**Title:** GENES ENCODING A FAMILY OF POTASSIUM CHANNELS

**Abstract**

This invention relates generally to the potassium channel gene family. More particularly, the present invention relates to the cloning and characterization of potassium channel genes from *Drosophila melanogaster* and *Caenorhabditis elegans*. Other aspects of the present invention include methods of assaying substances to determine effects on cell growth. Also presented are methods of controlling nematode and insect pests by inhibiting potassium channels substantially homologous to those encoded by nucleotide sequences as described herein.
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GENES ENCODING A FAMILY OF POTASSIUM CHANNELS

Field of Invention

This invention relates generally to the potassium channel gene family. More particularly, the present invention relates to the cloning and characterization of potassium channel genes from Drosophila melanogaster and Caenorhabditis elegans.

Background of the Invention

Synthetic organic insecticides are primarily nerve poisons acting on the cholinergic system (organophosphorus compounds and methylcarbamates), the voltage-gated sodium channel (pyrethroids and DDT), and the GABA-gated chloride channel (cyclodienes and other polychlorocycloalkanes). Potassium channels comprise a large and diverse group of integral membrane proteins that determine the level of excitability and repolarization properties of neurons and muscle fibers [B. Hille, Ionic Channels of Excitable Membranes, Sinauer, Sunderland, MA (1984)].

The multiple essential functions encoded by the potassium channels make them excellent targets for new pesticides and animal and human therapeutics. Potassium channel diversity in the fruitfly Drosophila melanogaster results from an extended gene family coding for homologous proteins. Six genes encoding

Modulation of cardiac action potential by compounds that effect the behavior of potassium channels may be a useful treatment for serious heart conditions. In this regard, each of the potassium channels cloned from insects have corresponding versions in mammalian species, including, specifically, a delayed rectifier potassium channel homolog, RAK, cloned from rat cardiac tissue [M. Paulmichl, P. Nasmith, R. Hellmiss, K. Reed, W.A. Boyle, J.M. Neronne, E.G. Peralta, D.E. Clapham, *Proc. Natl. Acad. Sci USA* 88, 7892-7895 (1991)]. Thus, the RAK channel represents an important target of new drugs for the control of heart failure. The delayed rectifier potassium current in heart cells regulates the duration of the plateau of the cardiac action potential by countering the depolarizing,
inward calcium current. Delayed rectifier potassium currents characteristically are activated upon depolarization from rest, display a sigmoidal or delayed onset, and have a nonlinear, or rectifying, current-voltage relation. Several types of delayed potassium conductances have been identified in cardiac cells based on measured single-channel conductances. Heart rate and contractility are regulated by second messenger modification of delayed rectifier potassium conductances, and species differences in the shape of the plateau may be influenced by the type and level of channel expression.

On the basis of predicted membrane spanning topology, potassium channels may be subdivided into two distinct classes: voltage-gated, calcium-activated, and cyclic nucleotide-gated potassium channels that are composed of six membrane spanning domains (S1-S6) and a single pore forming domain (H5), and inward rectifying potassium channels that pass through the membrane twice and also contain a single pore forming region [Y. Kubo, E. Reuveny, P.A. Slesinger, Y.N. Jan, L.Y. Jan Nature 364, 802-806 (1993); Y. Kubo, T.J. Baldwin, Y.N. Jan, L.Y. Jan Nature 362, 127-133 (1993)]. Here, we report the cloning and functional expression in yeast of a novel Drosophila melanogaster potassium channel. Further, we identify a Caenorhabditis elegans homolog that constitutes the second member of a new family of potassium channels exhibiting a topological configuration unique among the known classes of potassium channels.

The yeast Saccharomyces cerevisiae is utilized as a model eukaryotic organism for the purpose of studying potassium transport mechanisms.
Due to the ease with which one can manipulate the genetic constitution of the yeast *Saccharomyces cerevisiae*, researchers have developed a detailed understanding of many complex biological pathways, including potassium transport. In yeast, high affinity potassium uptake is performed by the product of the *TRK1* gene [R.F. Gaber, C.A. Styles, G.R. Fink *Mol. Cell. Biol.* 8, 2848-2859 (1988)]. Mutant yeast strains lacking *trk1* function are incapable of growing in medium lacking high concentrations of potassium. Since potassium transport mechanisms are present in organisms as divergent as yeast and man, one could predict that expression of heterologous potassium channels in mutant cells might replace *trk1* function, and support growth on medium containing low potassium concentration. In this regard, plant potassium channels were shown to function in yeast and represent important targets for new herbicides [J.A Anderson, S.S. Huprikar, L.V. Kochian, W.J. Lucas, R.F. Gaber, *Proc. Natl. Acad. Sci USA* 89, 3736-3740 (1992); H. Sentenac, N. Bonnau, M. Minet, F. Lacroute, J.-M. Salmon, F. Gaynard, C. Grignon, *Science* 256, 663-665 (1992); D.P. Schachtman and J.I. Schroeder, *Nature* 370, 655-658]. Thus, we have employed this yeast expression system for cloning and expression of potassium channels from heterologous species, making it useful for discovery of new pesticides, and animal and human therapeutics. Discovery of such compounds will necessarily require screening assays of high specificity and throughput. For example, new pesticides directed at potassium channels require high selectivity for insect channels and low activity against non-insect species. Screening assays utilizing yeast strains genetically modified to
accommodate functional expression of heterologous potassium channels offer significant advantages in this area.

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Summary of the Invention

A first aspect of the present invention is the discovery of a new subclass of potassium channel genes and proteins encoded thereby. Potassium channels belonging to this new subclass comprise four hydrophobic domains capable of forming transmembrane helices, wherein a first pore-forming domain is interposed between the first and second transmembrane helices and a second pore-forming domain is interposed between the third and fourth transmembrane helices, and wherein each pore-forming domain contains a potassium selective peptide motif. In preferred embodiments, the peptide motif is selected from the group consisting of a Y/F-G dipeptide motif.

In certain preferred embodiments, the isolation and characterization of invertebrate (i.e. insect and nematode) potassium channel genes belonging to this new subclass is presented. In more preferred embodiments, the present invention provides for the isolation of complementary DNA fragments from Drosophila melanogaster and Caenorhabditis elegans which encode conserved amino acid sequence elements unique to this potassium channel gene family. A yeast expression technology is employed to clone cDNAs from Drosophila melanogaster and C. elegans and a hybridization approach is utilized to isolate additional cDNAs from Caenorhabditis elegans.

A second aspect of the present invention is a method of assaying substances to determine effects
on cell growth. Yeast cells of the kind described above are cultured in appropriate growth medium to cause expression of heterologous proteins, embedded in agar growth medium, and exposed to chemical compounds applied to the surface of the agar plates. Effects on the growth of embedded cells are found around compounds that have effects on the heterologous potassium channel.

A third aspect of the present invention is a method of controlling nematode and insect pests by inhibiting potassium channels substantially homologous to those encoded by nucleotide sequences as presented herein.

Brief Description of the Drawings

FIGURE 1. Growth of CY162 cells bearing pDmORF1. CY162 cells transformed with plasmids isolated from survivors of a primary library screen for plasmids that support the growth of CY162 on medium contain low potassium concentration. Six individual transformants of each plasmid-bearing strain are cultured in patches on the indicated medium. CY162 cells bearing pDmORF1 are found in the upper left-hand corner of each plate while pKAT1 containing cells are found in the lower right hand corner.

FIGURE 2A and 2B. DNA sequence and deduced amino acid sequence of Dm ORF1 [SEQ ID NOS:1 and 2]. The nucleotide sequence of the 2.4 kb cDNA revealed a single long open reading frame proximal to the GAL1 promoter. Segments corresponding to putative transmembrane (M1-M4) and pore-forming H5 domains in the predicted polypeptide are underlined. The single
amino-terminal asparagine linked glycosylation site is indicated by a G.

FIGURE 3A and 3B. DNA sequence and deduced amino acid sequence of the F22b7.7 segment of the *Caenorhabditis elegans* genome [SEQ ID NO: 3]. Segments corresponding to putative transmembrane (M1-M4) and pore-forming H5 domains in the predicted polypeptide are underlined.

FIGURE 4. Alignment of DmORF1 and F22b7.7 sequences. Protein-coding regions of DmORF1 [SEQ ID NO: 37] and F22b7.7 [SEQ ID NO: 38] (designated as CeORF-1 in this FIGURE) are compared using the protein sequence alignment algorithm in Genework DNA sequence analysis software. Identical amino acids are boxed.

FIGURE 5A. Comparison of the pore-forming domains of DmORF1 and F22b7.7. Amino acid sequences from the six cloned *Drosophila melanogaster* potassium channels and three inward rectifier channels [SEQ ID NOS: 7 through 21] are compared to DmORF1 and F22b7.7 within the pore-forming H5 regions. Amino acid identities are indicated by a vertical line and conserved substitutions indicated by a dot. Amino acid substitutions deemed acceptable are indicated.

FIGURE 5B. Hydropathy plot analysis of the DmORF1 and F22b7.7 polypeptide sequence. The Kyte-Doolittle hydropathy algorithm in the Geneworks DNA analysis software is used to predict the topology of DmORF1 and F22b7.7. The position of predicted membrane spanning domains (M1-M4) and pore-forming domains are indicated.
FIGURE 6. Predicted membrane spanning topology of DmORF1.

FIGURE 7. Heterologous potassium channel-dependent growth of plasmid bearing CY162 (trk1Δ) strains. CY162 bearing pYES2, pKAT1, pDmORF1, and pRATRAK are cultured at 30°C for four days on arginine phosphate agar medium containing 0 mM, 0.2 mM, or 100 mM added KCl.

FIGURE 8. Inhibition of growth of yeast cells containing heterologous potassium channels. CY162 cells (10^5) bearing the indicated plasmids are plated in arginine phosphate agar medium containing 0.2 mM potassium chloride. Sterile filter disks were placed on the surface of the agar and saturated with 20 ml of a 1 M solution of potassium channel blocking compound. Clockwise from upper left-hand corner is BaCl_2, CsCl, TEA, and RbCl. KCl is applied to the center disk.

