HUMAN ION CHANNELS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority of Application Serial No. 09/460,602, filed 1999 December 14, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention is directed, in part, to nucleic acid molecules encoding ion channels, the novel polypeptides of these human ion channels, and assays for screening compounds that bind to these polypeptides and/or modulate their activities.

BACKGROUND OF THE INVENTION

Ion channels are "molecular gates" that regulate the flow of ions into and out of cells. Ion flow plays an important role in all brain cell communication necessary for learning and memory. Additionally, ion flow is important in many physiological processes including, but not limited to, heart rate and body movement. Aberrations in ion channels have been implicated in, amongst other disorders, epilepsy, schizophrenia, Alzheimer's disease, migraine, arrhythmia, diabetes, and stroke damage. Ions flow down their electrochemical gradient through the ion channels (passive transport). The core of the channel is hydrophilic, and contains a part of the protein, the selectivity filter, which recognizes only certain ions and allows them to pass through. Channels are named by the ion(s) they allow to pass. Examples of ion channels include, but are not limited to, calcium channels, potassium channels, sodium channels, chloride channels, *etc*. An

additional component of the channel is the gate. Only when the gate is open can the ions recognized by the selectivity filter pass through the channel. Gates open in response to a variety of stimuli, including, but not limited to, changes in membrane potential or the presence of certain chemicals outside or inside the cell. Channel names often also include an indication of what controls the gate: *e.g.*, "voltage-gated calcium channel." Presently, more than 50 different types of ion channels have been identified.

Communication between neurons is achieved by the release of neurotransmitters into the synapse. These neurotransmitters then activate receptors on the post-synaptic neuron. Many such receptors contain pores to rapidly conduct ions, such as sodium, calcium, potassium, and chloride, into the neuron. These pores, or channels, are made of protein subunits that are members of the family of proteins generally referred to as neurotransmitter-gated ion channel proteins. Included in this family are the serotonin 5-HT3 receptor, the gamma-aminobutyric-acid (GABA) receptor subunits, including gamma-1, rho-3, and beta-like, and the acetylcholine receptor protein subunits, including alpha-9 chain, epsilon chain, and beta-2 chain.

The neurotransmitter-gated ion channel superfamily includes 5-HT3, $GABA_A$, glutamate, glycine, and nicotinic acetylcholine receptor families. Within this superfamily, functional receptors are formed by homo- or heteropentamers of subunits having four transmembrane domains and an extracellular ligand-binding domain. The transmembrane domains of these receptors contribute to the formation of an ion pore.

Serotonin, also known as 5-hydroxytryptamine or 5-HT, is a biogenic amine that functions as a neurotransmitter, a mitogen and a hormone (Conley, E.C. (1995) The Ion Channels FactsBook Vol. I. Extracellular Ligand-Gated Channels, Academic Press, London and San Diego. pp. 426). Serotonin activates a large number of receptors, most of which are coupled to activation of G-proteins. However, 5-HT3 receptors are structurally distinct and belong to the neurotransmitter-gated ion channel superfamily. 5-HT3 receptors are expressed both pre- and post-synaptically on central and peripheral neurons. Post-synaptic 5-HT3 receptors achieve their effects by inducing excitatory potentials in the post-synaptic neuron, whereas pre-synaptic 5-HT3 receptors modulate the release of other neurotransmitters from the pre-synaptic neuron (Conley, 1995). 5-HT3 receptors have important roles in pain reception, cognition, cranial motor neuron activity, sensory processing and modulation of affect (Conley, 1995). Thus, ligands or drugs that modulate 5-HT3 receptors may be useful in treating pain, neuropathies, migraine, cognitive

disorders, learning and memory deficits, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, emesis, cranial neuropathies, sensory deficits, anxiety, depression, schizophrenia, and other affective disorders.

Nicotinic acetylcholine receptors (AChR) are distinguished from other acetylcholine receptors by their affinity for nicotine and their structure—homo- or hetero-pentamers like all members of the neurotransmitter-gated ion channel superfamily. Nicotinic AChRs are found at the neuromuscular junction on skeletal muscle and on peripheral and central neurons. These receptors form nonselective cation channels and therefore induce excitatory currents when activated. Nicotinic AChRs are receptors for anesthetics, sedatives, and hallucinogens (Conley, 1995), and certain ligands have shown improvements in learning and memory in animals (Levin *et al.*, Behavioral Pharmacology, 1999, 10:675-780). Thus, ligands or drugs that modulate nicotinic AChRs could be useful for anesthesia, sedation, improving learning and memory, improving cognition, schizophrenia, anxiety, depression, attention deficit hyperactivity disorder, and addiction or smoking cessation. Expression of AChR subunits is regulated during development enabling the design of ligands or drugs specifically targeted for particular developmental stages or diseases.

The neurotransmitter γ-aminobutyric acid (GABA) activates a family of neurotransmitter-gated ion channels ($GABA_A$) and a family of G protein-coupled receptors ($GABA_B$) (Conley, 1995). $GABA_A$ receptors form chloride channels that induce inhibitory or hyperpolarizing currents when stimulated by GABA or $GABA_A$ receptor agonists (Conley, 1995). $GABA_A$ receptors are modulated by benzodiazepines, barbiturates, picrotoxin, and bicucuilline (Conley, 1995). Thus, ligands or drugs that modulate $GABA_A$ receptors could be useful in sedation, anxiety, epilepsy, seizures, alcohol addiction or withdrawal, panic disorders, pre-menstrual syndrome, migraine, and other diseases characterized by hyper-excitability of central or peripheral neurons. The pharmacology of $GABA_A$ receptors is affected by changing the subunit composition of the receptor. GABA receptor rho subunits are relatively specifically expressed in the retina (Cutting *et al.*, 1991, Proc. Natl. Acad. Sci. USA, 88:2673-7), and the pharmacology of rho receptor homomultimers resembles that of so-called $GABA_C$ receptors (Shimada *et al.*, 1992, Mol. Pharmacol. 41:683-7). Therefore, GABA receptors consisting of rho subunits may be useful targets for discovering ligands or drugs to treat visual defects, macular degeneration, glaucoma, and other retinal disorders.

Compounds that modify the activity of these channels may also be useful for the control of neuromotor diseases including epilepsy and neurodegenerative diseases including Parkinson's and Alzheimer's. Also compounds that modulate the activity of these channels may treat diseases including but not limited to cardiovascular arrhythmias, stroke, and endocrine and muscular disorders.

Therefore, ion channels may be useful targets for discovering ligands or drugs to treat many diverse disorders and defects, including schizophrenia, depression, anxiety, attention deficit hyperactivity disorder, migraine, stroke, ischemia, and neurodegenerative disease such as Alzheimer's disease, Parkinson's disease, glaucoma and macular degeneration. In addition compounds which modulate ion channels can be used for the treatment of cardiovascular diseases including ischemia, congestive heart failure, arrhythmia, high blood pressure and restenosis.

These and other aspects of the invention are described below.

SUMMARY OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule that comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence homologous to a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, or a fragment thereof. The nucleic acid molecule encodes at least a portion of ion-x (wherein x is 1, 2a, 2b, 3, 4a, 4b, 5, 6, and 7). In some embodiments, the nucleic acid molecule comprises a sequence that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, or a fragment thereof. In some embodiments, the nucleic acid molecule comprises a sequence homologous to a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, or a fragment thereof. In some embodiments, the nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, and fragments thereof.

According to some embodiments, the present invention provides vectors which comprise the nucleic acid molecule of the invention. In some embodiments, the vector is an expression vector.

According to some embodiments, the present invention provides host cells which comprise the vectors of the invention. In some embodiments, the host cells comprise expression vectors.

The present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence complementary to at least a portion of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, said portion comprising at least 10 nucleotides.

The present invention provides a method of producing a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, or a homolog or fragment thereof. The method comprising the steps of introducing a recombinant expression vector that includes a nucleotide sequence that encodes the polypeptide into a compatible host cell, growing the host cell under conditions for expression of the polypeptide and recovering the polypeptide.

The present invention provides an isolated antibody which binds to an epitope on a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, or a homolog or fragment thereof.

The present invention provides an method of inducing an immune response in a mammal against a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, or a homolog or fragment thereof. The method comprises administering to a mammal an amount of the polypeptide sufficient to induce said immune response.

The present invention provides a method for identifying a compound which binds ion-x. The method comprises the steps of: contacting ion-x with a compound and determining whether the compound binds ion-x.

The present invention provides a method for identifying a compound which binds a nucleic acid molecule encoding ion-x. The method comprises the steps of contacting said nucleic acid molecule encoding ion-x with a compound and determining whether said compound binds said nucleic acid molecule.

The present invention provides a method for identifying a compound which modulates the activity of ion-x. The method comprises the steps of contacting ion-x with a compound and determining whether ion-x activity has been modulated.

The present invention provides a method of identifying an animal homolog of ion-x. The method comprises the steps screening a nucleic acid database of the animal with a

sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, or a portion thereof and determining whether a portion of said library or database is homologous to said sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, or portion thereof.

The present invention provides a method of identifying an animal homolog of ion-x. The methods comprises the steps screening a nucleic acid library of the animal with a nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, or a portion thereof; and determining whether a portion of said library or database is homologous to said sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9,SEQ ID NO:49 and SEQ ID NO:51, or a portion thereof.

Another aspect of the present invention relates to methods of screening a human subject to diagnose a disorder affecting the brain or genetic predisposition therefor. The methods comprise the steps of assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression, or biological activity of at least one ion channel that is expressed in the brain. The ion channels comprise an amino acid sequence selected from the group consisting of: SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:50 and allelic variants thereof. A diagnosis of the disorder or predisposition is made from the presence or absence of the mutation. The presence of a mutation altering the amino acid sequence, expression, or biological activity of the ion channel in the nucleic acid correlates with an increased risk of developing the disorder.

The present invention further relates to methods of screening for an ion-1 or ion-3 mental disorder genotype in a human patient. The methods comprise the steps of providing a biological sample comprising nucleic acid from the patient, in which the nucleic acid includes sequences corresponding to allelles of ion-1 or ion-3. The presence of one or more mutations in the ion-1 allelle or the ion-3 allelle is detected indicative of a mental disorder genotype. In some embodiments, the mental disorder includes, but is not limited to, schizophrenia, affective disorders, ADHD/ADD (*i.e.*, Attention Deficit-Hyperactivity Disorder/Attention Deficit Disorder), and neural disorders such as Alzheimer's disease, Parkinson's disease, migraine, and senile dementia as well as

depression, anxiety, bipolar disease, epilepsy, neuritis, neurasthenia, neuropathy, neuroses, and the like.

The present invention provides kits for screening a human subject to diagnose a mental disorder or a genetic predisposition therefor. The kits include an oligonucleotide useful as a probe for identifying polymorphisms in a human ion-1 gene or a human ion-3 gene. The oligonucleotide comprises 6-50 nucleotides in a sequence that is identical or complementary to a sequence of a wild type human ion-1 or ion-3 gene sequence or ion-1 or ion-3 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution. The kit also includes a media packaged with the oligonucleotide. The media contains information for identifying polymorphisms that correlate with a mental disorder or a genetic predisposition therefor, the polymorphisms being identifiable using the oligonucleotide as a probe.

The present invention further relates to methods of identifying ion channel allelic variants that correlates with mental disorders. The methods comprise the steps of providing biological samples that comprise nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny, and detecting in the nucleic acid the presence of one or more mutations in an ion channel that is expressed in the brain. The ion channel comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:50, and allelic variants thereof. The nucleic acid includes sequences corresponding to the gene or genes encoding ion-x. The one or more mutations detected indicate an allelic variant that correlates with a mental disorder.

The present invention further relates to purified polynucleotides comprising nucleotide sequences encoding alleles of ion-1 or ion-3 from a human with a mental disorder. The polynucleotide hybridizes to the complement of SEQ ID NO:49 or of SEQ ID NO:51 under the following hybridization conditions: (a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate and (b) washing 2 times for 30 minutes at 60°C in a wash solution comprising 0.1x SSC and 1% SDS. The polynucleotide that encodes ion-1 or ion-3 amino

acid sequence of the human differs from SEQ ID NO:50 or from SEQ ID NOS:16 or 17 by at least one residue.

The present invention also provides methods for identifying a modulator of biological activity of ion-x comprising the steps of contacting a cell that expresses ion-x in the presence and in the absence of a putative modulator compound and measuring ion-x biological activity in the cell. The decreased or increased ion-x biological activity in the presence versus absence of the putative modulator is indicative of a modulator of biological activity.

As used herein, the term "biological activity" of an ion channel refers to the native activity of the ion channel. Activities of ion channels include, but are not limited to, the ability to bind or be affected by certain compounds, and the ability to transport ions from one side of the membrane to the other side.

The present invention further provides methods to identify compounds useful for the treatment of mental disorders. The methods comprise the steps of contacting a composition comprising ion-1 with a compound suspected of binding ion-1 or contacting a composition comprising ion-3 with a compound suspected of binding ion-3. The binding between ion-1 and the compound suspected of binding ion-1 or between ion-3 and the compound suspected of binding ion-3 is detected. Compounds identified as binding ion-1 or ion-3 are candidate compounds useful for the treatment of mental disorders.

The present invention further provides methods for identifying a compound useful as a modulator of binding between ion-x and a binding partner of ion-x. The methods comprise the steps of contacting the binding partner and a composition comprising ion-x in the presence and in the absence of a putative modulator compound and detecting binding between the binding partner and ion-x. Decreased or increased binding between the binding partner and ion-x in the presence of the putative modulator, as compared to binding in the absence of the putative modulator is indicative a modulator compound useful for the treatment of mental disorders.

The present invention further provides chimeric receptors comprising at least a portion of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, said portion comprising at least 10 nucleotides.

These and other aspects of the invention are described in greater detail below.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides, *inter alia*, isolated and purified polynucleotides that encode human ion channels or a portion thereof, vectors containing these polynucleotides, host cells transformed with these vectors, processes of making ion channels and subunits, methods of using the above polynucleotides and vectors, isolated and purified ion channels and subunits, methods of screening compounds which modulate ion channel activity, and compounds that modulate ion channel activity.

Definitions

Various definitions are made throughout this document. Most words have the meaning that would be attributed to those words by one skilled in the art. Words specifically defined either below or elsewhere in this document have the meaning provided in the context of the present invention as a whole and as typically understood by those skilled in the art.

As used herein, the phrase "ion channel" refers to an entire channel that allows the movement of ions across a membrane, as well as to subunit polypeptide chains that comprise such a channel. As the ion channels of the present inventions are ligand-gated, the ion channels are also referred to as "receptors." Those of skill in the art will recognize that ion channels are made of subunits. As used herein, the term "subunit" refers to any component portion of an ion channel, including but not limited to the beta subunit and other associated subunits.

"Synthesized" as used herein and understood in the art, refers to polynucleotides produced by purely chemical, as opposed to enzymatic, methods. "Wholly" synthesized DNA sequences are therefore produced entirely by chemical means, and "partially" synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means.

By the term "region" is meant a physically contiguous portion of the primary structure of a biomolecule. In the case of proteins, a region is defined by a contiguous portion of the amino acid sequence of that protein.

The term "domain" is herein defined as referring to a structural part of a biomolecule that contributes to a known or suspected function of the biomolecule. Domains may be co-extensive with regions or portions thereof; domains may also incorporate a portion of a biomolecule that is distinct from a particular region, in addition to all or part of that region. Examples of ion channel domains include, but are not limited

to, the extracellular (*i.e.*, N-terminal), transmembrane and cytoplasmic (*i.e.*, C-terminal) domains, which are co-extensive with like-named regions of ion channels; and each of the loop segments (both extracellular and intracellular loops) connecting adjacent transmembrane segments.

As used herein, the term "activity" refers to a variety of measurable indicia suggesting or revealing binding, either direct or indirect; affecting a response, *i.e.*, having a measurable affect in response to some exposure or stimulus, including, for example, the affinity of a compound for directly binding a polypeptide or polynucleotide of the invention. Activity can also be determined by measurement of downstream enzyme activities, and downstream messengers such as K^+ ions, Ca^{2+} ions, Na^+ ions, Cl^- ions, cyclic AMP, and phospholipids after some stimulus or event. For example, activity can be determined by measuring ion flux. As used herein, the term "ion flux" includes ion current. Activity can also be measured by measuring changes in membrane potential using electrodes or voltage-sensitive dyes, or by measuring neuronal or cellular activity such as action potential duration or frequency, the threshold for stimulating action potentials, long-term potentiation, or long-term inhibition.

As used herein, the term "protein" is intended to include full length and partial fragments of proteins. The term "protein" may be used, herein, interchangeably with "polypeptide." Thus, as used herein, the term "protein" includes polypeptide, peptide, oligopeptide, or amino acid sequence.

As used herein, the term "chimeric receptor" is intended to refer to a receptor comprising portions of more than one type of receptor. As a non-limiting example, a chimeric receptor may comprise the pore-forming transmembrane domain of an alpha7 nicotinic acetylcholine receptor and the extracellular domain of the alpha10 nicotinic acetylcholine receptor. Chimeric receptors of the present invention are not limited to hybrids of related receptors; chimeric receptors may also include, for example, the pore-forming transmembrane domain of an alpha7 nicotinic acetylcholine receptor and the extracellular domain of the GABA receptor. Chimeric receptors may also include portions of known wild-type receptors and portions of artificial receptors.

As used herein, the term "antibody" is meant to refer to complete, intact antibodies, Fab fragments, and $F(ab)_2$ fragments thereof. Complete, intact antibodies include monoclonal antibodies such as murine monoclonal antibodies, polyclonal antibodies,

chimeric antibodies, humanized antibodies, and recombinant antibodies identified using phage display.

As used herein, the term "binding" means the physical or chemical interaction between two proteins, compounds or molecules (including nucleic acids, such as DNA or RNA), or combinations thereof. Binding includes ionic, non-ionic, hydrogen bonds, Van der Waals, hydrophobic interactions, etc. The physical interaction, the binding, can be either direct or indirect, indirect being through or due to the effects of another protein, compound or molecule. Direct binding refers to interactions that do not take place through or due to the effect of another protein, compound or molecule, but instead are without other substantial chemical intermediates. Binding may be detected in many different manners. As a non-limiting example, the physical binding interaction between an ion channel of the invention and a compound can be detected using a labeled compound. Alternatively, functional evidence of binding can be detected using, for example, a cell transfected with and expressing an ion channel of the invention. Binding of the transfected cell to a ligand of the ion channel that was transfected into the cell provides functional evidence of binding. Other methods of detecting binding are well known to those of skill in the art.

As used herein, the term "compound" means any identifiable chemical or molecule, including, but not limited to a small molecule, peptide, protein, sugar, nucleotide, or nucleic acid. Such compound can be natural or synthetic.

As used herein, the term "complementary" refers to Watson-Crick base-pairing between nucleotide units of a nucleic acid molecule.

As used herein, the term "contacting" means bringing together, either directly or indirectly, a compound into physical proximity to a polypeptide or polynucleotide of the invention. The polypeptide or polynucleotide can be present in any number of buffers, salts, solutions, etc. Contacting includes, for example, placing the compound into a beaker, microtiter plate, cell culture flask, or a microarray, such as a gene chip, or the like, which contains either the ion channel polypeptide or fragment thereof, or nucleic acid molecule encoding an ion channel or fragment thereof.

As used herein, the phrase "homologous nucleotide sequence," or "homologous amino acid sequence," or variations thereof, refers to sequences characterized by a homology, at the nucleotide level or amino acid level, of at least about 60%, more preferably at least about 70%, more preferably at least about 80%, more preferably at least

about 90%, and most preferably at least about 95% to the entirety of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, or to at least a portion of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, which portion encodes a functional domain of the encoded polypeptide, or to SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50. Homologous nucleotide sequences include those sequences coding for isoforms of ion channel proteins. Such isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. Homologous nucleotide sequences include nucleotide sequences encoding for an ion channel protein of a species other than human, including, but not limited to, mammals. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. Although the present invention provides particular sequences, it is understood that the invention is intended to include within its scope other human allelic variants and non-human forms of the ion channels described herein.

Homologous amino acid sequences include those amino acid sequences which contain conservative amino acid substitutions in SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, as well as polypeptides having ion channel activity. A homologous amino acid sequence does not, however, include the sequence of known polypeptides having ion channel activity. Percent homology can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WI), which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, **1981**, *2*, 482-489, which is incorporated herein by reference in its entirety) using the default settings.

As used herein, the term "percent homology" and its variants are used interchangeably with "percent identity" and "percent similarity."

As used herein, the term "isolated" nucleic acid molecule refers to a nucleic acid molecule (DNA or RNA) that has been removed from its native environment. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules.

As used herein, the terms "modulates" or "modifies" means an increase or decrease in the amount, quality, or effect of a particular activity or protein.

The term "preventing" refers to decreasing the probability that an organism contracts or develops an abnormal condition.

The term "treating" refers to having a therapeutic effect and at least partially alleviating or abrogating an abnormal condition in the organism.

The term "therapeutic effect" refers to the inhibition or activation factors causing or contributing to the abnormal condition. A therapeutic effect relieves to some extent one or more of the symptoms of the abnormal condition. In reference to the treatment of abnormal conditions, a therapeutic effect can refer to one or more of the following: (a) an increase in the proliferation, growth, and/or differentiation of cells; (b) inhibition (*i.e.*, slowing or stopping) of cell death; (c) inhibition of degeneration; (d) relieving to some extent one or more of the symptoms associated with the abnormal condition; and (e) enhancing the function of the affected population of cells. Compounds demonstrating efficacy against abnormal conditions can be identified as described herein.

The term "abnormal condition" refers to a function in the cells or tissues of an organism that deviates from their normal functions in that organism. An abnormal condition can relate to cell proliferation, cell differentiation, cell signaling, or cell survival. An abnormal condition may also include obesity, diabetic complications such as retinal degeneration, and irregularities in glucose uptake and metabolism, and fatty acid uptake and metabolism.

Abnormal cell proliferative conditions include cancers such as fibrotic and mesangial disorders, abnormal angiogenesis and vasculogenesis, wound healing, psoriasis, diabetes mellitus, and inflammation.

Abnormal differentiation conditions include, but are not limited to, neurodegenerative disorders, slow wound healing rates, and slow tissue grafting healing rates. Abnormal cell signaling conditions include, but are not limited to, psychiatric disorders involving excess neurotransmitter activity.

Abnormal cell survival conditions may also relate to conditions in which programmed cell death (apoptosis) pathways are activated or abrogated. A number of protein kinases are associated with the apoptosis pathways. Aberrations in the function of any one of the protein kinases could lead to cell immortality or premature cell death.

The term "administering" relates to a method of incorporating a compound into cells or tissues of an organism. The abnormal condition can be prevented or treated when the cells or tissues of the organism exist within the organism or outside of the organism.

Cells existing outside the organism can be maintained or grown in cell culture dishes. For cells harbored within the organism, many techniques exist in the art to administer compounds, including (but not limited to) oral, parenteral, dermal, injection, and aerosol applications. For cells outside of the organism, multiple techniques exist in the art to administer the compounds, including (but not limited to) cell microinjection techniques, transformation techniques and carrier techniques.

The abnormal condition can also be prevented or treated by administering a compound to a group of cells having an aberration in ion channel in an organism. The effect of administering a compound on organism function can then be monitored. The organism is preferably a mouse, rat, rabbit, guinea pig or goat, more preferably a monkey or ape, and most preferably a human.

By "amplification" it is meant increased numbers of DNA or RNA in a cell compared with normal cells. "Amplification" as it refers to RNA can be the detectable presence of RNA in cells, since in some normal cells there is no basal expression of RNA. In other normal cells, a basal level of expression exists, therefore in these cases amplification is the detection of at least 1 to 2-fold, and preferably more, compared to the basal level.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues which has a sufficient number of bases to be used in a polymerase chain reaction (PCR). This short sequence is based on (or designed from) a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having at least about 10 nucleotides and as many as about 50 nucleotides, preferably about 15 to 30 nucleotides. They are chemically synthesized and may be used as probes.

As used herein, the term "probe" refers to nucleic acid sequences of variable length, preferably between at least about 10 and as many as about 6,000 nucleotides, depending on use. They are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. They may be single- or double-stranded and are carefully designed to have specificity in PCR, hybridization membrane-based, or ELISA-like technologies.

As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a probe, primer, or oligonucleotide will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences will hybridize with specificity to their proper complements at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present in excess, at T_m, 50% of the probes are hybridized to their complements at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for longer probes, primers or oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

The amino acid sequences are presented in the amino (N) to carboxy (C) direction, from left to right. The N-terminal α-amino group and the C-terminal β-carboxy groups are not depicted in the sequence. The nucleotide sequences are presented by single strands only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or amino acids are represented by their three letters code designations.

Polynucleotides

The present invention provides purified and isolated polynucleotides (*e.g.*, DNA sequences and RNA transcripts, both sense and complementary antisense strands, both single- and double-stranded, including splice variants thereof) that encode unknown ion channels. These genes are described herein and designated herein collectively as ion-x (where x is 1, 2a, 2b, 3, 4a, 4b, 5, 6, and 7). That is, these genes and gene products are described herein and designated herein as ion-1, ion-2a, ion-2b, ion-3, ion-4a, ion-4b, ion-5, ion-6, and ion-7. Table 1 below identifies the novel gene sequence ion-x designation, the SEQ ID NO: of the gene sequence, and the SEQ ID NO: of the polypeptide encoded thereby.

Table 1

ion	Nucleotide Sequence (SEQ ID NO:)	Amino acid Sequence (SEQ ID NO:)
1	1, 49	10, 11, 50
2a	2	12, 13
2b	3	14, 15
3	4, 51	16, 17
4a	5	18, 19
4b	6	20, 21
5	7	22, 23, 24, 25, 26, 27, 28
6	8	29, 30
7	9	31, 32

When a specific ion-x is identified (for example ion-5), it is understood that only that specific ion channel is being referred to.

As described in Example 11 below, the genes encoding as ion-1 (nucleic acid sequence SEQ ID NO:1, SEQ ID NO:49, amino acid sequence SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:50), ion-2a (nucleic acid sequence SEQ ID NO:2, amino acid sequence SEQ ID NO:12, SEQ ID NO:13), ion-2b (nucleic acid sequence SEQ ID NO:3, amino acid sequence SEQ ID NO:14, SEQ ID NO:15), ion-3 (nucleic acid sequence SEQ ID NO:4, SEQ ID NO:51, amino acid sequence SEQ ID NO:16, SEQ ID NO:17), ion-4a (nucleic acid sequence SEQ ID NO:5, amino acid sequence SEQ ID NO:18, SEQ ID NO:19), ion-4b (nucleic acid sequence SEQ ID NO:6, amino acid sequence SEQ ID NO:20, SEQ ID NO:21), ion-5 (nucleic acid sequence SEQ ID NO:7, amino acid sequence SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28), ion-6 (nucleic acid sequence SEQ ID NO:8, amino acid sequence SEQ ID NO:29, SEQ ID NO:30), and ion-7 (nucleic acid sequence SEQ ID NO:9, amino acid sequence SEQ ID NO:31, SEQ ID NO:32)..

Ion-1 (nucleic acid sequence SEQ ID NO:1, SEQ ID NO:49, amino acid sequence SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:50), ion-2a (nucleic acid sequence SEQ ID NO:2, amino acid sequence SEQ ID NO:12, SEQ ID NO:13), ion-2b (nucleic acid sequence SEQ ID NO:3, amino acid sequence SEQ ID NO:14, SEQ ID NO:15), ion-3 (nucleic acid sequence SEQ ID NO:4, SEQ ID NO:51, amino acid sequence SEQ ID NO:16, SEQ ID NO:17), ion-5 (nucleic acid sequence SEQ ID NO:7, amino acid sequence SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28), and ion-7 (nucleic acid sequence SEQ ID NO:9, amino acid

sequence SEQ ID NO:31, SEQ ID NO:32) have been detected in brain tissue indicating that these ion-x proteins are neuroreceptors.

The invention provides purified and isolated polynucleotides (*e.g.*, cDNA, genomic DNA, synthetic DNA, RNA, or combinations thereof, whether single- or double-stranded) that comprise a nucleotide sequence encoding the amino acid sequence of the polypeptides of the invention. Such polynucleotides are useful for recombinantly expressing the receptor and also for detecting expression of the receptor in cells (*e.g.*, using Northern hybridization and *in situ* hybridization assays). Such polynucleotides also are useful in the design of antisense and other molecules for the suppression of the expression of ion-x in a cultured cell, a tissue, or an animal; for therapeutic purposes; or to provide a model for diseases or conditions characterized by aberrant ion-x expression. Specifically excluded from the definition of polynucleotides of the invention are entire isolated, non-recombinant native chromosomes of host cells. A preferred polynucleotide has a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, which correspond to naturally occurring ion-x sequences. It will be appreciated that numerous other polynucleotide sequences exist that also encode ion-x having sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, due to the well-known degeneracy of the universal genetic code.

The invention also provides a purified and isolated polynucleotide comprising a nucleotide sequence that encodes a mammalian polypeptide, wherein the polynucleotide hybridizes to a polynucleotide having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, or the non-coding strand complementary thereto, under the following hybridization conditions:

(a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate; and

(b) washing 2 times for 30 minutes each at 60°C in a wash solution comprising 0.1% SSC, 1% SDS. Polynucleotides that encode a human allelic variant are highly preferred.

The present invention relates to molecules which comprise the gene sequences that encode the ion channels; constructs and recombinant host cells incorporating the gene sequences; the novel ion-x polypeptides encoded by the gene sequences; antibodies to the polypeptides and homologs; kits employing the polynucleotides and polypeptides, and methods of making and using all of the foregoing. In addition, the present invention

relates to homologs of the gene sequences and of the polypeptides and methods of making and using the same.

Genomic DNA of the invention comprises the protein-coding region for a polypeptide of the invention and is also intended to include allelic variants thereof. It is widely understood that, for many genes, genomic DNA is transcribed into RNA transcripts that undergo one or more splicing events wherein intron (*i.e.*, non-coding regions) of the transcripts are removed, or "spliced out." RNA transcripts that can be spliced by alternative mechanisms, and therefore be subject to removal of different RNA sequences but still encode an ion-x polypeptide, are referred to in the art as splice variants which are embraced by the invention. Splice variants comprehended by the invention therefore are encoded by the same original genomic DNA sequences but arise from distinct mRNA transcripts. Allelic variants are modified forms of a wild-type gene sequence, the modification resulting from recombination during chromosomal segregation or exposure to conditions which give rise to genetic mutation. Allelic variants, like wild type genes, are naturally occurring sequences (as opposed to non-naturally occurring variants that arise from *in vitro* manipulation).

The invention also comprehends cDNA that is obtained through reverse transcription of an RNA polynucleotide encoding ion-x (conventionally followed by second strand synthesis of a complementary strand to provide a double-stranded DNA).

Preferred DNA sequences encoding human ion-x polypeptides are set out in sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51. A preferred DNA of the invention comprises a double stranded molecule along with the complementary molecule (the "non-coding strand" or "complement") having a sequence unambiguously deducible from the coding strand according to Watson-Crick base-pairing rules for DNA. Also preferred are other polynucleotides encoding the ion-x polypeptide of sequences selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, which differ in sequence from the polynucleotides of sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, by virtue of the well-known degeneracy of the universal nuclear genetic code.

The invention further embraces other species, preferably mammalian, homologs of the human ion-x DNA. Species homologs, sometimes referred to as "orthologs," in general, share at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least

65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology with human DNA of the invention. Generally, percent sequence "homology" with respect to polynucleotides of the invention may be calculated as the percentage of nucleotide bases in the candidate sequence that are identical to nucleotides in the ion-x sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

Polynucleotides of the invention permit identification and isolation of polynucleotides encoding related ion-x polypeptides, such as human allelic variants and species homologs, by well-known techniques including Southern and/or Northern hybridization, and polymerase chain reaction (PCR). Examples of related polynucleotides include human and non-human genomic sequences, including allelic variants, as well as polynucleotides encoding polypeptides homologous to ion-x and structurally related polypeptides sharing one or more biological, immunological, and/or physical properties of ion-x. Non-human species genes encoding proteins homologous to ion-x can also be identified by Southern and/or PCR analysis and are useful in animal models for ion-x disorders. Knowledge of the sequence of a human ion-x DNA also makes possible through use of Southern hybridization or polymerase chain reaction (PCR) the identification of genomic DNA sequences encoding ion-x expression control regulatory sequences such as promoters, operators, enhancers, repressors, and the like. Polynucleotides of the invention are also useful in hybridization assays to detect the capacity of cells to express ion-x. Polynucleotides of the invention may also provide a basis for diagnostic methods useful for identifying a genetic alteration(s) in an ion-x locus that underlies a disease state or states, which information is useful both for diagnosis and for selection of therapeutic strategies.

According to the present invention, the ion-x nucleotide sequences disclosed herein may be used to identify homologs of the ion-x, in other animals, including but not limited to humans and other mammals, and invertebrates. Any of the nucleotide sequences disclosed herein, or any portion thereof, can be used, for example, as probes to screen databases or nucleic acid libraries, such as, for example, genomic or cDNA libraries, to identify homologs, using screening procedures well known to those skilled in the art. Accordingly, homologs having at least 50%, more preferably at least 60%, more

preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 100% homology with ion-x sequences can be identified.

