttt gac aac atc ctg ttt gcc atc ttg acc gtg ttc cag tgt atc Asn Phe Asp Asn Ile Leu Phe Ala Ile Leu Thr Val Phe Gln Cys Ile 300 305 310			1024
acc atg gag ggc tgg act gac atc ctc tac aat aca aat gat gcg gcc Thr Met Glu Gly Trp Thr Asp Ile Leu Tyr Asn Thr Asn Asp Ala Ala 315 320 325			1072
ggc aac acg tgg aac tgg ttg tac ttc atc ccc ctc atc atc att ggc Gly Asn Thr Trp Asn Trp Leu Tyr Phe Ile Pro Leu Ile Ile Ile Gly 330 335 340			1120
tcc ttc ttc atg ctc aac ctg gtg ctc ggt gtg ctt tca gga gag ttt Ser Phe Phe Met Leu Asn Leu Val Leu Gly Val Leu Ser Gly Glu Phe 345 350 355			1168

gcc	aaa	gag	cgg	gag	cga	gtc	gag	aac	cgc	cgt	gcc	ttc	ctg	aag	ctc	1216
Ala	Lys	Glu	Arg	Glu	Arg	Val	Glu	Asn	Arg	Arg	Ala	Phe	Leu	Lys	Leu	
360					365				370						375	
cgc	agg	cag	cag	cag	att	gag	cga	gaa	ctg	aat	ggg	tac	ttg	gag	tgg	1264
Arg	Arg	Gln	Gln	Gln	Ile	Glu	Arg	Glu	Leu	Asn	Gly	Tyr	Leu	Glu	Trp	
			380					385					390			
atc	ttc	aag	gcg	gag	gaa	gtc	atg	ttg	gca	gag	gag	gac	aag	aac	gca	1312
Ile	Phe	Lys	Ala	Glu	Glu	Val	Met	Leu	Ala	Glu	Glu	Asp	Lys	Asn	Ala	
			395					400					405			
gaa	gag	aag	tcc	cct	ttg	gat	gtg	ttg	aag	aga	gct	gct	acc	aag	aag	1360
Glu	Glu	Lys	Ser	Pro	Leu	Asp	Val	Leu	Lys	Arg	Ala	Ala	Thr	Lys	Lys	
		410					415					420				
agc	cga	aat	gac	ctc	atc	cat	gca	gaa	gag	ggg	gag	gac	cgg	ttt	gta	1408
Ser	Arg	Asn	Asp	Leu	Ile	His	Ala	Glu	Glu	Gly	Glu	Asp	Arg	Phe	Val	
	425					430				435						
gac	ctc	tgt	gct	gct	ggg	tct	ccc	ttt	gct	cgt	gcc	agc	ctc	aag	agt	1456
Asp	Leu	Cys	Ala	Ala	Gly	Ser	Pro	Phe	Ala	Arg	Ala	Ser	Leu	Lys	Ser	
440					445					450					455	
ggg	aag	aca	gag	agc	tca	tcg	tac	ttc	cgg	agg	aag	gag	aag	atg	ttc	1504
Gly	Lys	Thr	Glu	Ser	Ser	Ser	Tyr	Phe	Arg	Arg	Lys	Glu	Lys	Met	Phe	
			460						465					470		
cgg	ttc	ctt	atc	cgt	cgt	atg	gtg	aaa	gca	cag	agc	ttc	tac	tgg	gtg	1552
Arg	Phe	Leu	Ile	Arg	Arg	Met	Val	Lys	Ala	Gln	Ser	Phe	Tyr	Trp	Val	
		475						480					485			
gta	ctg	tgc	gtg	gtg	gcc	ctg	aac	acg	ttg	tgt	gtg	gcc	atg	gta	cac	1600
Val	Leu	Cys	Val	Val	Ala	Leu	Asn	Thr	Leu	Cys	Val	Ala	Met	Val	His	
		490					495					500				
tat	aat	cag	cct	cag	cgg	ctt	acc	act	gca	ctg	tac	ttt	gca	gag	ttt	1648
Tyr	Asn	Gln	Pro	Gln	Arg	Leu	Thr	Thr	Ala	Leu	Tyr	Phe	Ala	Glu	Phe	
	505					510					515					
gtt	ttc	ctg	ggg	ctc	ttc	ctc	aca	gag	atg	tcc	ctg	aag	atg	tac	ggg	1696
Val	Phe	Leu	Gly	Leu	Phe	Leu	Thr	Glu	Met	Ser	Leu	Lys	Met	Tyr	Gly	
520					525					530					535	
cta	ggg	ccc	aga	agc	tac	ttc	cgg	tct	tcc	ttc	aac	tgc	ttt	gac	ttt	1744
Leu	Gly	Pro	Arg	Ser	Tyr	Phe	Arg	Ser	Ser	Phe	Asn	Cys	Phe	Asp	Phe	
				540					545					550		
ggg	gtg	att	gtg	ggg	agt	atc	ttt	gaa	gta	gtc	tgg	gct	gcc	atc	aag	1792
Gly	Val	Ile	Val	Gly	Ser	Ile	Phe	Glu	Val	Val	Trp	Ala	Ala	Ile	Lys	
			555					560					565			
cca	gga	acc	tcc	ttc	gga	atc	agt	gtg	ctg	cgg	gct	ctc	cga	ctg	ctg	1840
Pro	Gly	Thr	Ser	Phe	Gly	Ile	Ser	Val	Leu	Arg	Ala	Leu	Arg	Leu	Leu	
		570					575					580				
agg	att	ttc	aaa	gtc	acc	aag	tat	tgg	aac	tcc	ctg	agg	aac	ctg	gtt	1888
Arg	Ile	Phe	Lys	Val	Thr	Lys	Tyr	Trp	Asn	Ser	Leu	Arg	Asn	Leu	Val	
	585					590					595					