FIGURE 9A and 9B. DNA sequence and deduced amino acid sequence of CORK [SEQ ID NO: 36]. The nucleotide sequence of the 1.4 kb cDNA revealed a single long open reading frame proximal to the GAL1 promoter. Segments corresponding to pore-forming H5 domains in the predicted polypeptide are underlined. Asparagine-linked glycosylation sites are indicated by a G.

**Detailed Description of the Invention**

Nucleotide bases are abbreviated herein as follows: Ade; A-Adenine G-Guanine Ura; U-Uracil C-Cytosine T-Thymine

Amino acid residues are abbreviated herein to either three
letters or a single letter as follows:
Ala; A-Alanine Leu; L-Leucine
Arg; R-Arginine Lys; K-Lysine
Asn; N-Asparagine Met; M-Methionine
Asp; D-Aspartic acid Phe; F-Phenylalanine
Cys; C-Cysteine Pro; P-Proline
Gln; Q-Glutamine Ser; S-Serine
Glu; E-Glutamic acid Thr; T-Threonine
Gly; G-Glycine Trp; W-Tryptophan
His; H-Histidine Tyr; Y-Tyrosine
Ile; I-Isoleucine Val; V-Valine

The term "mammalian" as used herein refers to any mammalian species (e.g., human, mouse, rat, and monkey).

The term "heterologous" as used herein refers to DNA sequences, proteins, and other materials originating from organisms other than the organism used in the expression of the potassium channels or portions thereof, or described herein (e.g., mammalian, avian, amphibian, insect, plant), or combinations thereof not naturally found in yeast.

The terms "upstream" and "downstream" are used herein to refer to the direction of transcription and translation, with a sequence being transcribed or translated prior to another sequence being referred to as "upstream" of the latter.

The potassium channels of the present invention possess properties in common with known potassium channels including, voltage-gated channels, calcium activated channels, cyclic nucleotide gated channels, inward rectifier channels, and the like, and especially with regard to electrophysiological properties. Certain preferred channels exhibit inward and outward currents that are affected by potassium concentration, particularly characteristic of voltage-gated channels. The term "channel" and the
nucleotide sequences encoding same, is intended to encompass subtypes of the aforementioned classes of channels, and mutants, derivatives and homologs thereof.

The nucleotide sequences encoding the potassium channels or parts thereof may be expressed recombinantly, and utilized for a variety of reasons, the most notable of which is for screening of substances that modulate the activity of the potassium ion channels. Such substances, especially inhibitors of the activity of the potassium channels of the present invention, may be utilized as insecticides, antihelmenthics, drugs suitable for the control of heart failure, and the like.

Heterologous DNA sequences are typically expressed in a host by means of an expression vector. An expression vector is a replicable DNA construct in which a DNA sequence encoding the heterologous DNA sequence is operably linked to suitable control sequences capable of affecting the expression of a protein or protein subunit coded for by the heterologous DNA sequence in the intended host. Generally, control sequences include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and (optionally) sequences which control the termination of transcription and translation. Vectors useful for practicing the present invention include plasmids, viruses (including bacteriophage), and integratable DNA fragments (i.e., fragments integratable into the host genome by genetic recombination). The vector may replicate and function independently of the host genome, as in the case of a plasmid, or may integrate into the genome itself, as in the case of an integratable DNA fragment. Suitable vectors will contain replicon and control sequences which are derived from species compatible with the intended expression host. For example, a promoter operable in a host cell is
one which binds the RNA polymerase of that cell, and a ribosomal binding site operable in a host cell is one which binds the endogenous ribosomes of that cell.

DNA regions are operably associated when they are functionally related to each other. For example, a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of leader sequences, contiguous and in reading phase.

Transformed host cells of the present invention are cells which have been transformed or transfected with the vectors constructed using recombinant DNA techniques and express the protein or protein subunit coded for by the heterologous DNA sequences. In preferred embodiments, the transformed host cells are yeast. A variety of yeast cultures, and suitable expression vectors for transforming yeast cells, are known. See e.g., U.S. Patent No. 4,745,057; U.S. Patent No. 4,797,359; U.S. Patent No. 4,615,974; U.S. Patent No. 4,880,734; U.S. Patent No. 4,711,844; and U.S. Patent No. 4,865,989. Saccharomyces cerevisiae is the most commonly used among the yeasts, although a number of other yeast species are commonly available. See e.g., U.S. Patent No. 4,806,472 (Kluveromyces lactis and expression vectors therefore); 4,855,231 (Pichia pastoris and expression vectors therefore). A heterologous potassium channel may permit a yeast strain unable to grow in medium containing low potassium concentration to survive [CY162, for example, see J.A Anderson, S.S. Huprikar, L.V. Kochian, W.J. Lucas, R.F. Gaber, Proc. Natl. Acad. Sci USA 89, 3736-3740 (1992)]. Yeast vectors may contain an origin of replication from the endogenous 2 micron (2μ) yeast plasmid or an autonomously
replicating sequence (ARS) which confer on the plasmid the ability to replicate at high copy number in the yeast cell, centromeric (CEN) sequences which limit the ability of the plasmid to replicate at only low copy number in the yeast cell, a promoter, DNA encoding the heterologous DNA sequences, sequences for poly-adenylation and transcription termination, and a selectable marker gene. An exemplary plasmid is YRp7, (Stinchcomb et al., (1979) Nature 282, 39; Kingsman et al., (1979) Gene 7, 141; Tschemper et al., (1980) Gene 10, 157]. This plasmid contains the TRP1 gene, which provides a selectable marker for a mutant strain of yeast lacking the ability to grow in the absence tryptophan, for example ATCC No. 44076. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for metallothionein (YEpl52), 3-phosphoglycerate kinase [pPGKH, HITZERMAN et al., (1980) J. Biol. Chem. 255, 2073] or other glycolytic enzymes [pYSK153, Hess et al., (1968) J. Adv. Enzyme Reg. 7, 149]; and Holland et al., (1978) Biochemistry 17, 4900], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-fructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. HITZERMAN et al., EPO Publn. No. 73,657. Other promoters, which have the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2 (pAD4M), isocyttochrome C, acid phosphates, degradative enzymes associated with nitrogen metabolism, and the aforementioned metallothionein and glyceraldehyde-3-
phosphate dehydrogenase, as well as enzymes responsible for maltose and galactose (pYES2) utilization. Finally, in constructing suitable expression plasmids, the termination sequences associated with these genes may also be ligated into the expression vector 3' of the heterologous coding sequences to provide polyadenylation and termination of the mRNA.

In one embodiment of the present invention, a yeast expression system is described, wherein yeast cells bear heterologous potassium channels. In preferred embodiments, these channels are DmORF-1, CORK, or RAK. As noted above, transformed host cells of the present invention express the proteins or proteins subunit coded for by the heterologous DNA sequences. When expressed, the potassium channel is located in the host cell membrane (i.e., physically positioned therein in proper orientation for both the stereoselective binding of ligands and passage of potassium ions).

In certain preferred screening embodiments of the present invention, a transformed yeast cell is presented, containing a heterologous DNA sequence which codes for a rat cardiac delayed rectifier potassium channel, RAK, cloned into a suitable expression vector. RAK is capable of complementing the potassium-dependent phenotype of Saccharomyces cerevisiae strain CY162 on medium containing low potassium concentration.

The potassium channel subclass of the present invention is characterized in that the potassium channels have four hydrophobic domains capable of forming transmembrane helices. These channels are further characterized in that they comprise two pore-forming domains, one of which is interposed between said first helix and said second helix, and the other of which is interposed between said third helix and said fourth helix. The pore-forming domains
further contain a potassium selective motif which serves to confer upon the channel the ability to pass potassium ions to the exclusion of other ions, such as sodium, calcium, and the like. In certain preferred embodiments, this motif contains the peptide Y/G, and particularly in either a dipeptide or tripeptide motif, and frequently with Y/F-G bonding. In most preferred embodiments, the motif is selected from the group consisting of G-V-G, G-L-G, G-Y-G, G-F-G, and G-I-G.

In certain embodiments of the present invention, the potassium channel is positioned within a cell membrane in such a manner as to allow it to function as a modulator of the flow of potassium ions into and out of the cell. To best regulate this activity, at least one pore-forming domain may be positioned proximal to a exterior portion of the cell membrane.

In other embodiments, the potassium channels of the present invention further comprise an amino-terminal glycosylation site, and especially wherein that site is asparagine-linked.

Potassium channels belonging to the subclass as presented herein may be derived from a wide variety of animal species, both vertebrate and invertebrate. Using the yeast expression technology and other teachings as set forth herein, the present inventors have isolated a single 2463 base pair cDNA fragment from an invertebrate source, designated Dm ORF1 [SEQ ID NO: 1], by complementation of the potassium-dependent phenotype of Saccharomyces cerevisiae strain CY162 (trk1Δ) on medium containing low potassium concentration [J.A Anderson, S.S. Huprikar, L.V. Kochian, W.J. Lucas, R.F. Gaber, Proc. Natl. Acad. Sci USA 89, 3736-3740 (1992)]. Dm ORF1 contains a single long open reading frame encoding a protein of 618 amino acids [SEQ ID NO:2] that exhibits substantial amino acid identity to the pore-
forming regions of other potassium channels. The DmORF1 contains structural features that distinguish it from other classes of potassium channels, including four hydrophobic domains capable of forming transmembrane helices (M1-M4) and two putative pore forming H5 domains found between transmembrane helices M1 and M2, and M3 and M4. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity [L. Begenbotham, T. Abramson, R. MacKinnon, *Science* 258, 1152-1155, (1992)]. This work was expanded to clone a construct derived from *C. elegans* having a single open reading frame sufficient to encode a protein of 434 amino acids, designated pCORK.