The disclosure herein of polynucleotides encoding ion-x polypeptides makes readily available to the worker of ordinary skill in the art many possible fragments of the ion channel polynucleotide. Polynucleotide sequences provided herein may encode, as non-limiting examples, a native channel, a constitutive active channel, or a dominant-negative channel.

One preferred embodiment of the present invention provides an isolated nucleic acid molecule comprising a sequence homologous to a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, and fragments thereof. Another preferred embodiment provides an isolated nucleic acid molecule comprising a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49 and SEQ ID NO:51, and fragments thereof.

As used in the present invention, fragments of ion-x-encoding polynucleotides comprise at least 10, and preferably at least 12, 14, 16, 18, 20, 25, 50, or 75 consecutive nucleotides of a polynucleotide encoding ion-x. Preferably, fragment polynucleotides of the invention comprise sequences unique to the ion-x-encoding polynucleotide sequence, and therefore hybridize under highly stringent or moderately stringent conditions only (*i.e.*, "specifically") to polynucleotides encoding ion-x (or fragments thereof). Polynucleotide fragments of genomic sequences of the invention comprise not only sequences unique to the coding region, but also include fragments of the full-length sequence derived from introns, regulatory regions, and/or other non-translated sequences. Sequences unique to polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, *e.g.*, those made available in public sequence databases. Such sequences also are recognizable from Southern hybridization analyses to determine the number of fragments of genomic DNA to which a polynucleotide will hybridize. Polynucleotides of the invention can be labeled in a manner that permits their detection, including radioactive, fluorescent, and enzymatic labeling.

Fragment polynucleotides are particularly useful as probes for detection of full-length or fragments of ion-x polynucleotides. One or more polynucleotides can be

included in kits that are used to detect the presence of a polynucleotide encoding ion-x, or used to detect variations in a polynucleotide sequence encoding ion-x.

The invention also embraces DNAs encoding ion-x polypeptides that hybridize under moderately stringent or high stringency conditions to the non-coding strand, or complement, of the polynucleotides set forth in a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

Exemplary highly stringent hybridization conditions are as follows: hybridization at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1 X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel *et al.* (Eds.), Protocols in Molecular Biology, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, *et al.*, (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

With the knowledge of the nucleotide sequence information disclosed in the present invention, one skilled in the art can identify and obtain nucleotide sequences which encode ion-x from different sources (*i.e.*, different tissues or different organisms) through a variety of means well known to the skilled artisan and as disclosed by, for example, Sambrook *et al.*, "Molecular cloning: a laboratory manual", Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), which is incorporated herein by reference in its entirety.

For example, DNA that encodes ion-x may be obtained by screening mRNA, cDNA, or genomic DNA with oligonucleotide probes generated from the ion-x gene sequence information provided herein. Probes may be labeled with a detectable group, such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with procedures known to the skilled artisan and used in conventional hybridization assays, as described by, for example, Sambrook *et al.*

A nucleic acid molecule comprising any of the ion-x nucleotide sequences described above can alternatively be synthesized by use of the polymerase chain reaction

(PCR) procedure, with the PCR oligonucleotide primers produced from the nucleotide sequences provided herein. See U.S. Patent Numbers 4,683,195 to Mullis *et al.* and 4,683,202 to Mullis. The PCR reaction provides a method for selectively increasing the concentration of a particular nucleic acid sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotide probes to serve as primers for the template-dependent, polymerase mediated replication of a desired nucleic acid molecule.

A wide variety of alternative cloning and *in vitro* amplification methodologies are well known to those skilled in the art. Examples of these techniques are found in, for example, Berger *et al.*, *Guide to Molecular Cloning Techniques*, Methods in Enzymology 152, Academic Press, Inc., San Diego, CA (Berger), which is incorporated herein by reference in its entirety.

Automated sequencing methods can be used to obtain or verify the nucleotide sequence of ion-x. The ion-x nucleotide sequences of the present invention are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. An error in a sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation.

The nucleic acid molecules of the present invention, and fragments derived therefrom, are useful for screening for restriction fragment length polymorphism (RFLP) associated with certain disorders, as well as for genetic mapping.

The polynucleotide sequence information provided by the invention makes possible large-scale expression of the encoded polypeptide by techniques well known and routinely practiced in the art.

Vectors

Another aspect of the present invention is directed to vectors, or recombinant expression vectors, comprising any of the nucleic acid molecules described above. Vectors are used herein either to amplify DNA or RNA encoding ion-x and/or to express DNA which encodes ion-x. Preferred vectors include, but are not limited to, plasmids, phages, cosmids, episomes, viral particles or viruses, and integratable DNA fragments (*i.e.*, fragments integratable into the host genome by homologous recombination). Preferred viral particles include, but are not limited to, adenoviruses, baculoviruses, parvoviruses, herpesviruses, poxviruses, adeno-associated viruses, Semliki Forest viruses, vaccinia viruses, and retroviruses. Preferred expression vectors include, but are not limited to, pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). Other expression vectors include, but are not limited to, pSPORT™ vectors, pGEM™ vectors (Promega), pPROEXvectors™ (LTI, Bethesda, MD), Bluescript™ vectors (Stratagene), pQE™ vectors (Qiagen), pSE420™ (Invitrogen), and pYES2™(Invitrogen).

Expression constructs preferably comprise ion-x-encoding polynucleotides operatively linked to an endogenous or exogenous expression control DNA sequence and a transcription terminator. Expression control DNA sequences include promoters, enhancers, operators, and regulatory element binding sites generally, and are typically selected based on the expression systems in which the expression construct is to be utilized. Preferred promoter and enhancer sequences are generally selected for the ability to increase gene expression, while operator sequences are generally selected for the ability to regulate gene expression. Expression constructs of the invention may also include sequences encoding one or more selectable markers that permit identification of host cells bearing the construct. Expression constructs may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell. Preferred constructs of the invention also include sequences necessary for replication in a host cell.

Expression constructs are preferably utilized for production of an encoded protein, but may also be utilized simply to amplify an ion-x-encoding polynucleotide sequence. In preferred embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Autonomously replicating recombinant expression constructs such as plasmid and viral DNA vectors incorporating polynucleotides of the invention are also provided. Preferred expression vectors are replicable DNA constructs in which a DNA sequence

encoding ion-x is operably linked or connected to suitable control sequences capable of effecting the expression of the ion-x in a suitable host. DNA regions are operably linked or connected when they are functionally related to each other. For example, a promoter is operably linked or connected to a coding sequence if it controls the transcription of the sequence. Amplification vectors do not require expression control domains, but rather need only the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. The need for control sequences in the expression vector will vary depending upon the host selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding and sequences which control the termination of transcription and translation.

Preferred vectors preferably contain a promoter that is recognized by the host organism. The promoter sequences of the present invention may be prokaryotic, eukaryotic or viral. Examples of suitable prokaryotic sequences include the P_R and P_L promoters of bacteriophage lambda (The bacteriophage Lambda, Hershey, A. D., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1973), which is incorporated herein by reference in its entirety; Lambda II, Hendrix, R. W., Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (1980), which is incorporated herein by reference in its entirety); the trp, recA, heat shock, and lacZ promoters of *E. coli* and the SV40 early promoter (Benoist *et al. Nature*, 1981; *290*, 304-310, which is incorporated herein by reference in its entirety). Additional promoters include, but are not limited to, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney virus, cytomegalovirus immediate early promoter, Epstein Barr virus, Rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine, and human metalothionein.

Additional regulatory sequences can also be included in preferred vectors. Preferred examples of suitable regulatory sequences are represented by the Shine-Dalgarno of the replicase gene of the phage MS-2 and of the gene cII of bacteriophage lambda. The Shine-Dalgarno sequence may be directly followed by DNA encoding ion-x and result in the expression of the mature ion-x protein.

Moreover, suitable expression vectors can include an appropriate marker that allows the screening of the transformed host cells. The transformation of the selected host

is carried out using any one of the various techniques well known to the expert in the art and described in Sambrook *et al.*, *supra*.

An origin of replication can also be provided either by construction of the vector to include an exogenous origin or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter may be sufficient. Alternatively, rather than using vectors which contain viral origins of replication, one skilled in the art can transform mammalian cells by the method of co-transformation with a selectable marker and ion-x DNA. An example of a suitable marker is dihydrofolate reductase (DHFR) or thymidine kinase (*see*, U.S. Patent No. 4,399,216).

Nucleotide sequences encoding ion-x may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Sambrook *et al.*, *supra* and are well known in the art. Methods for construction of mammalian expression vectors are disclosed in, for example, Okayama *et al.*, *Mol. Cell. Biol.*, **1983**, *3*, 280, Cosman *et al.*, *Mol. Immunol.*, **1986**, *23*, 935, Cosman *et al.*, *Nature*, **1984**, *312*, 768, EP-A-0367566, and WO 91/18982, each of which is incorporated herein by reference in its entirety.

Host cells

According to another aspect of the invention, host cells are provided, including prokaryotic and eukaryotic cells, comprising a polynucleotide of the invention (or vector of the invention) in a manner that permits expression of the encoded ion-x polypeptide. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell that are well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. Expression systems of the invention include bacterial, yeast, fungal, plant, insect, invertebrate, vertebrate, and mammalian cells systems.

The invention provides host cells that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. As stated

above, such host cells are useful for amplifying the polynucleotides and also for expressing the ion-x polypeptide or fragment thereof encoded by the polynucleotide.

In still another related embodiment, the invention provides a method for producing an ion-x polypeptide (or fragment thereof) comprising the steps of growing a host cell of the invention in a nutrient medium and isolating the polypeptide or variant thereof from the cell or the medium. Because ion-x is a membrane spanning channel, it will be appreciated that, for some applications, such as certain activity assays, the preferable isolation may involve isolation of cell membranes containing the polypeptide embedded therein, whereas for other applications a more complete isolation may be preferable.

According to some aspects of the present invention, transformed host cells having an expression vector comprising any of the nucleic acid molecules described above are provided. Expression of the nucleotide sequence occurs when the expression vector is introduced into an appropriate host cell. Suitable host cells for expression of the polypeptides of the invention include, but are not limited to, prokaryotes, yeast, and eukaryotes. If a prokaryotic expression vector is employed, then the appropriate host cell would be any prokaryotic cell capable of expressing the cloned sequences. Suitable prokaryotic cells include, but are not limited to, bacteria of the genera *Escherichia*, *Bacillus*, *Salmonella*, *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

If an eukaryotic expression vector is employed, then the appropriate host cell would be any eukaryotic cell capable of expressing the cloned sequence. Preferably, eukaryotic cells are cells of higher eukaryotes. Suitable eukaryotic cells include, but are not limited to, non-human mammalian tissue culture cells and human tissue culture cells. Preferred host cells include, but are not limited to, insect cells, HeLa cells, Chinese hamster ovary cells (CHO cells), African green monkey kidney cells (COS cells), human 293 cells, and murine 3T3 fibroblasts. Propagation of such cells in cell culture has become a routine procedure (*see*, Tissue Culture, Academic Press, Kruse and Patterson, eds. (1973), which is incorporated herein by reference in its entirety).

In addition, a yeast host may be employed as a host cell. Preferred yeast cells include, but are not limited to, the genera *Saccharomyces*, *Pichia*, and *Kluveromyces*. Preferred yeast hosts are *S. cerevisiae* and *P. pastoris*. Preferred yeast vectors can contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replication sequence (ARS), a promoter region, sequences for polyadenylation, sequences for

transcription termination, and a selectable marker gene. Shuttle vectors for replication in both yeast and *E. coli* are also included herein.

Alternatively, insect cells may be used as host cells. In a preferred embodiment, the polypeptides of the invention are expressed using a baculovirus expression system (*see*, Luckow *et al.*, *Bio/Technology*, **1988**, *6*, 47, Baculovirus Expression Vectors: A Laboratory Manual, O'Rielly *et al.* (Eds.), W.H. Freeman and Company, New York, **1992**, and U.S. Patent No. 4,879,236, each of which is incorporated herein by reference in its entirety). In addition, the MAXBAC™ complete baculovirus expression system (Invitrogen) can, for example, be used for production in insect cells.

Host cells of the invention are a valuable source of immunogen for development of antibodies specifically immunoreactive with ion-x. Host cells of the invention are also useful in methods for the large-scale production of ion-x polypeptides wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells, or from the medium in which the cells are grown, by purification methods known in the art, *e.g.*, conventional chromatographic methods including immunoaffinity chromatography, receptor affinity chromatography, hydrophobic interaction chromatography, lectin affinity chromatography, size exclusion filtration, cation or anion exchange chromatography, high pressure liquid chromatography (HPLC), reverse phase HPLC, and the like. Still other methods of purification include those methods wherein the desired protein is expressed and purified as a fusion protein having a specific tag, label, or chelating moiety that is recognized by a specific binding partner or agent. The purified protein can be cleaved to yield the desired protein, or can be left as an intact fusion protein. Cleavage of the fusion component may produce a form of the desired protein having additional amino acid residues as a result of the cleavage process.

Knowledge of ion-x DNA sequences allows for modification of cells to permit, or increase, expression of endogenous ion-x. Cells can be modified (*e.g.*, by homologous recombination) to provide increased expression by replacing, in whole or in part, the naturally occurring ion-x promoter with all or part of a heterologous promoter so that the cells express ion-x at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to endogenous ion-x encoding sequences. (See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955.) It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA

(*e.g.*, ada, dhfr, and the multifunctional CAD gene which encodes carbamoyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the ion-x coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the ion-x coding sequences in the cells.

Knock-outs

The DNA sequence information provided by the present invention also makes possible the development (*e.g.*, by homologous recombination or "knock-out" strategies; see Capecchi, *Science 244*:1288-1292 (1989), which is incorporated herein by reference) of animals that fail to express functional ion-x or that express a variant of ion-x. Such animals (especially small laboratory animals such as rats, rabbits, and mice) are useful as models for studying the *in vivo* activities of ion-x and modulators of ion-x.

Antisense

Also made available by the invention are anti-sense polynucleotides that recognize and hybridize to polynucleotides encoding ion-x. Full-length and fragment anti-sense polynucleotides are provided. Fragment antisense molecules of the invention include (i) those that specifically recognize and hybridize to ion-x RNA (as determined by sequence comparison of DNA encoding ion-x to DNA encoding other known molecules). Identification of sequences unique to ion-x encoding polynucleotides can be deduced through use of any publicly available sequence database, and/or through use of commercially available sequence comparison programs. After identification of the desired sequences, isolation through restriction digestion or amplification using any of the various polymerase chain reaction techniques well known in the art can be performed. Anti-sense polynucleotides are particularly relevant to regulating expression of ion-x by those cells expressing ion-x mRNA.

Antisense nucleic acids (preferably 10 to 30 base-pair oligonucleotides) capable of specifically binding to ion-x expression control sequences or ion-x RNA are introduced into cells (*e.g.*, by a viral vector or colloidal dispersion system such as a liposome). The antisense nucleic acid binds to the ion-x target nucleotide sequence in the cell and prevents transcription and/or translation of the target sequence. Phosphorothioate and methylphosphonate antisense oligonucleotides are specifically contemplated for therapeutic use by the invention. Locked nucleic acids are also specifically contemplated for therapeutic use by the present invention. (See, for example, Wahlestedt *et al.*, Proc.

Natl. Acad. Sci. USA, Vol. 97, Issue 10, 5633-5638, May 9, 2000, which is incorporated by reference in its entirety) The antisense oligonucleotides may be further modified by adding poly-L-lysine, transferrin polylysine, or cholesterol moieties at their 5' end. Suppression of ion-x expression at either the transcriptional or translational level is useful to generate cellular or animal models for diseases/conditions characterized by aberrant ion-x expression.

Antisense oligonucleotides, or fragments of nucleotide sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, or sequences complementary or homologous thereto, derived from the nucleotide sequences of the present invention encoding ion-x are useful as diagnostic tools for probing gene expression in various tissues. For example, tissue can be probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiography techniques to investigate native expression of this enzyme or pathological conditions relating thereto. Antisense oligonucleotides are preferably directed to regulatory regions of sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, or mRNA corresponding thereto, including, but not limited to, the initiation codon, TATA box, enhancer sequences, and the like.

Transcription factors

The ion-x sequences taught in the present invention facilitate the design of novel transcription factors for modulating ion-x expression in native cells and animals, and cells transformed or transfected with ion-x polynucleotides. For example, the Cys_2-His_2 zinc finger proteins, which bind DNA via their zinc finger domains, have been shown to be amenable to structural changes that lead to the recognition of different target sequences. These artificial zinc finger proteins recognize specific target sites with high affinity and low dissociation constants, and are able to act as gene switches to modulate gene expression. Knowledge of the particular ion-x target sequence of the present invention facilitates the engineering of zinc finger proteins specific for the target sequence using known methods such as a combination of structure-based modeling and screening of phage display libraries (Segal *et al.*, Proc. Natl. Acad. Sci. (USA) 96:2758-2763 (1999); Liu *et al.*, Proc. Natl. Acad. Sci. (USA) 94:5525-5530 (1997); Greisman *et al.*, Science 275:657-661 (1997); Choo *et al.*, J. Mol. Biol. 273:525-532 (1997)). Each zinc finger domain usually recognizes three or more base pairs. Since a recognition sequence of 18 base pairs is generally sufficient in length to render it unique in any known genome, a zinc finger

protein consisting of 6 tandem repeats of zinc fingers would be expected to ensure specificity for a particular sequence (Segal *et al.*) The artificial zinc finger repeats, designed based on ion-x sequences, are fused to activation or repression domains to promote or suppress ion-x expression (Liu *et al.)* Alternatively, the zinc finger domains can be fused to the TATA box-binding factor (TBP) with varying lengths of linker region between the zinc finger peptide and the TBP to create either transcriptional activators or repressors (Kim *et al.*, Proc. Natl. Acad. Sci. (USA) 94:3616-3620 (1997). Such proteins and polynucleotides that encode them, have utility for modulating ion-x expression *in vivo* in both native cells, animals and humans; and/or cells transfected with ion-x -encoding sequences. The novel transcription factor can be delivered to the target cells by transfecting constructs that express the transcription factor (gene therapy), or by introducing the protein. Engineered zinc finger proteins can also be designed to bind RNA sequences for use in therapeutics as alternatives to antisense or catalytic RNA methods (McColl *et al.*, Proc. Natl. Acad. Sci. (USA) 96:9521-9526 (1997); Wu *et al.*, Proc. Natl. Acad. Sci. (USA) 92:344-348 (1995)). The present invention contemplates methods of designing such transcription factors based on the gene sequence of the invention, as well as customized zinc finger proteins, that are useful to modulate ion-x expression in cells (native or transformed) whose genetic complement includes these sequences.

Polypeptides

The invention also provides purified and isolated mammalian ion-x polypeptides encoded by a polynucleotide of the invention. Presently preferred is a human ion-x polypeptide comprising the amino acid sequence set out in sequences selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, or fragments thereof comprising an epitope specific to the polypeptide. By "epitope specific to" is meant a portion of the ion-x receptor that is recognizable by an antibody that is specific for the ion-x, as defined in detail below.

Although the sequences provided are particular human sequences, the invention is intended to include within its scope other human allelic variants; non-human mammalian forms of ion-x, and other vertebrate forms of ion-x.

It will be appreciated that extracellular epitopes are particularly useful for generating and screening for antibodies and other binding compounds that bind to receptors such as ion-x. Thus, in another preferred embodiment, the invention provides a purified and isolated polypeptide comprising at least one extracellular domain of ion-x.

Purified and isolated polypeptides comprising the extracellular domain of ion-x are highly preferred. Also preferred is a purified and isolated polypeptide comprising an ion-x fragment selected from the group consisting of the extracellular domain of ion-x, a transmembrane domain of ion-x, the cytoplasmic region of ion-x, and fusions thereof. Such fragments may be continuous portions of the native receptor. However, it will also be appreciated that knowledge of the ion-x gene and protein sequences as provided herein permits recombining of various domains that are not contiguous in the native protein. Using a FORTRAN computer program called "tmtrest.all" [Parodi *et al.*, Comput. Appl. Biosci. 5:527-535 (1994)], ion-x was shown to contain transmembrane-spanning domains.

The invention also embraces polypeptides that have at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55% or at least 50% identity and/or homology to the preferred polypeptide of the invention. Percent amino acid sequence "identity" with respect to the preferred polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the ion-x sequence after aligning both sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Percent sequence "homology" with respect to the preferred polypeptide of the invention is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the ion-x sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and also considering any conservative substitutions as part of the sequence identity.

In one aspect, percent homology is calculated as the percentage of amino acid residues in the smaller of two sequences which align with identical amino acid residue in the sequence being compared, when four gaps in a length of 100 amino acids may be introduced to maximize alignment [Dayhoff, in Atlas of Protein Sequence and Structure, Vol. 5, p. 124, National Biochemical Research Foundation, Washington, D.C. (1972), incorporated herein by reference].

Polypeptides of the invention may be isolated from natural cell sources or may be chemically synthesized, but are preferably produced by recombinant procedures involving host cells of the invention. Use of mammalian host cells is expected to provide for such post-translational modifications (*e.g.*, glycosylation, truncation, lipidation, and

phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of ion-x polypeptides are embraced by the invention.

The invention also embraces variant (or analog) ion-x polypeptides. In one example, insertion variants are provided wherein one or more amino acid residues supplement an ion-x amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the ion-x amino acid sequence. Insertional variants with additional residues at either or both termini can include, for example, fusion proteins and proteins including amino acid tags or labels.

Insertion variants include ion-x polypeptides wherein one or more amino acid residues are added to an ion-x acid sequence or to a biologically active fragment thereof.

Variant products of the invention also include mature ion-x products, *i.e.*, ion-x products wherein leader or signal sequences are removed, with additional amino terminal residues. The additional amino terminal residues may be derived from another protein, or may include one or more residues that are not identifiable as being derived from specific proteins. Ion-x products with an additional methionine residue at position -1 (Met^{-1}-ion-x) are contemplated, as are variants with additional methionine and lysine residues at positions -2 and -1 (Met^{-2}-Lys^{-1}-ion-x). Variants of ion-x with additional Met, Met-Lys, Lys residues (or one or more basic residues in general) are particularly useful for enhanced recombinant protein production in bacterial host cells.

The invention also embraces ion-x variants having additional amino acid residues that result from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of a glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at position -1 after cleavage of the GST component from the desired polypeptide. Variants that result from expression in other vector systems are also contemplated.

Insertional variants also include fusion proteins wherein the amino terminus and/or the carboxy terminus of ion-x is/are fused to another polypeptide.

In another aspect, the invention provides deletion variants wherein one or more amino acid residues in an ion-x polypeptide are removed. Deletions can be effected at one or both termini of the ion-x polypeptide, or with removal of one or more non-terminal amino acid residues of ion-x. Deletion variants, therefore, include all fragments of an ion-x polypeptide.

The invention also embraces polypeptide fragments of sequences selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, wherein the fragments maintain biological (*e.g.*, ligand binding and/or ion trafficking) and/or immunological properties of a ion-x polypeptide.

In one preferred embodiment of the invention, an isolated nucleic acid molecule comprises a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence homologous to a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, and fragments thereof, wherein the nucleic acid molecule encodes at least a portion of ion-x. In a more preferred embodiment, the isolated nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, and fragments thereof.

As used in the present invention, polypeptide fragments comprise at least 5, 10, 15, 20, 25, 30, 35, or 40 consecutive amino acids of a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50. Preferred polypeptide fragments display antigenic properties unique to, or specific for, human ion-x and its allelic and species homologs. Fragments of the invention having the desired biological and immunological properties can be prepared by any of the methods well known and routinely practiced in the art.

In one embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:1. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:1. Preferably, the invention provides fragments of SEQ ID NO:1 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:1, may include more than one portion of SEQ ID NO:1, or may include repeated portions of SEQ ID NO:1. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the serotonin 5-HT3 receptor.

In another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:2. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:2. Preferably, the invention provides fragments of SEQ ID NO:2 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:2, may include more than one portion of SEQ ID NO:2, or may include repeated portions of SEQ ID NO:2. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the GABA receptor gamma-1 subunit.

In yet another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:3. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:3. Preferably, the invention provides fragments of SEQ ID NO:3 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:3, may include more than one portion of SEQ ID NO:3, or may include repeated portions of SEQ ID NO:3. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the GABA receptor gamma-1 subunit.

In still another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:4. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:4. Preferably, the invention provides fragments of SEQ ID NO:4 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:4, may include more than one portion of SEQ ID NO:4, or may include repeated portions of SEQ ID NO:4. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the acetylcholine receptor alpha-9 chain.

In another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:5. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:5. Preferably, the invention provides fragments of SEQ ID NO:5 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:5, may include more than one portion of SEQ ID NO:5, or may include repeated portions of SEQ ID NO:5. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the GABA receptor rho-3 subunit.

In yet another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:6. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:6. Preferably, the invention provides fragments of SEQ ID NO:6 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:6, may include more than one portion of SEQ ID NO:6, or may include repeated portions of SEQ ID NO:6. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the GABA receptor rho-3 subunit.

In still another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:7. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:7. Preferably, the invention provides fragments of SEQ ID NO:7 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:7, may include more than one portion of SEQ ID NO:7, or may include repeated portions of SEQ ID NO:7. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the acetylcholine receptor epsilon chain.

In yet another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:8. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:8. Preferably, the invention provides fragments of SEQ ID NO:8 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:8, may include more than one portion of SEQ ID NO:8, or may include repeated portions of SEQ ID NO:8. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the GABA receptor beta-like subunit.

In still another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:9. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:9. Preferably, the invention provides fragments of SEQ ID NO:9 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:9, may include more than one portion of SEQ ID NO:9, or may include repeated portions of SEQ ID NO:9. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the acetylcholine receptor beta-2 chain.

In another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:49. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:49. Preferably, the invention provides fragments of SEQ ID NO:49 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. The fragment can be located within any portion of SEQ ID NO:49, may include more than one portion of SEQ ID NO:49, or may include repeated portions of SEQ ID NO:49. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the GABA receptor rho-3 subunit.

In still another embodiment of the invention, the nucleic acid molecule comprises SEQ ID NO:51. Alternatively, the nucleic acid molecule comprises a fragment of SEQ ID NO:51. Preferably, the invention provides fragments of SEQ ID NO:51 which comprise at least 14 and preferably at least 16, 18, 20, 25, 50, or 75 consecutive nucleotides. In a more preferred embodiment, the invention provides fragments of SEQ ID NO:51 which comprise at least 1963 and more preferably at least 1965, 1970, 1975, 2000, or 2005 consecutive nucleotides. In an even more preferred embodiment, the invention provides fragments of SEQ ID NO:51 which are not set forth in Genbank Accession Number AF199235 (*e.g.* Lustig, L.R., Heil, H. and Fuchs, P.A., Identification of a novel human nicotinic acetylcholine receptor subunit from inner ear and lymphoid tissue, Direct Submission to Genbank). The fragment can be located within any portion of SEQ ID NO:51, may include more than one portion of SEQ ID NO:51, or may include repeated portions of SEQ ID NO:51. In a preferred embodiment, the nucleic acid molecule comprises a sequence related to the alpha10 nicotinic acetylcholine receptor.

In still another aspect, the invention provides substitution variants of ion-x polypeptides. Substitution variants include those polypeptides wherein one or more amino acid residues of an ion-x polypeptide are removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature; however, the invention embraces substitutions that are also non-conservative. Conservative substitutions for this purpose may be defined as set out in Tables 2, 3, or 4 below.

Variant polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table 2 (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 9/6/96), immediately below.

Table 2

Conservative Substitutions I

SIDE CHAIN CHARACTERISTIC	AMINO ACID
Aliphatic	
Non-polar	G A P I L V
Polar - uncharged	C S T M N Q
Polar - charged	D E K R
Aromatic	H F W Y
Other	N Q D E

Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY, NY (1975), pp.71-77] as set out in Table 3, below.

Table 3
Conservative Substitutions II

SIDE CHAIN CHARACTERISTIC	AMINO ACID
Non-polar (hydrophobic)	
A. Aliphatic:	A L I V P
B. Aromatic:	F W
C. Sulfur-containing:	M
D. Borderline:	G
Uncharged-polar	
A. Hydroxyl:	S T Y
B. Amides:	N Q
C. Sulfhydryl:	C
D. Borderline:	G
Positively Charged (Basic):	K R H
Negatively Charged (Acidic):	D E

As still another alternative, exemplary conservative substitutions are set out in Table 4, below.

Table 4
Conservative Substitutions III

Original Residue	Exemplary Substitution
Ala (A)	Val, Leu, Ile
Arg (R)	Lys, Gln, Asn
Asn (N)	Gln, His, Lys, Arg
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp

His (H)	Asn, Gln, Lys, Arg
Ile (I)	Leu, Val, Met, Ala, Phe,
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, Gln, Asn
Met (M)	Leu, Phe, Ile
Phe (F)	Leu, Val, Ile, Ala
Pro (P)	Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser
Val (V)	Ile, Leu, Met, Phe, Ala

It should be understood that the definition of polypeptides of the invention is intended to include polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. By way of example, the modifications may be covalent in nature, and include for example, chemical bonding with polymers, lipids, other organic, and inorganic moieties. Such derivatives may be prepared to increase circulating half-life of a polypeptide, or may be designed to improve the targeting capacity of the polypeptide for desired cells, tissues, or organs. Similarly, the invention further embraces ion-x polypeptides that have been covalently modified to include one or more water-soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol. Variants that display ligand binding properties of native ion-x and are expressed at higher levels, as well as variants that provide for constitutively active receptors, are particularly useful in assays of the invention; the variants are also useful in providing cellular, tissue and animal models of diseases/conditions characterized by aberrant ion-x activity.

In a related embodiment, the present invention provides compositions comprising purified polypeptides of the invention. Preferred compositions comprise, in addition to the polypeptide of the invention, a pharmaceutically acceptable (*i.e.*, sterile and non-toxic) liquid, semisolid, or solid diluent that serves as a pharmaceutical vehicle, excipient, or medium. Any diluent known in the art may be used. Exemplary diluents include, but are not limited to, water, saline solutions, polyoxyethylene sorbitan monolaurate, magnesium stearate, methyl- and propylhydroxybenzoate, talc, alginates, starches, lactose, sucrose, dextrose, sorbitol, mannitol, glycerol, calcium phosphate, mineral oil, and cocoa butter.

Variants that display ligand binding properties of native ion-x and are expressed at higher levels, as well as variants that provide for constitutively active receptors, are

particularly useful in assays of the invention; the variants are also useful in assays of the invention and in providing cellular, tissue and animal models of diseases/conditions characterized by aberrant ion-x activity.

Antibodies

Also comprehended by the present invention are antibodies (*e.g.*, monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, including compounds which include CDR sequences which specifically recognize a polypeptide of the invention) specific for ion-x or fragments thereof. Preferred antibodies of the invention are human antibodies that are produced and identified according to methods described in WO93/11236, published June 20, 1993, which is incorporated herein by reference in its entirety. Antibody fragments, including Fab, Fab', $F(ab')_2$, and F_v, are also provided by the invention. The term "specific for," when used to describe antibodies of the invention, indicates that the variable regions of the antibodies of the invention recognize and bind ion-x polypeptides exclusively (*i.e.*, are able to distinguish ion-x polypeptides from other known ion channel polypeptides by virtue of measurable differences in binding affinity, despite the possible existence of localized sequence identity, homology, or similarity between ion-x and such polypeptides). It will be understood that specific antibodies may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and, in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow *et al.* (Eds.), Antibodies A Laboratory Manual; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6. Antibodies that recognize and bind fragments of the ion-x polypeptides of the invention are also contemplated, provided that the antibodies are specific for ion-x polypeptides. Antibodies of the invention can be produced using any method well known and routinely practiced in the art.

The invention provides an antibody that is specific for the ion-x of the invention. Antibody specificity is described in greater detail below. However, it should be emphasized that antibodies that can be generated from polypeptides that have previously been described in the literature and that are capable of fortuitously cross-reacting with ion-

x (*e.g.*, due to the fortuitous existence of a similar epitope in both polypeptides) are considered "cross-reactive" antibodies. Such cross-reactive antibodies are not antibodies that are "specific" for ion-x. The determination of whether an antibody is specific for ion-x or is cross-reactive with another known receptor is made using any of several assays, such as Western blotting assays, that are well known in the art. For identifying cells that express ion-x and also for modulating ion-x -ligand binding activity, antibodies that specifically bind to an extracellular epitope of the ion-x are preferred.

In one preferred variation, the invention provides monoclonal antibodies. Hybridomas that produce such antibodies also are intended as aspects of the invention. In yet another variation, the invention provides a humanized antibody. Humanized antibodies are useful for *in vivo* therapeutic indications.

In another variation, the invention provides a cell-free composition comprising polyclonal antibodies, wherein at least one of the antibodies is an antibody of the invention specific for ion-x. Antisera isolated from an animal is an exemplary composition, as is a composition comprising an antibody fraction of an antisera that has been resuspended in water or in another diluent, excipient, or carrier.

In still another related embodiment, the invention provides an anti-idiotypic antibody specific for an antibody that is specific for ion-x.

It is well known that antibodies contain relatively small antigen binding domains that can be isolated chemically or by recombinant techniques. Such domains are useful ion-x binding molecules themselves, and also may be reintroduced into human antibodies, or fused to toxins or other polypeptides. Thus, in still another embodiment, the invention provides a polypeptide comprising a fragment of an ion-x-specific antibody, wherein the fragment and the polypeptide bind to the ion-x. By way of non-limiting example, the invention provides polypeptides that are single chain antibodies and CDR-grafted antibodies.