gtt tcc ctc ctc aac tcc atg aag tcc atc atc agc ctt ctc ttc ctg	1936
Val Ser Leu Leu Asn Ser Met Lys Ser Ile Ile Ser Leu Leu Phe Leu	
600 605 610 615	
ctt ttc ctt ttc att gtg gtc ttc gct ctg ttg ggg atg cag ctg ttt	1984
Leu Phe Leu Phe Ile Val Val Phe Ala Leu Leu Gly Met Gln Leu Phe	
620 625 630	
ggg gga cag ttc aac ttt caa gat gag act cca acc acc aat ttt gat	2032
Gly Gly Gln Phe Asn Phe Gln Asp Glu Thr Pro Thr Thr Asn Phe Asp	
635 640 645	
acc ttc cca gct gcc atc ctc act gtg ttt cag att ctg aca gga gag	2080
Thr Phe Pro Ala Ala Ile Leu Thr Val Phe Gln Ile Leu Thr Gly Glu	
650 655 660	
gac tgg aat gca gtc atg tat cat ggg att gag tca caa gga gga gtc	2128
Asp Trp Asn Ala Val Met Tyr His Gly Ile Glu Ser Gln Gly Gly Val	
665 670 675	
agc aaa ggc atg ttt tca tcc ttt tac ttc atc gtc ctg aca ctg ttt	2176
Ser Lys Gly Met Phe Ser Ser Phe Tyr Phe Ile Val Leu Thr Leu Phe	
680 685 690 695	
gga aac tac acc ctg ttg aac gtt ttc ttg gcc att gct gtg gac aac	2224
Gly Asn Tyr Thr Leu Leu Asn Val Phe Leu Ala Ile Ala Val Asp Asn	
700 705 710	
ctt gcc aat gcc cag gag ttg acc aag gat gaa gag gag atg gaa gag	2272
Leu Ala Asn Ala Gln Glu Leu Thr Lys Asp Glu Glu Glu Met Glu Glu	
715 720 725	
gca gcc aat cag aag ctt gct ctt cag aag gcc aaa gaa gta gct gaa	2320
Ala Ala Asn Gln Lys Leu Ala Leu Gln Lys Ala Lys Glu Val Ala Glu	
730 735 740	
gtc agc ccc atg tct gct gcc aac atc tcc att gct gcc agg cag cag	2368
Val Ser Pro Met Ser Ala Ala Asn Ile Ser Ile Ala Ala Arg Gln Gln	
745 750 755	
aac tcg gcc aag gcg cgc tca gta tgg gag cag cgg gcc agt cag cta	2416
Asn Ser Ala Lys Ala Arg Ser Val Trp Glu Gln Arg Ala Ser Gln Leu	
760 765 770 775	
agg ctc cag aac ctg cgt gcc agc tgt gag gca ctg tac agt gag atg	2464
Arg Leu Gln Asn Leu Arg Ala Ser Cys Glu Ala Leu Tyr Ser Glu Met	
780 785 790	
gac ccg gag gag cgc ctg cgt tat gcc agc acg cgc cac gtg agg cca	2512
Asp Pro Glu Glu Arg Leu Arg Tyr Ala Ser Thr Arg His Val Arg Pro	
795 800 805	
gac atg aag aca cac atg gac cga ccc cta gtg gtg gaa cct ggt cgg	2560
Asp Met Lys Thr His Met Asp Arg Pro Leu Val Val Glu Pro Gly Arg	
810 815 820	
gat ggc ctg cgg gga ccc gcc ggg aac aag tca aag cct gag ggc acg	2608
Asp Gly Leu Arg Gly Pro Ala Gly Asn Lys Ser Lys Pro Glu Gly Thr	
825 830 835	
gag gcc acc gaa ggt gcg gat cca cca cgc cga cac cac cgg cat cgt	2656