A search of the GENBANK database for DNA and protein sequences similar to DmORF1 revealed several cloned potassium channel sequences including a putative protein coding DNA sequence, F22b7.7, reported in the *Caenorhabditis elegans* genome sequencing project [R. Wilson, R. Ainscough, K Anderson, et al. *Nature* 368, 32-38 (1994)]. The DNA sequence contained a single long open reading frame sufficient to encode a protein of 336 amino acids (predicted MW 38.5 kDa) with substantial homology to known potassium channel sequences.

Using the hybridization approach, a cDNA sequence designated CeORF1 [SEQ ID NO: 38] was isolated by probing a *Caenorhabditis elegans* cDNA library with oligonucleotides designed using F22b7.7 DNA sequences [T.N. Davis and J. Thorner *Meth. Enzymol.* 139, 246-262 (1987)]. CeORF1 contains a single long open reading frame encoding a protein that exhibits substantial amino acid identity to pore-forming regions of other potassium channels.

CeORF1 and pCORK each contain structural features similar to DmORF1, including two putative pore forming H5 domains. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity [L.
Heginbotham, T. Abramson, R. MacKinnon, Science 258, 1152-1155, (1992)]. These features form the basis of the designation of a new sub-family of potassium channels comprising DmORF1, CORK, and CeORF1.

Other aspects of the present invention relate to methods of modulating potassium channel activity, by affecting the ability of such channel to allow the flow of ions into, through, or out of a cellular membrane, and particularly when these ions are potassium ions. Certain substances whether biological or chemical in nature, may be applied to cell membranes having as an integral part of their structure, one or more potassium channels comprising the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 36, or RAK, in an amount and for a time sufficient to affect the ability of the potassium channel to so regulate the flow of ions. Substances that are potassium channel blockers will inhibit the ability of the channel to regulate the flow of such ions. Substances that enhance such ability may be considered potassium channel "activators."

Substances that modulate the activity of RAK may do so by modulation of cardiac action potential, upward or downward.

Application of such substances may take the form of in vitro, ex vivo, or in vivo application, each in a formulation suitable to deliver the substance to the cell membrane and to sustain such delivery for a time sufficient to allow the substance to interact with the membrane. Appropriate formulations, concentrations of substances, application time, and other relevant parameters may be established by utilizing, inter alia, known assays for measuring ion channel current flow. Another suitable endpoint one skilled in the art may utilize in optimizing these parameters, especially in the case of potassium channel blockers, is "cell death". Such assays may be performed in vitro and extrapolated to in vivo conditions.
or in some cases may be easily established directly in vivo, as for example, by applying the substance directly to a test sample comprising the target insect pest (whole organism) and noting the appropriate parameters at which an acceptable per cent of insect death is attained.

In certain preferred embodiments, methods of selectively inhibiting insect pests are presented by applying to such insect pests a substance capable of selectively inhibiting the activity of a potassium channel contained in the cells of such insect, and comprising the amino acid sequence of SEQ ID NO:2, or a potassium channel substantially homologous thereto. In the most preferred embodiments, the inhibitor will inhibit the activity of the aforementioned potassium channel without inhibition of other, non-homologous potassium channels that may be present in species other than the targeted insect pest. It is envisioned that such other species may also be present at the site of application of the inhibitor, such as in a garden, crop, or other site wherein it is desired to control insect pests. In other preferred embodiments, methods of selectively inhibiting nematode pests are presented much in the same manner as discussed for control of insect pests, by applying to such pests a substance capable of selectively inhibiting the activity of a potassium channel contained in the cells of such pest, and comprising the the amino acid sequence of SEQ ID NO:4 or SEQ ID NO: 36, or potassium channels substantially homologous thereto.

The following Examples are provided to further illustrate various aspects of the present invention. They are not to be construed as limiting the invention.

Example 1

Recombinant expression library screening.

Isolation of expression plasmids encoding heterologous potassium channels. CY162 cells are transformed with plasmid DNA from each library to give 3 x 10⁶ transformants from each library on SCD-ura (synthetic complete dextrose (2 %) medium containing all necessary nutritional supplements except uracil) containing 0.1 M KCl agar medium. Transformants are replica-plated to SCG-ura (synthetic complete galactose (2 %) medium containing all necessary nutritional supplements except uracil) agar medium. Colonies that grow on this selective agar medium are transferred to SCG-ura agar medium to obtain single colonies clones and while reassaying suppression of the potassium-dependent phenotype. Plasmid DNA is isolated from surviving colonies and used to transform CY162. Six individual transformant strains containing one plasmid, pDmORF1, that confers the potassium independent phenotype is cultured on
SCD-ura and SCG-ura medium along with CY162 strains bearing pkAT1, which encodes a plant inward rectifier potassium channel that supports the growth of CY162 on selective medium (FIGURE 1). The plasmid bearing strains exhibit potassium-independent growth on both dextrose and galactose containing medium. Growth on dextrose is likely due to basal level of transcription leading to sufficient potassium channel expression to support growth.

**Example 2**

**DNA sequence analysis of DmORF1.** Plasmids that confer suppression of the potassium-dependent phenotype are subjected to automated DNA sequence analysis performed by high temperature cycle sequencing (Applied Biosystems). Geneworks DNA sequence analysis software (Intelligenetics) is used to align raw DNA sequence information and to identify open reading frames. The DNA sequence of the 2.4 kb insert in pDmORF1 is displayed in FIGURE 2A and 2B [SEQ ID NO:1]. The 5' untranslated sequences of the cDNA contain long poly A and poly T tracts not likely to be found in protein coding regions. The first ATG proximal to the 5' end is present in a consensus Drosophila melanogaster translational initiation site [D.R. Cavener Nucleic Acids Res., 15, 1353-1361 (1987)], consistent with the designation of this site as the translational start site. A single long open reading frame sufficient to encode a protein of 618 amino acids (predicted MW 68 kDa) is encoded in pDmORF1. A consensus polyadenylation site, AATCAA, occurs at position 2093-2098 in 3' untranslated sequences. The DmORF1 contains structural features that distinguish it from other classes of potassium channels, including four hydrophobic domains capable of forming transmembrane helices (M1-M4) and two pore forming H5 domains found between transmembrane helices.

Example 3

Identification of *Caenorhabditis elegans* sequences homologous to DmORF1. A search of the GENBANK database protein sequences similar to DmORF1 reveals significant matches with several known potassium channel sequences. The closest match is to a putative protein coding DNA sequence, F22b7.7, reported in the *Caenorhabditis elegans* genome sequencing project [R. Wilson, R. Ainscough, K. Anderson, *et al., Nature* 368, 32-38 (1994)]. The DNA sequence and predicted amino acid sequence assembled from putative exons recognized by a GENBANK exon identification algorithm is displayed in FIGURE 3A and 3B [SEQ ID NOS:3 and 4]. The DNA sequence contains a single long open reading frame sufficient to encode a protein of 336 amino acids (predicted MW 38.5 kDa) with substantial homology to known potassium channel sequences. The F22b7.7 sequence contains structural features that distinguish it from other classes of potassium channels, including three of four hydrophobic domains capable of forming transmembrane helices (M1-M4) identified in DmORF1 and two pore forming H5 domains found between transmembrane helices a predicted M1 and M2, and M3 and M4. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity [L. Heginbotham, T. Abramson, R. MacKinnon, *Science* 258, 1152-1155, (1992)]. The lack of an amino terminal transmembrane domain homologous to DmORF1 M1 in the F22b7.7 sequence may be due to failure of the search algorithm to identify exon(s)
encoding the amino terminus. Alternatively, an amino terminal coding sequence may be added by trans-splicing, which occurs frequently in *Caenorhabditis elegans*.

**Example 4**

Cloning and DNA sequence analysis of CeORF1.

Oligonucleotides corresponding to DNA sequences encoding the two pore forming domains of F22b7.7 are synthesized using an Applied Biosystems DNA synthesizer.

F22b7.7-B2-1:
5'TCCATTTTCTTTGCGTAGCTGCTACTACCACAGTACGGTAAATCCA [SEQ ID NO:5].

F22b7.7-B2-2:
5'TCATTCTACTGGTCCTTCATTACAATGACTACTGTCGCGGTGTTTTGGCGACTTG [SEQ ID NO:6]. The oligos were labelled at their 5' ends with $^{32}$P using a 5'-end labelling kit according to manufacturers instructions (New England Nuclear). The labelled oligos are pooled and used to screen 6 x 10^5 plaques from a λZAP-Caenorhabditis elegans cDNA library (obtained from Clontech) by published methods [T.N. Davis and J. Thorner *Meth. Enzymol*. 139, 246-262 (1987)]. Hybridization is at 42°C for 16 hours. Positive clones are plaque-purified by twice repeating the hybridization screening process.

Plasmid DNAs, excised from phage DNA according to manufacturers instructions, are subjected to automated DNA sequence analysis performed by high temperature cycle sequencing (Applied Biosystems). Geneworks DNA sequence analysis software (Intelligenetics) is used to align raw DNA sequence data and to identify open reading frames.

**Example 5**

Comparison of the putative proteins encoded by DmORF1 and F22b7.7. Predicted amino acid sequences of DmORF1 and
F22b7.7 are aligned and displayed in FIGURE 4 [SEQ ID NOS:37 and 38]. Only limited overall amino acid homology is exhibited by these two proteins with regions of greatest homology existing in the pore forming H2-1 and H2-2 domains. FIGURE 5A shows a comparison of the pore forming domains of DmORF1 and F22b7.7 with those of the known Drosophila melanogaster potassium channel and inward rectifier sequences [SEQ ID NOS:7 through 21]. Amino acid identities greater than 50% are observed with all potassium channel sequences. FIGURE 5B shows hydropathy plot analysis of DmORF1 and F22b7.7. The two proteins, which show remarkable topological similarity through their length, are predicted to be composed of four membrane-spanning hydrophobic domains (M1-M4), and two pore forming H2 domains. These data suggest the predicted topology shown in FIGURE 6. Both proteins are predicted to span the membrane four times with amino and carboxyl termini residing within the cell. This topology places the single amino-terminal asparagine-linked glycosylation site and H2 domains on the cell exterior permitting permeation of the membrane by the pore forming domains from the outside, an absolute requirement for the formation of a functional potassium channel.