Non-human antibodies may be humanized by any of the methods known in the art. In one method, the non-human CDRs are inserted into a human antibody or consensus antibody framework sequence. Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

Antibodies of the invention are useful for, *e.g.*, therapeutic purposes (by modulating activity of ion-x), diagnostic purposes to detect or quantitate ion-x, and purification of ion-x. Kits comprising an antibody of the invention for any of the purposes

described herein are also comprehended. In general, a kit of the invention also includes a control antigen for which the antibody is immunospecific.

Compositions

Mutations in the ion-x gene that result in loss of normal function of the ion-x gene product underlie ion-x -related human disease states. The invention comprehends gene therapy to restore ion-x activity to treat those disease states. Delivery of a functional ion-x gene to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly viral vectors (*e.g.*, adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (*e.g.*, liposomes or chemical treatments). See, for example, Anderson, *Nature*, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, *Science, 244*: 1275-1281 (1989); Verma, *Scientific American*: 68-84 (1990); and Miller, *Nature, 357*: 455-460 (1992). Alternatively, it is contemplated that in other human disease states, preventing the expression of, or inhibiting the activity of, ion-x will be useful in treating disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of ion-x.

Another aspect of the present invention is directed to compositions, including pharmaceutical compositions, comprising any of the nucleic acid molecules or recombinant expression vectors described above and an acceptable carrier or diluent. Preferably, the carrier or diluent is pharmaceutically acceptable. Suitable carriers are described in the most recent edition of *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference in its entirety. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The formulations are sterilized by commonly used techniques.

Also within the scope of the invention are compositions comprising polypeptides, polynucleotides, or antibodies of the invention that have been formulated with, *e.g.*, a pharmaceutically acceptable carrier.

The invention also provides methods of using antibodies of the invention. For example, the invention provides a method for modulating ligand binding of an ion-x comprising the step of contacting the ion-x with an antibody specific for the ion-x, under conditions wherein the antibody binds the receptor.

Ion channels that may be expressed in the brain, such as ion-x, provide an indication that aberrant ion-x signaling activity may correlate with one or more neurological or psychological disorders. The invention also provides a method for treating a neurological or psychiatric disorder comprising the step of administering to a mammal in need of such treatment an amount of an antibody-like polypeptide of the invention that is sufficient to modulate ligand binding to an ion-x in neurons of the mammal. Ion-x may also be expressed in many tissues, including but not limited to, kidney, colon, small intestine, stomach, testis, placenta, adrenal gland, peripheral blood leukocytes, bone marrow, retina, ovary, fetal brain, fetal liver, heart, spleen, liver, lung, muscle, thyroid gland, uterus, prostate, skin, salivary gland, and pancreas. Tissues where specific ion-x of the present invention are expressed are identified in the Examples below.

Kits

The present invention is also directed to kits, including pharmaceutical kits. The kits can comprise any of the nucleic acid molecules described above, any of the polypeptides described above, or any antibody which binds to a polypeptide of the invention as described above, as well as a negative control. The kit preferably comprises additional components, such as, for example, instructions, solid support, reagents helpful for quantification, and the like.

In another aspect, the invention features methods for detection of a polypeptide in a sample as a diagnostic tool for diseases or disorders, wherein the method comprises the steps of: (a) contacting the sample with a nucleic acid probe which hybridizes under hybridization assay conditions to a nucleic acid target region of a polypeptide having a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50, said probe comprising the nucleic acid sequence encoding the polypeptide, fragments thereof, and the complements of the sequences and fragments; and (b) detecting the presence or amount of the probe:target region hybrid as an indication of the disease.

In preferred embodiments of the invention, the disease is selected from the group consisting of thyroid disorders (*e.g.* thyreotoxicosis, myxoedema); renal failure; inflammatory conditions (*e.g.*, Crohn's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (*e.g.*, pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder,

post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome; attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy, *etc.*); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases and disorders (*e.g.*, type 2 diabetes, obesity, anorexia, hypotension, hypertension, thrombosis, myocardial infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); and sexual dysfunction, among others.

As described above and in Example 11 below, the genes encoding ion-1 (nucleic acid sequence SEQ ID NO:1, SEQ ID NO:49, amino acid sequence SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:50), ion-2a (nucleic acid sequence SEQ ID NO:2, amino acid sequence SEQ ID NO:12, SEQ ID NO:13), ion-2b (nucleic acid sequence SEQ ID NO:3, amino acid sequence SEQ ID NO:14, SEQ ID NO:15), ion-3 (nucleic acid sequence SEQ ID NO:4, SEQ ID NO:51, amino acid sequence SEQ ID NO:16, SEQ ID NO:17), ion-5 (nucleic acid sequence SEQ ID NO:7, amino acid sequence SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28), and ion-7 (nucleic acid sequence SEQ ID NO:9, amino acid sequence SEQ ID NO:31, SEQ ID NO:32) have been detected in brain tissue indicating that these ion-x proteins are neuroreceptors. Kits may be designed to detect either expression of polynucleotides encoding these proteins or the proteins themselves in order to identify tissue as being neurological. For example, oligonucleotide hybridization kits can be provided which include a container having an oligonucleotide probe specific for the ion-x-specific DNA and optionally, containers with positive and negative controls and/or instructions. Similarly, PCR kits can be provided which include a container having primers specific for the ion-x-specific sequences, DNA and optionally, containers with size markers, positive and negative controls and/or instructions.

Hybridization conditions should be such that hybridization occurs only with the genes in the presence of other nucleic acid molecules. Under stringent hybridization conditions only highly complementary nucleic acid sequences hybridize. Preferably, such

conditions prevent hybridization of nucleic acids having 1 or 2 mismatches out of 20 contiguous nucleotides. Such conditions are defined supra.

The diseases for which detection of genes in a sample could be diagnostic include diseases in which nucleic acid (DNA and/or RNA) is amplified in comparison to normal cells. By "amplification" is meant increased numbers of DNA or RNA in a cell compared with normal cells.

The diseases that could be diagnosed by detection of nucleic acid in a sample preferably include central nervous system and metabolic diseases. The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The samples used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample that is compatible with the method utilized.

Alternatively, immunoassay kits can be provided which have containers container having antibodies specific for the ion-x protein and optionally, containers with positive and negative controls and/or instructions.

Kits may also be provided useful in the identification of ion-x binding partners such as natural ligands, neurotransmitters, or modulators (agonists or antagonists). Substances useful for treatment of disorders or diseases preferably show positive results in one or more *in vitro* assays for an activity corresponding to treatment of the disease or disorder in question. Substances that modulate the activity of the polypeptides preferably include, but are not limited to, antisense oligonucleotides, agonists and antagonists, and inhibitors of protein kinases.

Methods of inducing immune response

Another aspect of the present invention is directed to methods of inducing an immune response in a mammal against a polypeptide of the invention by administering to the mammal an amount of the polypeptide sufficient to induce an immune response. The amount will be dependent on the animal species, size of the animal, and the like but can be determined by those skilled in the art.

Methods of identifying ligands

The invention also provides assays to identify compounds that bind ion-x. One such assay comprises the steps of: (a) contacting a composition comprising an ion-x with a

compound suspected of binding ion-x; and (b) measuring binding between the compound and ion-x. In one variation, the composition comprises a cell expressing ion-x on its surface. In another variation, isolated ion-x or cell membranes comprising ion-x are employed. The binding may be measured directly, *e.g.*, by using a labeled compound, or may be measured indirectly by several techniques, including measuring ion trafficking of ion-x induced by the compound.

Specific binding molecules, including natural ligands and synthetic compounds, can be identified or developed using isolated or recombinant ion-x products, ion-x variants, or preferably, cells expressing such products. Binding partners are useful for purifying ion-x products and detection or quantification of ion-x products in fluid and tissue samples using known immunological procedures. Binding molecules are also manifestly useful in modulating (*i.e.*, blocking, inhibiting or stimulating) biological activities of ion-x, especially those activities involved in signal transduction.

The DNA and amino acid sequence information provided by the present invention also makes possible identification of binding partner compounds with which an ion-x polypeptide or polynucleotide will interact. Methods to identify binding partner compounds include solution assays, *in vitro* assays wherein ion-x polypeptides are immobilized, and cell-based assays. Identification of binding partner compounds of ion-x polypeptides provides candidates for therapeutic or prophylactic intervention in pathologies associated with ion-x normal and aberrant biological activity.

The invention includes several assay systems for identifying ion-x-binding partners. In solution assays, methods of the invention comprise the steps of (a) contacting an ion-x polypeptide with one or more candidate binding partner compounds and (b) identifying the compounds that bind to the ion-x polypeptide. Identification of the compounds that bind the ion-x polypeptide can be achieved by isolating the ion-x polypeptide/binding partner complex, and separating the binding partner compound from the ion-x polypeptide. An additional step of characterizing the physical, biological, and/or biochemical properties of the binding partner compound is also comprehended in another embodiment of the invention. In one aspect, the ion-x polypeptide/binding partner complex is isolated using an antibody immunospecific for either the ion-x polypeptide or the candidate binding partner compound.

In still other embodiments, either the ion-x polypeptide or the candidate binding partner compound comprises a label or tag that facilitates its isolation, and methods of the

invention to identify binding partner compounds include a step of isolating the ion-x polypeptide/binding partner complex through interaction with the label or tag. An exemplary tag of this type is a poly-histidine sequence, generally around six histidine residues, that permits isolation of a compound so labeled using nickel chelation. Other labels and tags, such as the FLAG® tag (Eastman Kodak, Rochester, NY), well known and routinely used in the art, are embraced by the invention.

In one variation of an *in vitro* assay, the invention provides a method comprising the steps of (a) contacting an immobilized ion-x polypeptide with a candidate binding partner compound and (b) detecting binding of the candidate compound to the ion-x polypeptide. In an alternative embodiment, the candidate binding partner compound is immobilized and binding of ion-x is detected. Immobilization is accomplished using any of the methods well known in the art, including covalent bonding to a support, a bead, or a chromatographic resin, as well as non-covalent, high affinity interactions such as antibody binding, or use of streptavidin/biotin binding wherein the immobilized compound includes a biotin moiety. Detection of binding can be accomplished (i) using a radioactive label on the compound that is not immobilized, (ii) using of a fluorescent label on the non-immobilized compound, (iii) using an antibody immunospecific for the non-immobilized compound, (iv) using a label on the non-immobilized compound that excites a fluorescent support to which the immobilized compound is attached, as well as other techniques well known and routinely practiced in the art.

The invention also provides cell-based assays to identify binding partner compounds of an ion-x polypeptide. In one embodiment, the invention provides a method comprising the steps of contacting an ion-x polypeptide expressed on the surface of a cell with a candidate binding partner compound and detecting binding of the candidate binding partner compound to the ion-x polypeptide. In a preferred embodiment, the detection comprises detecting a calcium flux or other physiological event in the cell caused by the binding of the molecule.

Another aspect of the present invention is directed to methods of identifying compounds that bind to either ion-x or nucleic acid molecules encoding ion-x, comprising contacting ion-x, or a nucleic acid molecule encoding the same, with a compound, and determining whether the compound binds ion-x or a nucleic acid molecule encoding the same. Binding can be determined by binding assays which are well known to the skilled artisan, including, but not limited to, gel-shift assays, Western blots, radiolabeled

competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis, ELISA, and the like, which are described in, for example, *Current Protocols in Molecular Biology*, **1999**, John Wiley & Sons, NY, which is incorporated herein by reference in its entirety. The compounds to be screened include (which may include compounds which are suspected to bind ion-x, or a nucleic acid molecule encoding the same), but are not limited to, extracellular, intracellular, biologic or chemical origin. The methods of the invention also embrace ligands, especially neuropeptides, that are attached to a label, such as a radiolabel (*e.g.*, ^{125}I, ^{35}S, ^{32}P, ^{33}P, ^{3}H), a fluorescence label, a chemiluminescent label, an enzymic label and an immunogenic label. Modulators falling within the scope of the invention include, but are not limited to, non-peptide molecules such as non-peptide mimetics, non-peptide allosteric effectors, and peptides. The ion-x polypeptide or polynucleotide employed in such a test may either be free in solution, attached to a solid support, borne on a cell surface or located intracellularly or associated with a portion of a cell. One skilled in the art can, for example, measure the formation of complexes between ion-x and the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between ion-x and its substrate caused by the compound being tested.

In another embodiment of the invention, high throughput screening for compounds having suitable binding affinity to ion-x is employed. Briefly, large numbers of different small peptide test compounds are synthesized on a solid substrate. The peptide test compounds are contacted with ion-x and washed. Bound ion-x is then detected by methods well known in the art. Purified polypeptides of the invention can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the protein and immobilize it on the solid support.

Generally, an expressed ion-x can be used for HTS binding assays in conjunction with its defined ligand, in this case the corresponding neuropeptide that activates it. The identified peptide is labeled with a suitable radioisotope, including, but not limited to, ^{125}I, ^{3}H, ^{35}S or ^{32}P, by methods that are well known to those skilled in the art. Alternatively, the peptides may be labeled by well-known methods with a suitable fluorescent derivative (Baindur *et al.*, *Drug Dev. Res.*, **1994**, *33*, 373-398; Rogers, *Drug Discovery Today*, **1997**, *2*, 156-160). Radioactive ligand specifically bound to the receptor in membrane

preparations made from the cell line expressing the recombinant protein can be detected in HTS assays in one of several standard ways, including filtration of the receptor-ligand complex to separate bound ligand from unbound ligand (Williams, *Med. Res. Rev.*, **1991**, *11*, 147-184; Sweetnam *et al.*, *J. Natural Products*, **1993**, *56*, 441-455). Alternative methods include a scintillation proximity assay (SPA) or a FlashPlate format in which such separation is unnecessary (Nakayama, *Cur. Opinion Drug Disc. Dev.*, **1998**, *1*, 85-91 Bossé *et al.*, *J. Biomolecular Screening*, **1998**, *3*, 285-292.). Binding of fluorescent ligands can be detected in various ways, including fluorescence energy transfer (FRET), direct spectrophotofluorometric analysis of bound ligand, or fluorescence polarization (Rogers, *Drug Discovery Today*, **1997**, *2*, 156-160; Hill, *Cur. Opinion Drug Disc. Dev.*, **1998**, *1*, 92-97).

Other assays may be used to identify specific ligands of a ion-x receptor, including assays that identify ligands of the target protein through measuring direct binding of test ligands to the target protein, as well as assays that identify ligands of target proteins through affinity ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Alternatively, such binding interactions are evaluated indirectly using the yeast two-hybrid system described in Fields *et al.*, Nature, 340:245-246 (1989), and Fields *et al.*, Trends in Genetics, 10:286-292 (1994), both of which are incorporated herein by reference. The two-hybrid system is a genetic assay for detecting interactions between two proteins or polypeptides. It can be used to identify proteins that bind to a known protein of interest, or to delineate domains or residues critical for an interaction. Variations on this methodology have been developed to clone genes that encode DNA binding proteins, to identify peptides that bind to a protein, and to screen for drugs. The two-hybrid system exploits the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA binding domain that binds to an upstream activation sequence (UAS) of a reporter gene, and is generally performed in yeast. The assay requires the construction of two hybrid genes encoding (1) a DNA-binding domain that is fused to a first protein and (2) an activation domain fused to a second protein. The DNA-binding domain targets the first hybrid protein to the UAS of the reporter gene; however, because most proteins lack an activation domain, this DNA-binding hybrid protein does not activate transcription of the reporter gene. The second hybrid protein, which contains the activation domain, cannot by itself activate expression of the reporter gene because it does not bind the UAS. However, when both hybrid

proteins are present, the noncovalent interaction of the first and second proteins tethers the activation domain to the UAS, activating transcription of the reporter gene. For example, when the first protein is an ion channel gene product, or fragment thereof, that is known to interact with another protein or nucleic acid, this assay can be used to detect agents that interfere with the binding interaction. Expression of the reporter gene is monitored as different test agents are added to the system. The presence of an inhibitory agent results in lack of a reporter signal.

The yeast two-hybrid assay can also be used to identify proteins that bind to the gene product. In an assay to identify proteins that bind to an ion-x receptor, or fragment thereof, a fusion polynucleotide encoding both an ion-x receptor (or fragment) and a UAS binding domain (*i.e.*, a first protein) may be used. In addition, a large number of hybrid genes each encoding a different second protein fused to an activation domain are produced and screened in the assay. Typically, the second protein is encoded by one or more members of a total cDNA or genomic DNA fusion library, with each second protein-coding region being fused to the activation domain. This system is applicable to a wide variety of proteins, and it is not even necessary to know the identity or function of the second binding protein. The system is highly sensitive and can detect interactions not revealed by other methods; even transient interactions may trigger transcription to produce a stable mRNA that can be repeatedly translated to yield the reporter protein.

Other assays may be used to search for agents that bind to the target protein. One such screening method to identify direct binding of test ligands to a target protein is described in U.S. Patent No. 5,585,277, incorporated herein by reference. This method relies on the principle that proteins generally exist as a mixture of folded and unfolded states, and continually alternate between the two states. When a test ligand binds to the folded form of a target protein (*i.e.*, when the test ligand is a ligand of the target protein), the target protein molecule bound by the ligand remains in its folded state. Thus, the folded target protein is present to a greater extent in the presence of a test ligand which binds the target protein, than in the absence of a ligand. Binding of the ligand to the target protein can be determined by any method that distinguishes between the folded and unfolded states of the target protein. The function of the target protein need not be known in order for this assay to be performed. Virtually any agent can be assessed by this method as a test ligand, including, but not limited to, metals, polypeptides, proteins, lipids, polysaccharides, polynucleotides and small organic molecules.

Another method for identifying ligands of a target protein is described in Wieboldt *et al.*, Anal. Chem., 69:1683-1691 (1997), incorporated herein by reference. This technique screens combinatorial libraries of 20-30 agents at a time in solution phase for binding to the target protein. Agents that bind to the target protein are separated from other library components by simple membrane washing. The specifically selected molecules that are retained on the filter are subsequently liberated from the target protein and analyzed by HPLC and pneumatically assisted electrospray (ion spray) ionization mass spectroscopy. This procedure selects library components with the greatest affinity for the target protein, and is particularly useful for small molecule libraries.

Other embodiments of the invention comprise using competitive screening assays in which neutralizing antibodies capable of binding a polypeptide of the invention specifically compete with a test compound for binding to the polypeptide. In this manner, the antibodies can be used to detect the presence of any peptide that shares one or more antigenic determinants with ion-x. Radiolabeled competitive binding studies are described in A.H. Lin *et al. Antimicrobial Agents and Chemotherapy*, **1997**, vol. 41, no. 10. pp. 2127-2131, the disclosure of which is incorporated herein by reference in its entirety.

As described above and in Example 11 below, the genes encoding ion-1 (nucleic acid sequence SEQ ID NO:1, SEQ ID NO:49, amino acid sequence SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:50), ion-2a (nucleic acid sequence SEQ ID NO:2, amino acid sequence SEQ ID NO:12, SEQ ID NO:13), ion-2b (nucleic acid sequence SEQ ID NO:3, amino acid sequence SEQ ID NO:14, SEQ ID NO:15), ion-3 (nucleic acid sequence SEQ ID NO:4, SEQ ID NO:51, amino acid sequence SEQ ID NO:16, SEQ ID NO:17), ion-5 (nucleic acid sequence SEQ ID NO:7, amino acid sequence SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28), and ion-7 (nucleic acid sequence SEQ ID NO:9, amino acid sequence SEQ ID NO:31, SEQ ID NO:32) have been detected in brain tissue indicating that these ion-x proteins are neuroreceptors. Accordingly, natural binding partners of these molecules include neurotransmitters.

Identification of modulating agents

The invention also provides methods for identifying a modulator of binding between a ion-x and an ion-x binding partner, comprising the steps of: (a) contacting an ion-x binding partner and a composition comprising an ion-x in the presence and in the absence of a putative modulator compound; (b) detecting binding between the binding

partner and the ion-x; and (c) identifying a putative modulator compound or a modulator compound in view of decreased or increased binding between the binding partner and the ion-x in the presence of the putative modulator, as compared to binding in the absence of the putative modulator.

Ion-x binding partners that stimulate ion-x activity are useful as agonists in disease states or conditions characterized by insufficient ion-x signaling (*e.g.*, as a result of insufficient activity of an ion-x ligand). Ion-x binding partners that block ligand-mediated ion-x signaling are useful as ion-x antagonists to treat disease states or conditions characterized by excessive ion-x signaling. In addition ion-x modulators in general, as well as ion-x polynucleotides and polypeptides, are useful in diagnostic assays for such diseases or conditions.

In another aspect, the invention provides methods for treating a disease or abnormal condition by administering to a patient in need of such treatment a substance that modulates the activity or expression of a polypeptide having a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50.

Agents that modulate (*i.e.*, increase, decrease, or block) ion-x activity or expression may be identified by incubating a putative modulator with a cell containing an ion-x polypeptide or polynucleotide and determining the effect of the putative modulator on ion-x activity or expression. The selectivity of a compound that modulates the activity of ion-x can be evaluated by comparing its effects on ion-x to its effect on other ion channel compounds. Selective modulators may include, for example, antibodies and other proteins, peptides, or organic molecules that specifically bind to an ion-x polypeptide or an ion-x -encoding nucleic acid. Modulators of ion-x activity will be therapeutically useful in treatment of diseases and physiological conditions in which normal or aberrant ion-x activity is involved. Ion-x polynucleotides, polypeptides, and modulators may be used in the treatment of such diseases and conditions as infections, such as viral infections caused by HIV-1 or HIV-2; thyroid disorders (*e.g.* thyreotoxicosis, myxoedema); renal failure; inflammatory conditions (*e.g.*, Crohn's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (*e.g.*, pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder, post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome;

attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy, *etc.*); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases and disorders (*e.g.*, type 2 diabetes, obesity, anorexia, hypotension, hypertension, thrombosis, myocardial infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); and sexual dysfunction, among others. Ion-x polynucleotides and polypeptides, as well as ion-x modulators, may also be used in diagnostic assays for such diseases or conditions.

Methods of the invention to identify modulators include variations on any of the methods described above to identify binding partner compounds, the variations including techniques wherein a binding partner compound has been identified and the binding assay is carried out in the presence and absence of a candidate modulator. A modulator is identified in those instances where binding between the ion-x polypeptide and the binding partner compound changes in the presence of the candidate modulator compared to binding in the absence of the candidate modulator compound. A modulator that increases binding between the ion-x polypeptide and the binding partner compound is described as an enhancer or activator, and a modulator that decreases binding between the ion-x polypeptide and the binding partner compound is described as an inhibitor.

The invention also comprehends high-throughput screening (HTS) assays to identify compounds that interact with or inhibit biological activity (*i.e.*, affect enzymatic activity, binding activity, *etc.*) of an ion-x polypeptide. HTS assays permit screening of large numbers of compounds in an efficient manner. Cell-based HTS systems are contemplated to investigate ion-x receptor-ligand interaction. HTS assays are designed to identify "hits" or "lead compounds" having the desired property, from which modifications can be designed to improve the desired property. Chemical modification of the "hit" or "lead compound" is often based on an identifiable structure/activity relationship between the "hit" and the ion-x polypeptide.

Another aspect of the present invention is directed to methods of identifying compounds which modulate (*i.e.*, increase or decrease) activity of ion-x comprising contacting ion-x with a compound, and determining whether the compound modifies

activity of ion-x. The activity in the presence of the test compared is measured to the activity in the absence of the test compound. One of skill in the art can, for example, measure the activity of the ion channel polypeptide using electrophysiological methods, described *infra*. Where the activity of the sample containing the test compound is higher than the activity in the sample lacking the test compound, the compound will have increased activity. Similarly, where the activity of the sample containing the test compound is lower than the activity in the sample lacking the test compound, the compound will have inhibited activity.

The activity of the polypeptides of the invention can also be determined by, as non-limiting examples, the ability to bind or be activated by certain ligands, including, but not limited to, known neurotransmitters, agonists and antagonists, including but not limited to serotonin, acetylcholine, nicotine, and GABA. Alternatively, the activity of the ion channels can be assayed by examining activity such as ability to bind or be affected by calcium ions, hormones, chemokines, neuropeptides, neurotransmitters, nucleotides, lipids, odorants, and photons. In various embodiments of the method, the assay may take the form of an ion flux assay, a membrane potential assay, a yeast growth assay, a cAMP assay, an inositol triphosphate assay, a diacylglycerol assay, an Aequorin assay, a Luciferase assay, a FLIPR assay for intracellular Ca^{2+} concentration, a mitogenesis assay, a MAP Kinase activity assay, an arachidonic acid release assay (*e.g.*, using [^{3}H]-arachidonic acid), and an assay for extracellular acidification rates, as well as other binding or function-based assays of activity that are generally known in the art

Another potentially useful assay to examine the activity of ion channels is electrophysiology, the measurement of ion permeability across the cell membrane. This technique is described in, for example, Electrophysiology; A Practical Approach, DI Wallis editor, IRL Press at Oxford University Press, (1993), and Voltage and patch Clamping with Microelectrodes, Smith *et al.*, eds., Waverly Press, Inc for the American Physiology Society (1985), each of which is incorporated by reference in its entirety.

Another assay to examine the activity of ion channels is through the use of the FLIPR Fluorometric Imaging Plate Reader system, developed by Dr. Vince Groppi of the Pharmacia Corporation to perform cell-based, high-throughput screening (HTS) assays measuring, for example, membrane potential. Changes in plasma membrane potential correlate with the modulation of ion channels as ions move into or out of the cell. The FLIPR system measures such changes in membrane potential. This is accomplished by

loading cells expressing an ion channel gene with a cell-membrane permeant fluorescent indicator dye suitable for measuring changes in membrane potential such as diBAC (bis-(1,3-dibutylbarbituric acid)pentamethine oxonol, Molecular Probes). Thus the modulation of ion channel activity can be assessed with FLIPR and detected as changes in the emission spectrum of the diBAC dye.

The present invention is particularly useful for screening compounds by using ion-x in any of a variety of drug screening techniques. The compounds to be screened include (which may include compounds which are suspected to modulate ion-x activity), but are not limited to, extracellular, intracellular, biologic or chemical origin. The ion-x polypeptide employed in such a test may be in any form, preferably, free in solution, attached to a solid support, borne on a cell surface or located intracellularly. One skilled in the art can, for example, measure the formation of complexes between ion-x and the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between ion-x and its substrate caused by the compound being tested.

The activity of ion-x polypeptides of the invention can be determined by, for example, examining the ability to bind or be activated by chemically synthesized peptide ligands. Alternatively, the activity of ion-x polypeptides can be assayed by examining their ability to bind calcium ions, hormones, chemokines, neuropeptides, neurotransmitters, nucleotides, lipids, odorants, and photons. Alternatively, the activity of the ion-x polypeptides can be determined by examining the activity of effector molecules including, but not limited to, adenylate cyclase, phospholipases and ion channels. Thus, modulators of ion-x polypeptide activity may alter ion channel function, such as a binding property of a channel or an activity such as ion selectivity. In various embodiments of the method, the assay may take the form of an ion flux assay, a yeast growth assay, a cAMP assay, an inositol triphosphate assay, a diacylglycerol assay, an Aequorin assay, a Luciferase assay, a FLIPR assay for intracellular Ca^{2+} concentration, a mitogenesis assay, a MAP Kinase activity assay, an arachidonic acid release assay (*e.g.*, using [^{3}H]-arachidonic acid), and an assay for extracellular acidification rates, as well as other binding or function-based assays of ion-x activity that are generally known in the art. Ion-x activity can be determined by methodologies that are used to assay for FaRP activity, which is well known to those skilled in the art. Biological activities of ion-x receptors according to the invention include, but are not limited to, the binding of a natural or an

unnatural ligand, as well as any one of the functional activities of ion channels known in the art.

The modulators of the invention exhibit a variety of chemical structures, which can be generally grouped into non-peptide mimetics of natural ion channel ligands, peptide and non-peptide allosteric effectors of ion channels, and peptides that may function as activators or inhibitors (competitive, uncompetitive and non-competitive) (*e.g.*, antibody products) of ion channels. The invention does not restrict the sources for suitable modulators, which may be obtained from natural sources such as plant, animal or mineral extracts, or non-natural sources such as small molecule libraries, including the products of combinatorial chemical approaches to library construction, and peptide libraries. Examples of organic modulators of ion channels are GABA, serotonin, acetylcholine, nicotine, glutamate, glycine, NMDA, and kainic acid.

Other assays can be used to examine enzymatic activity including, but not limited to, photometric, radiometric, HPLC, electrochemical, and the like, which are described in, for example, *Enzyme Assays: A Practical Approach*, eds., R. Eisenthal and M. J. Danson, **1992**, Oxford University Press, which is incorporated herein by reference in its entirety.

The use of cDNAs encoding ion channels in drug discovery programs is well known; assays capable of testing thousands of unknown compounds per day in high-throughput screens (HTSs) are thoroughly documented. The literature is replete with examples of the use of radiolabelled ligands in HTS binding assays for drug discovery (see Williams, *Medicinal Research Reviews*, **1991**, *11*, 147-184.; Sweetnam, *et al.*, *J. Natural Products*, **1993**, *56*, 441-455 for review). Recombinant receptors are preferred for binding assay HTS because they allow for better specificity (higher relative purity); provide the ability to generate large amounts of receptor material, and can be used in a broad variety of formats (see Hodgson, *Bio/Technology*, **1992**, *10*, 973-980; each of which is incorporated herein by reference in its entirety).

A variety of heterologous systems is available for functional expression of recombinant receptors that are well known to those skilled in the art. Such systems include bacteria (Strosberg, *et al.*, *Trends in Pharmacological Sciences*, **1992**, *13*, 95-98), yeast (Pausch, *Trends in Biotechnology*, **1997**, *15*, 487-494), several kinds of insect cells (Vanden Broeck, *Int. Rev. Cytology*, **1996**, *164*, 189-268), amphibian cells (Jayawickreme *et al.*, *Current Opinion in Biotechnology*, **1997**, *8*, 629-634) and several mammalian cell lines (CHO, HEK293, COS, etc.; see Gerhardt, *et al.*, *Eur. J. Pharmacology*, **1997**, *334*, 1-

23). These examples do not preclude the use of other possible cell expression systems, including cell lines obtained from nematodes (PCT application WO 98/37177).

In preferred embodiments of the invention, methods of screening for compounds that modulate ion-x activity comprise contacting test compounds with ion-x and assaying for the presence of a complex between the compound and ion-x. In such assays, the ligand is typically labeled. After suitable incubation, free ligand is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular compound to bind to ion-x.

Examples of such biological responses include, but are not limited to, the following: the ability to survive in the absence of a limiting nutrient in specifically engineered yeast cells (Pausch, *Trends in Biotechnology*, **1997**, *15*, 487-494); changes in intracellular Ca^{2+} concentration as measured by fluorescent dyes (Murphy, *et al.*, *Cur. Opinion Drug Disc. Dev.*, **1998**, *1*, 192-199). Fluorescence changes can also be used to monitor ligand-induced changes in membrane potential or intracellular pH; an automated system suitable for HTS has been described for these purposes (Schroeder, *et al.*, *J. Biomolecular Screening*, **1996**, *1*, 75-80). Melanophores prepared from *Xenopus laevis* show a ligand-dependent change in pigment organization in response to heterologous ion channel activation; this response is adaptable to HTS formats (Jayawickreme *et al.*, *Cur. Opinion Biotechnology*, **1997**, *8*, 629-634). Assays are also available for the measurement of common second messengers, including cAMP, phosphoinositides and arachidonic acid, but these are not generally preferred for HTS.

In another embodiment of the invention, permanently transfected CHO cells could be used for the preparation of membranes which contain significant amounts of the recombinant receptor proteins; these membrane preparations would then be used in receptor binding assays, employing the radiolabeled ligand specific for the particular receptor. Alternatively, a functional assay, such as fluorescent monitoring of ligand-induced changes in internal Ca^{2+} concentration or membrane potential in permanently transfected CHO cells containing each of these receptors individually or in combination would be preferred for HTS. Equally preferred would be an alternative type of mammalian cell, such as HEK293 or COS cells, in similar formats. More preferred would be permanently transfected insect cell lines, such as *Drosophila* S2 cells. Even more preferred would be recombinant yeast cells expressing the *Drosophila melanogaster*

receptors in HTS formats well known to those skilled in the art (*e.g.*, Pausch, *Trends in Biotechnology*, **1997**, *15*, 487-494).

The invention contemplates a multitude of assays to screen and identify inhibitors of ligand binding to ion-x. In one example, the ion-x is immobilized and interaction with binding partner is assessed in the presence and absence of a candidate modulator such as an inhibitor compound. In another example, interaction between the ion-x and its binding partner is assessed in a solution assay, both in the presence and absence of a candidate inhibitor compound. In either assay, an inhibitor is identified as a compound that decreases binding between the ion-x and its binding partner. Another contemplated assay involves a variation of the dihybrid assay wherein an inhibitor of protein/protein interactions is identified by detection of a positive signal in a transformed or transfected host cell, as described in PCT publication number WO 95/20652, published August 3, 1995.

