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(54) Title: POTASSIUM CHANNELS, NUCLEOTIDE SEQUENCES ENCODING THEM, AND METHODS OF USING SAME

(57) Abstract: This invention relates generally to a new family of potassium channels, whose molecular architecture is characterized by four membrane spanning domains and two putative pore forming domains. More particularly, the present invention relates to the cloning and characterization of mutants of this family of distinct transmembrane potassium ion channels which confer improved inward potassium flux under acidic conditions, characterization of such channels, newly identified polynucleotide sequences, polypeptides encoded by such sequences, expression vectors capable of heterologous expression of such polynucleotide sequences, transformed host cells containing the expression vectors, and assay methods and kits therefor for determining the expression of heterologous nucleotide sequences encoding all or a portion of said potassium channels in host cells, chromosome mapping, diagnostic methodologies and kits therefor.

POTASSIUM CHANNELS, NUCLEOTIDE SEQUENCES ENCODING THEM, AND METHODS OF USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Serial No. 08/816,011 filed on March 11, 1997, which is a continuation-in-part of co-pending application PCT/US95/14364, filed on October 25, 1995, which is a continuation-in-part of U.S. application Serial No. 07/332,312, filed on October 31, 1994, now U.S. Patent No. 5,559,026, issued September 24, 1996.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates generally to a new family of potassium channels. More particularly, the present invention relates to the cloning and characterization of a family of distinct trans-membrane potassium ion channels, characterization of such channels, newly identified polynucleotide sequences, polypeptides encoded by such sequences, expression vectors capable of heterologous expression of such polynucleotide sequences, transformed host cells containing the expression vectors and assay methods for determining the expression of heterologous nucleotide sequences encoding all or a portion of said potassium channels in host cells, chromosome mapping, diagnostic methodologies and kits therefor.

Genes encoding potassium channels representative of this family were cloned from *Drosophila melanogaster, Caenorhabditis elegans,* human and mouse ESTs, and human brain, heart, and kidney cDNA libraries. More particularly, the invention arises in part from the determination that the DNA sequences of these genes encode a structurally distinct potassium channel whose molecular architecture is characterized by four membrane spanning domains and two putative pore forming domains.

Summary of the Related Art

Ion channels, which include sodium (Na^+), potassium (K^+), and calcium (Ca^{++}), are present in both eukaryotic and prokaryotic cells and control a variety of

physiological and pharmacological processes. Potassium channels comprise a large and diverse group of integral membrane proteins that are involved in the movement of potassium into and out of the cell. Such channels regulate the level of excitability and repolarization properties of neurons and muscle fibers (B. Hille, *Ionic Channels of Excitable Membranes,* 2d Ed., Sinauer, Sunderland, MA (1992)), and are implicated in a broad spectrum of processes in both excitable and non-excitable cells. In almost all cells, K^+ channels play a role in determining the resting electrical membrane potential by setting the membrane permeability to K^+ ions. Potassium currents have been shown to be more diverse than sodium or calcium currents and play a role in determining the way a cell responds to external stimuli.

Several classes of K^+ channels have been identified based on their pharmacological and electrophysiological properties. These include voltage-gated, ATP-sensitive, muscarinic-activated, S type, SK Ca^{++}-activated, Na^+-activated, and inward and/or outward rectifier types of K^+ channels. Prior to this work, and on the basis of membrane-spanning segments, potassium channels may be subdivided into topologically distinct classes. For example, one well-known class of voltage-gated, calcium activated, and/or cyclic nucleotide-gated-channels is composed of six membrane scanning domains (S I -S6) one of which contains repeated positive charges presumed to be involved in the voltage sensing of these channels and hence in their functional outward rectification and a single pore forming domain (H5 or P region). A second class may be described as an inward rectifying potassium channel that passes through the cellular membrane twice and also contains 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); see also American Cyanamid copending U.S. patent application # 08/431,928 filed on 6/28/1995 for a description of "HIRK").

The best characterized class of K^+ channels are the voltage-gated outward rectifying channels (the K_v family), the prototype being the protein which is coded for by the Shaker gene seen in *Drosophila melanogaster,* which is a voltage-gated channel. The proteins in this gene family contain a structural motif characterized by six membrane spanning segments (S1-S6), a putative voltage sensor (S4), and an S5-

S6 linker (H5 or P region) involved in ion conductance. A functional channel is assembled in the membrane via the association of four Shaker subunits, necessitating the presence of four P domains.