Example 6

Functional expression of a rat atrial delayed rectifier potassium channel in yeast. CY162 transformants containing plasmids pKAT1, which encodes a plant inward rectifier potassium channel, pRATRAK, which encodes a rat atrial delayed rectifier potassium channel, pDmORF1, and control plasmid pYES are cultured on arginine-phosphate-dextrose agar medium lacking ura medium [A. Rodriguez-Navarro and J. Ramos, J. Bacteriol. 159, 940-945, (1984)] containing various KCl concentrations (FIGURE 7). Strains containing pKAT1, pRATRAK, and pDmORF1 all support the growth of CY162
on medium containing a low concentration of potassium, while 5
pYES2 containing CY162 cells only grow on medium containing
a high potassium concentration, indicating that heterologous
potassium channels of several different types function to
provide high affinity potassium uptake.

pRATRAK is constructed by modifying the protein-coding
sequences of RATRAK to add 5' HindIII and 3' XbaI sites
using PCR. In addition, four A residues are added to the
sequences immediately 5' proximal to the initiator ATG to
provide a good yeast translational initiation site. The
modified fragment is cloned into the HindIII and XbaI sites
in the yeast expression vector pYES2 (Invitrogen), forming
pRATRAK.

Example 7

Bioassay of functional expression of heterologous potassium
channels

Yeast strains dependent on heterologous potassium
channels for growth should be sensitive to non-specific
potassium channel blocking compounds. To test the potassium
channel blocking properties of several compounds, a
convenient agar plate bioassay is employed. Strains
containing pKAT1, pRATRAK, pDmORF1, and pYES2 are plated in
arginine-phosphate-dextrose agar medium lacking ura and
containing various amounts of potassium chloride. Arginine-
phosphate-dextrose medium is used to avoid interference from
potassium and ammonium ions present in standard synthetic
yeast culture medium. Sterile filter disks were placed on
the surface of the agar and saturated with potassium channel
blocking ions CsCl, BaCl$_2$, and TEA. The growth of
heterologous potassium channel containing strains is
inhibited by potassium channel blocking ions, in a channel
dependent manner. DmORF1-dependent growth is blocked by
BaCl₂ but not by CsCl or TEA. KAT-dependent growth is blocked by BaCl₂, CsCl and TEA. RATRAK-dependent growth is blocked by BaCl₂, CsCl and TEA to a much greater extent than pKAT1, reflecting in part a slower growth rate of pRATRAK-containing cells. These observations confirm that these channels support the growth of the mutant yeast cells and demonstrate the efficacy of the yeast bioassay for screening for compounds that block potassium channel function. The control pYES-containing strain grows only around applied KCl and RbCl, a congener of KCl.

Example 8

Identification of compounds that alter potassium channel activity

Yeast strains made capable of growing on medium containing low potassium concentration by expression of heterologous potassium channels are used to screen libraries of chemical compounds of diverse structure for those that interfere with channel function. CY162 cells containing pKAT1, pRATRAK, pDmORF1, pCeORF1, and pYES2-TRK1 (10⁻⁴/ml) are plated in 200 ml of arginine-phosphate-dextrose agar medium lacking ura and containing 0.2 mM potassium chloride in 500 cm² plates. The CY162 cells bearing pYES2-TRK1 are included in the assay as a control to identify compounds that have non-specific effects on the yeast strain and are therefore not specifically active against the heterologous potassium channels. Samples of chemical compounds of diverse structure (2 µl of 10 mg/ml solution in DMSO) are applied to the surface of the hardened agar medium in a 24 x 24 array. The plates are incubated for 2 days at 30°C during which time the applied compounds radially diffuse into the agar medium. The effects of applied compounds on strains bearing heterologous potassium channel
genes are compared to the pYES2-TRK1 bearing strain. Compounds that cause a zone of growth inhibition around the point of application that is larger on plates containing cells bearing the heterologous potassium channels than that observed around the pYES2-TRK1 bearing strains are considered selective potassium channel blockers. Compounds that induce a zone of enhanced growth around the point of application that is larger on plates containing cells bearing the heterologous potassium channels than that observed around the pYES2-TRK1 bearing strains are considered selective potassium channel openers.

Example 9

DmORF1-induced currents in X. laevis oocytes assayed by two-electrode voltage clamp

DNA sequence analysis of the pDmORF insert strongly suggest that the protein encoded by the single long ORF possesses properties in common with known potassium channels. To test this hypothesis, the electrophysiological properties of the putative potassium channel encoded by DmORF was examined by expression in X. laevis oocytes. Currents were measured by two-electrode whole-cell voltage clamp. DNA sequences encoding the open reading frame of DmORF1 were amplified by polymerase chain reaction (PCR) using the following oligonucleotides:

MP023: ATAAAGCTTTAAATGTCGCCGGAATCGATGGAT [SEQ ID NO:22]
MP024: AGCTCTAGACCTCCATCTGGAAGCCCATGT [SEQ ID NO:23]

The full length PCR product was cloned into corresponding sites in pSP64 poly A (Promega), forming pMP147. Template DNA was linearized with EcoRI and RNA transcribed using the Message Machine (Ambion) in vitro transcription kit according to manufacturers instructions. A sample of the RNA was resolved in a MOPS-acetate-formaldehyde agarose gel
and RNA content was estimated by ethidium bromide staining. The remainder was stored on dry ice. *X. laevis* oocytes were isolated and injected with 50 nl of sterile TE containing 5-20 ng transcript according to published procedures. After three days, whole oocyte currents were recorded using a two-electrode voltage clamp. Electrodes contained 3M KCl and had resistances of 0.3-1.0 MΩ. Recordings were performed with constant perfusion at room temperature in the presence of either low (10 mM) or high (90 mM) potassium chloride.

Two electrode voltage clamp analysis of the DmORF1 gene product expressed in *X. laevis* oocytes demonstrates properties of a voltage- and potassium-dependent potassium channel. At low potassium concentrations, DmORF1 exhibited outward current at depolarizing potentials. At high potassium concentration, DmORF1 exhibits both inward and outward currents. The DmORF1 channel displays a high preference for potassium and shows cation selectivity in the rank order K>Rb>NH₄>Cs>Na>Li. Potassium currents were greatly attenuated by BaCl₂.

**Example 10**

Developmental regulation of DmORF1 expression in *D. melanogaster* determined by northern blotting analysis

Isolation of pDmORF1 from a *D. melanogaster* expression library strongly suggests that the insert contained within originated in mRNA from that species. Detailed understanding of the developmental regulation of DmORF1 expression should aid in determining strategies for use of DmORF1 as a target for novel insecticides. To characterize DmORF1 expression, northern blotting analysis of poly A RNA from various stages of the *D. melanogaster* life cycle was carried out.

*D. melanogaster* poly A⁺ RNA from embryo, larvae and
adult forms (Invitrogen, 5 µg) was resolved in a MOPS-acetate-formaldehyde agarose gel according to standard procedures. The gel was stained with ethidium bromide and photographed to mark the positions of 18 S and 28 S ribosomal RNAs used as molecular weight markers. RNA was transferred by capillary action to nitrocellulose with 10 x SSPE. The blot was air-dried, baked for one hour at 80°C, and prehybridized in 4x SSPE, 1% SDS, 2x Denhardt's, 0.1 % single stranded DNA at 68 °C for 2 hours.

A 2.4 kb XhoI fragment of DmORF1 was isolated from pDmORF1 and labeled with α-32P dCTP using the Ready-to-Go kit (Pharmacia) according to manufacturers instructions. The probe was denatured by heating to 100°C for 5 minutes followed by quenching in an ice water bath. The probe was added to the prehybridization solution and hybridization continued for 24 hours at 68 °C.

The blot was washed briefly with 2x SSPE, 0.1% SDS at room temperature followed by 0.5 x SSPE, 0.1 % SDS at 65 °C for 2 hours. The blot was air-dried and exposed to Reflection X-ray film (NEN) using an intensifying screen at -70 °C for 48 hours.

Northern blotting analysis indicates that the DmORF1 probe hybridizes to an mRNA species of approximately 2.8 kb isolated from D. melanogaster embryo, larvae, and adult forms. The length of the DmORF1 mRNA corresponds well with the length of the predicted ORF. Thus, the DmORF is expressed at all developmental stages in the life cycle of D. melanogaster.

**Example 11**

**Expression of the DmORF1 gene product in vitro.**

DNA sequence analysis of the pDmORF1 insert reveals a single long ORF with conserved amino acid sequence domains
in common with known potassium channels. The DNA sequence predicts an ORF sufficient to encode a protein of 618 amino acid in length. The DmORF1 polypeptide contains four segments of at least 20 hydrophobic amino acids in length suggesting that the segments span the plasma membrane. In addition, the DmORF1 protein sequence contains a putative N-linked glycosylation site (Asn-Thr-Thr) at amino acids 58-60. To confirm that a protein of the predicted size of DmORF is expressed from the insert in pDmORF1 and to test the proposition that DmORF1 is glycosylated, pDmORF1 was used as template to drive coupled in vitro transcription/translation.

Plasmid pMP147 was used as template to produce $^{35}$S-labeled DmORF gene product in vitro using a TnT coupled transcription-translation kit (Promega) according to manufacturers instructions. Glycosylation of the nascent DmORF1 polypeptide was accomplished by addition of canine pancreatic microsomes (Promega) to the transcription-translation reaction. Samples of glycosylated DmORF protein were treated with endoglycosidase H to remove added carbohydrate moieties. Aliquots were precipitated with TCA and collected on GF/C filters, washed with ethanol, dried and counted. Equivalent cpm's were resolved by SDS-PAGE. The gel was impregnated with soluble fluor Amplify (Amersham) and dried onto Whatman 3MM paper. The dried gel was exposed to Reflection X-ray film at room temperature.