Candidate modulators contemplated by the invention include compounds selected from libraries of either potential activators or potential inhibitors. There are a number of different libraries used for the identification of small molecule modulators, including: (1) chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules. Chemical libraries consist of random chemical structures, some of which are analogs of known compounds or analogs of compounds that have been identified as "hits" or "leads" in other drug discovery screens, some of which are derived from natural products, and some of which arise from non-directed synthetic organic chemistry. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of plants or marine organisms. Natural product libraries include polyketides, non-ribosomal peptides, and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed of large numbers of peptides, oligonucleotides, or organic compounds as a mixture. These libraries are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are non-peptide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see

Myers, Curr. Opin. Biotechnol. 8:701-707 (1997). Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to modulate activity.

Still other candidate inhibitors contemplated by the invention can be designed and include soluble forms of binding partners, as well as such binding partners as chimeric, or fusion, proteins. A "binding partner" as used herein broadly encompasses non-peptide modulators, as well as such peptide modulators as neuropeptides other than natural ligands, antibodies, antibody fragments, and modified compounds comprising antibody domains that are immunospecific for the expression product of the identified ion-x gene.

The polypeptides of the invention are employed as a research tool for identification, characterization and purification of interacting, regulatory proteins. Appropriate labels are incorporated into the polypeptides of the invention by various methods known in the art and the polypeptides are used to capture interacting molecules. For example, molecules are incubated with the labeled polypeptides, washed to remove unbound polypeptides, and the polypeptide complex is quantified. Data obtained using different concentrations of polypeptide are used to calculate values for the number, affinity, and association of polypeptide with the protein complex.

Labeled polypeptides are also useful as reagents for the purification of molecules with which the polypeptide interacts including, but not limited to, inhibitors. In one embodiment of affinity purification, a polypeptide is covalently coupled to a chromatography column. Cells and their membranes are extracted, and various cellular subcomponents are passed over the column. Molecules bind to the column by virtue of their affinity to the polypeptide. The polypeptide-complex is recovered from the column, dissociated and the recovered molecule is subjected to protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotides for cloning the corresponding gene from an appropriate cDNA library.

Alternatively, compounds may be identified which exhibit similar properties to the ligand for the ion-x of the invention, but which are smaller and exhibit a longer half time than the endogenous ligand in a human or animal body. When an organic compound is designed, a molecule according to the invention is used as a "lead" compound. The design of mimetics to known pharmaceutically active compounds is a well-known approach in the development of pharmaceuticals based on such "lead" compounds. Mimetic design, synthesis and testing are generally used to avoid randomly screening a large number of

molecules for a target property. Furthermore, structural data deriving from the analysis of the deduced amino acid sequences encoded by the DNAs of the present invention are useful to design new drugs, more specific and therefore with a higher pharmacological potency.

Comparison of the protein sequences of the present invention with the sequences present in all the available databases showed a significant homology with the transmembrane domains, including the pore domain, of ion channel proteins. Accordingly, computer modeling can be used to develop a putative tertiary structure of the proteins of the invention based on the available information of the transmembrane domain of other proteins. Thus, novel ligands based on the predicted structure of ion-x can be designed.

In a particular embodiment, the novel molecules identified by the screening methods according to the invention are low molecular weight organic molecules, in which case a composition or pharmaceutical composition can be prepared thereof for oral intake, such as in tablets. The compositions, or pharmaceutical compositions, comprising the nucleic acid molecules, vectors, polypeptides, antibodies and compounds identified by the screening methods described herein, can be prepared for any route of administration including, but not limited to, oral, intravenous, cutaneous, subcutaneous, nasal, intramuscular or intraperitoneal. The nature of the carrier or other ingredients will depend on the specific route of administration and particular embodiment of the invention to be administered. Examples of techniques and protocols that are useful in this context are, *inter alia*, found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A (ed.), 1980, which is incorporated herein by reference in its entirety.

The dosage of these low molecular weight compounds will depend on the disease state or condition to be treated and other clinical factors such as weight and condition of the human or animal and the route of administration of the compound. For treating human or animals, between approximately 0.5 mg/kg of body weight to 500 mg/kg of body weight of the compound can be administered. Therapy is typically administered at lower dosages and is continued until the desired therapeutic outcome is observed.

The present compounds and methods, including nucleic acid molecules, polypeptides, antibodies, compounds identified by the screening methods described herein, have a variety of pharmaceutical applications and may be used, for example, to treat or prevent unregulated cellular growth, such as cancer cell and tumor growth. In a particular

embodiment, the present molecules are used in gene therapy. For a review of gene therapy procedures, see *e.g.* Anderson, *Science*, **1992**, *256*, 808-813, which is incorporated herein by reference in its entirety.

The present invention also encompasses a method of agonizing (stimulating) or antagonizing an ion-x natural binding partner associated activity in a mammal comprising administering to said mammal an agonist or antagonist to one of the above disclosed polypeptides in an amount sufficient to effect said agonism or antagonism. One embodiment of the present invention, then, is a method of treating diseases in a mammal with an agonist or antagonist of the protein of the present invention comprises administering the agonist or antagonist to a mammal in an amount sufficient to agonize or antagonize ion-x-associated functions.

Exemplary diseases and conditions amenable to treatment based on the present invention include, but are not limited to, thyroid disorders (*e.g.* thyreotoxicosis, myxoedema); renal failure; inflammatory conditions (*e.g.*, Crohn's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (*e.g.*, pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder, post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome; attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy, *etc.*); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases and disorders (*e.g.*, type 2 diabetes, obesity, anorexia, hypotension, hypertension, thrombosis, myocardial infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); and sexual dysfunction, among others.

Compounds that can traverse cell membranes and are resistant to acid hydrolysis are potentially advantageous as therapeutics as they can become highly bioavailable after being administered orally to patients. However, many of these protein inhibitors only weakly inhibit function. In addition, many inhibit a variety of protein kinases and will therefore cause multiple side effects as therapeutics for diseases.

Methods of determining the dosages of compounds to be administered to a patient and modes of administering compounds to an organism are disclosed in U.S. Application Serial No. 08/702,282, filed August 23, 1996 and International patent publication number WO 96/22976, published August 1 1996, both of which are incorporated herein by reference in their entirety, including any drawings, figures or tables. Those skilled in the art will appreciate that such descriptions are applicable to the present invention and can be easily adapted to it.

The proper dosage depends on various factors such as the type of disease being treated, the particular composition being used and the size and physiological condition of the patient. Therapeutically effective doses for the compounds described herein can be estimated initially from cell culture and animal models. For example, a dose can be formulated in animal models to achieve a circulating concentration range that initially takes into account the IC_{50} as determined in cell culture assays. The animal model data can be used to more accurately determine useful doses in humans.

Plasma half-life and biodistribution of the drug and metabolites in the plasma, tumors and major organs can also be determined to facilitate the selection of drugs most appropriate to inhibit a disorder. Such measurements can be carried out. For example, HPLC analysis can be performed on the plasma of animals treated with the drug and the location of radiolabeled compounds can be determined using detection methods such as X-ray, CAT scan and MRI. Compounds that show potent inhibitory activity in the screening assays, but have poor pharmacokinetic characteristics, can be optimized by altering the chemical structure and retesting. In this regard, compounds displaying good pharmacokinetic characteristics can be used as a model.

Toxicity studies can also be carried out by measuring the blood cell composition. For example, toxicity studies can be carried out in a suitable animal model as follows: 1) the compound is administered to mice (an untreated control mouse should also be used); 2) blood samples are periodically obtained via the tail vein from one mouse in each treatment group; and 3) the samples are analyzed for red and white blood cell counts, blood cell composition and the percent of lymphocytes versus polymorphonuclear cells. A comparison of results for each dosing regime with the controls indicates if toxicity is present.

At the termination of each toxicity study, further studies can be carried out by sacrificing the animals (preferably, in accordance with the American Veterinary Medical

Association guidelines Report of the American Veterinary Medical Assoc. Panel on Euthanasia, Journal of American Veterinary Medical Assoc., 202:229-249, 1993). Representative animals from each treatment group can then be examined by gross necropsy for immediate evidence of metastasis, unusual illness or toxicity. Gross abnormalities in tissue are noted and tissues are examined histologically. Compounds causing a reduction in body weight or blood components are less preferred, as are compounds having an adverse effect on major organs. In general, the greater the adverse effect the less preferred the compound.

For the treatment of cancers the expected daily dose of a hydrophobic pharmaceutical agent is between 1 to 500 mg/day, preferably 1 to 250 mg/day, and most preferably 1 to 50 mg/day. Drugs can be delivered less frequently provided plasma levels of the active moiety are sufficient to maintain therapeutic effectiveness. Plasma levels should reflect the potency of the drug. Generally, the more potent the compound the lower the plasma levels necessary to achieve efficacy.

Ion-x mRNA transcripts may found in many tissues, including, but not limited to, brain, kidney, colon, small intestine, stomach, testis, placenta, adrenal gland, peripheral blood leukocytes, bone marrow, retina, ovary, fetal brain, fetal liver, heart, spleen, liver, kidney, lung, muscle, thyroid gland, uterus, prostate, skin, salivary gland, and pancreas. Tissues where specific ion-x mRNA transcripts are expressed are identified in the Examples, below.

Sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, and fragments thereof, will, as detailed above, enable screening the endogenous neurotransmitters/hormones/ligands which activate, agonize, or antagonize ion-x and for compounds with potential utility in treating disorders including, but not limited to, thyroid disorders (*e.g.* thyreotoxicosis, myxoedema); renal failure; inflammatory conditions (*e.g.*, Crohn's disease); diseases related to cell differentiation and homeostasis; rheumatoid arthritis; autoimmune disorders; movement disorders; CNS disorders (*e.g.*, pain including migraine; stroke; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, anxiety, generalized anxiety disorder, post-traumatic-stress disorder, depression, bipolar disorder, delirium, dementia, severe mental retardation; dyskinesias, such as Huntington's disease or Tourette's Syndrome; attention disorders including ADD and ADHD, and degenerative disorders such as Parkinson's, Alzheimer's; movement disorders, including ataxias, supranuclear palsy,

etc.); infections, such as viral infections caused by HIV-1 or HIV-2; metabolic and cardiovascular diseases and disorders (*e.g.*, type 2 diabetes, obesity, anorexia, hypotension hypertension, thrombosis, myocardial infarction, cardiomyopathies, atherosclerosis, *etc.*); proliferative diseases and cancers (*e.g.*, different cancers such as breast, colon, lung, *etc.*, and hyperproliferative disorders such as psoriasis, prostate hyperplasia, *etc.*); hormonal disorders (*e.g.*, male/female hormonal replacement, polycystic ovarian syndrome, alopecia, *etc.*); and sexual dysfunction, among others.

For example, ion-x may be useful in the treatment of respiratory ailments such as asthma, where T cells are implicated by the disease. Contraction of airway smooth muscle is stimulated by thrombin. Cicala *et al* (1999) Br J Pharmacol 126:478-484. Additionally, in bronchiolitis obliterans, it has been noted that activation of thrombin receptors may be deleterious. Hauck *et al.*(1999) Am J Physiol 277:L22-L29. Furthermore, mast cells have also been shown to have thrombin receptors. Cirino *et al* (1996) J Exp Med 183:821-827. Ion-x may also be useful in remodeling of airway structures in chronic pulmonary inflammation via stimulation of fibroblast procollagen synthesis. See, e.g., Chambers *et al.* (1998) Biochem J 333:121-127; Trejo *et al.* (1996) J Biol Chem 271:21536-21541.

In another example, increased release of sCD40L and expression of CD40L by T cells after activation of thrombin receptors suggests that ion-x may be useful in the treatment of unstable angina due to the role of T cells and inflammation. See Aukrust *et al.* (1999) Circulation 100:614-620.

A further example is the treatment of inflammatory diseases, such as psoriasis, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, and thyroiditis. Due to the tissue expression profile of ion-x, inhibition of thrombin receptors may be beneficial for these diseases. See, *e.g.*, Morris *et al.* (1996) Ann Rheum Dis 55:841-843. In addition to T cells, NK cells and monocytes are also critical cell types which contribute to the pathogenesis of these diseases. See, e.g., Naldini & Carney (1996) Cell Immunol 172:35-42; Hoffman & Cooper (1995) Blood Cells Mol Dis 21:156-167; Colotta *et al.* (1994) Am J Pathol 144:975-985.

Expression of ion-x in bone marrow and spleen may suggest that it may play a role in the proliferation of hematopoietic progenitor cells. See DiCuccio *et al.* (1996) Exp Hematol 24:914-918.

As another example, ion-x may be useful in the treatment of acute and/or traumatic brain injury. Astrocytes have been demonstrated to express thrombin receptors.

Activation of thrombin receptors may be involved in astrogliosis following brain injury. Therefore, inhibition of receptor activity may be beneficial for limiting neuroinflammation. Scar formation mediated by astrocytes may also be limited by inhibiting thrombin receptors. See, *e.g*, Pindon *et al.* (1998) Eur J Biochem 255:766-774; Ubl & Reiser. (1997) Glia 21:361-369; Grabham & Cunningham (1995) J Neurochem 64:583-591.

Ion-x receptor activation may mediate neuronal and astrocyte apoptosis and prevention of neurite outgrowth. Inhibition would be beneficial in both chronic and acute brain injury. See, *e.g.*, Donovan *et al.* (1997) J Neurosci 17:5316-5326; Turgeon *et al* (1998) J Neurosci 18:6882-6891; Smith-Swintosky *et al.* (1997) J Neurochem 69:1890-1896; Gill *et al.* (1998) Brain Res 797:321-327; Suidan *et al.* (1996) Semin Thromb Hemost 22:125-133.

The attached Sequence Listing contains the sequences of the polynucleotides and polypeptides of the invention and is incorporated herein by reference in its entirety.

As described above and in Example 11 below, the genes encoding ion-1 (nucleic acid sequence SEQ ID NO:1, SEQ ID NO:49, amino acid sequence SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:50), ion-2a (nucleic acid sequence SEQ ID NO:2, amino acid sequence SEQ ID NO:12, SEQ ID NO:13), ion-2b (nucleic acid sequence SEQ ID NO:3, amino acid sequence SEQ ID NO:14, SEQ ID NO:15), ion-3 (nucleic acid sequence SEQ ID NO:4, SEQ ID NO:51, amino acid sequence SEQ ID NO:16, SEQ ID NO:17), ion-5 (nucleic acid sequence SEQ ID NO:7, amino acid sequence SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28), and ion-7 (nucleic acid sequence SEQ ID NO:9, amino acid sequence SEQ ID NO:31, SEQ ID NO:32) have been detected in brain tissue indicating that these ion-x proteins are neuroreceptors. The identification of modulators such as agonists and antagonists is therefore useful for the identification of compounds useful to treat neurological diseases and disorders. Such neurological diseases and disorders, include, but are not limited to, schizophrenia, affective disorders, ADHD/ADD (*i.e.*, Attention Deficit-Hyperactivity Disorder/Attention Deficit Disorder), and neural disorders such as Alzheimer's disease, Parkinson's disease, migraine, and senile dementia as well as depression, anxiety, bipolar disease, epilepsy, neuritis, neurasthenia, neuropathy, neuroses, and the like.

Methods of Screening Human Subjects

Thus in yet another embodiment, the invention provides genetic screening procedures that entail analyzing a person's genome -- in particular their alleles for ion channels of the invention -- to determine whether the individual possesses a genetic characteristic found in other individuals that are considered to be afflicted with, or at risk for, developing a mental disorder or disease of the brain that is suspected of having a hereditary component. For example, in one embodiment, the invention provides a method for determining a potential for developing a disorder affecting the brain in a human subject comprising the steps of analyzing the coding sequence of one or more ion channel genes from the human subject; and determining development potential for the disorder in said human subject from the analyzing step.

More particularly, the invention provides a method of screening a human subject to diagnose a disorder affecting the brain or genetic predisposition therefor, comprising the steps of: (a) assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering the amino acid sequence, expression, or biological activity of at least one ion channel that is expressed in the brain, wherein the ion channel comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:10, 11, 12, 13, 14, 15, 16, 17, 22, 23, 24, 25, 26, 27, 28, 31, 32, and 50, or an allelic variant thereof, and wherein the nucleic acid corresponds to the gene encoding the ion channel; and (b) diagnosing the disorder or predisposition from the presence or absence of said mutation, wherein the presence of a mutation altering the amino acid sequence, expression, or biological activity of allele in the nucleic acid correlates with an increased risk of developing the disorder. In preferred variations, the ion channel is ion-1 or ion-3 comprising amino acid sequences set forth in SEQ ID NO:49 for ion-1 and SEQ ID NO:51 for ion-3, or an allelic variant thereof.

By "human subject" is meant any human being, human embryo, or human fetus. It will be apparent that methods of the present invention will be of particular interest to individuals that have themselves been diagnosed with a disorder affecting the brain or have relatives that have been diagnosed with a disorder affecting the brain.

By "screening for an increased risk" is meant determination of whether a genetic variation exists in the human subject that correlates with a greater likelihood of developing a disorder affecting the brain than exists for the human population as a whole, or for a relevant racial or ethnic human sub-population to which the individual belongs. Both

positive and negative determinations (*i.e.*, determinations that a genetic predisposition marker is present or is absent) are intended to fall within the scope of screening methods of the invention. In preferred embodiments, the presence of a mutation altering the sequence or expression of at least one ion-1 or ion-3 ion channel allele in the nucleic acid is correlated with an increased risk of developing the disorder, whereas the absence of such a mutation is reported as a negative determination.

The "assaying" step of the invention may involve any techniques available for analyzing nucleic acid to determine its characteristics, including but not limited to well-known techniques such as single-strand conformation polymorphism analysis (SSCP) [Orita *et al., Proc Natl. Acad. Sci. USA, 86*: 2766-2770 (1989)]; heteroduplex analysis [White *et al., Genomics, 12*: 301-306 (1992)]; denaturing gradient gel electrophoresis analysis [Fischer *et al., Proc. Natl. Acad. Sci. USA, 80*: 1579-1583 (1983); and Riesner *et al., Electrophoresis, 10*: 377-389 (1989)]; DNA sequencing; RNase cleavage [Myers *et al., Science, 230*: 1242-1246 (1985)]; chemical cleavage of mismatch techniques [Rowley *et al., Genomics, 30*: 574-582 (1995); and Roberts *et al., Nucl. Acids Res., 25*: 3377-3378 (1997)]; restriction fragment length polymorphism analysis; single nucleotide primer extension analysis [Shumaker *et al., Hum. Mutat., 7*: 346-354 (1996); and Pastinen *et al., Genome Res., 7*: 606-614 (1997)]; 5' nuclease assays [Pease *et al., Proc. Natl. Acad. Sci. USA, 91*:5022-5026 (1994)]; DNA Microchip analysis [Ramsay, G., *Nature Biotechnology, 16*: 40-48 (1999); and Chee *et al.*, U.S. Patent No. 5,837,832]; and ligase chain reaction [Whiteley *et al.*, U.S. Patent No. 5,521,065]. [See generally, Schafer and Hawkins, *Nature Biotechnology, 16*: 33-39 (1998).] All of the foregoing documents are hereby incorporated by reference in their entirety.

Thus, in one preferred embodiment involving screening ion-1 or ion-3 sequences, for example, the assaying step comprises at least one procedure selected from the group consisting of: (a) determining a nucleotide sequence of at least one codon of at least one ion-1 or ion-3 allele of the human subject; (b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; (c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and (d) performing a restriction endonuclease digestion to determine whether nucleic acid

from the human subject has a nucleotide sequence identical to or different from one or more reference sequences.

In a highly preferred embodiment, the assaying involves sequencing of nucleic acic to determine nucleotide sequence thereof, using any available sequencing technique. [See, *e.g.*, Sanger *et al., Proc. Natl. Acad. Sci. (USA), 74*: 5463-5467 (1977) (dideoxy chain termination method); Mirzabekov, *TIBTECH, 12*: 27-32 (1994) (sequencing by hybridization); Drmanac *et al., Nature Biotechnology, 16*: 54-58 (1998); U.S. Patent No. 5,202,231; and *Science, 260*: 1649-1652 (1993) (sequencing by hybridization); Kieleczawa *et al., Science, 258*: 1787-1791 (1992) (sequencing by primer walking); (Douglas *et al., Biotechniques, 14*: 824-828 (1993) (Direct sequencing of PCR products); and Akane *et al., Biotechniques 16*: 238-241 (1994); Maxam and Gilbert, *Meth. Enzymol., 65*: 499-560 (1977) (chemical termination sequencing), all incorporated herein by reference.] The analysis may entail sequencing of the entire ion-x gene genomic DNA sequence, or portions thereof; or sequencing of the entire seven transmembrane receptor coding sequence or portions thereof. In some circumstances, the analysis may involve a determination of whether an individual possesses a particular allelic variant, in which case sequencing of only a small portion of nucleic acid -- enough to determine the sequence of a particular codon characterizing the allelic variant -- is sufficient. This approach is appropriate, for example, when assaying to determine whether one family member inherited the same allelic variant that has been previously characterized for another family member, or, more generally, whether a person's genome contains an allelic variant that has been previously characterized and correlated with a mental disorder having a heritable component.

In another highly preferred embodiment, the assaying step comprises performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences. In a preferred embodiment, the hybridization involves a determination of whether nucleic acid derived from the human subject will hybridize with one or more oligonucleotides, wherein the oligonucleotides have nucleotide sequences that correspond identically to a portion of the ion-x gene sequence taught herein, or that correspond identically except for one mismatch. The hybridization conditions are selected to differentiate between perfect sequence complementarity and imperfect matches differing by one or more bases. Such hybridization experiments thereby can provide single nucleotide polymorphism sequence

information about the nucleic acid from the human subject, by virtue of knowing the sequences of the oligonucleotides used in the experiments.

Several of the techniques outlined above involve an analysis wherein one performs a polynucleotide migration assay, *e.g.*, on a polyacrylamide electrophoresis gel (or in a capillary electrophoresis system), under denaturing or non-denaturing conditions. Nucleic acid derived from the human subject is subjected to gel electrophoresis, usually adjacent tc (or co-loaded with) one or more reference nucleic acids, such as reference ion channel-encoding sequences having a coding sequence identical to all or a portion of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51 (or identical except for one known polymorphism). The nucleic acid from the human subject and the reference sequence(s) are subjected to similar chemical or enzymatic treatments and then electrophoresed under conditions whereby the polynucleotides will show a differential migration pattern, unless they contain identical sequences. [See generally Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, New York: John Wiley & Sons, Inc. (1987-1999); and Sambrook *et al.*, (eds.), Molecular Cloning, *A Laboratory Manual*, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press (1989), both incorporated herein by reference in their entirety.]

In the context of assaying, the term "nucleic acid of a human subject" is intended to include nucleic acid obtained directly from the human subject (*e.g.*, DNA or RNA obtained from a biological sample such as a blood, tissue, or other cell or fluid sample); and also nucleic acid derived from nucleic acid obtained directly from the human subject. By way of non-limiting examples, well known procedures exist for creating cDNA that is complementary to RNA derived from a biological sample from a human subject, and for amplifying DNA or RNA derived from a biological sample obtained from a human subject. Any such derived polynucleotide which retains relevant nucleotide sequence information of the human subject's own DNA/RNA is intended to fall within the definition of "nucleic acid of a human subject" for the purposes of the present invention.

In the context of assaying, the term "mutation" includes addition, deletion, and/or substitution of one or more nucleotides in the ion-x gene sequence (*e.g.*, as compared to the ion channel-encoding sequences set forth of SEQ ID NOS:1-9, SEQ ID NO:49, and SEQ ID NO:51) and other polymorphisms that occur in introns (where introns exist) and that are identifiable via sequencing, restriction fragment length polymorphism, or other techniques. The various activity examples provided herein permit determination of

whether a mutation modulates activity of the relevant receptor in the presence or absence of various test substances.

In a related embodiment, the invention provides methods of screening a person's genotype with respect to ion channels of the invention, and correlating such genotypes with diagnoses for disease or with predisposition for disease (for genetic counseling). For example, the invention provides a method of screening for an ion-1 or ion-3 mental disorder genotype in a human patient, comprising the steps of: (a) providing a biological sample comprising nucleic acid from the patient, the nucleic acid including sequences corresponding to said patient's ion-1 or ion-3 alleles; (b) analyzing the nucleic acid for the presence of a mutation or mutations; (c) determining an ion-1 or ion-3 genotype from the analyzing step; and (d) correlating the presence of a mutation in an ion-1 or ion-3 allele with a mental disorder genotype. In a preferred embodiment, the biological sample is a cell sample containing human cells that contain genomic DNA of the human subject. The analyzing can be performed analogously to the assaying described in preceding paragraphs. For example, the analyzing comprises sequencing a portion of the nucleic acid (e.g., DNA or RNA), the portion comprising at least one codon of the ion-1 or ion-3 alleles.

Although more time consuming and expensive than methods involving nucleic acid analysis, the invention also may be practiced by assaying protein of a human subject to determine the presence or absence of an amino acid sequence variation in ion channel protein from the human subject. Such protein analyses may be performed, *e.g.*, by fragmenting ion channel protein via chemical or enzymatic methods and sequencing the resultant peptides; or by Western analyses using an antibody having specificity for a particular allelic variant of the ion channel.

The invention also provides materials that are useful for performing methods of the invention. For example, the present invention provides oligonucleotides useful as probes in the many analyzing techniques described above. In general, such oligonucleotide probes comprise 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides that have a sequence that is identical, or exactly complementary, to a portion of a human ion channel gene sequence taught herein (or allelic variant thereof), or that is identical or exactly complementary except for one nucleotide substitution. In a preferred embodiment, the oligonucleotides have a sequence that corresponds in the

foregoing manner to a human ion channel coding sequence taught herein, and in particular the coding sequences set forth in SEQ ID NO:49, and SEQ ID NO:51. In one variation, ar oligonucleotide probe of the invention is purified and isolated. In another variation, the oligonucleotide probe is labeled, *e.g.*, with a radioisotope, chromophore, or fluorophore. In yet another variation, the probe is covalently attached to a solid support. [See generally Ausubel *et al.* and Sambrook *et al.*, *supra*.]

In a related embodiment, the invention provides kits comprising reagents that are useful for practicing methods of the invention. For example, the invention provides a kit for screening a human subject to diagnose a mental disorder or a genetic predisposition therefor, comprising, in association: (a) an oligonucleotide useful as a probe for identifying polymorphisms in a human ion-1 or ion-3 ion channel gene, the oligonucleotide comprising 6-50 nucleotides that have a sequence that is identical or exactly complementary to a portion of a human ion-1 or ion-3 gene sequence or ion-1 or ion-3 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution; and (b) a media packaged with the oligonucleotide containing information identifying polymorphisms identifiable with the probe that correlate with a mental disorder or a genetic predisposition therefor. Exemplary information-containing media include printed paper package inserts or packaging labels; and magnetic and optical storage media that are readable by computers or machines used by practitioners who perform genetic screening and counseling services. The practitioner uses the information provided in the media to correlate the results of the analysis with the oligonucleotide with a diagnosis. In a preferred variation, the oligonucleotide is labeled.

In still another embodiment, the invention provides methods of identifying those allelic variants of ion channels of the invention that correlate with mental disorders. For example, the invention provides a method of identifying an ion channel allelic variant that correlates with a mental disorder, comprising steps of: (a) providing a biological sample comprising nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny; (b) analyzing the nucleic acid for the presence of a mutation or mutations in at least ion channel that is expressed in the brain, wherein the ion channel comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:17, SEQ ID NO:22 to SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:50, or an allelic variant thereof, and wherein

the nucleic acid includes sequence corresponding to the gene or genes encoding the ion channel; (c) determining a genotype for the patient for the ion channel from said analyzing step; and (d) identifying an allelic variant that correlates with the mental disorder from the determining step. To expedite this process, it may be desirable to perform linkage studies in the patients (and possibly their families) to correlate chromosomal markers with disease states. The chromosomal localization data provided herein facilitates identifying an involved ion channel with a chromosomal marker.

The foregoing method can be performed to correlate ion channels of the invention to a number of disorders having hereditary components that are causative or that predispose persons to the disorder. For example, in one preferred variation, the ion channel comprises ion-1 having an amino acid sequence set forth in SEQ ID NO:49 or an allelic variant thereof.

Also contemplated as part of the invention are polynucleotides that comprise the allelic variant sequences identified by such methods, and polypeptides encoded by the allelic variant sequences, and oligonucleotide and oligopeptide fragments thereof that embody the mutations that have been identified. Such materials are useful in *in vitro* cell-free and cell-based assays for identifying lead compounds and therapeutics for treatment of the disorders. For example, the variants are used in activity assays, binding assays, and assays to screen for activity modulators described herein. In one preferred embodiment, the invention provides a purified and isolated polynucleotide comprising a nucleotide sequence encoding an ion channel allelic variant identified according to the methods described above; and an oligonucleotide that comprises the sequences that differentiate the allelic variant from the ion-1 or ion-3 sequences set forth in SEQ ID NOS:49 and 51. The invention also provides a vector comprising the polynucleotide (preferably an expression vector); and a host cell transformed or transfected with the polynucleotide or vector. The invention also provides an isolated cell line that is expressing the allelic variant ion channel polypeptide; purified cell membranes from such cells; purified polypeptide; and synthetic peptides that embody the allelic variation amino acid sequence. In one particular embodiment, the invention provides a purified polynucleotide comprising a nucleotide sequence encoding a ion-1 protein of a human that is affected with a mental disorder; wherein said polynucleotide hybridizes to the complement of SEQ ID NO:49 under the following hybridization conditions: (a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran

sulfate and (b) washing 2 times for 30 minutes at 60°C in a wash solution comprising 0.1x SSC and 1% SDS; and wherein the polynucleotide encodes a ion-1 amino acid sequence that differs from SEQ ID NO:50 by at least one residue.

An exemplary assay for using the allelic variants is a method for identifying a modulator of ion-x biological activity, comprising the steps of: (a) contacting a cell expressing the allelic variant in the presence and in the absence of a putative modulator compound; (b) measuring ion-x biological activity in the cell; and (c) identifying a putative modulator compound in view of decreased or increased ion-x biological activity in the presence versus absence of the putative modulator.

Additional features of the invention will be apparent from the following Examples. Examples 1, 2, 11, and portions of Example 3 are actual, while the remaining Examples are prophetic. Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

Table 2 contains the sequences of the polynucleotides and polypeptides of the invention, in addition to exemplary primers useful for cloning said sequences. "X" indicates an unknown amino acid or a gap (absence of amino acid(s)).