Glu 840	Ala	Thr	Glu	Gly	Ala 845	Asp	Pro	Pro	Arg	Arg 850	His	His	Arg	His	Arg 855	
gat	agg	gac	aag	acc	tca	gcc	tca	acc	cct	gct	gga	ggc	gaa	cag	gac	2704
Asp	Arg	Asp	Lys	Thr 860	Ser	Ala	Ser	Thr	Pro	Ala	Gly	Gly	Glu	Gln	Asp	
									865					870		
agg	aca	gac	tgc	cca	aag	gcc	gaa	agc	acc	gag	acc	ggg	gcc	cgg	gag	2752
Arg	Thr	Asp	Cys	Pro	Lys	Ala	Glu	Ser	Thr	Glu	Thr	Gly	Ala	Arg	Glu	
			875					880					885			
gaa	cgt	gcg	cgc	cct	cgt	cga	agt	cac	agc	aag	gag	gct	cca	ggg	gct	2800
Glu	Arg	Ala	Arg	Pro	Arg	Arg	Ser	His	Ser	Lys	Glu	Ala	Pro	Gly	Ala	
		890					895					900				
gac	aca	caa	gtg	cgt	tgt	gag	cgc	agt	aga	cgt	cac	cac	cgg	cgc	gga	2848
Asp	Thr	Gln	Val	Arg	Cys	Glu	Arg	Ser	Arg	Arg	His	His	Arg	Arg	Gly	
	905					910					915					
tcc	ccg	gag	gag	gcc	act	gaa	cgg	gaa	cct	cgg	cgc	cac	cgt	gcc	cac	2896
Ser	Pro	Glu	Glu	Ala	Thr	Glu	Arg	Glu	Pro	Arg	Arg	His	Arg	Ala	His	
920					925				930						935	
cgg	cac	gca	cag	gac	tca	agc	aag	gaa	ggc	aag	gag	ggc	act	gca	ccg	2944
Arg	His	Ala	Gln	Asp	Ser	Ser	Lys	Glu	Gly	Lys	Glu	Gly	Thr	Ala	Pro	
				940					945					950		
gtg	ctt	gta	ccc	aag	ggc	gag	cgt	cgc	gca	aga	cat	cga	ggc	ccg	cgt	2992
Val	Leu	Val	Pro	Lys	Gly	Glu	Arg	Arg	Ala	Arg	His	Arg	Gly	Pro	Arg	
			955				960						965			
acg	ggc	ccc	cgt	gag	aca	gag	aac	agt	gag	gag	ccc	aca	cgc	agg	cac	3040
Thr	Gly	Pro	Arg	Glu	Thr	Glu	Asn	Ser	Glu	Glu	Pro	Thr	Arg	Arg	His	
		970					975					980				
cgt	gca	aag	cat	aag	gtg	cca	cca	aca	ctt	gag	ccc	cca	gag	agg	gag	3088
Arg	Ala	Lys	His	Lys	Val	Pro	Pro	Thr	Leu	Glu	Pro	Pro	Glu	Arg	Glu	
	985					990				995						
gtt	gca	gag	aag	gag	agc	aac	gtg	gtg	gaa	ggg	gat	aag	gaa	act	cga	3136
Val	Ala	Glu	Lys	Glu	Ser	Asn	Val	Val	Glu	Gly	Asp	Lys	Glu	Thr	Arg	
1000				1005					1010					1015		
aat	cac	cag	ccc	aag	gaa	cct	cgc	tgt	gac	ctg	gag	gcc	att	gcg	gtt	3184
Asn	His	Gln	Pro	Lys	Glu	Pro	Arg	Cys	Asp	Leu	Glu	Ala	Ile	Ala	Val	
			1020					1025				1030				
aca	ggc	gtg	ggc	tct	ctg	cac	atg	ctg	ccc	agc	acc	tgt	ctc	cag	aaa	3232
Thr	Gly	Val	Gly	Ser	Leu	His	Met	Leu	Pro	Ser	Thr	Cys	Leu	Gln	Lys	
		1035					1040					1045				
gtg	gac	gaa	cag	cca	gag	gat	gca	gac	aac	cag	cgt	aat	gtc	acc	cgg	3280
Val	Asp	Glu	Gln	Pro	Glu	Asp	Ala	Asp	Asn	Gln	Arg	Asn	Val	Thr	Arg	
	1050					1055				1060						
atg	ggc	agt	cag	ccc	tca	gac	ccc	agc	acc	act	gtg	cat	gtc	cca	gtg	3328
Met	Gly	Ser	Gln	Pro	Ser	Asp	Pro	Ser	Thr	Thr	Val	His	Val	Pro	Val	
	1065				1070				1075							
aca	ctg	aca	ggc	cct	ccc	ggg	gag	gcc	act	gta	gtt	ccc	agt	gct	aac	3376
Thr	Leu	Thr	Gly	Pro	Pro	Gly	Glu	Ala	Thr	Val	Val	Pro	Ser	Ala	Asn	