Translation of the DmORF1 gene product in vitro produced a polypeptide of 68 kDa, consistent with the predicted molecular weight of the ORF. Translation of DmORF1 in the presence of canine pancreatic microsomes results in synthesis of a protein with reduced electrophoretic mobility, consistent with glycosylation of the nascent polypeptide. Treatment of glycosylated DmORF with EndoH increased its relative mobility as expected upon
removal of carbohydrate moieties. Thus, the pDmORF1 insert is capable of directing the expression of a glycoprotein with the expected molecular weight. EndoH treatment removes carbohydrate residues consistent with the sugar added through N-linked glycosylation.

**Example 12**

**High-affinity K⁺ uptake and selectivity of DmORF1 expressed in yeast.**

Expression of DmORF permits CY162 cells to grow on medium containing a low concentration of potassium, implying that DmORF1 supplies high affinity potassium uptake capacity. To characterize the potassium uptake properties of CY162 cells containing DmORF1, ⁸⁶Rb uptake studies were performed. Examination of the uptake of this potassium congener revealed important aspects of potassium uptake by DmORF1.

Yeast strains containing heterologous potassium-expression plasmids CY162-DmORF1, CY162-pKAT and the control strain CY162-pYES2 (Clontech) were cultured overnight in SC Gal-ura containing 0.1 M KCl. The cells were harvested, washed with sterile doubled distilled water and starved for K⁺ for 6 hours in Ca-MES buffer. Cells were washed again and distributed to culture tubes (10⁶ cells/tube) containing ⁸⁶RbCl in Ca-MES buffer. The tubes were incubated at room temperature, samples filtered at various time intervals and counted. ⁸⁶Rb uptake into cells was displayed. For Double Reciprocal analysis, ⁸⁶Rb was held constant and barium ions varied to determine Ki values.

The high-affinity potassium uptake capacity encoded by DmORF1 permits high-affinity uptake of the potassium congener, ⁸⁶Rb, as well. Barium inhibited ⁸⁶Rb uptake with a
Ki of μM as demonstrated in Double Reciprocal analysis. No high affinity $^{86}$Rb uptake is observed in control CY162-pYES2 cells and $^{86}$Rb uptake into CY162-pKAT cells is consistent with its published properties.

Example 13

Expression of Drosophila melanogaster potassium channels in yeast.

Voltage-gated potassium channel diversity in the fruitfly Drosophila melanogaster is encoded in large part by six genes, Shaker, Shab, Shal, Shaw, Eag, and Slo. Expression of these potassium channels in yeast will permit their introduction into screening assays for novel insecticidal compounds and facilitate characterization of their ion channel properties and sensitivity to compounds with activating and inhibitory properties.

DNA sequences encoding Drosophila melanogaster potassium channels were amplified by PCR using synthetic oligonucleotides that add 5' HindIII or Kpn I, sites and 3' XbaI, SphI, or XhoI sites:

Shaker 5':AAAAAGCTTTAAAAATGGCGAGCAACTCAC [SEQ ID NO:24]
Shaker 3':AAACTCGAGTCATACCTGTTGACT [SEQ ID NO:25]

Shab 5':AAAAAGCTTTAAAAATGGCGAGCACTCAC [SEQ ID NO:26]
Shab 3':AAAAAGCTTTAAAAATGGCGAGCACTCAC [SEQ ID NO:27]

Shal 5':AAAAAGCTTTAAAAATGGCGAGCACTCAC [SEQ ID NO:28]
Shal 3':TTTTCTAGACTACATCGGTGTTCTT [SEQ ID NO:29]

Shaw 5':AAAAAGCTTTAAAAATGAATTGATCGATCAAC [SEQ ID NO:30]
Shaw 3':AAATCTAGATTAGTGCAGACTGAA [SEQ ID NO:31]
Eag 5':AAAAAGCTTAAAAATGCCTGGCGGA [SEQ ID NO:32]
Eag 3':AAATCTAGAGGCTACAGGAAGTCC [SEQ ID NO:33]

Slo 5':GGGGGTCACAAAATGTCGGGGGTTTGAT [SEQ ID NO:34]
Slo 3':TTTTTTACTAGATCAAGAGTTATC [SEQ ID NO:35]

Plasmids used as templates for the PCR reactions were:
pBSc-DShakerH37, pBSc-dShab11, pBSc-dShal2+(A)₃₆, pBScMXT-
dShaw [A. Wei, M. Covarrubias, A. Butler, K. Baker, M. Pak,
L. Salkoff, Science 248, 599-603 (1990), provided by L.
Salkoff], pBScMXT-slo,v4 [N.S. Atkinson, G.A. Robertson, B.
Ganetzky, Science 253,551-555, (1991), provided by L.
Salkoff], and pBIMCH20 Eag [CH20] [J. Warmke, R. Drysdale,
B. Ganetzky, Science 252, 1560-1564 (1991), A. Bruggemann,
(1993), provided by B. Ganetzky].

Amplified fragments were digested with the appropriate
restriction endonucleases, purified using GeneClean (Bio
101), and ligated into corresponding sites in pYES2
(Invitrogen). CY162 cells were transformed with assembled
Drosophila melanogaster potassium channel expression
plasmids by the LiCl method and plated on SCD-ura containing
0.1M KCl agar medium. Selected transformants were tested
for growth on arginine-phosphate-galactose (2 %)/sucrose
(0.2 %)-ura agar medium containing 1-5 mM KCl. CY162 cells
containing pKAT1 or pDmORF1 were cultured as positive
controls and CY162 cells containing pYES2 were grown to
provide a negative control.

CY162 cells bearing Drosophila melanogaster potassium
channel expression plasmids survive under conditions in
which growth is dependent on functional potassium channel
expression. At potassium ion concentrations between 1-3 mM,
negative control CY162 cells containing pYES2 grow poorly.
Expression of the Drosophila melanogaster potassium channels
Shal, Shaw and Eag substantially improve growth of CY162. These results are consistent with the *Drosophila melanogaster* potassium channels providing high-affinity potassium uptake capacity. This capacity is apparently sufficient to replace the native high-affinity potassium transport capacity encoded by TRK1 which is lacking in CY162 (trk1 trk2) cells.

**Example 14**

Cloning of a novel *C. elegans* sequence with homology to potassium channels.

In order to expand the applicability of this technology to discover compounds with novel anhelmentic activity, CY162 cells were transformed with a pYES2-based yeast expression library constructed using cDNA synthesized from *C. elegans* mRNA (Invitrogen). Plasmid DNA isolated from yeast cells that survived the selection scheme described in EXAMPLE 1 were subjected to automated DNA sequence analysis performed by high temperature cycle sequencing (Applied Biosystems). Geneworks DNA sequence analysis software (Intelligenetics) is used to align raw DNA sequence information and to identify open reading frames. The DNA sequence of the 1.4 kb insert in pCORK is displayed in FIGURE 9A and 9B. The 5' untranslated sequences of the cDNA are present in this construct. A single long open reading frame sufficient to encode a protein of 434 amino acids (predicted MW 48 kDa) is predicted in pCORK [SEQ ID NO:38]. A consensus polyadenylation site, AATAAA, occurs at position 1359-1364 in 3' untranslated sequences and is followed by a tract of 15 consecutive A residues. The CORK ORF contains structural features that resemble pore forming H5 domains found in potassium channels. Two putative pore forming H5 domains (residues 76-39 and 150-162) contain the G-Y/F-G
(1) GENERAL INFORMATION:

(i) APPLICANT: American Cyanamid Company

(ii) TITLE OF INVENTION: Genes Encoding a Novel Family of Potassium Channels

(iii) NUMBER OF SEQUENCES: 38

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: American Cyanamid Company
(B) STREET: One Cyanamid Plaza
(C) CITY: Wayne
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07470-8426

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Matthews, Gale F.
(B) REGISTRATION NUMBER: 32,369
(C) REFERENCE/DOCKET NUMBER: 32,421-01 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-660-6329
(B) TELEFAX: 201-660-7160

(2) INFORMATION FOR SEQ ID NO:1:

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(A) LENGTH: 2441 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
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(B) LOCATION: 190..2043

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Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr

1   5

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**SUBSTITUTE SHEET (RULE 26)**
Arg Lys Pro Asp Pro Arg Trp Thr Thr Thr Ser Thr Arg Ser Arg Arg
575 580 585

CCT CCA GTC AAT CCT ATT TGC GCA ACG GAC GCG GTC CGC CAC CGC CCT
Pro Pro Val Asn Pro Ile Cys Ala Thr Asp Ala Val Arg His Arg Pro
590 595 600 605

TCG AAT CGA ATG GCA GCT TGG CCA GGC GGC GCC GGC GCC TAA CGA ACAT
Ser Asn Arg Met Ala Ala Trp Pro Ala Ala Ala Ala Gly
610 615

GGGCTTCCAG ATGAGGAGTG GAGCAACCC GCCATCGGCA TTTGGCGGCTG GAGCCTATCA
2113

ACGCAAAGGCC GCTGCTGGCA AGCGCGCAGG CGAGAGCATC TACACCCAGA ATCAAGCCCC
2173

ATCCGCTCGC CGGGCGACAG TGATATCCGC GCCGCCGACAC GCCCTGCCCC AGATGAGCAT
2233

GGGACGCGGC AGCTTGCAA CCAGTGGTTC TGGATCGGCC GCCATGGCGG CAGTGGCCGC
2293

GGGTGGTGCC AGCGCTCTTT CAGCTAAGCC ATCGGGATCA TCGTGAACCT CTGCTCCGG
2353

CCGAAACGAC ATATTTCTGG TTAGCTCCGA AAAAGATAGT AATGTCCTGG AGCGAGCCAC
2413

CATTGCGGAT CTGATTCTGG CGCTCGAG
2441

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 618 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr Ile Ser Tyr
1 5 10 15