TABLE 2

The following DNA sequence Ion1 <SEQ ID NO. 1 and SEQ ID NO:49> were identified in *H. sapiens*:

<SEQ ID NO. 1>

AGGTATGGGAGGGCTGAGTGGGGCTGATGGCATGCAGGAGCAAGGACCCGACTTTTGGAGGGCATAGGAGA
CTATTCAGGTCTGGTCTGAAACTACACAGAGGACTGGGTTAAAAATGAGGCGGTTGACAGGGCCACAAGGC
TGACTGAGAGCCTGACTGGTTTCTGGAGTTCTCTGGCAAAAAGAAGTCCAGACTGAAGTTTGCAGGTGAGC
ACCTGCCTAGGTGTTCCAGAGGCATATGACGGTGATGATGGAGGAGGCCATGAAGAGCAGGTAGAGGCGGA
AGAGCAGGGCGTCCATCGCGTGGCTGAACTGCACCCACAGCTCCACCGAGTGCTGCTTCTGGGCCTCGTGT
TCCCGCTGGGCCCTTGTCCATTCTGAGCCCCCTGTCAGCTCTGCCTCCCCAGGGCCTGGCATCTGCCCTGC
TGATACCTCTGGCTCCTTCACACCTACAGAAAGACAGAGACTCAGCCATGGGCTGCAAATGTCACCTGTGG
AGGGAGGGAGACAGGGAAGGAGGCAGGAGCAGAGAAGTGGAGGTGGGGGAAGAGGAAGTATGACTTCCCTC

ACCGGGCAGGTGGGTGGGGGGTGAGACCCGGGCCCTTATTTCCCTTCTGGGGCGCAGTGGGACAGCATCTC
CCTTGGCCGGTGCAGTGCAGCAGCAGGGAGTGGAGCCACCGAGGCAGAGGTAGG

<SEQ ID NO:49>
GCGGCCGCGAATTCGGCACGAGCCGGTCACCAACATCAGCGTCCCCACCCAAGTCAACATCTCCTTCGCGA
TGTCTGCCATCCTAGATGTGGTTTGGGATAACCCATTTATCAGCTGGAACCCAGAGGAATGTGAGGGCATC
ACGAAGATGAGTATGGCAGCCAAGAACCTGTGGCTCCCAGACATTTTCATCATTGAACTCATGGATGTGGA
TAAGACCCCAAAAGGCCTCACAGCATATGTAAGTAATGAAGGTCGCATCAGGTATAAGAAACCCATGAAGG
TGGACAGTATCTGTAACCTGGACATCTTCTACTTCCCCTTCGACCAGCAGAACTGCACACTCACCTTCAGC
TCATTCCTCTACACAGTGGACAGCATGTTGCTGGACATGGAGAAAGAAGTGTGGGAAATAACAGACGCATC
CCGGAACATCCTTCAGACCCATGGAGAATGGGAGCTCCTGGGCCTCAGCAAGGCCACCGCAAAGTTGTCCA
GGGGAGGCAACCTGTATGATCAGATCGTGTTCTATGTGGCCATCAGGCGCAGGCCCAGCCTCTATGTCATA
AACCTTCTCGTGCCCAGTGGCTTTCTGGTTGCCATCGATGCCCTCAGCTTCTACCTGCCAGTGAAAAGTGG
GAATCGTGTCCCATTCAAGATAACGCTCCTGCTGGGCTACAACGTCTTCCTGCTCATGATGAGTGACTTGC
TCCCCACCAGTGGCACCCCCCTCATCGGTGTCTACTTCGCCCTGTGCCTGTCCCTGATGGTGGGCAGCCT
GCTGGAGACCATCTTCATCACCCACCTGCTGCACGTGGCCACCACCCAGCCCCCACCCCTGCCTCGGTGGC
TCCACTCCCTGCTGCTCCACTGCAACAGCCCGGGGAGATGCTGTCCCACTGCGCCCCAGAAGGAAAATAAG
GGCCCGGGTCTCACCCCCACCCACCTGCCCGGTGTGAAGGAGCCAGAGGTATCAGCAGGGCAGATGCCGGG
CCCTGCGGAGGCAGAGCTGACAGGGGGCTCAGAATGGACAAGGGCCCAGCGGGAACACGAGGCCCAGAAGC
AGCACTCAGTGGAGCTGTGGTTGCAGTTCAGCCACGCGATGGACGCCATGCTCTTCCGCCTCTACCTGCTC
TTCATGGCCTCCTCTATCATCACCGTCATATGCCTCTGGAACACCTAGGCAGGTGCTCACCTGCCAACTTC
AGTCTGGAGCTTCTCTTGCCTCCAGGGACTGGCCAGGTCTCCCCCCTTTCCTGAGTACCAACTATCATATC
CCCAAAGATGACTGAGTCTCTGCTGTATTCCATGTATCCCAATCCGGTCCTGCTGATCAATTCCAATCCCA
GACATTTCTCCCTGTTCCTGCATTTTGTTGGCTTCCTTCAGTCCTACCATATGGTTCTAGGTCCCTCTTAC
GTCATCTGCATAGCAGACTATACCTCTTCTGTCCGCTGACCTCGACTCTAGATTGCGGCCGC

The following amino acid sequences <SEQ ID NOS. 10 and 11> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 1:

<SEQ ID NO: 10>
PTSASVAPLPAAALHRPREMLSHCAPEGKXGPGSHPPPTCPVREVILPLPPPPLLCSCLLPCLPPSTGDIC
SPWLSLCLSVGVKEPEVSAGQMPGPGEAELTGGSEWTRAQREHEAQKQHSVELWVQFSHAMDALLFRLYLL
FMASSIITVICLWNTXAGAHLQTSVWTSFCQRTPETSQALSQPCGPVNRLIFNPVLCVVSDQTXIVSYALQ
KSGPCSCMPSAPLSPPIP

<SEQ ID NO: 11>
GPGSHPPPTCPVREVILPLPPPPLLCSCLLPCLPPSTGDICSPWLSLCLSVGVKEPEVSAGQMPGPGEAEL
TGGSEWTRAQREHEAQKQHSVELWVQFSHAMDALLFRLYLLFMASSIITVICLWNT

The following amino acid sequence <SEQ ID NO:50> is a predicted amino acid sequence derived from the DNA sequence of SEQ ID NO:49:
<SEQ ID 49>
RPRIRHEPVTNISVPTQVNISFAMSAILDVVWDNPFISWNPEECEGITKMSMAAKNLWLPDIFIIELMDVD
KTPKGLTAYVSNEGRIRYKKPMKVDSICNLDIFYFPFDQQNCTLTFSSFLYTVDSMLLDMEKEVWEITDAS
RNILQTHGEWELLGLSKATAKLSRGGNLYDQIVFYVAIRRRPSLYVINLLVPSGFLVAIDALSFYLPVKSG
NRVPFKITLLLGYNVFLLMMSDLLPTSGTPLIGVYFALCLSLMVGSLLETIFITHLLHVATTQPPPLPRWL
HSLLLHCNSPGRCCPTAPQKENKGPGLTPTHLPGVKEPEVSAGQMPGPAEAELTGGSEWTRAQREHEAQKQ
HSVELWLQFSHAMDAMLFRLYLLFMASSIITVICLWNT

The following sequences <SEQ ID NOS: 33 and 34> are, respectively, forward and reverse primers for SEQ ID NO:1.
ion1.for <SEQ ID NO: 33>
CAGTTCAGCCACGCGATGGA

ion1.rev <SEQ ID NO: 34>
GTTCCAGAGGCATATGACGGT

The following DNA sequence Ion2a <SEQ ID NO. 2> was identified in *H. sapiens*:

TAGATCCATGGTAAATGATATTTTGGTGAGTCAACTTTCTAAATGTATAAAAATATATTTTATTTTTCAGG
GGTATTTCATTTCTGCTTAATAGAATGTAACAAATGTTCTATTACAAAGCAAATTATAATATAAAACATGT
TATAATTGAAAATACTTGATTTTTTGAAATCAAGATTATTTTCATTACCTGTCAGTCTCCTAGAGTTTGCG
TTAAAGGAGCAAATTGATCTTTCCTTATGCTACTTTTTTGATGTCAAAAATTCATTTATTATTGTGCTCAG
ATGACTCCTGGTCTCCATCCTGGATCCACTCTGATTCCAATGAATAATATTTCTGTGCCGCAAGAAGATGA
TTATGGGTATCAGTGTTTGGAGGGCAAAGATTGTGCCAGCTTCTTCTGTTGCTTTGAAGACTGCAGAACAG
GATCTTGGAGGGAAGGAAGGATACACATACGCATTGCCAAAATTGACTCTTATTCTAGAATATTTTTCCCA
ACCGCTTTTGCCCTGTTCAACTTGGTTTATTGGGTTGGCTATCTTTACTTAT

The following amino acid sequences <SEQ ID NOS. 12 and 13> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 2:

<SEQ ID NO: 12>
DPWXMIFWXVNFLNVXKYILFFRGISFLLNRMXQMFYYKANYNIKHVIIENTXFFEIKIIFITCQSPRVCV
KGANXSFLMLLFXCQKFIYYCAQMTPGLHPGSTLIPMNNISVPQEDDYGYQCLEGKDCASFFCCFEDCRTG
SWREGRIHIRIAKIDSYSRIFFPTAFALFNLVYWVGYLYL

<SEQ ID NO: 13>
CQKFIYYCAQMTPGLHPGSTLIPMNNISVPQEDDYGYQCLEGKDCASFFCCFEDCRTGSWREGRIHIRIAK
IDSYSRIFFPTAFALFNLVYWVGYLYL

The following sequences <SEQ ID NOS: 35 and 36> are, respectively, forward and reverse primers for SEQ ID NO: 2.

ion2a.for <SEQ ID NO: 35>
GGATCCACTCTGATTCCAATGAA

ion2a.rev <SEQ ID NO: 36>
GATAGCCAACCCAATAAACCAAGT

The following DNA sequence Ion2b <SEQ ID NO. 3> was identified in *H. sapiens*:

ATAAAATTTTATAGCAGGGTGGGTTTCTAGAGGAAATCTTACTCAATTATTTGCACTGCAGGTTAAGAAAA
CCATAATCTTTATGCTGCAACCTGTTCTGCTTCAAAGGAAGAAAATCAAAGAATTTTTTCTCTTTGCTTTT
AGTCCTTTTCACATAATAATAACTGAGCTTAAAAAAGTATTGCCAAAGTATTTCACCATTTTATATTTTAG
CATGTGAAAGGAGCTCCACATTTTTGGTTTTGCAACTTTGAGAAATAAAAAATTAAGAATTGATTAAATAT
TAGTATGGAAAATAAATGAGAGCAACTACAGATTTTTAAACCAATATTACCTTAGAGTATACAGAACTCGT
CCATCATTCCAAATTCGAAGCAGACGATTAGGAGTTGTTATCCAGTGAGCATCAGATTTTCTTGAGTTTCT
GAAGAAAGTGTCAGGAATCCAAATTTTTCCAACCATATTACTGTTAAGCATAAGCACTTTCATGGTACTAT
TGAATTTTAAACGACTGTCAAACCAGGTTTGGGCAAAAATTATATCTATTGGATATTCCTAAAATATAAGA
AGAGTAACAACATATTAGTAAAGCTACTATTTTAGTTGTTTTTCTCGAAAGTTTG

The following amino acid sequences <SEQ ID NOS. 14 and 15> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 3:

<SEQ ID NO: 14>
NFREKQLKXXLYXYVVTLLIFXEYPIDIIFAQTWFDSRLKFNSTMKVLMLNSNMVGKIWIPDTFFRNSRKS
DAHWITTPNRLLRIWNDGRVLYTLRXYWFKNLXLLSFIFHTNIXSILNFLFLKVAKPKMWSSFHMLKYKMV
KYFGNTFLSSVIIMXKGLKAKRKNSLIFFLXSRTGCSIKIMVFLTCSANNXVRFPLETHPAIKFY

<SEQ ID NO: 15>
EYPIDIIFAQTWFDSRLKFNSTMKVLMLNSNMVGKIWIPDTFFRNSRKSDAHWITTPNRLLRIWNDGRVLY
TLR

The following DNA sequences Ion3 <SEQ ID NO. 4 and SEQ ID NO:51> were identified in *H. sapiens*:

<SEQ ID NO. 4>
CCTGGCACACAGCAAGCAGTCGACAGATTTTGCCTATCATTAGGATCTGGGGATACTGATGTTCCATCATC
AAGGGCCAAGTCGTGAGGGGTGTTCTCCCTGGAAGGAGCTAATCCTTTCCCCTCAGTCTTAAAATGAGGGC
ACGTTCCCAGGACGCCCCCCTCTCACTTTCTGCAGTGGGGCCGTTCCGCAGACCCAGGCCCTGTCGCGGCC
CCGCCCTGGGGGGACCCCAGCGCATCGTCAGGTCCCCCCGCGCCCCCGCTGCTCACCGATGAGCGGCACGC
TCTCGGCCGGTGGCATGCTCTCGGCCAGCAGCAACTGGAAGACGGTGAGCGCCAGCAGCACGGTGACGCCC
AGCGACACCTTCTCGCCTGAGTCGGCAGGCAGGTGGAAGGCGAGCGGCGCAAGCAGCGAGATGAGCACGCA
GGGCAGCAGCAGGTTGCACAC

<SEQ ID NO. 51>
GTATGCCTGTATGTGCTTTTACTTCTGAAGTCCAGCCAACATTATTTCTCCTTCCTTTCTGTCTTCCTGC
CATGTCTTCTGTACTTTTGGAAACTATGCACTTGTGCAGACATTGTGCTCAATACTTTGTTTCTTCAGAT
GCCATCATTAATGAGAACTATGACTACCTGAAGGGGTTCTTGGAAGACCTGGCACCTCCAGAGCGCAGCA
GCCTAATTCAGGATTGGGAAACATCTGGGCTTGTTTACCTGGACTATATTAGAGTCATTGAAATGCTCCG
CCATATACAGCAGGTACCTGAGATCCTGAAACTGCTGCCTGATTTTCCTTTTCTCAGGCCCTTAAATCTT
CAGATACCTCACAAGGCCTTAGTATACACTTGAGAATGCACTGACAGAGATAGCACTGTCAAAGCAGGCA
TCTTGCTGAGGCTCATTTGATATAACCGTTTCTGACAGCTATATCGAAACTTAAAAATGCTATTTTATGT
TGATTACCAACTAGTATGTGCAATAGACATTCCTGAGGCTTGTCCATAGACAGTCTCTTCCCCTTGTTCA
GTCCTAGTTTGAGTGAGAAGCCCAAAGATGAGAGATAAAATAAGAATGGAGATTTGGTGAGGGTGAGGAT
AGCTGTTTTACACATCATTTGGCATGTTTTAAAATTGCAAATATGGGTTTTAAAGTCAATGTCTTCGGTC
AGTTTTTTTTTTTTTTTTTTTGAAACAGAGTCTTGCTCTGTCATCCAGGCTGGAGTGCAGTGGTGTGATC
GTGGCTCACTGCAACCTCTGCCTCCCAGTCTTAAGTGAGTCTCATGCCCCAGCCTCCCAAGTAGCTGGGA
GTATAGGGTGTGTGCCACCACACGCAGCTAACTTTTGTATTTTTAGTAGAGATAGTGTTTCACCACATTG
GCCAGGCTGGTCTTGAATTCCTGGCCTCAAGTGATCGGCCCACCTTGGCCTCCCAAATTGCTGGGATTAC
AGGCATGAGCTTACCGCACGCCTGCACGCAGCCTTAAGGTCAGTCTTTGTAGTCGTAAAATGAGTCTCCA
CTGCTTGCTTATGGTGCAAAAACCAAACTCATTATAATAAATATAGGATTCAAGTCCTTTTAGAGGCTTT
TACCTTTCCTGCCTTACTCCTACCACTCTTATTCCACGTTCCAGCCTTGCTAGCCTGCTGTACTCACACT
AAATTACTTCTGGTGTTTCTAACAAACCATATTATGTTCCACACTACCTAGCACACTTAAACTCATCCTT
TTAAGATCTAGGTTGCTGTTACCCCTACTTCTCTCTGCTTTTCCCCAGAGATAATTAATTGCACTTTCTT

ACTACCAAGATACTTAGTACATTATTCTACTAGTGCACCTGTCAGACCATATTGTAGTTACTTATTCATA
TTTTCAGGTTGCTATAAGCCCCTTTTGGGAAGGTCTTTTACGGTTACAGGCAATAGAGTGTAGAGGTTAA
CAGCTCAAGTTCTGGAAGCAGACTTATAGATTCAATTTGTGGCTTCCAAATTCACTGGCTATGTAATCTT
GAGCAAGTTAACTACCTCTCTCGTCTGAAAGAAAAAGGTGGGTGGGGTAGACAGTAGTACAGATTATAGT
TCATATTGACAGATTTTCACAAAGATTAAACAAAATCTACATGAAGTGTTTTACATAGTACCTTTCATGT
ACTAAATGCCTTTTTTTTTTTTTTTTAGGCAGAGTTTTACTCTGTCACCCAGGCTGGAGGGCAGTGGCA
CAATCTCAGCTCACTGCATCTCCCAGCTTCAAACAATTCTCCTGCCTCAGCCTCCCCAGTAGCTGGGATT
ACAGGCGTGTGCCACCACACCCAGCTTTGTGTGTGTGTGTGTGTATTTTAGTAGAGACGGGGTTTCACCA
TGTTGGCCAGGCTGGTCTCAAACTCCTGACCTCGGCCTCTACTAAATGCTTAATAATTGTTATCTATTAT
TATCCTCATCAAAGTTCCAACTCCTAGTACAGTGCCTGGCACATAATAGACATAATTCAATGTTTGCTGT
ACTTTTAGTATGAATCAAGAACATCATTTCTAAATAATCACTTGAAGAAACCACTTTCTCATTGAATATT
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GCTGGATGTGGTGGTGCATGACTGTAATCCTAGTACTTTGGGAGGCTGAGGCAGGAGGATTGCTTGAGAC
CAGCCTGAGCAACATAGCGAGACCCCCCTTCTCCCAAAAAAAAAAAAAAAAAAACCTATGTTACATAAAA
ACTCTCTAGTATTATCTTGTTCTGCTTCTTCTCCTTACCCTACATGTCACCATGTAAATCTCCTTTGAAT
TCCCACCTTTGGGGGTTTTAGCTGTCTTCTCTTTGCCTGGAAAGCTGAGCTCTCTCCTTGTTATTCAGGT
CTCAATTTAAATATGACCTCCTTAAAGAAGCCTCTCTTGGTCCTCCAGTCTCAAGTAGCTATCCAGTTTC
TCTCTGCCACATCCACCTGTTTAAATTATCTACATGGCTTGTGATTTTTCAGGATTTATTACTGTTTTGT
GTTTTCTTATTTATTTTCTATCAGTTTCATGAGAGCAAATAACCTGTCTTGCTCTTGATCCTCCTGCCCC
CTGCACACAGCTTTTTTGGTGTTTTAGAAAAGGCTATAAACTTGGAGTCAGGGGACCTAAATTAAATGTT
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TCTTGATTTTTGGTGCTGTGTTATTAAAGCTTGCTGTATAGTTCGGGATCTCAAGACTTTTCCTAGTCCA
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GTGTAGAGTCTATAGGTGGGATTGGAGTGATAGGAACTCCCCTTGGATTAATTGGCTTCTCTGCTTCTTT
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GTGGTGCTGAGTCTGCATCATCCTCCTGATAGAACCTCCGACTCAACACCAGACCCTCAGCGAGTCCCTT
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CCCTTACCCAGTCCTATCTGCGAGAACTGGCTGTTGGGAGCCTGTGAGCCCCAGGCACTTTGCATCACAGT
CACATGCCCATTCACACCACACAGAGGTTCCCTGCCTTGTTTGGATTGGCACTGTTTGCCATTCTCTGGG
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TGGTATTGGCTTTCCCTTTGTATTTTTGAGCCTTCTTACCCTGAGGATCTTTATGGTGGCCTTGTTTGAT
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GAGCAAGAAAAAAAAGGTTCTCAATCAAAGGTTTATCATAGAAGCCATGTTGTGCATAAAAGAGAATATC
AACTTCCAGTTCAAGATAAGGGTGATGAACAATCTCTTCTTTTTTTTTTTTTTTTTGAGACAGAGTCTC
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GGAACCAGTTACCTCAACATTCGATAGATCTGTGGAATCTACAACATTCAAATAACTTATTTTCTCAACA
GAACCCAAAGTTAACAGAGGTCTGGAGAATTAAATATTGGAATAATTAAGCAAAGGCCTGCAGAGTATCT
GCTCTTTTTAGATGTTTCATCTTTAGCTCAGTTTTGTTAATTTGTATTTCCAGAAAATTGTTCCAGATTT
TTTGTTATTCAAATAACCAGTCCTTAGACGTATTAATCAATTTTACTGGAGTTCTGTATAATCTTAATTT
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CAGCCTAGGCAACATAGGGAGACCTTGTCTCTTAAAAAATGAACAAAAATTAGCTGGGTGGTGTGCACCT
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CAGGGAATCACTGGCATATCCACGATGCCCAAAGTCATGGCTATGGATGTGATTGCCAGGGAAGTATGTG
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GGCACCGTGCTAGACAGTGCCTTGCACTATCAGGTTATCTCATTTAAATCCTTGCAGTTTTCAAGATGAG
TACTGTTAATCCCATTACCAGATTGAGAGAACAGAAACCCAGAGAGTTTAAATATCTTACCCAAGTGAGA
GCGCTCATAAGACAGGGCCGAAAGTGACTGAATGCTTGCCCTCTTCTCCCACACTGCCCACAGTGTTTGG
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CCGGAGCCACCACCTCAGCCTGGGCCTTCTGCTTCTGTTTCTACTCCCTGCAGGTATGAAGCTCAGTACA
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GAGCACTGAAAGGTTTCAGCAGCATACTAGCCTGGTCAGATGTGCATGCTTAAAAGCGTGGTCCTGGGGC
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GGAGAGGGTAGACTCAAGACGATGTTTCGCAAAATCTGGAGGATTTGAATGATCTGACAGGATCTGGAGA
TTAACTGGATTCCCCGAGGAGGAGGCAGCAGAGGGAGAAGGGAGGGATAATTCCCAGATTTCCAACTAAG
GTCACTGTTGAAGCTGAGGGCATGCTATTTGCTGAGATAGGGAACATGGGATAGGAAGCAGGTTTCAGAG
CATGGAGGGAGGAAGCGAGTTCATATTTTGGACCCTCAGAGTTGAAGGTGCCTGTGGGAAACTCAGGAGT
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ACAGGATTCATGGGAACAATGTTTAGGTCAGGGATTGAGGAAAATTTGTGTCAGTAAAGCCTGGGGAAGT
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GGAAACAGGTTTGAGAGATACTGGAGGGGCAGAGCAGTGGGATTTAGAATCCCTGGGTGAAAGTCTGGA
CTCTTGTGGCTTATTTGGGCCCCTCTAGCATTTGTGGAGAGGCAGGCAGACTCCAGGTCCTTGAAAAGGG
GAGGGTGGAGGAGAAATTTGTCAGCCTGGCGCCAGAAGATAGTACCAGTTCACTCCATGGCCTTTACCTC

ATGTGTCCCTGCAGGCAGGCCAGGGAGGAACTAGAGCCACAGCTAGAGCAAGAGAAGGCAGACACCAGGA
GGACACTCATAAGGACAGGGCCCCAGCCCTGGGAGTGGAGGGTGTGAGCAGAGGCCCTGGGACTAGGGCC
TGGGATGGACAACCCTCCTTACTGACCCTCCAGAGTGCCTGGGAGCTGAGGGCCGGCTGGCTCTCAAGCT
GTTCCGTGACCTCTTTGCCAACTACACAAGTGCCCTGAGACCTGTGGCAGACACAGACCAGACTCTGAAT
GTGACCCTGGAGGTGACACTGTCCCAGATCATCGACATGGTGCGTTGTGGTGGTGGTACAGCTGTGGAGT
CTTACCTGTCACAGTGTCAAGAAATGAAGGGGTGAGAGACTGGGATTATTCTCCATGGAATTTCTTTTCT
GTAAATGTTAATATTAACAAAGGTAGCAGTTACAAACTGTTGGGTACTGACTGTTGGGTACTGAGTATTG
GGTGCCTACCTCGTGCCCAATATTTTGTTCACCTGAACTTACTGAATCCCTGCTAAGCAGGGATTCTCAC
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GCACAGACAGGGCAGGATATGCATATACATGTCCACATCTCTGCCATTCCCTGCCCCCACTAGGATGAAC
GGAACCAGGTGCTGACCCTGTATCTGTGGATACGGCAGGAGTGGACAGATGCCTACCTACGATGGGACCC
CAATGCCTATGGTGGCCTGGATGCCATCCGCATCCCCAGCAGTCTTGTGTGGCGGCCAGACATCGTACTC
TATAACAAGTACTGCCTATCTGGGCCCCTCCTCTCTCTTACCCCTCTCTAGACTTGCCCTTAGCTGTGGG
GGTGTAGTGATCCCCTCTCCCTACCACATAACCTGGTTGCCACGCTGCCCTGGAAGCTTTTCCCCAGGAC
CCTTCTAAGCTGCCAAGCACTCAGCCCCTCCATGGCACCCCCACTTTAGGCTATCCCAGGCCAGCCCAGG
CTGAACGTCTCCTCGGAACCTACTGTGTGGTCCAGGGCAGATGTCTGAATCACAAGGGCCTCTCTAGGGC
ACACTTTTAGCTCTAAGTCTCTCAGGGCTCCCCCAAGAGCCTGTCTAAGGGTCTCTTTCCTCCAGGACAT
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ACCAACGTGGTCCTGCGCCACGATGGCGCCGTGCGCTGGGACGCGCCGGCCATCACGCGCAGCTCGTGCC
GCGTGGATGTAGCAGCCTTCCCGTTCGACGCCCAGCACTGCGGCCTGACGTTCGGCTCCTGGACTCACGG
CGGGCACCAACTGGATGTGCGGCCGCGCGGCGCTGCAGCCAGCCTGGCGGACTTCGTGGAGAACGTGGAG
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GACGTCACCTTCACGCTGCTGCTGCGCCGCCGCGCCGCCGCCTACGTGTGCAACCTGCTGCTGCCCTGCG
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GCCGCGACAGGGCCTGGGTCTGCGGAACGGCCCCACTGCAGAAAGTGAGAGGGGGGCGTCCTGGGAACGT
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TGATGATGGAACATCAGTATCCCCAGATCCTAATGACAGGCAAAATCTGTCGACTGCTTGCTGTGTGCCA
GGCACTCCCCTAAGCACTTGACCTTTATTAACTCAGGTAAGCATCACCACAAACCTAGGAAGTAGGTCCT
CTGGGTATCCCATTTGTACAAAAAGGATTCGTATCTTGCCCCAGCTCATGCCCGTCGTTATTTGAGAGCG
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GATGACCCACGTGTAGGAATGAAGGATGGGGAGAACTCGGCCCTTACCTCCTTCCTGCTTCCATCCATGG
GGCTTGGAGGGTCTGGAGAGCTTCATGGTGGGCTTATTTCCATTTGTGCAGAGGTGGCTGGGAAGCTCAG
GAACCACAGGCTTTTGTTTTGAGTCAATTGGCTTTCTCTCTCTCTTGCAGGGAAGTACTACATGGCCACT
ATGACCATGGTCACATTCTCAACAGCACTCACCATCCTTATCATGAACCTGCATTACTGTGGTCCCAGTG
TCCGCCCAGTGCCAGCCTGGGCTAGGGCCCTCCTGCTGGGACACCTGGCACGGGGCCTGTGCGTGCGGGA
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GGCCCGTGTGATGGACCGCTTCTTCCTGGCCATCTTCTTCTCCATGGCCCTGGTCATGAGCCTCCTGGTG
CTGGTGCAGGCCCTGTGAGGGCTGGGACTAAGTCACAGGGATCTGCTGCAGCCACAGCTCCTCCAGAAAG
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CCTGCCACTCCCCTAATTCCAAAAAAAAGAGGAACTCTACAAAGGCCAAGATCACAGAGTACAGTCTTGG
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CCTGCTGAGGGGCTCAGGGAAAAGGGTCGGGGATTCTCAGTCGAGTTTCCAGAGCAGGAGGCCCTACAGA
CATTTGGCCCCAAATCCCTGACTCAATAAAGTAAGCGTGTACCTAGCACCTCCTCGATGCCCTGTGTTAC
CCATGAGGTCTGTGGTAGTGGAAGCTGGGGGTCCAGGTCTGTCTACTTCAGGTCTCATGGCCGCTGGCGC
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TGTCACTAAAGTTGGTATATAACCCCCCACTGCTAAATTTGACTAGCTTAAAAAAAAAAAAGAACTTAGGC
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CCAGAGTTCCCTGCAGATGGGATTGACAGGAATCTTGGTTACACTGAAAGCACACATGGCCAACATCCTC
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GAGGCTACAGTGAGCTGTGGTGGTGCCACTGCACTCCAACCTGGGTGACAGACTGAGACCACGTCTCAAA
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TCCAGAGGTCATTGGGTCTAACTTAACTGCCTTCTATTGCCACAAATAAGGTGCTGCAGAGTGGGATGAA
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TGAGCAAATAATTTGTCTCTGGGTCTCACTTGACCATATAAGGGGGGTAATGCCTCCTGTTCTGCCTCCT
TCCCATAGATTACTGTGCAGTAAAGATGAGATGAGATGATGATGAGATGAGATGAGATGAGATGAGATGA
GATGAGATGAGATGAGATGAGATGAGATGATGAGATGAGATGATGAGATGAGATGAGATGAGATGAGATG
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TCCTGGTGTGGTGGTTCAGAGTTTGGCTCTTGAGCCAGGCTCTCTGGACTCCACTTCTTAGTAGCTGGGT
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AGAGTTTCGCTCTTGTTGCCCAGGCTGGAGTGCAATGGCACAATCATGGCTCACAGCAACCTCCGCCTCC
CAGGTTCAAGAGATTCTCCTGCCTCAGTCTCCCGAGTAGCTGGGATTACAGGCATGCGCCACCACGCCCG
GCTAATTTTGTATTTTTAGTAGAGACGGGGTTTCTCCATGTTGGTCAGGCTGGTCTCGAACTCCTGACCT
CAGGTGATCTGCCCATCTTGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCACACCCGGCCTCT
CTTTGCCCCTTTGTGCTTTGGTACTTTCATCTGCAGAACAGAGGTGATGACAGTACCACTGGGGTGTGGT
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AGTTCTGAAGGACAAAGGAGTTCTGAAGGCTTGGGGAGGAGCTGCTGTTTCTTCCCTGGAAATGGCCCAT
TCCCACCTAGAAACATGGTGGCCTGGGTAGGCCTTGGCACACCAAGTGTCCGAGGGAAGAGAAGAGTCAT
AGCTGGGGATCATCTGGTCCAATTTGCTTATTATACACACAGAGAAACTGAGGCACAGAGAAAGAATGGG
TTGGTCGTAGAGAAAGTTAGAGCAGAGCCTGGACTAGAGCCCAGGCCTCCAGCACCAAAAGCCTGGCCTC
ATGGCCTTCAAAGGTGGGTTTGAGGGAGCCCTGAGGGCAGTAACAGAGACAGTGGGTTCTGCACTGGGAG
GCAGAGAAGGACCAAAGGAGGACTTTGTGGGGAGCAGCCCTTCTGTCCCTCACCTCAGTGCAGCCTGAAT
CTCTCAGGGGCCTGATCAGTGGCCTTTTCCTGCAAGGGATAGGCAGATCCAGGCTGGAGAGCAGGTGTCC
CTGCTCCCTCAACCATCTGCTCTCCCACACACTCATCTCCTGGCTAAGGCTGGCAACCCCCAAGGTGCCA
CTTCAGCTAGTGCACTTTTTTTTATTATTAATGCAGTTGTTTCCTTATAAAAGATTCAGGTGGGCCGGGC
ACGGTGGCTGACACCTGTAATCCCAGCACTTTGGGAGGCCGAGGTGGATGGATCACCTGAGGTCAGGAGT

TCAAGACCAGCCTGGCCAACATGGTGAAACCCCATCTCTACTAAAAATACAAAAAATTAGCCGGGCTTGG
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GGAGGTTGCAGTGAGCCGAGATTGTGCCATTGCACTCCAGCCTGGGCAACAAGAGTGAAACTCTGTCTCA
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AGTCCCACAGGTGCCTGCAGCCCTTACCTGAATTATCCAGATGGCAAGGCCCAGACTTGCACTTCTTGTC
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CTCCTGATGTTGTCCAAGATAGGAAGTAAAGCCCATAGCCCTTCAGAAATTAAGAACCTGGGCCCATCCT
CATGGTTCTTCTTCTGGCCTGTGCTGGGGACATGAACAGGAGGAGCATCCACCACTTCCTGACCACAGCC
TGAGCTGGACCTTAGGGGCACAGCACCCAACTGCTGTCTCCTTGCCCCCACCACCCCACCCAGCACACCC
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GGGACACAGGAGATAGGGGCTTCGGTTTGCCAAAGTCCCTGGTGGATGTGGAAGGTCCACTTTCCGCACA
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CAGCCTCACGGTGGCCCGGGGCCCTGCTGGCCGGAAGCGCAGGCCGTGCACACCTCGGAACACCTGGTGG
CACCGGGGTGGACGCTGGCCGCTGCCCAGGAGCTGCAGGGCCTCAGTCAGCAGGAAATGGAGTGTCTTGA
AGGAGAAGTGGTGGAGGTAGTGGGCCCGGGAGCGGCCCGCCTCACGCACGGCTGCATTGAACTCCTTGTG
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GATGGACGGGTGGGGCTGAGACTCCACTCTGGCCACCTGGCCTGACGCTCCTGCCATTGGCTGCTTGCCA
GTGTCCAGCTGTCTGCATACACCTGGTTGGCCTGGAACTCCGTGTGGTTGAGATCCGGGAGAGCAGCTGT
CATGGCAGCAGCACAGCCAGCGTACTGGTCATCAAAGGAGGCCAGGGCCATGTCCAGCTGAATCTCTTGA
GAGAAGAGGTCTCGTCGTGTGATGGGGTGGCTCTGGGCCTGAGGGGACAGGAGTAGCAGGGACTGAGAGG
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CAAACACACCTGGTGGGGAAGGGGATTGGGAACCTCTGGCTGTAATTTCCCCAAGACTAGCATCTGGAGC
TGTCCCCTTGGGCTGAGTGATCCCCAGGGGAAGCGTCGGGCATTCTTTCCTCTCTCTCTTTCTCCCTCCA
GGGTTCAGAAGAAGCCGATGGCTCAGTTCCCTGCTGGGGTGGGAACAGTGGGGGATGCCCATACCTGAAG
TGCTTCCATGAGGCCCACAGACACAAGAAGCAGAGACATCATAGCAGGCATCTGCATGCTGGTGACCCTG
GGCCAGTTGCTGTCTCTTTTTGGGTCTCAGTTTCCTCATCTGGAAAATTGCAGTGTTAATCTGTATAGTT
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TGGAGGCTCAGAAGAGAGAGGACCCTTCTTGCTGAAAGGAACATGAGTGACTTCCTGGAGGAGTGACGTG
AACAGGTCTTGTAGGATGAACAGGAGACTAAGATGTCAGCAAGTGGAGACTGGGAGAGAAAAGACTGGTG
TTTGGGTGGGAAAGGACTTTTGTGCAGCAGGAATGGAAAAAGCAAAGGTATTGGAGGTGGGAACTGAGGA
TGTAAAGAGAGAAAGACATGTCATCTAGGCTGGCAGAGCTTGCGGGGTAGGAACGTTGGGAAGATGGGCC
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ACAGGGGCAGATCCAGGAGTGTTCAGCTGGTGGGGCCTTAGCAGGATCTCCAGGGACAGACTTAGAGCAG
GGCTTGGTCCACCAGTTGCCCCCACTCCCTGCAGTCCTCTTGTGGAATGAGCCTTCGGTGCTTCCAGGGA
GGGACAGATATAAACCCCGGGTGCTGGGGGAAGAGGGGATCAGAAGAGCAGGAGAAGACAGAGGATACCA
GTTTCCCTAAGAGAAGCAGCAGGAACCACAAGCCTTCCACACCCTCTTTGCTGGGGGACAGGCGGAGTGC
CCGAAGTGGTTCCAGGAAGAGGGTGTCCAGGCATTGGGTCTGGATTGGAGCAGGCAGTTTCCTTTTTTTT
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GTGATCTCGGCGCACTGCATCCTCCACCTCCTGAGTTCAAGTGATTCTTTTGCCTCAGCCTCCGGAGTAG
CTGGGACTAGAGGTGCCCGCCTCCACACCCAGCTAATTTTTGTATTTTTAGTAAAGACGGGGTTTCACCA
TGCTGGCCAGGATGGTCTCGAACTCCTGACCTCAGGTGATCCGCCCGCCTCAGCCTCCCAAAATGTTGGG
ATTACAGGTGTGAGCCACCATGCCGGCCTTATTGTCATTTTTTTAAGAACTGAAAGGAAACATGCTTACA
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TCTCCCTGCTCACATAATGGCAGCTTGGTTTATCTCATGGTATAAACCATAATTAACCATTTCATACTTA
TGAACACTTAGGTTTCTTCCCTAATTTTAAAATATTATACATAATACTACAGTGAATATTTAGGTATATA
AATCCTTCTCTATGTGTGTGCATGTTTTTATAGGAAAGATTTTGAGAAGTAAAATGAGATTTAAAGAATA
TGAATATTTTTTATTTTGACAGAAACTGCCACCCCACCAACAATGGATGAGAGTGCCCTTTATTCCACAT
CTTTGCCAGTGCTGAATATGATTGATCTCTTTTTAATTTCCATTTAACTGGTAGGAAAAATGGTATCTAC
TTTGTTTGTTTGTTTGAGGCAGGGTCTTGCTCTGTTGCCCAGGCTGGAGTGCAGTGGCACAATCATAGCT