1080	1085	1090	1095	
acg gac ctg gaa ggc caa gcg gag ggc aag aag gag gca gag gct gac				3424
Thr Asp Leu Glu Gly Gln Ala Glu Gly Lys Lys Glu Ala Glu Ala Asp				
1100		1105	1110	
gat gtg ctg aga aga ggc ccc agg ccc atc gtt ccc tac agt tcc atg				3472
Asp Val Leu Arg Arg Gly Pro Arg Pro Ile Val Pro Tyr Ser Ser Met				
1115		1120	1125	
ttc tgc ctc agc ccc acc aac cta ctc cgt cgc ttc tgc cat tac att				3520
Phe Cys Leu Ser Pro Thr Asn Leu Leu Arg Arg Phe Cys His Tyr Ile				
1130		1135	1140	
gtg acc atg cgg tac ttt gag atg gtg att ctt gtg gtc atc gcc ttg				3568
Val Thr Met Arg Tyr Phe Glu Met Val Ile Leu Val Val Ile Ala Leu				
1145		1150	1155	
agc agc att gcc ctg gct gct gag gat ccc gtg cgg acc gac tca ttc				3616
Ser Ser Ile Ala Leu Ala Ala Glu Asp Pro Val Arg Thr Asp Ser Phe				
1160		1165	1170	1175
cgg aac aat gct ctg aag tac atg gac tac atc ttt aca gga gtc ttc				3664
Arg Asn Asn Ala Leu Lys Tyr Met Asp Tyr Ile Phe Thr Gly Val Phe				
1180		1185	1190	
acc ttt gag atg gtc ata aag atg ata gac ttg ggc ctg ctg ctg cac				3712
Thr Phe Glu Met Val Ile Lys Met Ile Asp Leu Gly Leu Leu Leu His				
1195		1200	1205	
cct ggg gcc tac ttc cgg gac ctg tgg aac att ctg gac ttc att gtt				3760
Pro Gly Ala Tyr Phe Arg Asp Leu Trp Asn Ile Leu Asp Phe Ile Val				
1210		1215	1220	
gtc agt gga gcc ctg gtg gca ttt gca ttc tca gga tcc aaa ggg aaa				3808
Val Ser Gly Ala Leu Val Ala Phe Ala Phe Ser Gly Ser Lys Gly Lys				
1225		1230	1235	
gac atc aat acc atc aag tct ctg aga gtc ctg cga gtc ctg cgg ccc				3856
Asp Ile Asn Thr Ile Lys Ser Leu Arg Val Leu Arg Val Leu Arg Pro				
1240		1245	1250	1255
ctc aag acc atc aag cgg ctg cct aaa ctc aag gct gtg ttt gac tgt				3904
Leu Lys Thr Ile Lys Arg Leu Pro Lys Leu Lys Ala Val Phe Asp Cys				
1260		1265	1270	
gtg gtg aac tct ctg aag aat gtc ttg aac atc ctg atc gtc tac atg				3952
Val Val Asn Ser Leu Lys Asn Val Leu Asn Ile Leu Ile Val Tyr Met				
1275		1280	1285	
ctc ttc atg ttt ata ttt gcc gtc atc gcc gtc caa ctc ttc aaa ggg				4000
Leu Phe Met Phe Ile Phe Ala Val Ile Ala Val Gln Leu Phe Lys Gly				
1290		1295	1300	
aag ttc ttt tac tgc act gat gag tcc aag gag ctg gag cgg gac tgc				4048
Lys Phe Phe Tyr Cys Thr Asp Glu Ser Lys Glu Leu Glu Arg Asp Cys				
1305		1310	1315	
agg ggt cag tat ttg gat tat gag aag gaa gag gta gaa gcc cag cca				4096
Arg Gly Gln Tyr Leu Asp Tyr Glu Lys Glu Glu Val Glu Ala Gln Pro				
1320		1325	1330	1335