Leu Met Phe Gly Ala Ala Ile Tyr Tyr His Ile Glu His Gly Glu Glu
20 25 30

Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Glu Ala Ala Asn Glu
35 40 45

Tyr Leu Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr Gln Asp Glu
50 55 60

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

Pro Thr Tyr Asp Thr Pro Tyr Thr Trp Thr Phe Tyr His Ala Phe
85 90 95

Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn Ile Ser
100 105 110

Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr Ser Val Ile
115 120 125

Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly Glu Tyr Phe
130 135

Gly Arg Thr Phe Glu Ala Tyr Arg Arg Tyr Lys Lys Tyr Lys Met
145 150 155 160

Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu Ile Thr Thr
165 170 175

SUBSTITUTE SHEET (RULE 26)
Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu Val Leu Pro 180
Cys Val Gly Val His Leu Leu Arg Glu Leu Gly Leu Ser Ser Ile Ser 195
Leu Tyr Tyr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe Gly Asp Tyr 210
Val Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Gly Gly Trp Phe 225
Val Val Tyr Gln Ile Phe Val Ile Val Trp Phe Ile Phe Ser Leu Gly 245
Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu Gln Ser Lys 260
Lys Leu Ala Tyr Leu Glu Gln Gln Leu Ser Ser Asn Leu Lys Ala Thr 275
Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val Gly Tyr Leu Arg 290
Arg Met Leu Asn Glu Leu Tyr Ile Leu Lys Val Lys Pro Val Tyr Thr 305
Asp Val Asp Ile Ala Tyr Thr Leu Pro Arg Ser Asn Ser Cys Pro Asp 325
Leu Ser Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser Arg Lys Arg 340
Ala Phe Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg Glu Ala Gly 355
Met Val His Ala Asn Ser Asp Thr Asp Leu Thr Lys Leu Asp Arg Glu 370
Lys Thr Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr Asp Leu Leu 385
Ala Lys Val Val Ala Leu Ala Leu Thr Val Lys Pro Pro Pro Ala Glu 405
Gln Glu Asp Ala Ala Leu Tyr Gly Gly Tyr His Gly Phe Ser Asp Ser 420
Gln Ile Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn Glu Phe Thr 435
Ser Pro Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe Asn Leu Glu 450
Ala Pro Arg Trp Gln Ser Glu Arg Pro Leu Arg Ser Ser His Asn Glu 465
Trp Thr Trp Ser Gly Asp Asn Gln Gln Ile Gln Glu Ala Phe Asn Gln 485
Arg Tyr Lys Gly Gln Glu Arg Ala Asn Gly Ala Ala Asn Ser Thr Met 500
Val His Leu Glu Pro Asp Ala Leu Glu Glu Gln Leu Arg Asn Asn His 515
Arg Val Pro Val Ala Ser Arg Ser Ser Pro Cys Arg Met Val Cys Asp 530
Val  Cys  Phe  Pro  Ser  Arg  Arg  Ser  Thr  Pro  Arg  Arg  Ile  Trp  Ser  Ala  
545  550  555  560
Ser  Cys  Pro  Trp  Ser  Arg  Tyr  Pro  Arg  Val  Ser  Ser  Arg  Arg  Lys  Pro  
565  570  575
Asp  Pro  Arg  Trp  Thr  Thr  Thr  Ser  Thr  Arg  Ser  Arg  Pro  Pro  Val  
580  585  590
Asn  Pro  Ile  Cys  Ala  Thr  Asp  Ala  Val  Arg  His  Arg  Pro  Ser  Asn  Arg  
595  600  605
Met  Ala  Ala  Trp  Pro  Ala  Ala  Ala  Ala  Gly  
610  615

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1011 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1008

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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ATG  TCC  GAT  CAG  CTG  TTT  GTC  GCA  TTT  GAG  AAG  TAT  TTC  TTG  ACG  AGT  
Met  Ser  Asp  Gln  Leu  Phe  Val  Ala  Phe  Glu  Lys  Tyr  Phe  Leu  Thr  Ser  1  5  10  15

AAC  GAG  GTC  AAG  AAG  AAT  GCA  GCA  ACC  GAG  ACA  TGG  ACA  TTT  TCA  TCG  
Asn  Glu  Val  Lys  Lys  Asn  Ala  Ala  Thr  Glu  Thr  Trp  Thr  Phe  Ser  Ser  20  25  30

TCC  ATT  TTC  TTT  GCC  GTA  ACC  GTC  GTC  ACT  ACC  ATC  GGA  TAC  GGT  AAT  
Ser  Ile  Phe  Phe  Ala  Val  Thr  Val  Val  Thr  Tyr  Gly  Tyr  Gly  Asn  35  40  45

CCA  GTT  CCA  GTG  ACA  AAC  ATT  GGA  CGG  ATA  TGG  ATG  ATA  TTG  TTC  TCC  
Pro  Val  Pro  Val  Thr  Asn  Ile  Glu  Arg  Ile  Thr  Cys  Ile  Leu  Phe  Ser  50  55  60

TTG  CTT  GGA  ATA  CCT  CTA  ACA  CTG  GTT  ACC  ATC  GCT  GAC  TTG  GCA  GGT  
Leu  Leu  Gly  Ile  Pro  Leu  Thr  Leu  Thr  Ile  Asp  Leu  Ala  Gly  65  70  75  80

AAA  TTC  CTA  TCT  GAA  CAT  CTT  GTT  TGG  TTT  TGG  TAT  GGA  AAC  TAT  TTG  
Lys  Phe  Leu  Ser  Glu  His  Leu  Val  Thr  Tyr  Gly  Asn  Tyr  Leu  Lys  85  90  95

TTA  AAA  TAT  CTC  ATA  TTG  TCA  CGA  CAT  CGA  AAA  GAA  CGG  AGA  GAG  CAC  
Leu  Lys  Tyr  Leu  Ile  Leu  Ser  Arg  His  Arg  Lys  Glu  Arg  Arg  Glu  His  100  105  110

GTT  TGT  GAG  CAC  TGT  CAC  AGT  CAT  GGA  ATG  GGG  CAT  GAT  AGT  AAT  ATC  
Val  Cys  Glu  His  Cys  His  Ser  His  Gly  Met  Gly  His  Asp  Met  Asn  Ile  115  120  125

GAG  GAA  AGA  ATT  CCT  GCA  TTC  CTG  GTA  TTA  GCT  ATT  CTG  ATA  GCA  
Glu  Glu  Arg  Ile  Pro  Ala  Phe  Leu  Val  Leu  Ala  Ile  Leu  Ile  Val  130  135  140

TAT  ACA  GCG  TTT  GGC  GGT  GTC  CTA  ATG  TCA  AAA  TTA  GAG  CGG  TGG  TCT  
Tyr  Thr  Ala  Phe  Gly  Gly  Val  Met  Ser  Lys  Leu  Glu  Pro  Trp  Ser  145  150  155
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SUBSTITUTE SHEET (RULE 26)
(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 336 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Asp Gln Leu Phe Val Ala Phe Glu Lys Tyr Phe Leu Thr Ser
1   5  10  15
Asn Glu Val Lys Asn Ala Ala Thr Glu Thr Trp Thr Phe Ser Ser
20  25  30
Ser Ile Phe Ala Val Thr Val Val Thr Ile Gly Tyr Gly Asn
35  40  45

SUBSTITUTE SHEET (RULE 26)
Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Cys Ile Leu Phe Ser 50
Leu Leu Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Asp Leu Ala Gly 65 70 75
Lys Phe Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys 85 90
Leu Lys Tyr Leu Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His 100 105 110
Val Cys Glu His Cys His Ser His Gly Met Gly His Asp Met Asn Ile 115 120 125
Glu Glu Lys Arg Ile Pro Ala Phe Leu Val Leu Ala Ile Leu Ile Val 135 140
Tyr Thr Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser 145 150 155 160
Phe Phe Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly 165 170 175
Phe Gly Asp Leu Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu 180 185 190
Leu Tyr Ile Ile Leu Gly Lys Phe Ser Met Lys Lys Gln Lys Phe 195 200 205
Lys Ile Phe Leu Gly Leu Ala Ile Thr Thr Met Cys Ile Asp Leu Val 210 215 220
Gly Val Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln 225 230 235 240
Asp Ala Arg Ser Ala Leu Ala Val Val Gly Gly Lys Val Val Leu Val 245 250 255
Ser Glu Leu Tyr Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser 260 265 270
Arg Glu Ala Phe Ile Val Glu Asn Leu Tyr Val Ser Lys His Ile Ile 275 280 285
Pro Phe Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr Ile Asp Gln Thr 290 295 300
Ala Asp Ala Ala Thr Ile Thr Ser Ser Ser Ser Ala Ile Asp Met Gln 305 310 315 320
Ser Cys Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys 325 330 335

(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
TCCATTTTCT TTGCGTAAAC GCTCGTCAC ACCATCGGAT ACGGTAATCC A

SUBSTITUTE SHEET (RULE 26)
(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCATTCTACT GGTCCCTCAT TACAATGACT ACTGTCCGGTT TTGGCGACTT G

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ala Phe Leu Phe Ser Ile Glu Thr Gln Thr Thr Ile Gly Tyr Gly Phe
1 5 10
Arg Cys Val Thr Asp Glu Cys Pro
15
20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ala Phe Leu Phe Ser Leu Glu Thr Gln Val Thr Ile Gly Tyr Gly Phe
1 5 10 15
Arg Cys Val Thr Glu Gln Cys Ala
20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ala Phe Leu Phe Ile Glu Thr Glu Ala Thr Ile Gly Tyr Gly Tyr
1 5 10 15
Arg Tyr Ile Thr Asp His Cys Pro
20