CACTGCAGCCTTGACCTCCTGAGCTCAATCGATCCTCCTGCCTCAGCCTCCCGAGTAGCTGGGAGTACAG
GCACACATCACGATGCCTGGCTTATTTTTATATTTTTTGTAGAGGTGGGGTTTTGCCGTGTTGCCCAGGC
TGATCTCGAATTCCTGGGCTCAAGCATTCTACCCACCTTGGCCTCCCAAAGTGCTGGGATTACAGGCGTG
AGCCACAGCTCCCAGCCTCTGTTTTCTTTCTGTACACAAATGGTAATATAGTCAATGGGTCTTTATGTTT
TGGAATCTGATAAAAGCTGAAACTTCCCTTCAGAAAATGAATATATGCGCCTTCACACAAATGTTACATA
AATATCAAGGTGGTTATGCCTCTGCCCCCAATCTCATTTAGGTTAAGCGTCGCTG

The following amino acid sequences <SEQ ID NOS. 16 and 17> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 4:

<SEQ ID NO. 16>
VCNLLLPCVLISLLAPLAFHLPADSGEKVSLGVTVLLALTVFQLLLAESMPPAESVPLIGEQRGRGGTXRC
AGVPPGRGRDRAWVCGTAPLQKVRGGRPGNVPSFXDXGERISSFQGEHPSRLGPXXWNISIPRSXXXAKSV
DCLLCAR

<SEQ ID NO. 17>
VCNLLLPCVLISLLAPLAFHLPADSGEKVSLGVTVLLALTVFQLLLAESMPPAESVPLIGEQRGRGGT

The following sequences <SEQ ID NOS: 37 and 38> are, respectively, forward and reverse primers for SEQ ID NO: 4.

ion3.for <SEQ ID NO: 37>
GCGTGCTCATCTCGCTGCTT

ion3.rev <SEQ ID NO: 38>
TCACCGATGAGCGGCACGCT

The following DNA sequence Ion4a <SEQ ID NO. 5> was identified in *H. sapiens*:

ATCCAAGGAAACTAAAAACAAATGGGGACTAACAGCCTGGAGTCAGGCCTGTGACAGTGAGGGGATGCTAT
GGTGTCACTCTGAGGCCTGGCTTAACACTCTAAGAGAATGTACACAAATATGGGAGCAGCTATCTGGGGAG
TTTCAATTCATTGTGTGGGCACAAGATCCATACTATACTAGTCATCAGGGTCTAACTTTTAGAGATTCTTT
TTCCTCCTCCTAAAAGTGTGTGTATGATCAGTCCATTGGCAAACATATTTTTATCACCTAATATGTACATG
TCATTGGAGTAGGCACTAAGGATACAGAGCCACATAAGACATGGTTATAGAACTCATTGAGCTTACAAGAG
CTTATTACACTTACAAGACTGATATTTTCATGTTTTAGATGCCTACAATGAGGATGACCTAATGCTATACT
GGAAACACGGAAACAAGTCCTTAAATACTGAAGAACATATGTCCCTTTCTCAGTTCTTCATTGAAGACTTC
AGTGCATCTAGTGGATTAGCTTTCTATAGCAGCACAGGTACAGCATTTTACATGGGTGATTCATCAGCATT
TATTGGACATCTACTGTTTGCAAAGCACCACAACATG

The following amino acid sequences <SEQ ID NOS. 18 and 19> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 5:

<SEQ ID NO. 18>
PRKLKTNGDXQPGVRPVTVRGCYGVTLRPGLTLXENVHKYGSSYLGSFNSLCGHKIHTILVIRVXLLEILF
PPPKSVCMISPLANIFLSPNMYMSLEXALRIQSHIRHGYRTHXAYKSLLHLQDXYFHVLDAYNEDDLMLYW
KHGNKSLNTEEHMSLSQFFIEDFSASSGLAFYSSTGTAFYMGDSSAFIGHLLFAKHHNM

<SEQ ID NO. 19>
YFHVLDAYNEDDLMLYWKHGNKSLNTEEHMSLSQFFIEDFSASSGLAFYSSTGTAFYMGDSSAFIGHLLFA
KHHNM

The following sequences <SEQ ID NOS: 39 and 40> are, respectively, forward and reverse primers for SEQ ID NO: 5.

ion4a.for <SEQ ID NO: 39>
GCCTACAATGAGGATGACCTA

ion4a.rev <SEQ ID NO: 40>
CAGTAGATGTCCAATAAATGCTGA

The following DNA sequence Ion4b <SEQ ID NO. 6> was identified in *H. sapiens*:

GTAATTATCATGATGTTCTACAGTGTTCCCCACTAGAAATCCATTAGAAGGAAAATAGAAGAGTAGAAAAG
GAATGAGAATTCTAATCAAGGTTAGAATGAAGAGGATGGAAGAGAGCACAGCAATCATGACCCTATGATTA
ATCAAAGTAGGAGACATAAATAACATACATAATTAAAATGATTTATTAAAACACTAGTGATTTTGACACTG
CCGAGTTTCTGTCTTTTCAGAAAAAGAGCAATATCCCATGAAGAAACTTACCATGTTAGTCTCTGAAATGC
TGTCAATGCTTTCAACATGGACATCTATACCTACTGGCACTGGAGACCCTTAAGAAAGAAGAATGGTTAAT
ATGCTGAGGAATGCATTATAATCATTTTAAAACCATTTAAAACAGAGAGATTAATTCTCTTGGACAGCAAC
TGAATTACTGTTAAAGTTTTTTTAAACAACAAATTTCTCCATTATTCATTGAATCAGTTATTATATACTCA
ATTATTATAATAAAGGCACATGTGTAAATAAATGGTATTCTAATAATCATTACTCATTTGCTTGGGATCAT
GTCAATAATTTCCTCCTCTAGTATAAGAGTGGTGCCTCCAGTTTTCTTTTTTTTTTT

The following amino acid sequences <SEQ ID NOS. 20 and 21> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 6:

<SEQ ID NO. 20>
KKKRKLEAPLLYXRRKLLTXSQANEXXLLEYHLFTHVPLLXXLSIXXLIQXIMEKFVVXKNFNSNSVAVQE
NXSLCFKWFXNDYNAFLSILTILLSXGSPVPVGIDVHVESIDSISETNMVSFFMGYCSFSEKTETRQCQNH
XCFNKSFXLCMLFMSPTLINHRVMIAVLSSILFILTLIRILIPFLLFYFPSNGFLVGNTVEHHDNY

<SEQ ID NO. 21>
GSPVPVGIDVHVESIDSISETNMVSFFMGYCSFSEKTETRQCQNH

The following DNA sequence Ion5 <SEQ ID NO. 7> was identified in *H. sapiens*:

TTTCCCTCCTGCTGACCCCTGGACTTGGGGCCAGACCTACACACGCCAAGGAATGGGCACACACCATTCCT
CTTGTGAAGTTCACAAAATACAGATTGGTCAGCAGCCGGAAAGGATCATAATGCTGTGGTGGCAGCAGCCT
GCTTTTTCAAAATCAATTTCCCCTGGAGATGGGTGGAAAGTTGAAGTTGTAGTCGGTGCGCGCTAAGGCTG
GATACCCAGCGGGTAGGGGAGATCGGACACTCGGTTCAAGTAGGCCACGATGAGATAAGGTTGGAGCCCAG
GCTGAAGAGCACCCGAGCGACCCAGAAGCAGATGCCGTCACTTCCTGGGGAAGGGTCGGCACAAACAGTCC
TTAAAGGGGCAGCTGCAGGAGCCAGTGGCACGGGAGACAGTGGGGGCGCCTCTGCCGCGCTCCATCCGCCT
CTGGCTCCTGTCCAACCTCGCCGATGGCGTCCTGGCCTCTCGTGTCCTGCCCTCTCATGTTCTTGGCCACG
AAGTTCACGGCATCCCCACAGCAGCGTATCTCCGGGGCGGCAGTGCCCAGGCTCTGGTGAGGCAGCCGGCA
GGAAGTAGGCTAGCAGCACCAAGCCTGAGATGAGCACGCAGGGAACCATGATG

The following amino acid sequences <SEQ ID NOS. 22-28> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 7:

<SEQ ID NO. 22>
FPSCXPLDLGPDLHTPRNGHTPFLLXSSQNTDWSAAGKDHNAVVAAACFFKINFPWRWVESXSCSRCALRL
DTQRVGEIGHSVQVGHDEIRLEPRLKSTRATQKQMPSLPGEGSAQTVLKGAAAGASGTGDSGGASAALHPP
LAPVQPRRWRPGLSCPALSCSWPRSSRHPHSSVSPGRQCPGSGEAAGRKXASSTKPEMSTQGTMM

<SEQ ID NO. 23>
FPPADPWTWGQTYTRQGMGTHHSSCEVHKIQIGQQPERIIMLWWQQPAFSKSISPGDGWKVEVVVGARXGW
IPSGXGRSDTRFKXATMRXGWSPGXRAPERPRSRCRHFLGKGRHKQSLKGQLQEPVARETVGAPLPRSIRL
WLLSNLADGVLASRVLPSHVLGHEVHGIPTAAYLRGGSAQALVRQPAGSRLAAPSLRXARREPX

<SEQ ID NO. 24>
SLLLTPGLGARPTHAKEWAHTIPLVKFTKYRLVSSRKGSXCCGGSSLLFQNQFPLEMGGKLKLXSVRAKAG
YPAGRGDRTLGSSRPRXDKVGAQAEEHPSDPEADAVTSWGRVGTNSPXRGSCRSQWHGRQWGRLCRAPSAS

GSCPTSPMASWPLVSCPLMFLATKFTASPQQRISGAAVPRLWXGSRQEVGXQHQAXDEHAGNHD

<SEQ ID NO. 25>
HHGSLRAHLRLGAASLLPAGCLTRAWALPPRRYAAVGMPXTSWPRTXEGRTREARTPSARLDRSQRRMERG
RGAPTVSRATGSCSCPFKDCLCRPFPRKXRHLLLGRSGALQPGLQPYLIVAYLNRVSDLPYPLGIQPXRAP
TTTSTFHPSPGEIDFEKAGCCHHSIMILSGCXPICILXTSQEEWCVPIPWRVXVWPQVQGSAGGK

<SEQ ID NO. 26>
IMVPCVLISGLVLLAYFLPAASPEPGHCRPGDTLLWGCRELRGQEHERAGHERPGRHRRGWTGARGGWSAA
EAPPLSPVPLAPAAAPLRTVCADPSPGSDGICFWVARVLFSLGSNLISSWPTXTECPISPTRWVSSLSAHR
LQLQLSTHLQGKLILKKQAAATTALXSFPAADQSVFCELHKRNGVCPFLGVCRSGPKSRGQQEG

<SEQ ID NO. 27>
SWFPACSSQAWCCXPTSCRLPHQSLGTAAPEIRCCGDAVNFVAKNMRGQDTRGQDAIGEVGQEPEADGARQ
RRPHCLPCHWLLQLPLXGLFVPTLPQEVTASASGSLGCSSAWAPTLSHRGLLEPSVRSPLPAGYPALARTD
YNFNFPPISRGNXFXKSRLLPPQHYDPFRLLTNLYFVNFTRGMVCAHSLACVGLAPSPGVSRRE

<SEQ ID NO. 28>
IMVPCVLISGLVLLAYFLPAXXQSLGTAAPEIRCCGDAVNFVAKNMRGQDXXDGICFWVARVLFSLGSNLI
XXAYLNRVSDLPYPLGIQP

The following sequences <SEQ ID NOS: 41 and 42> are, respectively, forward and reverse primers for SEQ ID NO: 7.

ion5.for <SEQ ID NO: 41>
CATCATGGTTCCCTGCGTGCT

ion5.rev <SEQ ID NO: 42>
GTCCTGCCCTCTCATGTTCTT

The following DNA sequence Ion6 <SEQ ID NO. 8> was identified in *H. sapiens*:

TCTTGGACACATCTTAATGTGGCCTGAATTGTTCATTCTTATTTTAAAAGTCTTTCTATTTCTCTTTGGAA
GTTATGGAATAACGGATGGAGAAATGAAGAGATGGGATTCCAAGTGGAGAGATGGATAATCCAAACAGTCA
CATGTAGGAGGGAAAACATAATTTGGGGGACATTTTCAAGCACAAATAATAAATTAAAAAGAAATCTTGGT
TATTTTTTGTTTGACACATTCCTCCCTTTTGAGTGCAAAAGAAAACATGTGTTAAAGAAGCAGTTCTGCCA
TAATGTAGCCTGGACCTACATCTGACTCCCAGTAATTGAATTGCCCAGTTCCTTGACCTGCAACATTGATG
GCGATGCAACTGCCCTGAGGCAGAAGTGGCTACCTGTCCACCAAGCGCCACGCACTGCCTGCCTTATTGAA
TGTAGATCCCGAGGCAAAGACTACATTTCCCATGCTCCCTTGCTCTGAGGTGGAGTCATGTGATGGATTCC
CGCCAATGGGGTGATGTGATGAATTCCCATCAAT

The following amino acid sequences <SEQ ID NOS. 29 and 30> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 8:

<SEQ ID NO. 29>
SWTHLNVAXIVHSYFKSLSISLWKLWNNGWRNEEMGFQVERWIIQTVTCRRENIIWGTFSSTNNKLKRNLG
YFLFDTFLPFECKRKHVLKKQFCHNVAWTYIXLPVIELPSSLTCNIDGDATALRQKWLPVHQAPRTACLIE
CRSRGKDYISHAPLLXGGVMXWIPANGVMXXIPIN

<SEQ ID NO. 30>
IVHSYFKSLSISLWKLWNNGWRNEEMGFQVERWIIQTVTCRRENIIWGTFSSTNNKLKRNLGYFLFDTFLP
FECKRKHVLKKQFCHNVAWTYI

The following sequences <SEQ ID NOS: 43 and 44> are, respectively, forward and reverse primers for SEQ ID NO: 8.

ion6.for <SEQ ID NO: 43>
AGGAGGGAAAACATAATTTGGGGGA

ion6.rev <SEQ ID NO: 44>
AGGGAGGAATGTGTCAAACAAA

The following DNA sequence Ion7 <SEQ ID NO. 9> was identified in *H. sapiens*:

CTTCTGAATGTTTAGCTTTTTGACTCTTTTGTAACAGCATACAGCTTAAAACACAAACACATTGTATAGCT
TTACAAAAGTATTTTTTCTTTCTTTTTGCCTTTATTCTATAAGTGTTGGTCTATTTTTAATTTTTTTTGTT
TTTTACTTTTTAGGTTTTTTGTTAAAAATGAAGACACAAACATACACATTTGCCTAGGCCTACACAGGGTC
AAGATCATAAATATCACTGCCTTCCATCTCCACATCTTGTCCTACAGAAGGTCTTCATGGTCAATAACACA
CATGGAGTTGTCATTTCTTATGATAATGCCTTCTTCTGGAATCCTCCTGAAGAATCTGCCTGAGGCCATTT
TATAAACAGTTTTTTTTAAATAAGTGGAATGAGTATACTCCAAAATAATGATAAAATACAGTATAATAAAT
ACATAAACCACTAAAAATTAGTTAGTATCACTATCAAGTATTATGTACTCTACATAATTGTATTGCTATAC
TGTTATGCAACTGGTAGTGCAGTAGCTATGTTTACATCAGTATCACTACAAACAAATGAGTAATGCATTGC
ACTATGATGTAATGACAATAGCTATGATGTCACTGGGTGGCAGGAATTTTTCAGCTCCATTTTCTTATGGG
ATCACTATCATACATGTGGCCCATC

The following amino acid sequences <SEQ ID NOS. 31 and 32> are predicted amino acid sequences derived from the DNA sequence of SEQ ID NO. 9:

<SEQ ID NO. 31>
SECLAFXLFCNSIQLKTQTHCIALQKYFFFLFAFILXVLVYFXFFLFFTFXVFCXKXRHKHTHLPRPTQGQ
DHKYHCLPSPHLVLQKVFMVNNTHGVVISYDNAFFWNPPEESAXGHFINSFFXISGMSILQNNDKIQYNKY
INHXKLVSITIKYYVLYIIVLLYCYATGSAVAMFTSVSLQTNEXCIALXCNDNSYDVTGWQEFFSSIFLWD
HYHTCGPX

<SEQ ID NO. 32>
RHKHTHLPRPTQGQDHKYHCLPSPHLVLQKVFMVNNTHGVVISYDNAFFWNPPEESA

The following sequences <SEQ ID NOS: 45 and 46> are, respectively, forward and reverse primers for SEQ ID NO: 9.

ion7.for <SEQ ID NO: 45>
CCTACACAGGGTCAAGATCAT

ion7.rev <SEQ ID NO: 46>
AGGAGGATTCCAGAAGAAGGCAT

EXAMPLES

Example 1: Identification of Ion Channel Sequences in GenBank/EMBL

A brief description of the searching mechanism follows. The BLAST algorithm, Basic Local Alignment Search Tool, is suitable for determining sequence similarity (Altschul *et al.*, *J. Mol. Biol.*, 1990, *215*, 403-410, which is incorporated herein by reference in its entirety). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring

sequence pair (HSPs) by identifying short words of length "W" in the query sequence that either match or satisfy some positive valued threshold score "T" when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension for the word hits in each direction are halted when: 1) the cumulative alignment score falls off by the quantity X from its maximum achieved value; 2) the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or 3) the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff *et al.*, *Proc. Natl. Acad. Sci. USA*, **1992**, *89*,10915-19, which is incorporated herein by reference in its entirety) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm (Karlin *et al.*, *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 5873-5787, which is incorporated herein by reference in its entirety) and Gapped BLAST (Altschul *et al.*, *Nuc. Acids Res.*, **1997**, *25*, 3389-3402, which is incorporated herein by reference in its entirety) perform a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to an ion channel gene or cDNA if the smallest sum probability in comparison of the test nucleic acid to an ion channel nucleic acid is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The Celera database was searched with the NCBI program BLAST (Altschul *et al.*, *Nuc. Acids Res.*, 1997, *25*, 3389, which is incorporated herein by reference in its entirety), using the known protein sequences of ion channels from the SWISSPROT database as query sequences to find patterns suggestive of novel ion channels. Specifically, one of the BLAST programs TBLASTN was used to compare protein sequences to the DNA database dynamically translated in six reading frames. Alternatively, a second search strategy was developed using a hidden Markov model (HMM)(Krogh, A., Brown, B.,

Mian, IS., Sjolander, K and D.Haussler, Hidden Markov models in computational biology: applications to protein modeling. J Mol Biol 1994, 235;1501-1531)) to query tht nucleotide database translated in six reading frames. HMMs, as used herein, describe the probability distribution of conserved sequence when compared to a related protein family. Because of this different search algorithm, the use of HMMs may yield different and possibly more relevant results than are generated by the BLAST search. Positive hits were further analyzed with the program BLASTX against the non-redundant protein and nucleotide databases maintained at NCBI to determine which hits were most likely to encode novel ion channels, using the standard (default) parameters. This search strategy, together with the insight of the inventors, identified SEQ NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51 as candidate sequences.

Example 2: Detection of Open Reading Frames and Prediction of the Primary Transcript for Ion Channels

The predictions of the primary transcript and mature mRNA were made manually. Consensus sequences found in textbooks (*i.e.*, Lodish, H. *et al. Molecular Cell Biology*, 1997, ISBN: 0-7167-2380-8) and regions of similarity to known ion channels were used to discover the primary transcripts of the ion channel polypeptides.

Example 3:Cloning of ion channel cDNA

To isolate cDNA clones encoding full length ion channel proteins, DNA fragments corresponding to a portion of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, or complementary nucleotide sequence thereof, can be used as probes for hybridization screening of a phage, phagemid, or plasmid cDNA library. The DNA fragments are amplified by PCR. The PCR reaction mixture of 50 µl contains polymerase mixture (0.2 mM dNTPs, 1x PCR Buffer and 0.75 µl Expand High Fidelity Polymerase (Roche Biochemicals)), 100ng to1µg of human cDNA, and 50 pmoles of forward primer and 50 pmoles of reverse primer. Primers may be readily designed by those of skill in the art based on the nucleotide sequences provided herein. Amplification is performed in an Applied Biosystems PE2400 thermocycler using for example, the following program: 95°C for 15 seconds, 52°C for 30 seconds and 72°C for 90 seconds; repeated for 25 cycles. The actual PCR conditions will depend, for example on the physical characteristics of the oligonucleotide primers and the length of the PCR product. The amplified product can be

separated from the plasmid by agarose gel electrophoresis, and purified by Qiaquick™ gel extraction kit (Qiagen).

A lambda phage library containing cDNAs cloned into lambda ZAPII phage-vector is plated with *E. coli* XL-1 blue host, on 15 cm LB-agar plates at a density of 50,000 pfu per plate, and grown overnight at 37°C; (plated as described by Sambrook *et al.*, *supra*). Phage plaques are transferred to nylon membranes (Amersham Hybond NJ), denatured for 2 minutes in denaturation solution (0.5 M NaOH, 1.5 M NaCl), renatured for 5 minutes in renaturation solution (1 M Tris pH 7.5, 1.5 M NaCl), and washed briefly in 2xSSC (20x SSC: 3 M NaCl, 0.3 M Na-citrate). Filter membranes are dried and incubated at 80°C for 120 minutes to cross-link the phage DNA to the membranes.

The membranes are hybridized with a DNA probe prepared as described above. A DNA fragment (25 ng) is labeled with α-^{32}P-dCTP (NEN) using Rediprime™ random priming (Amersham Pharmacia Biotech), according to manufacturers instructions. Labelled DNA is separated from unincorporated nucleotides by S200 spin columns (Amersham Pharmacia Biotech), denatured at 95°C for 5 minutes and kept on ice. The DNA-containing membranes (above) are pre-hybridized in 50 ml ExpressHyb™ (Clontech) solution at 68°C for 90 minutes. Subsequently, the labeled DNA probe is added to the hybridization solution, and the probe is left to hybridize to the membranes at 68°C for 70 minutes. The membranes are washed five times in 2x SSC, 0.1% SDS at 42°C for 5 minutes each, and finally washed 30 minutes in 0.1x SSC, 0.2% SDS. Filters are exposed to Kodak XAR film (Eastman Kodak Company, Rochester, N.Y., USA) with an intensifying screen at –80°C for 16 hours. One positive colony is isolated from the plates, and replated with about 1000 pfu on a 15 cm LB plate. Plating, plaque lift to filters, and hybridization are performed as described above. About four positive phage plaques may be isolated form this secondary screening.

cDNA containing plasmids (pBluescript SK-) are rescued from the isolated phages by *in vivo* excision by culturing XL-1 blue cells co-infected with the isolated phages and with the Excision helper phage, as described by the manufacturer (Stratagene). XL-blue cells containing the plasmids are plated on LB plates and grown at 37°C for 16 hours. Colonies (18) from each plate are re-plated on LB plates and grown. One colony from each plate is stricken onto a nylon filter in an ordered array, and the filter is placed on a LB plate to raise the colonies. The filter is hybridized with a labeled probe as described

above. About three positive colonies are selected and grown up in LB medium. Plasmid DNA is isolated from the three clones by Qiagen Midi Kit (Qiagen) according to the manufacturer's instructions. The size of the insert is determined by digesting the plasmid with the restriction enzymes NotI and SalI, which establishes an insert size.

The clones are sequenced directly using an ABI377 fluorescence-based sequencer (Perkin-Elmer/Applied Biosystems Division, PE/ABD, Foster City, CA) and the ABI PRISM™ Ready Dye-Deoxy Terminator kit with Taq FSTM polymerase. Each ABI cycle sequencing reaction contains about 0.5 μg of plasmid DNA. Cycle-sequencing is performed using an initial denaturation at 98°C for 1 minute, followed by 50 cycles using the following parameters: 98°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 60°C for 4 minutes. Temperature cycles and times are controlled by a Perkin-Elmer 9600 thermocycler. Extension products are purified using Centriflex™ gel filtration cartridges (Advanced Genetic Technologies Corp., Gaithersburg, MD). Each reaction product is loaded by pipette onto the column, which is centrifuged in a swinging bucket centrifuge (Sorvall model RT6000B tabletop centrifuge) at 1500 x *g* for 4 minutes at room temperature. Column-purified samples are dried under vacuum for about 40 minutes and dissolved in 5 μl of DNA loading solution (83% deionized formamide, 8.3 mM EDTA, and 1.6 mg/ml Blue Dextran). The samples are heated to 90°C for three minutes and loaded into the gel sample wells for sequence analysis using the ABI377 sequencer. Sequence analysis is performed by importing ABI377 files into the Sequencer program (Gene Codes, Ann Arbor, MI). Generally, sequence reads of up to about 700 bp are obtained. Potential sequencing errors are minimized by obtaining sequence information from both DNA strands and by re-sequencing difficult areas using primers annealing at different locations until all sequencing ambiguities are removed.

Ion1

Using oligonucleotide primers (5' AAAAGGCCTCACAGCATATG 3' (SEQ ID NO:47); and 5' AGCGTGCAGTTTTGCTGGTC 3' (SEQ ID NO:48)) based on SEQ ID NO:1, a human smooth muscle cDNA library was screened using the polymerase chain reaction (Altshul *et al.*). One cDNA was sequenced and found to comprise a longer cDNA corresponding to ion-1. Based on homology to the 5-HT3 receptor, the cDNA is nearly full-length, but does not contain the translation initiation codon. The DNA sequence for this clone is set forth as SEQ ID NO:49, and the deduced amino acid sequence is set forth as SEQ ID NO:50. The predicted protein has homology to the 5-HT3 receptor and to

nicotinic acetylcholine receptors. Therefore, the protein of SEQ ID NO:50 is likely a receptor for serotonin, acetylcholine, or nicotine, or any combination thereof.

The cDNA set forth in SEQ ID NO:49 contains all four transmembrane domains, including the pore domain, of this ion channel. The portion of the cDNA encoding these four transmembrane domains is sufficient for use as part of a chimeric receptor. Those skilled in the art can identify several ways to clone said portion of the cDNA in frame with an extracellular domain of other ligand-gated ion channels (*e.g.*, the extracellular domain of the alpha10 nicotinic acetylcholine receptor).

The cDNA of SEQ ID NO:49 can be used to express the protein of SEQ ID NO:50 by subcloning the cDNA into a suitable mammalian expression vector (*e.g.* pcDNA3.1) and transfecting the vector into mammalian cells (*e.g.* HEK293 or SHSY-5Y cells). Activity of this channel in the presence of neurotransmitters (*e.g.* serotonin or acetylcholine) or compounds can be measured by methods described *supra* and *infra*.

Ion-3

A full-length cDNA containing ion-3 sequence (SEQ ID NO:4) has been published (GenBank accession number AF199235) and has been named the alpha10 nicotinic acetylcholine receptor (nAChR). This sequence was used to search the Celera database of human genomic sequences using the BLAST algorithm. The sequence of the human genomic DNA region containing the alpha10 nAChR gene was discovered and is set forth in SEQ ID NO:51. The sequence 5' of the first exon contains the promoter region of the gene. The sequence set forth in SEQ ID NO:51, or a portion thereof, can be used to design zinc-finger proteins or polyamides or other compounds capable of binding to specific DNA sequences. These proteins, polyamides, or compounds can also be used to regulate the expression of the alpha10 nAChR. The proteins, polyamides, or compounds can decrease transcription of the gene by binding to sites that block access of transcription factors to the alpha10 nAChR gene. Alternatively, transcriptional activation domains can be added to the proteins, polyamides, or compounds such that their binding to the alpha10 nAChR gene results in increased transcription of mRNA encoding the receptor. The alpha10 nAChR sequence set forth in SEQ ID NO:51 can be introduced as a transgene into animals, *e.g.* mice, to express the receptor in cell types as dictated by its native promoter sequence or by a promoter fused to alpha10 nAChR sequence 5' of the transcription initiation site.

Example 4: Northern Blot Analysis

Ion channel expression patterns can be determined through northern blot analysis of mRNA from different cell and tissue types. Typically, "blots" of isolated mRNA from such cells or tissues are prepared by standard methods or purchased, from commercial suppliers, and are subsequently probed with nucleotide probes representing a fragment of the polynucleotide encoding the ion channel polypeptide.

Those skilled in the art are familiar with standard PCR protocols for the generation of suitable probes using pairs of sense and antisense orientation oligonucleotide primers derived from SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51. During the PCR process, the probe is labeled radioactively with the use of α^{32}P-dCTP by Rediprime™ DNA labeling system (Amersham Pharmacia) so as to permit detection during analysis. The probe is further purified on a Nick Column (Amersham Pharmacia).

A multiple human tissue northern blot from Clontech (Human II # 7767-1) is used in hybridization reactions with the probe to determine which tissues express ion channels. Pre-hybridization is carried out at 42°C for 4 hours in 5x SSC, 1x Denhardt's reagent, 0.1% SDS, 50% formamide, 250 µg/ml salmon sperm DNA. Hybridization is performed overnight at 42°C in the same mixture with the addition of about $1.5x10^6$ cpm/ml of labelled probe. The filters are washed several times at 42°C in 0.2x SSC, 0.1% SDS. Filters were exposed to Kodak XAR film (Eastman Kodak Company, Rochester, N.Y., USA) with an intensifying screen at –80°C, allowing analysis of mRNA expression.

Example 5: Expression of Ion Channel Polypeptides in Mammalian Cells

1. Expression of ion channel polypeptides in 293 cells

For expression of ion channel polypeptides in mammalian cells 293 (transformed human, primary embryonic kidney cells), a plasmid bearing the relevant ion channel coding sequence is prepared, using vector pCDNA6 (Invitrogen). Vector pCDNA6 contains the CMV promoter and a blasticidin resistant gene for selection of stable transfectants. Many other vectors can be used containing, for example, different promoters, epitope tags for detection and/or purification of the protein, and resistance genes. The forward primer for amplification of this ion channel polypeptide encoding cDNA is determined by procedures as well known in the art and preferably contains a 5' extension of nucleotides to introduce a restriction cloning site not present in the ion channel cDNA sequence, for example, a *HindIII* restriction site and nucleotides matching

the ion channel nucleotide sequence. The reverse primer is also determined by procedures known in the art and preferably contains a 5' extension of nucleotides to introduce a restriction cloning site not present in the ion channel cDNA sequence, for example, an XhoI restriction site, and nucleotides corresponding to the reverse complement of the ion channel nucleotide sequence. The PCR conditions are determined by the physical properties of the oligonucleotide primer and the length of the ion channel gene. The PCR product is gel purified and cloned into the HindIII-XhoI sites of the vector.

The plasmid DNA is purified using a Qiagen plasmid mini-prep kit and transfected into, for example, 293 cells using DOTAP transfection media (Boehringer Mannhein, Indianapolis, IN). Transiently transfected cells are tested for ion channel activity and expression after 24-48 hours by established techniques of electrophysiology Electrophysiology, A Practical Approach, DI Wallis editor, IRL Press at Oxford University Press, (1993), and Voltage and patch Clamping with Microelectrodes, TG Smith, H Lecar, SJ Redman and PW Gage, eds., Waverly Press, Inc for the American Physiology Society (1985). This provides one means by which ion channel activity can be characterized.

DNA is purified using Qiagen chromatography columns and transfected into 293 cells using DOTAP transfection media (Boehringer Mannheim, Indianapolis, IN). Transiently transfected cells are tested for expression after 24 hours of transfection, using Western blots probed with anti-His and anti-ion channel peptide antibodies. Permanently transfected cells are selected with Zeocin and propagated. Production of the recombinant protein is detected from both cells and media by western blots probed with anti-His, anti-Myc or anti-ion channel peptide antibodies.