agg cag tgg aag aaa tat gac ttc cac tat gac aat gtg ctc tgg gcc	4144
Arg Gln Trp Lys Lys Tyr Asp Phe His Tyr Asp Asn Val Leu Trp Ala	
1340 1345 1350	
ttg ctg act ctg ttt acg gtg tcc aca gga gag ggg tgg ccc atg gtg	4192
Leu Leu Thr Leu Phe Thr Val Ser Thr Gly Glu Gly Trp Pro Met Val	
1355 1360 1365	
ctg aaa cac tct gtg gac gcc acc tat gag gag cag ggg cca agc ccc	4240
Leu Lys His Ser Val Asp Ala Thr Tyr Glu Glu Gln Gly Pro Ser Pro	
1370 1375 1380	
ggg ttt cgg atg gag ctt tcc atc ttc tat gtg gtc tac ttt gtg gtc	4288
Gly Phe Arg Met Glu Leu Ser Ile Phe Tyr Val Val Tyr Phe Val Val	
1385 1390 1395	
ttc cct ttt ttc ttt gtc aac atc ttt gtg gcc ttg atc atc atc acc	4336
Phe Pro Phe Phe Phe Val Asn Ile Phe Val Ala Leu Ile Ile Ile Thr	
1400 1405 1410 1415	
ttc cag gag cag ggg gac aag gtg atg tct gag tgc agt ctg gaa aag	4384
Phe Gln Glu Gln Gly Asp Lys Val Met Ser Glu Cys Ser Leu Glu Lys	
1420 1425 1430	
aat gag agg gct tgc att gac ttt gcc atc agc gcc aaa ccc ctg aca	4432
Asn Glu Arg Ala Cys Ile Asp Phe Ala Ile Ser Ala Lys Pro Leu Thr	
1435 1440 1445	
cgg tac atg cct cag aac aag cag tgc ttc cag tat aag aca tgg aca	4480
Arg Tyr Met Pro Gln Asn Lys Gln Ser Phe Gln Tyr Lys Thr Trp Thr	
1450 1455 1460	
ttt gtg gtc tct cca ccc ttt gag tac ttc att atg gcc atg ata gcc	4528
Phe Val Val Ser Pro Pro Phe Glu Tyr Phe Ile Met Ala Met Ile Ala	
1465 1470 1475	
ctc aac aca gtg gtg ctg atg atg aag ttc tac gat gcc cct tat gag	4576
Leu Asn Thr Val Val Leu Met Met Lys Phe Tyr Asp Ala Pro Tyr Glu	
1480 1485 1490 1495	
tac gag ctg atg ctg aag tgc ttg aac atc gtc ttc aca tcc atg ttc	4624
Tyr Glu Leu Met Leu Lys Cys Leu Asn Ile Val Phe Thr Ser Met Phe	
1500 1505 1510	
tct ctg gag tgc atc ctg aag atc atc gcc ttc ggg gtg ttg aac tac	4672
Ser Leu Glu Cys Ile Leu Lys Ile Ile Ala Phe Gly Val Leu Asn Tyr	
1515 1520 1525	
ttc aga gat gcc tgg aac gtc ttt gac ttt gtc act gtt ttg gga agt	4720
Phe Arg Asp Ala Trp Asn Val Phe Asp Phe Val Thr Val Leu Gly Ser	
1530 1535 1540	
att act gat att tta gta acg gag att gcg gaa acg aac aac ttc atc	4768
Ile Thr Asp Ile Leu Val Thr Glu Ile Ala Glu Thr Asn Asn Phe Ile	
1545 1550 1555	
aac ttg agc ttc ctt cgc ctc ttc cgg gca gca cgg ctg atc aag ctg	4816
Asn Leu Ser Phe Leu Arg Leu Phe Arg Ala Ala Arg Leu Ile Lys Leu	
1560 1565 1570 1575	