(2) INFORMATION FOR SEQ ID NO: 10:

SUBSTITUTE SHEET (RULE 26)
(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Phe Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn
  1  5  10
Ile Ser Pro Thr Thr Phe Ala Gly  
  20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ala Phe Trp Trp Ala Val Val Thr Met Thr Thr Val Gly Tyr Gly Asp
  1  5  10  15
Met Thr Pro Val Gly Phe Trp Gly  
  20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Phe Trp Tyr Thr Ile Val Thr Met Thr Thr Leu Gly Tyr Gly Asp
  1  5  10  15
Met Val Pro Glu Thr Ile Ala Gly  
  20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Phe Trp Trp Ala Gly Ile Thr Met Thr Thr Val Gly Tyr Gly Asp
  1  5  10  15
Ile Cys Pro Thr Thr Ala Leu Gly  
  20
(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 24 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Leu Trp Trp Ala Leu Val Thr Met Thr Thr Val Gly Tyr Gly Asp
1      5
      10
Met Ala Pro Lys Thr Tyr Ile Gly
20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 24 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Leu Tyr Phe Thr Met Thr Cys Met Thr Ser Val Gly Phe Gly Asn
1      5
      10
      15
Val Ala Ala Glu Thr Asp Asn Glu
20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 24 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Cys Val Tyr Phe Leu Ile Val Thr Met Ser Thr Val Gly Tyr Gly Asp
1      5
      10
      15
Val Tyr Cys Glu Thr Val Leu Gly
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 24 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ser Leu Tyr Thr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe Gly Asp
1      5
      10
      15

SUBSTITUTE SHEET (RULE 26)
Tyr Val Pro Thr Phe Gly Ala Asn
20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
Ala Phe Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn
1 5 10
Ile Ser Pro Thr Thr Phe Ala Gly
20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
Ser Ile Phe Phe Ala Val Thr Val Val Thr Thr Ile Gly Tyr Gly Asn
1 5 10
Pro Val Pro Val Thr Asn Thr Gly
20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
Ser Leu Tyr Thr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe Gly Asp
1 5 10
Tyr Val Pro Thr Phe Gly Ala Asn
20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly Asp
1      5
Leu Met Pro Arg Arg Asp Gly Tyr
10     15
20
(2) INFORMATION FOR SEQ ID NO:22:
(xi) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
ATAAAGCTTA AAAATGTGC CGAATCGATG GAT

(2) INFORMATION FOR SEQ ID NO:23:
(xi) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
AGCTCTAGAC CTCCATCTGG AAGCCCATGT

(2) INFORMATION FOR SEQ ID NO:24:
(xi) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
AAAAAGCTTA AAAATGGCACA CATCAGC

(2) INFORMATION FOR SEQ ID NO:25:
(xi) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
AAACTCGAGT CATACCTGTG GACT

(2) INFORMATION FOR SEQ ID NO:26:
(xi) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
AAAAGCTTA AAATGGTCGG GCAATTG

(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:27:
AAAAGCATGC TCAATCGGAT GGCC

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:28:
AAAAGCTTAA AAATGGCCTC GGGCGGC

(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:29:
TTTTCTAGAC TACATCGTTG TCT

(2) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTION: SEQ ID NO:30:
AAAAGCTTA AAATGACTCT GATCAAC

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAATCTAGAT TAGTCGAAAC TGAA

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAAAAGCTTA AAATGCCTGG CGGA

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

AAATCTAGAG GCTACAGGAA GTCC

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 27 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGGGTACCA AAATGTGGGG GTGTGAT

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 25 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TTTTCTAGA TCAAGAGTTA TCATC

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 1529 amino acids
   (B) TYPE: amino acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Asx Asp Asp Ala His Asx Asp Asp Ala Asx His Ala Asx His Ala Asp
  1  5 10
Asx Ala Asp His Asp His Ala His Asx Asp Ala Asx His 30
  20  25
Asx Ala His Ala His Ala Asp Asp Asp Ala Ala His Ala His His
  35  40  45
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  50  55  60
Asx Asp Asp Ala His Asx Asx Ala Asx His Ala Ala Asx 80
  65  70  75
Asp Asx Asx Asx Asx Asx Ala Asp His Asp His Asp His Asp
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Asp Ala Ala His Asx Asp Asp Ala Ala Ala His Asp His Ala
  100  105  110
Ala His Ala Ala His Asx Ala Ala Asx Asp Ala His Asx Asp Ala
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  130  135  140
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  145  150  155  160
Asp Ala Asp Ala Asx Ala Ala Asp His Ala Asx Ala Ala His
  165  170  175
His His Asp His Asx His Ala Asx His Asp Asp His Asx Asp His
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His Ala Ala His Ala His Ala Ala Ala Ala Ala Ala Ala Ala
1475 1480 1485
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1490 1495 1500
Asx Asx Asp Asx His Asx Asp Ala Asx Asp Ala Ala Ala Ala
1505 1510 1515 1520 1525

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 479 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr Ile Ser Tyr
1 5 10 15
Leu Met Phe Gly Ala Ala Ile Tyr Tyr His Ile Glu His Gly Glu Glu
20 25 30
Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Glu Ile Ala Ile Asn Glu
35 40 45
Tyr Leu Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr Gln Asp Glu
50 55 60
Ile Leu Gln Arg Ile Ser Asp Tyr Cys Asp Lys Pro Val Thr Leu Pro
65 70 75 80
Pro Thr Tyr Asp Thr Pro Tyr Thr Trp Phe Tyr His Ala Phe
85 90
Phe Phe Ala Phe Thr Val Cys Ser Thr Val Gly Tyr Gly Asn Ile Ser
100 105 110
Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr Ser Val Ile
115 120 125
Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly Glu Tyr Phe
130 135 140
Gly Arg Thr Phe Glu Ala Ile Tyr Arg Arg Tyr Lys Tyr Lys Met
145 150 155 160
Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu Ile Thr Thr
165 170 175
Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu Val Leu Pro
180 185 190
Cys Val Gly Val His Leu Leu Arg Glu Leu Gly Leu Ser Ser Ile Ser
195 200 205
Leu Tyr Tyr Ser Tyr Val Thr Ile Thr Thr Ile Gly Phe Gly Asp Tyr
210 215 220
Val Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Gly Gly Trp Phe 225 230 235 240
Val Val Tyr Gln Ile Phe Val Ile Val Trp Phe Ile Phe Ser Leu Gly 245 250 255
Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu Gln Ser Lys 260 265 270
Lys Leu Ala Tyr Leu Glu Gln Gln Leu Ser Ser Asn Leu Lys Ala Thr 275 280 285
Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val Gly Tyr Leu Arg 290 295 300
Arg Met Leu Asn Glu Leu Tyr Ile Leu Lys Val Lys Pro Val Tyr Thr 305 310 315 320
Asp Val Asp Ile Ala Tyr Thr Leu Pro Arg Ser Asn Ser Pro Leu Ser 325 330 335
Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser Arg Lys Arg Ala Phe 340 345 350
Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg Glu Ala Gly Met Val 355 360 365
His Ala Asn Ser Asp Thr Asp Leu Thr Lys Leu Asp Arg Glu Lys Thr 370 375 380
Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr Asp Leu Leu Ala Lys 385 390 395 400
Val Val Asn Ala Leu Ala Thr Val Lys Pro Pro Pro Ala Leu Gln Glu 405 410 415
Asp Ala Ala Leu Tyr Gly Gly Tyr His Gly Phe Ser Asp Ser Gln Ile 420 425 430
Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn Glu Phe Thr Ser Pro 435 440 445
Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe Asn Leu Glu Ala Pro 450 455 460
Arg Trp Gln Ser Glu Arg Pro Leu Arg Ser Ser His Asn Glu Trp 465 470 475

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 335 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
Met Ser Asp Gln Leu Phe Val Ala Phe Glu Lys Tyr Phe Leu Thr Ser 1 5 10 15
Asn Glu Val Lys Lys Asn Ala Ala Thr Glu Thr Trp Thr Phe Ser Ser 20 25 30
Ser Ile Phe Phe Ala Val Thr Val Val Thr Thr Ile Gly Tyr Gly Asn 35 40 45
Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Ile Leu Phe Ser Leu
55
Ile Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Leu Ala Gly Lys Phe
70
Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys Leu Lys
85
Tyr Leu Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His Val Cys
105
Glu His Cys His Ser His Gly Met Gly His Asp Met Asn Ile Glu Glu
115
Lys Arg Ile Pro Ala Phe Leu Val Leu Ala Ile Leu Ile Val Tyr Thr
130
Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser Phe Phe
145
Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly
160
Asp Leu Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu Leu Tyr
175
Ile Ile Leu Gly Lys Phe Ser Met Lys Lys Gln Lys Phe Lys Ile
190
Phe Leu Gly Leu Ala Ile Thr Thr Met Cys Ile Asp Leu Val Gly Val
205
Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln Asp Ala
220
Arg Ser Ala Leu Ala Val Gly Gly Lys Val Val Leu Val Ser Glu
235
Leu Tyr Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser Arg Glu
250
Ala Phe Ile Val Glu Asn Leu Tyr Val Ser Lys His Ile Ile Pro Phe
265
Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr Ile Asp Gln Thr Ala Asp
280
Ala Ala Thr Ile Ser Thr Ser Ser Ser Ala Ile Asp Met Gln Ser Cys
305
Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys Xaa
330
What is claimed is:

1. A potassium channel comprising four hydrophobic domains capable of forming transmembrane helices, wherein
   (i) a first pore-forming domain is interposed between a first and a second transmembrane helix; and
   (ii) a second pore-forming domain is interposed between a third and a fourth transmembrane helix.

2. The potassium channel of claim 1 wherein each pore-forming domain comprises a potassium selective peptide motif.

3. The potassium channel of claim 2 wherein the peptide motif is selected from the group consisting of a Y/F-G dipeptide motif and a G-Y/F-G tripeptide motif.