2. Expression of ion channel polypeptides in COS cells

For expression of ion channel polypeptides in COS7 cells, a polynucleotide molecule having a nucleotide of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, or complementary nucleotide sequences thereof, can be cloned into vector p3-CI. This vector is a pUC18-derived plasmid that contains the HCMV (human cytomegalovirus) intron located upstream from the bGH (bovine growth hormone) polyadenylation sequence and a multiple cloning site. In addition, the plasmid contains the dhrf (dihydrofolate reductase) gene which provides selection in the presence of the drug methotrexane (MTX) for selection of stable transformants. Many other vectors can

be used containing, for example, different promoters, epitope tags for detection and/or purification of the protein, and resistance genes.

The forward primer is determined by procedures known in the art and preferably contains a 5' extension which introduces an XbaI restriction site for cloning, followed by nucleotides which correspond to a nucleotide sequence given in SEQ ID NO:1 to SEQ ID NO:9, and SEQ ID NO:49, and SEQ ID NO:51, or portion thereof. The reverse primer is also determined by methods well known in the art and preferably contains a 5'- extension of nucleotides which introduces a SalI cloning site followed by nucleotides which correspond to the reverse complement of a nucleotide sequence given in SEQ ID NOS:1-9, and SEQ ID NO:49 , and SEQ ID NO:51, or portion thereof.

The PCR consists of an initial denaturation step of 5 min at 95°C, 30 cycles of 30 sec denaturation at 95°C, 30 sec annealing at 58°C and 30 sec extension at 72°C, followed by 5 min extension at 72°C. The PCR product is gel purified and ligated into the XbaI and SalI sites of vector p3-CI. This construct is transformed into *E. coli* cells for amplification and DNA purification. The DNA is purified with Qiagen chromatography columns and transfected into COS 7 cells using Lipofectamine™ reagent (Gibco/BRL), following the manufacturer's protocols. Forty-eight and 72 hours after transfection, the media and the cells are tested for recombinant protein expression.

Ion channel polypeptides expressed in cultured COS cells can be purified by disrupting cells via homogenization and purifying membranes by centrifugation, solubilizing the protein using a suitable detergent, and purifying the protein by, for example, chromatography. Purified ion channel is concentrated to 0.5 mg/ml in an Amicon concentrator fitted with a YM-10 membrane and stored at -80°C.

Example 6: Expression of Ion Channel Polypeptides in Insect Cells

For expression of ion channel polypeptides in a baculovirus system, a polynucleotide molecule having a sequence selected from the group consisting of SEQ ID NOS:1-9, SEQ ID NO:49, and SEQ ID NO:51, or a portion thereof, or complement thereof, is amplified by PCR. The forward primer is determined by methods known in the art and preferably constitutes a 5' extension adding a NdeI cloning site, followed by nucleotides which corresponding to a nucleotide sequence provided in SEQ ID NOS:1-9, and SEQ ID NO:49, and SEQ ID NO:51, or a portion thereof. The reverse primer is also determined by methods known in the art and preferably constitutes a 5' extension which

introduces a KpnI cloning site, followed by nucleotides which correspond to the reverse complement of a nucleotide sequence provided in SEQ ID NOS:1-9, SEQ ID NO:49, and SEQ ID NO:51, or a portion thereof.

The PCR product is gel purified, digested with NdeI and KpnI, and cloned into the corresponding sites of vector pACHTL-A (Pharmingen, San Diego, CA). The pAcHTL expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV), and a 10XHis tag upstream from the multiple cloning site. A protein kinase site for phosphorylation and a thrombin site for excision of the recombinant protein preceding the multiple cloning site is also present. Of course, many other baculovirus vectors can be used in place of pAcHTL-A, such as pAc373, pVL941 and pAcIM1. Other suitable vectors for the expression of ion channel polypeptides can be used, provided that such vector constructs include appropriately located signals for transcription, translation, and trafficking, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow *et al.*, *Virology*, **1989**, *170*, 31-39, among others.

The virus is grown and isolated using standard baculovirus expression methods, such as those described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

In a preferred embodiment, pAcHLT-A containing the gene encoding the ion channel polypeptides is introduced into baculovirus using the "BaculoGold " transfection kit (Pharmingen, San Diego, CA) using methods provided by the manufacturer. Individual virus isolates are analyzed for protein production by radiolabeling infected cells with ^{35}S-methionine at 24 hours post infection. Infected cells are harvested at 48 hours post infection, and the labeled proteins are visualized by SDS-PAGE autoradiography. Viruses exhibiting high expression levels can be isolated and used for scaled up expression.

For expression of the ion channel polypeptides in Sf9 insect cells, a polynucleotide molecule having a sequence of SEQ ID NOS:1-9, SEQ ID NO:49, and SEQ ID NO:51, or a portion thereof, is amplified by PCR using the primers and methods described above for baculovirus expression. The ion channel polypeptide encoding cDNA insert is cloned into vector pAcHLT-A (Pharmingen), between the NdeI and KpnI sites (after elimination of an internal NdeI site). DNA is purified using Qiagen chromatography columns. Preliminary Western blot experiments from non-purified plaques are tested for the presence of the

recombinant protein of the expected size which reacts with the poly-His tag antibody. Because ion channel polypeptides are integral membrane proteins, preparation of the protein sample is facilitated using detergent extraction. Results are confirmed after further purification and expression optimization in HiG5 insect cells.

Example 7: Interaction Trap/Two-Hybrid System

In order to assay for ion channel polypeptide-interacting proteins, the interaction trap/two-hybrid library screening method can be used. This assay was first described in Fields, *et al.*, *Nature*, **1989**, *340*, 245, which is incorporated herein by reference in its entirety. A protocol is published in *Current Protocols in Molecular Biology* 1999, John Wiley & Sons, NY, and Ausubel, F.M. *et al.* 1992, *Short Protocols in Molecular Biology*, 4th ed., Greene and Wiley-Interscience, NY, both of which are incorporated herein by reference in their entirety. Kits are available from Clontech, Palo Alto, CA (Matchmaker Two Hybrid System 3).

A fusion of the nucleotide sequences encoding all or a partial ion channel polypeptide and the yeast transcription factor GAL4 DNA-binding domain (DNA-BD) is constructed in an appropriate plasmid (*i.e.*, pGBKT7), using standard subcloning techniques. Similarly, a GAL4 active domain (AD) fusion library is constructed in a second plasmid (*i.e.*, pGADT7) from cDNA of potential ion channel polypeptide-binding proteins (for protocols on forming cDNA libraries, see Sambrook *et al.*, *supra*. The DNA-BD/ ion channel fusion construct is verified by sequencing, and tested for autonomous reporter gene activation and cell toxicity, both of which would prevent a successful two-hybrid analysis. Similar controls are performed with the AD/library fusion construct to ensure expression in host cells and lack of transcriptional activity. Yeast cells are transformed (*ca.* 10^5 transformants/mg DNA) with both the ion channel and library fusion plasmids according to standard procedure (Ausubel, *et al.*, *supra*). In vivo binding of DNA-BD/ ion channel with AD/library proteins results in transcription of specific yeast plasmid reporter genes (*i.e.*, lacZ, HIS3, ADE2, LEU2). Yeast cells are plated on nutrient-deficient media to screen for expression of reporter genes. Colonies are dually assayed for β-galactosidase activity upon growth in Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) supplemented media (filter assay for β-galactosidase activity is described in Breeden, *et al.*, *Cold Spring Harb. Symp. Quant. Biol.*, **1985**, *50*, 643, which is incorporated herein by reference in its entirety). Positive AD-library plasmids are rescued

from transformants and reintroduced into the original yeast strain as well as other strains containing unrelated DNA-BD fusion proteins to confirm specific ion channel polypeptide/library protein interactions. Insert DNA is sequenced to verify the presence of an open reading frame fused to GAL4 AD and to determine the identity of the ion channel polypeptide-binding protein.

Example 8: FRET Analysis of Protein-Protein Interactions Involving Ion Channel Polypeptides

In order to assay for ion channel polypeptide-interacting proteins, fluorescence resonance energy transfer (FRET) methods can be used. An example of this type of assay is described in Mahajan NP, *et al.*, *Nature Biotechnology,* **1998**, *16*, 547, which is incorporated herein by reference in its entirety. This assay is based on the fact that when two fluorescent moieties having the appropriate excitation/emission properties are brought into close proximity, the donor fluorophore, when excited, can transfer its energy to the acceptor fluorophore whose emission is measured. The emission spectrum of the donor must overlap with the absorption spectrum of the acceptor while overlaps between the two absorption spectra and between the two emission spectra, respectively, should be minimized. An example of a useful donor/acceptor pair is Cyan Fluorescent Protein (CFP)/Yellow Fluorescent Protein (YFP) (Tsien, RY (1998), *Annual Rev Biochem* **67**, 509-544, which is incorporated by reference in its entirety).

A fusion of the nucleotide sequences encoding whole or partial ion channel polypeptides and CFP is constructed in an appropriate plasmid, using standard subcloning techniques. Similarly, a nucleotide encoding a YFP fusion of the possibly interacting target protein is constructed in a second plasmid. The CFP/ion channel polypeptide fusion construct is verified by sequencing. Similar controls are performed with the YFP/target protein construct. The expression of each protein can be monitored using fluorescence techniques (*e.g.*, fluorescence microscopy or fluorescence spectroscopy). Host cells are transformed with both the CFP/ ion channel polypeptide and YFP/target protein fusion plasmids according to standard procedure. *In situ* interactions between CFP/ion channel polypeptide and the YFP/target protein are detected by monitoring the YFP fluorescence after exciting the CFP fluorophore. The fluorescence is monitored using fluorescence microscopy or fluorescence spectroscopy. In addition, changes in the interaction due to *e.g.*, external stimuli are measured using time-resolved fluorescence techniques.

Alternatively, a YFP fusion library may be constructed from cDNA of potential ion channel polypeptide-binding proteins (for protocols on forming cDNA libraries, see Sambrook *et al.*, *supra*). Host cells are transformed with both the CFP/ion channel polypeptide and YFP fusion library plasmids. Clones exhibiting FRET are then isolated and the protein interacting with an ion channel polypeptide is identified by rescuing and sequencing the DNA encoding the YFP/target fusion protein.

Example 9: Assays to Identify Modulators of Ion Channel Activity

Set forth below are several nonlimiting assays for identifying modulators (agonists and antagonists) of ion channel activity. Although the following assays typically measure calcium flux, it is contemplated that measurement of other ions may be made. Among the modulators that can be identified by these assays are natural ligand compounds of the ion channel; synthetic analogs and derivatives of natural ligands; antibodies, antibody fragments, and/or antibody-like compounds derived from natural antibodies or from antibody-like combinatorial libraries; and/or synthetic compounds identified by high-throughput screening of libraries; and the like. All modulators that bind ion channel are useful for identifying such ion channels in tissue samples (*e.g.*, for diagnostic purposes, pathological purposes, and the like). Agonist and antagonist modulators are useful for up-regulating and down-regulating ion channel activity, respectively, to treat disease states characterized by abnormal levels of ion channels. The assays may be performed using single putative modulators, and/or may be performed using a known agonist in combination with candidate antagonists (or visa versa).

A. Aequorin Assays

In one assay, cells (*e.g.*, CHO cells) are transiently co-transfected with both an ion channel expression construct and a construct that encodes the photoprotein apoaequorin. In the presence of the cofactor coelenterazine, apoaequorin will emit a measurable luminescence that is proportional to the amount of intracellular (cytoplasmic) free calcium. (See generally, Cobbold, *et al.* "Aequorin measurements of cytoplasmic free calcium," *In*: McCormack J.G. and Cobbold P.H., eds., *Cellular Calcium: A Practical Approach*, Oxford:IRL Press (1991); Stables *et al.*, Analytical Biochemistry 252: 115-26 (1997); and Haugland, Handbook of Fluorescent Probes and Research Chemicals. Sixth edition. Eugene OR: Molecular Probes (1996).), each of which is incorporated by reference in its entirety.

In one exemplary assay, ion channel nucleic acid is subcloned into the commercial expression vector pzeoSV2 (Invitrogen) and transiently co-transfected along with a construct that encodes the photoprotein apoaquorin (Molecular Probes, Eugene, OR) into CHO cells using the transfection reagent FuGENE 6 (Boehringer-Mannheim) and the transfection protocol provided in the product insert.

The cells are cultured for 24 hours at 37°C in MEM (Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin and 10 μg/ml streptomycin, at which time the medium is changed to serum-free MEM containing 5 μM coelenterazine (Molecular Probes, Eugene, OR). Culturing is then continued for two additional hours at 37°C. Subsequently, cells are detached from the plate using VERSENE (Gibco/BRL), washed, and resuspended at 200,000 cells/ml in serum-free MEM.

Dilutions of candidate ion channel modulator compounds are prepared in serum-free MEM and dispensed into wells of an opaque 96-well assay plate at 50 μl/well. Plates are then loaded onto an MLX microtiter plate luminometer (Dynex Technologies, Inc., Chantilly, VA). The instrument is programmed to dispense 50μl cell suspensions into each well, one well at a time, and immediately read luminescence for 15 seconds. Dose-response curves for the candidate modulators are constructed using the area under the curve for each light signal peak. Data are analyzed with SlideWrite, using the equation for a one-site ligand, and EC_{50} values are obtained. Changes in luminescence caused by the compounds are considered indicative of modulatory activity.

B. Intracellular calcium measurement using FLIPR

Changes in intracellular calcium levels are another recognized indicator of ion channel activity, and such assays can be employed to screen for modulators of ion channel activity. For example, CHO cells stably transfected with an ion channel expression vector are plated at a density of 4×10^4 cells/well in Packard black-walled, 96-well plates specially designed to discriminate fluorescence signals emanating from the various wells on the plate. The cells are incubated for 60 minutes at 37°C in modified Dulbecco's PBS (D-PBS) containing 36 mg/L pyruvate and 1 g/L glucose with the addition of 1% fetal bovine serum and one of four calcium indicator dyes (Fluo-3™ AM, Fluo-4™ AM, Calcium Green™-1 AM, or Oregon Green™ 488 BAPTA-1 AM), each at a concentration of 4 μM. Plates are washed once with modified D-PBS without 1% fetal bovine serum and incubated for 10 minutes at 37°C to remove residual dye from the cellular membrane.

In addition, a series of washes with modified D-PBS without 1% fetal bovine serum is performed immediately prior to activation of the calcium response.

A calcium response is initiated by the addition of one or more candidate receptor agonist compounds, calcium ionophore A23187 (10μM; positive control), or ATP (4μM; positive control). Fluorescence is measured by Molecular Device's FLIPR with an argon laser (excitation at 488nm). (See, *e.g.*, Kuntzweiler *et al.*, Drug Development Research, 44(1):14-20 (1998)). The F-stop for the detector camera was set at 2.5 and the length of exposure was 0.4 milliseconds. Basal fluorescence of cells was measured for 20 seconds prior to addition of candidate agonist, ATP, or A23187, and the basal fluorescence level was subtracted from the response signal. The calcium signal is measured for approximately 200 seconds, taking readings every two seconds. Calcium ionophore A23187 and ATP increase the calcium signal 200% above baseline levels.

C. Extracellular Acidification Rate

In yet another assay, the effects of candidate modulators of ion channel activity are assayed by monitoring extracellular changes in pH induced by the test compounds. (*See, e.g.*, Dunlop *et al.*, Journal of Pharmacological and Toxicological Methods 40(1):47-55 (1998).) In one embodiment, CHO cells transfected with an ion channel expression vector are seeded into 12 mm capsule cups (Molecular Devices Corp.) at 4×10^5 cells/cup in MEM supplemented with 10% fetal bovine serum, 2mM L-glutamine, 10 U/ml penicillin, and 10 μg/ml streptomycin. The cells are incubated in this medium at 37°C in 5% CO_2 for 24 hours.

Extracellular acidification rates are measured using a Cytosensor microphysiometer (Molecular Devices Corp.). The capsule cups are loaded into the sensor chambers of the microphysiometer and the chambers are perfused with running buffer (bicarbonate-free MEM supplemented with 4mM L-glutamine, 10 units/ml penicillin, 10 μg/ml streptomycin, 26mM NaCl) at a flow rate of 100μl/minute. Candidate agonists or other agents are diluted into the running buffer and perfused through a second fluid path. During each 60-second pump cycle, the pump is run for 38 seconds and is off for the remaining 22 seconds. The pH of the running buffer in the sensor chamber is recorded during the cycle from 43-58 seconds, and the pump is re-started at 60 seconds to start the next cycle. The rate of acidification of the running buffer during the recording time is calculated by the Cytosoft program. Changes in the rate of acidification are calculated by subtracting the baseline value (the average of 4 rate measurements immediately before

addition of a modulator candidate) from the highest rate measurement obtained after addition of a modulator candidate. The selected instrument detects 61mV/pH unit. Modulators that act as agonists of the ion channel result in an increase in the rate of extracellular acidification compared to the rate in the absence of agonist. This response is blocked by modulators which act as antagonists of the ion channel.

Example 10: High throughput screening for modulators of ion channels using FLIPR

One method to identify compounds that modulate the activity of an ion channel polypeptide is through the use of the FLIPR Fluorometric Imaging Plate Reader system. This system was developed by Dr. Vince Groppi of the Pharmacia Corporation to perform cell-based, high-throughput screening (HTS) assays measuring, for example, membrane potential. Changes in plasma membrane potential correlate with the modulation of ion channels as ions move into or out of the cell. The FLIPR system measures such changes in membrane potential. This is accomplished by loading cells expressing an ion channel gene with a cell-membrane permeant fluorescent indicator dye suitable for measuring changes in membrane potential such as diBAC (bis-(1,3-dibutylbarbituric acid) pentamethine oxonol, Molecular Probes). Thus the modulation of ion channel activity is assessed with FLIPR and detected as changes in the emission spectrum of the diBAC dye.

As an example, COS cells that have been transfected with an ion channel gene of interest are bathed in diBAC. Due to the presence of both endogenous potassium channels in the cells as well as the transfected channel, the addition of 30mM extracellular potassium causes membrane depolarization which results in an increase in diBAC uptake by the cell, and thus an overall increase in fluorescence. When cells are treated with a potassium channel opener, such as chromakalim, the membrane is hyperpolarized, causing a net outflow of diBAC, and thus a reduction in fluorescence. In this manner the effect of unknown test compounds on membrane potential can be assessed using this assay.

Example 11: Tissue Expression Profiling

Tissue specific expression of the cDNAs encoding ions 1-5 and 7 was detected using a PCR-based system. BLAST results containing the protein sequence alignments obtained from searches of the Celera genomic DNA databases were used to estimate where intron/exon boundaries existed. Oligonucleotide primer pairs were designed based on this information to amplify 60 to 800 bp fragments of the predicted coding sequences. Primers

were synthesized by Sigma-Genosys, resuspended in water and the concentration determined by absorbence at 260nm and the concentration adjusted to 25 or 50 μM with 10mM TrisHCl pH 8.0.

Primer pairs were tested by PCR using genomic DNA as a template in a 100 μL reaction mixture containing: 0.5μM each forward and reverse primer, 1x PCR buffer II (Perkin-Elmer), 1.5mM $MgCl_2$ (Perkin-Elmer), 0.2mM each dNTP (Gibco-BRL), 0.5μg human genomic DNA (Clontech) and 5 units AmpliTaq Gold (Perkin-Elmer) with the following thermocycling conditions in a Perkin-Elmer 9600 thermocycler: one cycle of 95°C for 10 minutes; 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute; one cycle of 72°C for 10 minutes followed by a 4°C soak. Products were analyzed on 2% agarose gels containing 0.5μg/mL ethidium bromide run in Tris-Acetate EDTA running buffer (Gibco-BRL). Several randomly selected PCR products were purified using a Qiagen PCR Clean-up Kit and sequenced to confirm amplification of the desired target.

Primer pairs that passed the design and testing phase were used to amplify predicted exon sequences from cDNAs using PCR from human tissue cDNA panels obtained from OriGene (Rockville, MD). Expression profiling PCR reactions were set-up as described above except that two concentrations of cDNA were used (1.0 or 0.1μL of cDNA) in place of genomic DNA. The amplification conditions used were as follows: one cycle of 95°C for 10 minutes, 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, one cycle of 72°C for 10 minutes and completed with a 4°C soak. PCR products were analyzed on 2% TAE agarose gels containing 0.5μg/mL ethidium bromide.

Ion-1

The forward primer used was to detect expression of ion-1 was:

5' CAGTTCAGCCACGCGATGGA 3'(SEQ ID NO:33), and, the reverse primer was:

5' GTTCCAGAGGCATATGACGGT 3'(SEQ ID NO:34). This primer set will prime the synthesis of a 90 base pair fragment in the presence of the appropriate cDNA.

Ion-1 mRNA was detected in brain, kidney, colon, small intestine, stomach, testis, placenta, adrenal gland, peripheral blood leukocytes, bone marrow, and retina. This indicates that compounds modulating the activity of ion-1 may be useful in the treatment of diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, anxiety, migraine, epilepsy, obesity, bipolar and other mood disorders, inflammatory bowel disease, diarrhea or constipation, asthma, arthritis, leukemias and lymphomas, neurodegeneration, or retinal degeneration.

Ion-2a

The forward primer used was to detect expression of ion-2a was:

5' GGATCCACTCTGATTCCAATGAA 3'(SEQ ID NO:35), and, the reverse primer was:

5' GATAGCCAACCCAATAAACCAAGT 3'(SEQ ID NO:36). This primer set will prime the synthesis of a 246 base pair fragment in the presence of the appropriate cDNA.

Ion-2a mRNA was detected in brain, testis, ovary, fetal brain, and retina. This highly specific pattern of expression indicates that compounds modulating the activity of ion-2a may be useful in the treatment of diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, anxiety, migraine, epilepsy, obesity, bipolar and other mood disorders, neurodegeneration, or retinal degeneration, spermatogenesis, oogenesis, and other fertility disorders.

Ion-3

The forward primer used was to detect expression of ion-3 was:

5' GCGTGCTCATCTCGCTGCTT 3'(SEQ ID NO: 37), and, the reverse primer was:

5' TCACCGATGAGCGGCACGCT 3'(SEQ ID NO: 38). This primer set will prime the synthesis of a 160 base pair fragment in the presence of the appropriate cDNA.

Ion-3 mRNA was detected in brain, heart, spleen, liver, kidney, small intestine, lung, muscle, thyroid gland, adrenal gland, ovary, uterus, prostate, skin, fetal brain, fetal liver, stomach, testis, placenta, adrenal gland, peripheral blood leukocytes, bone marrow, and retina. This indicates that compounds modulating the activity of ion-3 may be useful in the treatment of diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, anxiety, migraine, epilepsy, obesity, bipolar and other mood disorders, cardiomyopathies, arrhythmias, hyper- or hypo-thyroidism, deficits in uterine

contractility, hyperprostatism, inflammatory bowel disease, diarrhea or constipation, asthma, arthritis, leukemias and lymphomas, neurodegeneration, or retinal degeneration.

Ion-4a

The forward primer used was to detect expression of ion-4a was:

5' GCCTACAATGAGGATGACCTA 3'(SEQ ID NO:39), and, the reverse primer was:

5' CAGTAGATGTCCAATAAATGCTGA 3'(SEQ ID NO:40). This primer set will prime the synthesis of a 189 base pair fragment in the presence of the appropriate cDNA.

Ion4a mRNA was detected in peripheral blood leukocytes and retina. This pattern of expression indicates that compounds modulating the activity of ion-4a may be useful in the treatment of diseases including inflammatory bowel disease, asthma, arthritis, leukemias and lymphomas, or retinal degeneration.

Ion-5

The forward primer used was to detect expression of ion-5 was:

5' CATCATGGTTCCCTGCGTGCT 3'(SEQ ID NO:41), and, the reverse primer was:

5' GTCCTGCCCTCTCATGTTCTT 3'(SEQ ID NO:42). This primer set will prime the synthesis of a 152 base pair fragment in the presence of the appropriate cDNA.

Ion-5 mRNA was detected in testis, ovary, peripheral blood leukocytes, bone marrow, fetal brain, and retina. This indicates that compounds modulating the activity of ion-5 may be useful in the treatment of diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, anxiety, migraine, epilepsy, obesity, bipolar and other mood disorders, inflammatory bowel disease, asthma, arthritis, leukemias and lymphomas, neurodegeneration, or retinal degeneration, spermatogenesis, oogenesis, and other fertility disorders.

Ion-6

The following ion-6 primer pair did not amplify the expected product from human tissue cDNA panels obtained from OriGene.

ion6.for AGGAGGGAAAACATAATTTGGGGGA (SEQ ID NO:43)
ion6.rev AGGGAGGAATGTGTCAAACAAA = (SEQ ID NO:44)

Ion-7

The forward primer used was to detect expression of ion-7 was:

5' CCTACACAGGGTCAAGATCAT 3'(SEQ ID NO:45), and, the reverse primer was:

5' AGGAGGATTCCAGAAGAAGGCAT 3'(SEQ ID NO:46). This primer set will prime the synthesis of a 132 base pair fragment in the presence of the appropriate cDNA.

Ion-7 mRNA was detected in brain, heart, spleen, liver, kidney, small intestine, lung, muscle, thyroid gland, adrenal gland, ovary, uterus, prostate, skin, fetal brain, fetal liver, stomach, testis, placenta, colon, salivary gland, pancreas, adrenal gland, peripheral blood leukocytes, bone marrow, and retina. This indicates that compounds modulating the activity of ion-7 may be useful in the treatment of diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, anxiety, migraine, epilepsy, obesity, bipolar and other mood disorders, cardiomyopathies, arrhythmias, hyper- or hypo-thyroidism, deficits in uterine contractility, hyperprostatism, inflammatory bowel disease, diarrhea or constipation, diabetes, asthma, arthritis, leukemias and lymphomas, neurodegeneration, or retinal degeneration.

Example 12: Chimeric Receptors

A chimeric receptor can be used to measure the activity of ligand binding when the ligand's native receptor activity is not amenable to easy measurement. Such chimera may consist of a ligand-binding domain of one receptor fused to the pore-forming domain of another receptor. A useful example of such a chimera can be found in WO 00/73431 A2.

The pore-forming transmembrane domain of ion1 (SEQ ID NO:49) can be fused with the extracellular domain of the alpha7 nicotinic acetylcholine receptor to form a chimeric receptor that binds alpha7 receptor ligands but passes current like that of ion1. To generate this chimera, PCR primers are designed to amplify the 5' region of the alpha7 receptor (GenBank accession number U62436) with a region of overlap with ion1 on the 3'-most primer. Respectively, the primers are:

GGGAATTCGGGACTCAACATGCGCTGC (SEQ ID NO:52) and:

CATAGAGGCTGGGCCTGCGGCGCATGGTCACTGTGAAGG (SEQ ID NO:53). Likewise, PCR primers are designed to amplify the 3' region of ion1 (SEQ ID NO:49) with a region of overlap with alpha7 on the 5'-most primer:

CCTTCACAGTGACCATGCGCCGCAGGCCCAGCCTCTATG (SEQ ID NO:54), and: GGGCGGCCGCCCTAGGTGTTCCAGAGGCA (SEQ ID NO:55). PCR

is performed using the appropriate cDNA clone (U62436 or ion1) as a template using Platinum Taq polymerase (Life Technologies, Gaithersburg, MD) according to the manufacturer's instructions. The PCR products from these two reactions are then diluted 1:1000 and pooled in a second PCR mixture with primers:

CCTTCACAGTGACCATGCGCCGCAGGCCCAGCCTCTATG (SEQ ID NO:56), and: CATAGAGGCTGGGCCTGCGGCGCATGGTCACTGTGAAGG (SEQ ID NO:57) to generate the final chimeric cDNA by splice-overlap PCR. These primers also add an EcoRI restriction site to the 5' end and a NotI site to the 3' end to facilitate subcloning into pcDNA3.1 (Invitrogen). The PCR product is ligated into pcDNA3.1 and transformed into competent *E. coli* (Life Technologies, Gaithersburg, MD). Isolated *E. coli* colonies selected on ampicillin-containing medium are isolated and expanded. The DNA from the plasmid in *E. coli* is isolated and sequenced to verify that the expected sequences are obtained. The DNA is then transformed into mammalian cells such as SH-EP1 cells using cationic lipid transfection reagent. Cells that are stably transformed are selected in the presence of 800 micrograms/ml geneticin. These cells are then assayed as described *supra* for changes in intracellular calcium or changes in membrane potential in response to ligands, *e.g.* nicotine.

As those skilled in the art will appreciate, numerous changes and modifications may be made to the preferred embodiments of the invention without departing from the spirit of the invention. It is intended that all such variations fall within the scope of the invention. The entire disclosure of each publication cited herein is hereby incorporated by reference.

What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence that is at least 90% homologous to a sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID N0: 32, and SEQ ID NO:50; said nucleic acid molecule encoding at least a portion of ion-x.

2. The isolated nucleic acid molecule of claim 1 comprising a sequence that encodes a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO: 32, and SEQ ID NO: 50.

3. The isolated nucleic acid molecule of claim 1 comprising a sequence at least 90% homologous to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, SEQ ID NO: 49 and SEQ ID NO: 51.

4. The isolated nucleic acid molecule of claim 1 comprising a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, SEQ ID NO: 49 and SEQ ID NO: 51.

5. The isolated nucleic acid molecule of claim 4 comprising a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

6. The isolated nucleic acid molecule of claim 4 wherein said nucleotide sequence is selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

7. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule is DNA.

8. The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule is RNA.

9. An expression vector comprising a nucleic acid molecule of any one of claims 1 to 5.

10. The expression vector of claim 9 wherein said nucleic acid molecule comprises a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

11. The expression vector of claim 9 wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

12. The expression vector of claim 9 wherein said vector is a plasmid.

13. The expression vector of claim 9 wherein said vector is a viral particle.

14. The expression vector of claim 13 wherein said vector is selected from the group consisting of adenoviruses, baculoviruses, parvoviruses, herpesviruses, poxviruses, adeno-associated viruses, Semliki Forest viruses, vaccinia viruses, and retroviruses.

15. The expression vector of claim 9 wherein said nucleic acid molecule is operably connected to a promoter selected from the group consisting of simian virus 40, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney virus, cytomegalovirus immediate early promoter, Epstein Barr virus, rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine, and human metalothionein.

16. A host cell transformed with an expression vector of claim 10.

17. The transformed host cell of claim 16 wherein said cell is a bacterial cell.

18. The transformed host cell of claim 17 wherein said bacterial cell is E. coli.

19. The transformed host cell of claim 16 wherein said cell is yeast.

20. The transformed host cell of claim 19 wherein said yeast is S. cerevisiae.

21. The transformed host cell of claim 16 wherein said cell is an insect cell.

22. The transformed host cell of claim 21 wherein said insect cell is S. frugiperda.

23. The transformed host cell of claim 16 wherein said cell is a mammalian cell.

24. The transformed host cell of claim 23 wherein mammalian cell is selected from the group consisting of chinese hamster ovary cells, HeLa cells, African green monkey kidney cells, human 293 cells, and murine 3T3 fibroblasts.

25. An isolated nucleic acid molecule comprising a nucleotide sequence complementary to at least a portion of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, said portion comprising at least 10 nucleotides.

26. The nucleic acid molecule of claim 25 wherein said molecule is an antisense oligonucleotide directed to a region of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

27. The nucleic acid molecule of claim 26 wherein said oligonucleotide is directed to a regulatory region of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

28. The nucleic acid molecule of claim 25 wherein said molecule is an antisense oligonucleotide directed to a region of nucleotide sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51.

29. A composition comprising a nucleic acid molecule of any one of claims 1 to 5 or 25 and an acceptable carrier or diluent.

30. A composition comprising a recombinant expression vector of claim 9 and an acceptable carrier or diluent.

31. A method of producing a polypeptide that comprises a sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 32, and SEQ ID NO: 50, said method comprising the steps of:
a) introducing a recombinant expression vector of claim 9 into a compatible host cell;
b) growing said host cell under conditions for expression of said polypeptide; and
c) recovering said polypeptide.

32. The method of claim 31 wherein said host cell is lysed and said polypeptide is recovered from the lysate of said host cell.

33. The method of claim 31 wherein said polypeptide is recovered by purifying the culture medium without lysing said host cell.

34. An isolated polypeptide encoded by a nucleic acid molecule of claim 1.

35. The polypeptide of claim 34 wherein said polypeptide comprises a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50.

36. The polypeptide of claim 34 wherein said polypeptide comprises an amino acid sequence at least 90% homologous to a sequence selected from the group consisting of to SEQ ID NO: 32, and SEQ ID NO: 50.

37. The polypeptide of claim 34 wherein said sequence at least 90% homologous to a sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 32, and SEQ ID NO: 50, comprises at least one conservative amino acid substitution compared to the sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID N0: 32, and SEQ ID NO: 50.

38. The polypeptide of claim 34 wherein said polypeptide comprises a fragment of a polypeptide with a sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50.

39. The polypeptide of claim 34 wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NOS:10-17, 22-28, 31, 32, and SEQ ID NO:50.

40. A composition comprising a polypeptide of claim 34 and an acceptable carrier or diluent.

41. An isolated antibody which binds to an epitope on a polypeptide of claim 34.

42. The antibody of claim 41 wherein said antibody is a monoclonal antibody.

43. A composition comprising an antibody of claim 41 and an acceptable carrier or diluent.

44. A method of inducing an immune response in a mammal against a polypeptide of claim 34 comprising administering to said mammal an amount of said polypeptide sufficient to induce said immune response.