ctt cgc cag ggc tac acc atc cgc atc ttg tta tgg acc ttt gtc cag	4864
Leu Arg Gln Gly Tyr Thr Ile Arg Ile Leu Leu Trp Thr Phe Val Gln	
1580 1585 1590	
tcc ttt aag gcg ctg ccc tac gtg tgc ctc ctc att gcc atg ctg ttc	4912
Ser Phe Lys Ala Leu Pro Tyr Val Cys Leu Leu Ile Ala Met Leu Phe	
1595 1600 1605	
ttc atc tac gcc atc atc ggc atg cag gtt ttt gga aac att gcc ctt	4960
Phe Ile Tyr Ala Ile Ile Gly Met Gln Val Phe Gly Asn Ile Ala Leu	
1610 1615 1620	
gat gat ggc acc agc atc aac cga cac aac aac ttc cgg aca ttt ctg	5008
Asp Asp Gly Thr Ser Ile Asn Arg His Asn Asn Phe Arg Thr Phe Leu	
1625 1630 1635	
caa gcc tta atg ctg ttg ttc agg agt gcc act ggg gag gcc tgg cac	5056
Gln Ala Leu Met Leu Leu Phe Arg Ser Ala Thr Gly Glu Ala Trp His	
1640 1645 1650 1655	
gaa atc atg ctg tct tgc ctg ggc aac cgg gcc tgc gac cca cat gcc	5104
Glu Ile Met Leu Ser Cys Leu Gly Asn Arg Ala Cys Asp Pro His Ala	
1660 1665 1670	
aac gcc agc gaa tgc ggg agc gac ttt gcc tat ttt tat ttt gtc tcc	5152
Asn Ala Ser Glu Cys Gly Ser Asp Phe Ala Tyr Phe Tyr Phe Val Ser	
1675 1680 1685	
ttc atc ttc ctc tgt tcc ttt ctg atg ctg aac ctc ttt gtt gct gtg	5200
Phe Ile Phe Leu Cys Ser Phe Leu Met Leu Asn Leu Phe Val Ala Val	
1690 1695 1700	
atc atg gac aat ttc gaa tac ctc acg cgg gat tct tcc atc cta ggg	5248
Ile Met Asp Asn Phe Glu Tyr Leu Thr Arg Asp Ser Ser Ile Leu Gly	
1705 1710 1715	
ccg cac cac ctc gat gaa ttc att cgc gtc tgg gct gaa tac gac cca	5296
Pro His His Leu Asp Glu Phe Ile Arg Val Trp Ala Glu Tyr Asp Pro	
1720 1725 1730 1735	
gct gcg tgt ggg cgc atc agt tac aat gac atg ttt gag atg ctg aaa	5344
Ala Ala Cys Gly Arg Ile Ser Tyr Asn Asp Met Phe Glu Met Leu Lys	
1740 1745 1750	
cac atg tcc cca cct ctg ggt ttg ggg aag aaa tgc ccg gct cga gtt	5392
His Met Ser Pro Pro Leu Gly Leu Gly Lys Lys Cys Pro Ala Arg Val	
1755 1760 1765	
gca tac aag cgc ctg gtt cga atg aac atg ccc ata tcc aat gag gac	5440
Ala Tyr Lys Arg Leu Val Arg Met Asn Met Pro Ile Ser Asn Glu Asp	
1770 1775 1780	
atg acg gta cac ttt aca tcc aca ctg atg gcc ctc atc cgg acg gca	5488
Met Thr Val His Phe Thr Ser Thr Leu Met Ala Leu Ile Arg Thr Ala	
1785 1790 1795	
ctg gag atc aag ctt gcc cca gcg ggg aca aaa cag cac caa tgt gat	5536
Leu Glu Ile Lys Leu Ala Pro Ala Gly Thr Lys Gln His Gln Cys Asp	
1800 1805 1810 1815	
gct gag ctg agg aag gag atc tct tct gtg tgg gct aat ctg ccc cag	5584

Ala Glu Leu Arg Lys Glu Ile Ser Ser Val Trp Ala Asn Leu Pro Gln	
1820 1825 1830	
aag act ctg gac tta ctg gtg cca ccc cac aaa cct gac gag atg aca	5632
Lys Thr Leu Asp Leu Leu Val Pro Pro His Lys Pro Asp Glu Met Thr	
1835 1840 1845	
gtg ggg aag gtc tat gcg gct ctc atg ata ttt gac ttc tac aaa cag	5680
Val Gly Lys Val Tyr Ala Ala Leu Met Ile Phe Asp Phe Tyr Lys Gln	
1850 1855 1860	
aac aaa acc acc aga gat cag act cac caa gct cct gga ggc ctg tcc	5728
Asn Lys Thr Thr Arg Asp Gln Thr His Gln Ala Pro Gly Gly Leu Ser	
1865 1870 1875	
cag atg ggt cct gtt tcc ctg ttc cat cct ctg aag gcc acc ctg gag	5776
Gln Met Gly Pro Val Ser Leu Phe His Pro Leu Lys Ala Thr Leu Glu	
1880 1885 1890 1895	
cag aca cag ccc gct gtg ctc cga gga gct cgg gtt ttc ctt cga caa	5824
Gln Thr Gln Pro Ala Val Leu Arg Gly Ala Arg Val Phe Leu Arg Gln	
1900 1905 1910	
aag agt gca act tcc ctc agc aat ggg ggc gcc ata caa acc cag gaa	5872
Lys Ser Ala Thr Ser Leu Ser Asn Gly Gly Ala Ile Gln Thr Gln Glu	
1915 1920 1925	
agt ggc atc aag gag tcc ctg tcc tgg ggc acg cag agg acc cag gac	5920
Ser Gly Ile Lys Glu Ser Leu Ser Trp Gly Thr Gln Arg Thr Gln Asp	
1930 1935 1940	
gta ctt tat gag gcc aga gca cct cta gaa cgt ggc cat tct gca gag	5968
Val Leu Tyr Glu Ala Arg Ala Pro Leu Glu Arg Gly His Ser Ala Glu	
1945 1950 1955	
atc cct gtg ggg cag cca gga gca ctg gct gta gat gtc cag atg cag	6016
Ile Pro Val Gly Gln Pro Gly Ala Leu Ala Val Asp Val Gln Met Gln	
1960 1965 1970 1975	
aac atg aca ttg aga gga ccg gat ggg gag ccc cag cct ggc ctg gag	6064
Asn Met Thr Leu Arg Gly Pro Asp Gly Glu Pro Gln Pro Gly Leu Glu	
1980 1985 1990	
agc caa ggc cga gcg gcc tct atg cca cgc ctg gcg gca gaa aca cag	6112
Ser Gln Gly Arg Ala Ala Ser Met Pro Arg Leu Ala Ala Glu Thr Gln	
1995 2000 2005	
ccg gcc cct aat gcc agc ccc atg aag cgc tcc atc tcc aca ctg gct	6160
Pro Ala Pro Asn Ala Ser Pro Met Lys Arg Ser Ile Ser Thr Leu Ala	
2010 2015 2020	
cca cgc ccg cat ggg act cag ctt tgc aac aca gtc ctg gac cgg cca	6208
Pro Arg Pro His Gly Thr Gln Leu Cys Asn Thr Val Leu Asp Arg Pro	
2025 2030 2035	
cct cct agc cag gtg tcc cat cac cac cac cac cgc tgc cac cgg cgc	6256
Pro Pro Ser Gln Val Ser His His His His His Arg Cys His Arg Arg	
2040 2045 2050 2055	
agg gac aag aag cag agg tcc ctg gaa aag ggg ccc agc ctg tct gtt	6304
Arg Asp Lys Lys Gln Arg Ser Leu Glu Lys Gly Pro Ser Leu Ser Val	