4. The potassium channel of claim 3 wherein at least one pore-forming domain is positioned proximal to an exterior portion of a cell membrane.

5. The potassium channel of claim 4 further comprising an amino-terminal glycosylation site.

6. The potassium channel of claim 5 wherein said glycosylation site is asparagine-linked.

7. The potassium channel of claim 6 characterized in that it belongs to a class of invertebrates.

8. The potassium channel of claim 7 characterized in that
it is insect-derived.

9. The potassium channel of claim 7 characterized in that it is nematode-derived.

10. An isolated nucleotide sequence capable of encoding DmORF-1.

11. The isolated nucleotide sequence of Claim 10 comprising the nucleotide sequence depicted in Seq. I.D. No. 1.

12. An isolated nucleotide sequence capable of encoding CORK.

13. The isolated nucleotide sequence of Claim 12 encoding for the protein depicted in Sequence I.D. No. 36.

14. An expression vector capable of expressing a heterologous potassium channel in a cell membrane of a yeast cell comprising the nucleotide sequence of Claim 10.

15. An expression vector capable of expressing a heterologous, potassium channel in a cell membrane of a yeast cell comprising the nucleotide sequence of Claim 11.

16. An expression vector capable of expressing a heterologous potassium channel in a cell membrane of a yeast cell comprising the nucleotide sequence of Claim 12.

17. An expression vector capable of expressing a heterologous potassium channel in a cell membrane of a yeast cell wherein the potassium channel comprises the amino acid sequence of Claim 13.
18. A transformed yeast cell comprising the nucleotide sequences of Claims 10, 11, 12 or 13.

19. A transformed yeast cell comprising the expression vector of claims 14, 15, 16 or 17.

20. A method of assaying substances to determine effects on cell growth, the method comprising the steps of:

a. preparing cultures of yeast cells in liquid medium lacking uracil, the liquid medium consisting of a concentration of KCl adequate to support growth of potassium-dependent mutant strains;

b. plating the yeast cells in uracil-free agar medium, the agar medium consisting of sufficient KCl to selectively support growth of potassium-dependent mutant strains containing a heterologous potassium channel of claim 1;

c. applying substances to the agar plate;

d. incubating the agar plate to permit growth; and

e. identifying zones of growth around the substances, wherein the level of growth indicates whether or not activity of the heterologous potassium channel has been modulated as compared to control.

21. The yeast cell of Claim 20 further comprising a nucleotide sequence encoding RAK, or a nucleotide sequence of Claim 10, 11, 12 or 13.

22. The method of claim 20, wherein said effect on cell

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growth is modulated by activation of the potassium channel.

23. The method of claim 20, wherein said effect on cell growth is modulated by inhibition of said potassium channel.

24. A method of selectively inhibiting insect pests by applying to such insect pests a substance capable of inhibiting a potassium channel substantially homologous to that encoded by the nucleotide sequence of claim 10.

25. A method of selectively inhibiting nematode pests by applying to such pests a substance capable of inhibiting a potassium channel substantially homologous to that encoded by the nucleotide sequence of claim 12.

26. A method of modulating the activity of a potassium channel positioned in a cellular membrane and comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 36, by contacting said cellular membrane with a substance, in an amount and for a period of time sufficient to inhibit the ability of potassium ions to pass through said channel.
SC galactose, 100 mM KCl

SC glucose, 0 mM KCl

SC galactose, 0 mM KCl

SC glucose, 100 mM KCl

FIG. 1
FIG. 2A
FIG. 3A
FIG. 3B
FIG. 4

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FIG. 5A
1) SHAKER

2) INWARD RECTIFIER

3) ORF1

FIG. 6
100 mM KCl

pORF1  pKAT1
pYES2  pRATRAK

0.2 mM KCl  0 mM KCl

FIG. 7
FIG. 8
230  Tyr His Tyr His Glu Lys Gly Met Glu Ile Arg Glu Lys Ala Glu Thr Asp Arg Pro Ser Pro Ser Ile Leu
TAC CAC TAT CAC CAT CAA AAA GGA ATG GAA ATT CGC GAA AAG GCG GAA ACC GAC AGA CCG TCT CCA TCC ATT CTT
250

260  Trp Thr Thr Phe Thr Asn Cys Tyr Gly Glu Leu Phe Asn Val Trp Phe Cys Phe Ala Val Thr Leu Thr Ile Phe
TGG ACC ACA TTC ACA AAC TGT TAT GGG CAA CTC TTC AAT GTT TGG TTC TGC TTT GCC GTT ACT CTC ACA ATC TTC
270

280  Pro Val Met Met Thr Val Thr Thr Arg Gly Asp Ser Gly Phe Leu Asn Lys Ile Met Ser Glu Asn Asp Glu Ile
CCT GTT ATG ATG ACC GTT ACC ACT CGT GGA GAT TCC GCC TTC CTA AAC AAA ATT ATG TCT GAA AAC GAT GAA ATC
300

310  Tyr Thr Leu Leu Thr Ser Phe Leu Val Phe Asn Leu Phe Ala Ala Ile Gly Ser Ile Val Ala Ser Lys Ile His
TAC ACT TTG CTC ACA AGT TTC CTC GTC TTC AAT TTG TTC GCT GCC GGC ATT GGA TCC ATA GGT TCC TCC AAG ATT CAC
320

330  Trp Pro Thr Pro Arg Tyr Leu Lys Phe Ala Ile Ile Leu Arg Ala Leu Phe Ile Pro Phe Phe Phe Cys Asn
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340

360  Tyr Arg Val Gln Thr Arg Ala Tyr Pro Val Phe Phe Glu Ser Thr Asp Ile Phe Val Ile Gly Gly Ile Ala Met
TAT CTG GTG CAG ACG CGT TAT CCT GCT TTC TTT GAG TCT ACT GAC ATT TTT GTG GAT GGA ATT GCC ATG
370

390  Ser Phe Ser His Gly Tyr Leu Ser Ala Leu Ala Met Gly Tyr Thr Pro Asn Val Val Pro Ser His Tyr Ser Arg
TCT TTT TCA CAT GGA TAC CTC AGC GCT CTG GCA ATG GAA TAC ACT CCA AAC GTC GTG CCA TCT CAC TAC AGA
400

410  Phe Ala Ala Glu Leu Ser Val Cys Thr Leu Met Val Gly Leu Leu Thr Gly Gly Leu Trp Pro Val Val Ile Glu
TTT GCC GCT CAG CTT TCC GTG ACT CTT ATG GTT GCC CTT CTC ACC GGT GGC CTG TGG CCC GTT GTT ATT GAG
420

430  His Phe Val Asp Lys Pro Ser Ile Leu
CAC TTC GTG GAC AAG CCA AGT ATC TTA TAA ATATTTATAGCATTAAGTATACATTGTTATATGGTTTTTTATTAAGCTGTGGAATAAA
434

ATAATTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 C12N15/81 C12N1/19 C12Q1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>BIOPHYS J, 63 (5). 1992. 1406-1411., MCCORMACK K ET AL 'TANDEM LINKAGE OF SHAKER POTASSIUM CHANNEL SUBUNITS DOES NOT ENSURE THE STOICHIOMETRY OF EXPRESSED CHANNELS' see the whole document</td>
<td>1</td>
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<tr>
<td>X</td>
<td>JOURNAL OF NEUROSCIENCE, 13 (11). 1993. 4669-4679., ZHONG Y ET AL 'Modulation of different K+ currents in Drosophila: A hypothetical role for the eag subunit in multimeric K+ channels' see the whole document</td>
<td>1</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:
  'A' document defining the general state of the art which is not considered to be of particular relevance
  'E' earlier document but published on or after the international filing date
  'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  'O' document referring to an oral disclosure, use, exhibition or other means
  'P' document published prior to the international filing date but later than the priority date claimed
  'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
  '*' document member of the same patent family

Date of the actual completion of the international search: 21 March 1996

Date of mailing of the international search report: 27 MARCH 1996

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340 2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer: Gurdjian, D

Form PCT/ISA-218 (second sheet) (July 1992)
<table>
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<tr>
<td>X</td>
<td>NATURE, vol. 345, 1990, pages 530-4, E.Y.ISACOFF ET AL. 'Evidence for the formation of heteromultimeric potassium channels in Xenopus oocytes'</td>
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<td>Y</td>
<td>see the whole document</td>
<td>20-26</td>
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<td>X</td>
<td>NATURE, vol. 368, March 1994, pages 32-38, R. WILSON ET AL. '2.2 mb of contiguous nucleotide sequence form chromosome III of c.elegans'</td>
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<td>Y</td>
<td>see abstract; table 2</td>
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<td>EP,A,0 615 976 (AMERICAN CYANAMID CO) 21 September 1994 see the whole document</td>
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<td>A</td>
<td>PROC NATL ACAD SCI U S A, 86 (12). 1989. 4372-4376 , KAMB A ET AL 'IDENTIFICATION OF GENES FROM PATTERN FORMATION TYROSINE KINASE AND POTASSIUM CHANNEL FAMILIES BY DNA AMPLIFICATION' see the whole document</td>
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<tr>
<td>A</td>
<td>US,A,5 356 775 (HEBERT STEVEN C ET AL) 18 October 1994 see the whole document</td>
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<td>P,X</td>
<td>NATURE (LONDON), 376 (6542). 1995. 690-695. KETCHUM K A ET AL 'A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem' see the whole document</td>
<td>1-13</td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **X** Claims Nos.: 23-26  
   because they relate to subject matter not required to be searched by this Authority, namely:
   
   **Remark:** Although claims 23-26 refer, at least partially as far it concerns a medical method, to a method of treatment of the human or animal body, the search has been carried out and has been based on the alleged effects of the composition.

2. **☐** Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. **☐** Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. **☐** As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. **☐** As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. **☐** As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. **☐** No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- **☐** The additional search fees were accompanied by the applicant's protest.
- **☐** No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
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