45. A method for identifying a compound which binds ion-x comprising the steps of:

a) contacting ion-x with a compound; and

b) determining whether said compound binds ion-x.

46. The method of claim 45 wherein the ion-x comprises an amino acid sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:32, and SEQ ID NO:50.

47. The method of claim 45 wherein binding of said compound to ion-x is determined by a protein binding assay.

48. The method of claim 45 wherein said protein binding assay is selected from the group consisting of a gel-shift assay, Western blot, radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis, and ELISA.

49. A compound identified by the method of claim 45.

50. A method for identifying a compound which binds a nucleic acid molecule encoding ion-x comprising the steps of:

a) contacting said nucleic acid molecule encoding ion-x with a compound; and

b) determining whether said compound binds said nucleic acid molecule.

51. The method of claim 50 wherein binding is determined by a gel-shift assay.

52. An antibody identified by the method of claim 50.

53. A method for identifying a compound which modulates the activity of ion-x comprising the steps of:

a) contacting ion-x with a compound; and

b) determining whether ion-x activity has been modulated.

54. The method of claim 53 wherein the ion-x comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:10 to SEQ ID NO:17, SEQ ID NO:22 to SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:50.

55. The method of claim 53 wherein said activity is neuropeptide binding.

56. The method of claim 53 wherein said activity is neuropeptide signaling.

57. An antibody identified by the method of claim 53.

58. A method of identifying an animal homolog of ion-x comprising the steps:
a) comparing the nucleic acid sequences of the animal with a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID N0: 9, SEQ ID N0: 49, and SEQ ID NO: 51, and
b) identifying nucleic acid sequences of the animal that are at least 90% homologous to said sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, SEQ ID N0: 49, and SEQ ID NO: 51.

59. The method of claim 58 wherein comparing the nucleic acid sequences of the animal with a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, SEQ ID NO: 49, and SEQ ID NO: 51, is performed by DNA hybridization.

60. The method of claim 58 wherein comparing the nucleic acid sequences of the animal with a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9, SEQ ID N0: 49, and SEQ ID NO: 51, is performed by computer homology search.

61. A method of screening a human subject to diagnose a disorder affecting the brain or genetic predisposition therefor, comprising the steps of:
(a) assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression, or biological activity of at least one ion channel that is expressed in the brain, wherein the ion channel comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID N0: 17, SEQ ID NO: 22 to SEQ ID NO: 28, SEQ ID N0: 31, SEQ ID NO: 32, and SEQ ID NO: 50, and wherein the nucleic acid corresponds to a gene encoding the ion channel; and
(b) diagnosing the disorder or predisposition from the presence or absence of said mutation, wherein the presence of a mutation altering the amino acid sequence,

expression, or biological activity of the ion channel correlates with an increased risk of developing the disorder.

62. A method according to claim 61, wherein the assaying step comprises at least one procedure selected from the group consisting of:

a) comparing nucleotide sequences from the human subject and reference sequences and determining a difference of either

at least a nucleotide of at least one codon between the nucleotide sequences from the human subject that encodes an ion-1 allele and an ion-1 reference sequence, or

at least a nucleotide of at least one codon between the nucleotide sequences from the human subject that encodes an ion-3 allele and an ion-3 reference sequence;

(b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences;

(c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and

(d) performing a restriction endonuclease digestion to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences.

63. A method according to claim 62 wherein the assaying step comprises: performing a polymerase chain reaction assay to amplify nucleic acid comprising ion-1 or ion-3 coding sequence, and determining nucleotide sequence of the amplified nucleic acid.

64. A method of screening for an ion-1 or ion-3 mental disorder genotype in a human patient, comprising the steps of:

(a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including sequences corresponding to allelles of ion-1 or ion-3; and

(b) detecting the presence of one or more mutations in the ion-1 allelle or the ion-3 allelle;

wherein the presence of a mutation in an ion-1 allelle or ion-3 allele is indicative of a mental disorder genotype.

65. The method according to claim 64 wherein said biological sample is a cell sample.

66. The method according to claim 64 wherein said detecting the presence of a mutation comprises sequencing at least a portion of said nucleic acid, said portion comprising at least one codon of said ion-1 or ion-3 alleles.

67. The method according to claim 64 wherein said nucleic acid is DNA.

68. The method according to claim 64 wherein said nucleic acid is RNA.

69. A kit for screening a human subject to diagnose a mental disorder or a genetic predisposition therefor, comprising, in association:

(a) an oligonucleotide useful as a probe for identifying polymorphisms in a human ion-1 gene or a human ion-3 gene, the oligonucleotide comprising 6-50 nucleotides in a sequence that is identical or complementary to a sequence of a wild type human ion-1 or ion-3 gene sequence or ion-1 or ion-3 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution; and

(b) a media packaged with the oligonucleotide, said media containing information for identifying polymorphisms that correlate with a mental disorder or a genetic predisposition therefor, the polymorphisms being identifiable using the oligonucleotide as a probe.

70. A method of identifying an ion channel allelic variant that correlates with a mental disorder, comprising steps of:

(a) providing a biological sample comprising nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny;

(b) detecting in the nucleic acid the presence of one or more mutations in an ion channel that is expressed in the brain, wherein the ion channel comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10 to SEQ ID NO: 17, SEQ ID NO: 22 to SEQ ID NO: 28, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 50, and wherein the nucleic acid includes sequence corresponding to the gene or genes encoding the ion channel;
wherein the one or more mutations detected indicates an allelic variant that correlates with a mental disorder.

71. A method according to claim 70, wherein the one or more ion channel is ion-1, ion-3.

72. A purified and isolated polynucleotide comprising a nucleotide sequence encoding an ion-1 or ion-3 allelic variant identified according to claim 70.

73. A host cell transformed or transfected with a polynucleotide according to claim 72 or with a vector comprising the polynucleotide.

74. A purified polynucleotide comprising a nucleotide sequence encoding ion-1 of a human with a mental disorder;
wherein said polynucleotide hybridizes to the complement of SEQ ID NO: 49 under the following hybridization conditions:
(a) hybridization for 16 hours at 42°C in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate and
(b) washing 2 times for 30 minutes at 60°C in a wash solution comprising 0.1x SSC and 1% SDS; and wherein the polynucleotide that encodes ion-1 amino acid sequence of the human differs from SEQ ID NO:49 by at least one nucleotide.

75. A vector comprising a polynucleotide according to claim 74.

76. A host cell that has been transformed or transfected with a polynucleotide according to claim 74 and that expresses the ion-1 protein encoded by the polynucleotide.

77. A host cell according to claim 76 that has been co-transfected with a polynucleotide encoding the ion-1 amino acid sequence set forth in SEQ ID NO:50 and that expresses the ion-1 protein having the amino acid sequence set forth in SEQ ID NO:50.

78. A method for identifying a modulator of biological activity of ion-1 or ion-3 comprising the steps of:

a) contacting a cell according to claim 76 in the presence and in the absence of a putative modulator compound;

b) measuring ion-1 or ion-3 biological activity in the cell;

wherein decreased or increased ion-1 or ion-3 biological activity in the presence versus absence of the putative modulator is indicative of a modulator of biological activity.

79. A method to identify compounds useful for the treatment of a mental disorder, said method comprising steps of:

(a) contacting a composition comprising ion-1 with a compound suspected of binding ion-1 or contacting a composition comprising ion-3 with a compound suspected of binding ion-3;

(b) detecting binding between ion-1 and the compound suspected of binding ion-1 or between ion-3 and the compound suspected of binding ion-3;

wherein compounds identified as binding ion-1 or ion-3 are candidate compounds useful for the treatment of a mental disorder.

80. A method for identifying a compound useful as a modulator of binding between ion-1 and a binding partner of ion-1 or between ion-3 and a binding partner of ion-3 comprising the steps of:

(a) contacting the binding partner and a composition comprising ion-1 or ion-3 in the presence and in the absence of a putative modulator compound;

(b) detecting binding between the binding partner and ion-1 or ion-3;

wherein decreased or increased binding between the binding partner and ion-1 or ion-3 in the presence of the putative modulator, as compared to binding in the

absence of the putative modulator is indicative a modulator compound useful for the treatment of a mental disorder.

81. A method according to claim 79 or 80 wherein the composition comprises a cell expressing ion-1 or ion-3 on its surface.

82. A method according to claim 81 wherein the composition comprises a cell transformed or transfected with a polynucleotide that encodes ion-1 or ion-3.

83. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence at least 90% homologous to SEQ ID NO: 50, said nucleic acid molecule encoding ion-1.

84. An isolated polypeptide encoded by a nucleic acid molecule of claim 83.

85. A chimeric receptor comprising at least a portion of a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:9, SEQ ID NO:49, and SEQ ID NO:51, said portion comprising at least 10 nucleotides.

86. The chimeric receptor of claim 85 wherein the chimeric receptor comprises at least a portion of SEQ ID NO:49.

SEQUENCE LISTING

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Roberds, Steven L.
Benjamin, Chris
Ruble, Cara L.
Karnovsky, Alla M.

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Leu Leu Phe Xaa Cys Gln Lys Phe Ile Tyr Tyr Cys Ala Gln Met Thr
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Asn Ser Val Ala Val Gln Glu Asn Xaa Ser Leu Cys Phe Lys Trp Phe
65 70 75 80

Xaa Asn Asp Tyr Asn Ala Phe Leu Ser Ile Leu Thr Ile Leu Leu Ser
85 90 95

Xaa Gly Ser Pro Val Pro Val Gly Ile Asp Val His Val Glu Ser Ile
100 105 110

Asp Ser Ile Ser Glu Thr Asn Met Val Ser Phe Phe Met Gly Tyr Cys
115 120 125

Ser Phe Ser Glu Lys Thr Glu Thr Arg Gln Cys Gln Asn His Xaa Cys
130 135 140

Phe Asn Lys Ser Phe Xaa Leu Cys Met Leu Phe Met Ser Pro Thr Leu
145 150 155 160

Ile Asn His Arg Val Met Ile Ala Val Leu Ser Ser Ile Leu Phe Ile
165 170 175

Leu Thr Leu Ile Arg Ile Leu Ile Pro Phe Leu Leu Phe Tyr Phe Pro
180 185 190

Ser Asn Gly Phe Leu Val Gly Asn Thr Val Glu His His Asp Asn Tyr
195 200 205

<210> 21
<211> 45
<212> PRT
<213> Homo sapiens

<400> 21

Gly Ser Pro Val Pro Val Gly Ile Asp Val His Val Glu Ser Ile Asp
1 5 10 15

Ser Ile Ser Glu Thr Asn Met Val Ser Phe Phe Met Gly Tyr Cys Ser

20 25 30

Phe Ser Glu Lys Thr Glu Thr Arg Gln Cys Gln Asn His
35 40 45

<210> 22
<211> 207
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 22

Phe Pro Ser Cys Xaa Pro Leu Asp Leu Gly Pro Asp Leu His Thr Pro
1 5 10 15

Arg Asn Gly His Thr Pro Phe Leu Leu Xaa Ser Ser Gln Asn Thr Asp
20 25 30

Trp Ser Ala Ala Gly Lys Asp His Asn Ala Val Val Ala Ala Ala Cys
35 40 45

Phe Phe Lys Ile Asn Phe Pro Trp Arg Trp Val Glu Ser Xaa Ser Cys
50 55 60

Ser Arg Cys Ala Leu Arg Leu Asp Thr Gln Arg Val Gly Glu Ile Gly
65 70 75 80

His Ser Val Gln Val Gly His Asp Glu Ile Arg Leu Glu Pro Arg Leu
85 90 95

Lys Ser Thr Arg Ala Thr Gln Lys Gln Met Pro Ser Leu Pro Gly Glu
100 105 110

Gly Ser Ala Gln Thr Val Leu Lys Gly Ala Ala Ala Gly Ala Ser Gly
115 120 125

Thr Gly Asp Ser Gly Gly Ala Ser Ala Ala Leu His Pro Pro Leu Ala
130 135 140

Pro Val Gln Pro Arg Arg Trp Arg Pro Gly Leu Ser Cys Pro Ala Leu
145 150 155 160

Ser Cys Ser Trp Pro Arg Ser Ser Arg His Pro His Ser Ser Val Ser
165 170 175

Pro Gly Arg Gln Cys Pro Gly Ser Gly Glu Ala Ala Gly Arg Lys Xaa
180 185 190

Ala Ser Ser Thr Lys Pro Glu Met Ser Thr Gln Gly Thr Met Met
195 200 205

<210> 23
<211> 206
<212> PRT

<213> Homo sapiens

<220>
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<223> Xaa is any nucleotide

<400> 23

Phe Pro Pro Ala Asp Pro Trp Thr Trp Gly Gln Thr Tyr Thr Arg Gln
1 5 10 15

Gly Met Gly Thr His His Ser Ser Cys Glu Val His Lys Ile Gln Ile
20 25 30

Gly Gln Gln Pro Glu Arg Ile Ile Met Leu Trp Trp Gln Gln Pro Ala
35 40 45

Phe Ser Lys Ser Ile Ser Pro Gly Asp Gly Trp Lys Val Glu Val Val
50 55 60

Val Gly Ala Arg Xaa Gly Trp Ile Pro Ser Gly Xaa Gly Arg Ser Asp
65 70 75 80

Thr Arg Phe Lys Xaa Ala Thr Met Arg Xaa Gly Trp Ser Pro Gly Xaa
85 90 95

Arg Ala Pro Glu Arg Pro Arg Ser Arg Cys Arg His Phe Leu Gly Lys
100 105 110

Gly Arg His Lys Gln Ser Leu Lys Gly Gln Leu Gln Glu Pro Val Ala
115 120 125

Arg Glu Thr Val Gly Ala Pro Leu Pro Arg Ser Ile Arg Leu Trp Leu
130 135 140

Leu Ser Asn Leu Ala Asp Gly Val Leu Ala Ser Arg Val Leu Pro Ser
145 150 155 160

His Val Leu Gly His Glu Val His Gly Ile Pro Thr Ala Ala Tyr Leu
165 170 175

Arg Gly Gly Ser Ala Gln Ala Leu Val Arg Gln Pro Ala Gly Ser Arg
180 185 190

Leu Ala Ala Pro Ser Leu Arg Xaa Ala Arg Arg Glu Pro Xaa
195 200 205

<210> 24
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<220>
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<223> Xaa is any nucleotide

<400> 24

Ser Leu Leu Leu Thr Pro Gly Leu Gly Ala Arg Pro Thr His Ala Lys
1 5 10 15

Glu Trp Ala His Thr Ile Pro Leu Val Lys Phe Thr Lys Tyr Arg Leu
20 25 30

Val Ser Ser Arg Lys Gly Ser Xaa Cys Cys Gly Gly Ser Ser Leu Leu
35 40 45

Phe Gln Asn Gln Phe Pro Leu Glu Met Gly Gly Lys Leu Lys Leu Xaa
50 55 60

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

Leu Gly Ser Ser Arg Pro Arg Xaa Asp Lys Val Gly Ala Gln Ala Glu
85 90 95

Glu His Pro Ser Asp Pro Glu Ala Asp Ala Val Thr Ser Trp Gly Arg
100 105 110

Val Gly Thr Asn Ser Pro Xaa Arg Gly Ser Cys Arg Ser Gln Trp His
115 120 125

Gly Arg Gln Trp Gly Arg Leu Cys Arg Ala Pro Ser Ala Ser Gly Ser
130 135 140

Cys Pro Thr Ser Pro Met Ala Ser Trp Pro Leu Val Ser Cys Pro Leu
145 150 155 160

Met Phe Leu Ala Thr Lys Phe Thr Ala Ser Pro Gln Gln Arg Ile Ser
165 170 175

Gly Ala Ala Val Pro Arg Leu Trp Xaa Gly Ser Arg Gln Glu Val Gly
180 185 190

Xaa Gln His Gln Ala Xaa Asp Glu His Ala Gly Asn His Asp
195 200 205

<210> 25
<211> 207
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 25

His His Gly Ser Leu Arg Ala His Leu Arg Leu Gly Ala Ala Ser Leu
1 5 10 15

Leu Pro Ala Gly Cys Leu Thr Arg Ala Trp Ala Leu Pro Pro Arg Arg
20 25 30

Tyr Ala Ala Val Gly Met Pro Xaa Thr Ser Trp Pro Arg Thr Xaa Glu

35 40 45

Gly Arg Thr Arg Glu Ala Arg Thr Pro Ser Ala Arg Leu Asp Arg Ser
50 55 60

Gln Arg Arg Met Glu Arg Gly Arg Gly Ala Pro Thr Val Ser Arg Ala
65 70 75 80

Thr Gly Ser Cys Ser Cys Pro Phe Lys Asp Cys Leu Cys Arg Pro Phe
85 90 95

Pro Arg Lys Xaa Arg His Leu Leu Leu Gly Arg Ser Gly Ala Leu Gln
100 105 110

Pro Gly Leu Gln Pro Tyr Leu Ile Val Ala Tyr Leu Asn Arg Val Ser
115 120 125

Asp Leu Pro Tyr Pro Leu Gly Ile Gln Pro Xaa Arg Ala Pro Thr Thr
130 135 140

Thr Ser Thr Phe His Pro Ser Pro Gly Glu Ile Asp Phe Glu Lys Ala
145 150 155 160

Gly Cys Cys His His Ser Ile Met Ile Leu Ser Gly Cys Xaa Pro Ile
165 170 175

Cys Ile Leu Xaa Thr Ser Gln Glu Glu Trp Cys Val Pro Ile Pro Trp
180 185 190

Arg Val Xaa Val Trp Pro Gln Val Gln Gly Ser Ala Gly Gly Lys
195 200 205

<210> 26
<211> 206
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 26

Ile Met Val Pro Cys Val Leu Ile Ser Gly Leu Val Leu Leu Ala Tyr
1 5 10 15

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

Thr Leu Leu Trp Gly Cys Arg Glu Leu Arg Gly Gln Glu His Glu Arg
35 40 45

Ala Gly His Glu Arg Pro Gly Arg His Arg Arg Gly Trp Thr Gly Ala
50 55 60

Arg Gly Gly Trp Ser Ala Ala Glu Ala Pro Pro Leu Ser Pro Val Pro
65 70 75 80

Leu Ala Pro Ala Ala Ala Pro Leu Arg Thr Val Cys Ala Asp Pro Ser
85 90 95

Pro Gly Ser Asp Gly Ile Cys Phe Trp Val Ala Arg Val Leu Phe Ser
100 105 110

Leu Gly Ser Asn Leu Ile Ser Ser Trp Pro Thr Xaa Thr Glu Cys Pro
115 120 125

Ile Ser Pro Thr Arg Trp Val Ser Ser Leu Ser Ala His Arg Leu Gln
130 135 140

Leu Gln Leu Ser Thr His Leu Gln Gly Lys Leu Ile Leu Lys Lys Gln
145 150 155 160

Ala Ala Ala Thr Thr Ala Leu Xaa Ser Phe Pro Ala Ala Asp Gln Ser
165 170 175

Val Phe Cys Glu Leu His Lys Arg Asn Gly Val Cys Pro Phe Leu Gly
180 185 190

Val Cys Arg Ser Gly Pro Lys Ser Arg Gly Gln Gln Glu Gly
195 200 205

<210> 27
<211> 206
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 27

Ser Trp Phe Pro Ala Cys Ser Ser Gln Ala Trp Cys Cys Xaa Pro Thr
1 5 10 15

Ser Cys Arg Leu Pro His Gln Ser Leu Gly Thr Ala Ala Pro Glu Ile
20 25 30

Arg Cys Cys Gly Asp Ala Val Asn Phe Val Ala Lys Asn Met Arg Gly
35 40 45

Gln Asp Thr Arg Gly Gln Asp Ala Ile Gly Glu Val Gly Gln Glu Pro
50 55 60

Glu Ala Asp Gly Ala Arg Gln Arg Arg Pro His Cys Leu Pro Cys His
65 70 75 80

Trp Leu Leu Gln Leu Pro Leu Xaa Gly Leu Phe Val Pro Thr Leu Pro
85 90 95

Gln Glu Val Thr Ala Ser Ala Ser Gly Ser Leu Gly Cys Ser Ser Ala
100 105 110

Trp Ala Pro Thr Leu Ser His Arg Gly Leu Leu Glu Pro Ser Val Arg
115 120 125

Ser Pro Leu Pro Ala Gly Tyr Pro Ala Leu Ala Arg Thr Asp Tyr Asn
130 135 140

Phe Asn Phe Pro Pro Ile Ser Arg Gly Asn Xaa Phe Xaa Lys Ser Arg
145 150 155 160

Leu Leu Pro Pro Gln His Tyr Asp Pro Phe Arg Leu Leu Thr Asn Leu
165 170 175

Tyr Phe Val Asn Phe Thr Arg Gly Met Val Cys Ala His Ser Leu Ala
180 185 190

Cys Val Gly Leu Ala Pro Ser Pro Gly Val Ser Arg Arg Glu
195 200 205

<210> 28
<211> 90
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 28

Ile Met Val Pro Cys Val Leu Ile Ser Gly Leu Val Leu Leu Ala Tyr
1 5 10 15

Phe Leu Pro Ala Xaa Xaa Gln Ser Leu Gly Thr Ala Ala Pro Glu Ile
20 25 30

Arg Cys Cys Gly Asp Ala Val Asn Phe Val Ala Lys Asn Met Arg Gly
35 40 45

Gln Asp Xaa Xaa Asp Gly Ile Cys Phe Trp Val Ala Arg Val Leu Phe
50 55 60

Ser Leu Gly Ser Asn Leu Ile Xaa Xaa Ala Tyr Leu Asn Arg Val Ser
65 70 75 80

Asp Leu Pro Tyr Pro Leu Gly Ile Gln Pro
85 90

<210> 29
<211> 177
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 29

Ser Trp Thr His Leu Asn Val Ala Xaa Ile Val His Ser Tyr Phe Lys

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Ser Leu Ser Ile Ser Leu Trp Lys Leu Trp Asn Asn Gly Trp Arg Asn
            20                  25                  30

Glu Glu Met Gly Phe Gln Val Glu Arg Trp Ile Ile Gln Thr Val Thr
        35                  40                  45

Cys Arg Arg Glu Asn Ile Ile Trp Gly Thr Phe Ser Ser Thr Asn Asn
    50                  55                  60

Lys Leu Lys Arg Asn Leu Gly Tyr Phe Leu Phe Asp Thr Phe Leu Pro
65                  70                  75                  80

Phe Glu Cys Lys Arg Lys His Val Leu Lys Lys Gln Phe Cys His Asn
                85                  90                  95

Val Ala Trp Thr Tyr Ile Xaa Leu Pro Val Ile Glu Leu Pro Ser Ser
            100                 105                 110

Leu Thr Cys Asn Ile Asp Gly Asp Ala Thr Ala Leu Arg Gln Lys Trp
        115                 120                 125

Leu Pro Val His Gln Ala Pro Arg Thr Ala Cys Leu Ile Glu Cys Arg
    130                 135                 140

Ser Arg Gly Lys Asp Tyr Ile Ser His Ala Pro Leu Leu Xaa Gly Gly
145                 150                 155                 160

Val Met Xaa Trp Ile Pro Ala Asn Gly Val Met Xaa Xaa Ile Pro Ile
                165                 170                 175

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<210> 30
<211> 93
<212> PRT
<213> Homo sapiens

<400> 30

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Ile Val His Ser Tyr Phe Lys Ser Leu Ser Ile Ser Leu Trp Lys Leu
1               5                   10                  15

Trp Asn Asn Gly Trp Arg Asn Glu Glu Met Gly Phe Gln Val Glu Arg
            20                  25                  30

Trp Ile Ile Gln Thr Val Thr Cys Arg Arg Glu Asn Ile Ile Trp Gly
        35                  40                  45

Thr Phe Ser Ser Thr Asn Asn Lys Leu Lys Arg Asn Leu Gly Tyr Phe
    50                  55                  60

Leu Phe Asp Thr Phe Leu Pro Phe Glu Cys Lys Arg Lys His Val Leu
65                  70                  75                  80

Lys Lys Gln Phe Cys His Asn Val Ala Trp Thr Tyr Ile
                85                  90
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<211> 221
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> Xaa is any nucleotide

<400> 31

Ser Glu Cys Leu Ala Phe Xaa Leu Phe Cys Asn Ser Ile Gln Leu Lys
1 5 10 15

Thr Gln Thr His Cys Ile Ala Leu Gln Lys Tyr Phe Phe Phe Leu Phe
20 25 30

Ala Phe Ile Leu Xaa Val Leu Val Tyr Phe Xaa Phe Phe Leu Phe Phe
35 40 45

Thr Phe Xaa Val Phe Cys Xaa Lys Xaa Arg His Lys His Thr His Leu
50 55 60

Pro Arg Pro Thr Gln Gly Gln Asp His Lys Tyr His Cys Leu Pro Ser
65 70 75 80

Pro His Leu Val Leu Gln Lys Val Phe Met Val Asn Asn Thr His Gly
85 90 95

Val Val Ile Ser Tyr Asp Asn Ala Phe Phe Trp Asn Pro Pro Glu Glu
100 105 110

Ser Ala Xaa Gly His Phe Ile Asn Ser Phe Phe Xaa Ile Ser Gly Met
115 120 125

Ser Ile Leu Gln Asn Asn Asp Lys Ile Gln Tyr Asn Lys Tyr Ile Asn
130 135 140

His Xaa Lys Leu Val Ser Ile Thr Ile Lys Tyr Tyr Val Leu Tyr Ile
145 150 155 160

Ile Val Leu Leu Tyr Cys Tyr Ala Thr Gly Ser Ala Val Ala Met Phe
165 170 175

Thr Ser Val Ser Leu Gln Thr Asn Glu Xaa Cys Ile Ala Leu Xaa Cys
180 185 190

Asn Asp Asn Ser Tyr Asp Val Thr Gly Trp Gln Glu Phe Phe Ser Ser
195 200 205

Ile Phe Leu Trp Asp His Tyr His Thr Cys Gly Pro Xaa
210 215 220

<210> 32
<211> 57
<212> PRT
<213> Homo sapiens

<400> 32

Arg His Lys His Thr His Leu Pro Arg Pro Thr Gln Gly Gln Asp His
1 5 10 15

Lys Tyr His Cys Leu Pro Ser Pro His Leu Val Leu Gln Lys Val Phe
20 25 30

Met Val Asn Asn Thr His Gly Val Val Ile Ser Tyr Asp Asn Ala Phe
35 40 45

Phe Trp Asn Pro Pro Glu Glu Ser Ala
50 55

<210> 33
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<212> DNA
<213> Homo sapiens

<400> 33
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<210> 34
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<213> Homo sapiens

<400> 34
gttccagagg catatgacgg t 21

<210> 35
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<400> 35
ggatccactc tgattccaat gaa 23

<210> 36
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<212> DNA
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<400> 36
gatagccaac ccaataaacc aagt 24

<210> 37
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<213> Homo sapiens

<400> 37
gcgtgctcat ctcgctgctt 20

<210> 38
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<400> 38
tcaccgatga gcggcacgct 20

<210> 39
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<212> DNA
<213> Homo sapiens

<400> 39
gcctacaatg aggatgacct a 21

<210> 40
<211> 24
<212> DNA
<213> Homo sapiens

<400> 40
cagtagatgt ccaataaatg ctga 24

<210> 41
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catcatggtt ccctgcgtgc t 21

<210> 42
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<400> 42
gtcctgccct ctcatgttct t 21

<210> 43
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<400> 43
aggagggaaa acataatttg gggga 25

<210> 44
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<400> 44
agggaggaat gtgtcaaaca aa 22

<210> 45
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<213> Homo sapiens

<400> 45
cctacacagg gtcaagatca t 21

<210> 46
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<400> 46
aggaggattc cagaagaagg cat 23

<210> 47
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<400> 47
aaaaggcctc acagcatatg 20

<210> 48
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<400> 48
agcgtgcagt tttgctggtc 20

<210> 49
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<400> 49
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cccagaggaa tgtgagggca tcacgaagat gagtatggca gccaagaacc tgtggctccc 180

agacattttc atcattgaac tcatggatgt ggataagacc ccaaaaggcc tcacagcata 240

tgtaagtaat gaaggtcgca tcaggtataa gaaacccatg aaggtggaca gtatctgtaa 300

cctggacatc ttctacttcc ccttcgacca gcagaactgc acactcacct tcagctcatt 360

cctctacaca gtggacagca tgttgctgga catggagaaa gaagtgtggg aaataacaga 420

cgcatcccgg aacatccttc agacccatgg agaatgggag ctcctgggcc tcagcaaggc 480

caccgcaaag ttgtccaggg gaggcaacct gtatgatcag atcgtgttct atgtggccat 540

caggcgcagg cccagcctct atgtcataaa ccttctcgtg cccagtggct ttctggttgc 600

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aacgctcctg ctgggctaca acgtcttcct gctcatgatg agtgacttgc tccccaccag 720

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acctcttctg tccgctgacc tcgactctag attgcggccg c 1481

<210> 50
<211> 393
<212> PRT
<213> Homo sapiens

<400> 50

Arg Pro Arg Ile Arg His Glu Pro Val Thr Asn Ile Ser Val Pro Thr
1 5 10 15

Gln Val Asn Ile Ser Phe Ala Met Ser Ala Ile Leu Asp Val Val Trp
20 25 30

Asp Asn Pro Phe Ile Ser Trp Asn Pro Glu Glu Cys Glu Gly Ile Thr
35 40 45

Lys Met Ser Met Ala Ala Lys Asn Leu Trp Leu Pro Asp Ile Phe Ile
50 55 60

Ile Glu Leu Met Asp Val Asp Lys Thr Pro Lys Gly Leu Thr Ala Tyr

65 70 75 80

Val Ser Asn Glu Gly Arg Ile Arg Tyr Lys Lys Pro Met Lys Val Asp
85 90 95

Ser Ile Cys Asn Leu Asp Ile Phe Tyr Phe Pro Phe Asp Gln Gln Asn
100 105 110

Cys Thr Leu Thr Phe Ser Ser Phe Leu Tyr Thr Val Asp Ser Met Leu
115 120 125

Leu Asp Met Glu Lys Glu Val Trp Glu Ile Thr Asp Ala Ser Arg Asn
130 135 140

Ile Leu Gln Thr His Gly Glu Trp Glu Leu Leu Gly Leu Ser Lys Ala
145 150 155 160

Thr Ala Lys Leu Ser Arg Gly Gly Asn Leu Tyr Asp Gln Ile Val Phe
165 170 175

Tyr Val Ala Ile Arg Arg Arg Pro Ser Leu Tyr Val Ile Asn Leu Leu
180 185 190

Val Pro Ser Gly Phe Leu Val Ala Ile Asp Ala Leu Ser Phe Tyr Leu
195 200 205

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

Gly Tyr Asn Val Phe Leu Leu Met Met Ser Asp Leu Leu Pro Thr Ser
225 230 235 240

Gly Thr Pro Leu Ile Gly Val Tyr Phe Ala Leu Cys Leu Ser Leu Met
245 250 255

Val Gly Ser Leu Leu Glu Thr Ile Phe Ile Thr His Leu Leu His Val
260 265 270

Ala Thr Thr Gln Pro Pro Pro Leu Pro Arg Trp Leu His Ser Leu Leu
275 280 285

Leu His Cys Asn Ser Pro Gly Arg Cys Cys Pro Thr Ala Pro Gln Lys
290 295 300

Glu Asn Lys Gly Pro Gly Leu Thr Pro Thr His Leu Pro Gly Val Lys
305 310 315 320

Glu Pro Glu Val Ser Ala Gly Gln Met Pro Gly Pro Ala Glu Ala Glu
325 330 335

Leu Thr Gly Gly Ser Glu Trp Thr Arg Ala Gln Arg Glu His Glu Ala
340 345 350

Gln Lys Gln His Ser Val Glu Leu Trp Leu Gln Phe Ser His Ala Met
355 360 365

Asp Ala Met Leu Phe Arg Leu Tyr Leu Leu Phe Met Ala Ser Ser Ile
370 375 380

Ile Thr Val Ile Cys Leu Trp Asn Thr
385 390

<210> 51
<211> 22735
<212> DNA
<213> Homo sapiens

<400> 51
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aatactttgt ttcttcagat gccatcatta atgagaacta tgactacctg aaggggttct 180

tggaagacct ggcacctcca gagcgcagca gcctaattca ggattgggaa acatctgggc 240

ttgtttacct ggactatatt agagtcattg aaatgctccg ccatatacag caggtacctg 300

agatcctgaa actgctgcct gattttcctt ttctcaggcc cttaaatctt cagatacctc 360

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cagtctttgt agtcgtaaaa tgagtctcca ctgcttgctt atggtgcaaa aaccaaactc 1080

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ttaagatcta ggttgctgtt acccctactt ctctctgctt ttccccagag ataattaatt 1320

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ctcggcctct actaaatgct taataattgt tatctattat tatcctcatc aaagttccaa 1980

ctcctagtac agtgcctggc acataataga cataattcaa tgtttgctgt acttttagta 2040

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gagtaattca ttcacacaac ctattatgga gaactcactg tatgccaagc actgtagtgg 2160

gtttggggaa tataaaggta aacagtatgt gttctgccct taccaaaata atgattttgt 2220

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