2060							2065							2070							
gac	aca	gaa	ggt	gca	cca	agt	act	gct	gca	gga	tct	ggc	ctg	ccc	cat	6352					
Asp	Thr	Glu	Gly	Ala	Pro	Ser	Thr	Ala	Ala	Gly	Ser	Gly	Leu	Pro	His						
2075							2080							2085							
gga	gaa	ggg	tcc	aca	ggc	tgc	cgg	cgg	gag	cgt	aag	caa	gag	cga	ggc	6400					
Gly	Glu	Gly	Ser	Thr	Gly	Cys	Arg	Arg	Glu	Arg	Lys	Gln	Glu	Arg	Gly						
2090							2095							2100							
cgg	tcc	cag	gag	cgg	agg	cag	ccc	tcc	tcc	tct	tct	tca	gag	aag	cag	6448					
Arg	Ser	Gln	Glu	Arg	Arg	Gln	Pro	Ser	Ser	Ser	Ser	Ser	Glu	Lys	Gln						
2105							2110							2115							
cgc	ttc	tat	tcc	tgt	gac	cgc	ttt	ggg	agc	cgg	gag	ccc	cca	caa	cct	6496					
Arg	Phe	Tyr	Ser	Cys	Asp	Arg	Phe	Gly	Ser	Arg	Glu	Pro	Pro	Gln	Pro						
2120							2125							2130							2135
aag	ccc	tcc	ctc	agt	agc	cac	ccc	ata	tcg	cca	aca	gcg	gca	cta	gag	6544					
Lys	Pro	Ser	Leu	Ser	Ser	His	Pro	Ile	Ser	Pro	Thr	Ala	Ala	Leu	Glu						
2140							2145							2150							
cca	gga	ccc	cac	ccg	cag	ggc	agt	ggg	tcc	gtt	aat	ggg	agc	ccc	ttg	6592					
Pro	Gly	Pro	His	Pro	Gln	Gly	Ser	Gly	Ser	Val	Asn	Gly	Ser	Pro	Leu						
2155							2160							2165							
atg	tca	aca	tct	ggg	gct	agc	acg	ccg	ggc	cga	ggg	ggg	cgg	agg	cag	6640					
Met	Ser	Thr	Ser	Gly	Ala	Ser	Thr	Pro	Gly	Arg	Gly	Gly	Arg	Arg	Gln						
2170							2175							2180							
ctc	ccc	cag	act	ccc	ctg	acc	cca	cgc	ccc	agc	atc	acc	tac	aag	acg	6688					
Leu	Pro	Gln	Thr	Pro	Leu	Thr	Pro	Arg	Pro	Ser	Ile	Thr	Tyr	Lys	Thr						
2185							2190							2195							
gcc	aat	tcc	tcg	cct	gtc	cac	ttt	gct	gag	ggg	cag	agt	ggc	ctt	cca	6736					
Ala	Asn	Ser	Ser	Pro	Val	His	Phe	Ala	Glu	Gly	Gln	Ser	Gly	Leu	Pro						
2200							2205							2210							2215
gcc	ttc	tcc	cct	ggc	cgt	ctc	agc	cgc	ggc	ctt	tct	gaa	cac	aat	gcc	6784					
Ala	Phe	Ser	Pro	Gly	Arg	Leu	Ser	Arg	Gly	Leu	Ser	Glu	His	Asn	Ala						
2220							2225							2230							
ctg	ctc	cag	aaa	gag	ccc	ctg	agc	cag	cct	cta	gct	tct	ggc	tcc	cgc	6832					
Leu	Leu	Gln	Lys	Glu	Pro	Leu	Ser	Gln	Pro	Leu	Ala	Ser	Gly	Ser	Arg						
2235							2240							2245							
att	ggc	tct	gac	cct	tac	cta	ggg	cag	cgt	ctg	gac	agt	gag	gcc	tct	6880					
Ile	Gly	Ser	Asp	Pro	Tyr	Leu	Gly	Gln	Arg	Leu	Asp	Ser	Glu	Ala	Ser						
2250							2255							2260							
gcc	cac	aac	ctg	cct	gag	gat	aca	ctc	acc	ttt	gaa	gag	gcc	gtg	gcc	6928					
Ala	His	Asn	Leu	Pro	Glu	Asp	Thr	Leu	Thr	Phe	Glu	Glu	Ala	Val	Ala						
2265							2270							2275							
acc	aac	tct	ggc	cgc	tcc	tcc	agg	act	tcc	tat	gtg	tcc	tcc	ctc	act	6976					
Thr	Asn	Ser	Gly	Arg	Ser	Ser	Arg	Thr	Ser	Tyr	Val	Ser	Ser	Leu	Thr						
2280							2285							2290							2295
tcc	caa	tcc	cac	cct	ctc	cgc	cgt	gta	ccc	aat	ggc	tac	cac	tgc	act	7024					
Ser	Gln	Ser	His	Pro	Leu	Arg	Arg	Val	Pro	Asn	Gly	Tyr	His	Cys	Thr						
2300							2305							2310							

ttg gga ctc agc acc ggc gtc cgg gcg cgg cac agc tac cac cac cca 7072
 Leu Gly Leu Ser Thr Gly Val Arg Ala Arg His Ser Tyr His His Pro
 2315 2320 2325

gac cag gat cac tgg tgc tag ctgcaccacg accacccatg caccagctcg 7123
 Asp Gln Asp His Trp Cys
 2330

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<211> 2333

<212> PRT

<213> Rattus norvegicus

<400> 13

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Gly	Gln	Gly	Gly	Leu	Pro	Pro	Gly	Gln	Arg	Val	Leu	Tyr	Lys	Gln	Ser
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Ile	Ala	Gln	Arg	Ala	Arg	Thr	Met	Ala	Leu	Tyr	Asn	Pro	Ile	Pro	Val
			50				55				60				
Lys	Gln	Asn	Cys	Phe	Thr	Val	Asn	Arg	Ser	Leu	Phe	Val	Phe	Ser	Glu
			65			70				75					80
Asp	Asn	Val	Val	Arg	Lys	Tyr	Ala	Lys	Arg	Ile	Thr	Glu	Trp	Pro	Pro
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Phe	Glu	Tyr	Met	Ile	Leu	Ala	Thr	Ile	Ile	Ala	Asn	Cys	Ile	Val	Leu
			100					105					110		
Ala	Leu	Glu	Gln	His	Leu	Pro	Asp	Gly	Asp	Lys	Thr	Pro	Met	Ser	Glu
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Arg	Leu	Asp	Asp	Thr	Glu	Pro	Tyr	Phe	Ile	Gly	Ile	Phe	Cys	Phe	Glu
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Ala	Gly	Ile	Lys	Ile	Ile	Ala	Leu	Gly	Phe	Val	Phe	His	Lys	Gly	Ser
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Tyr Leu Arg Asn Gly Trp Asn Val Met Asp Phe Val Val Val Leu Thr
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 Gly Ile Leu Ala Thr Ala Gly Thr Asp Phe Asp Leu Arg Thr Leu Arg
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 Ala Val Arg Val Leu Arg Pro Leu Lys Leu Val Ser Gly Ile Pro Ser
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 Leu Gln Val Val Leu Lys Ser Ile Met Lys Ala Met Val Pro Leu Leu
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 Gln Ile Gly Leu Leu Leu Phe Phe Ala Ile Leu Met Phe Ala Ile Ile
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 Pro Ala Arg Leu Cys Asp Ser Asp Thr Glu Cys Arg Glu Tyr Trp Pro
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 Pro Leu Ala Ser Gly Ser Arg Ile Gly Ser Asp Pro Tyr Leu Gly Gln
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 Thr Phe Glu Glu Ala Val Ala Thr Asn Ser Gly Arg Ser Ser Arg Thr
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 Ser Tyr Val Ser Ser Leu Thr Ser Gln Ser His Pro Leu Arg Arg Val
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 Pro Asn Gly Tyr His Cys Thr Leu Gly Leu Ser Thr Gly Val Arg Ala
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 Arg His Ser Tyr His His Pro Asp Gln Asp His Trp Cys
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