Another well characterized class of potassium channel proteins, the inward rectifier potassium channels (K_{ir} family) play a significant role in maintaining the resting potential of, and in controlling the excitability of a cell. These channels are characterized by two transmembrane domains and a pore-forming region and the lack of an S4 or voltage sensing region. Inward rectifying K^+ channels are generally characterized by two transmembrane domains and one pore-forming domain. The pore-forming domain is common to both groups of K^+ channels, the voltage-gated outward rectifier groups and the inward rectifying K^+ channels and is an essential element of the aqueous K^+-selective pore. A functional channel is assembled in the membrane via the association of four K_{ir} subunits, necessitating the presence of four P domains.

A potassium channel from *Saccharomyces cerevisiae,* designated Tok1, (Ketchum *et al.,* Nature 376:690-695 (1995)) or YORK (Lesage *et al.*, J. Biol. Chem 271:4183-4187 (1996)) has recently been identified and is characterized by the presence of two pore (2P) domains and an outward rectifying K^+ -selective current which is coupled to potassium equilibrium (Ketchum et *al.,* Nature 376:690-695 (1995)). In contrast to the other channels described, the yeast channel comprises eight transmembrane domains, such domains resembling an assembly of an inward rectifying K^+ channel of the K_{ir} family (two transmembrane domains) with an outward rectifying channel of the K_v family (six transmembrane domains).

A channel with four transmembrane domains and two pore-forming regions has recently been described by the present inventor (Goldstein, S. et *al., Proc. Natl. Acad. Sci. USA* 93:13256-13261 (1996) - "DmORFl" (also referred to as ORK1 or DORK)). Other investigators have described additional members of this potassium channel family (Fink, M. *el al., EMBO J.* 150:6854-6862 (1996) - "TREK"; Lesage *et al.*, *EMBO J.*, 15:1004-1011 (1996) - "TWIK-1"; Lesage F. *et al., FEBS Lett.* 402:28-32 (1997)). It has also been postulated that eight potassium channel families have

been revealed by the *C. elegans* genome project, Wei A., *et al., Neuropharmacology* 35 No. 7, 805-829 (1996).

SUMMARY OF THE INVENTION

A first aspect of the present invention is the discovery of a new family of potassium channel genes and proteins encoded thereby. Potassium channels belonging to this new family 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 the channels further contain various potassium selective peptide motifs. In preferred embodiments, the channels contain a GXG motif in the first pore-forming region and preferably in both pore-forming regions, wherein X is an amino acid selected from the group consisting of Y, F, V, I, M, and L, and particularly L or I. The channels preferably contain a further peptide motif in the P_1 and/or P_2 pore-forming regions, spanning several amino acids upstream of GXG, and particularly for about six (6) amino acids upstream of the first G. Thus, the preferred pore-forming region motif is XXXXXXGXG (SEQ ID NO:65), where X at positions 1, 4, and 5 are preferably the amino acids residues T or S, and X at position 6 is preferably I or V, and X at position 8 is an amino acid selected from the group consisting of Y, F, V, I, M, and L, again, with the amino acid residues L or I particularly preferred.

In further preferred embodiments, the channels display yet a second peptide motif, XXXXGXPX (SEQ ID NO:66), wherein X at position 1 is the amino acid residue Y or F, and preferably Y, and X at positions 2, 3, 4, and 6 are amino acid residues, wherein residues at position 2 are A, S, or G, with A or S preferred, and X at positions 3, 4, 6, and 8 are the amino acid residues M, I, V, L, F, or Y, with L or I particularly preferred. In certain embodiments, this motif is "YALLGIP" (SEQ ID NO:67). This second peptide motif is located downstream of P_1 generally about 12-25 amino acids downstream, and preferably about 16 amino acids downstream of P_1.

In certain preferred embodiments, the isolation and characterization of invertebrate (i.e. insect and nematode) potassium channel genes belonging to this new

family is presented. In more preferred embodiments, the present invention further provides the isolation and characterization of polynucleotides from invertebrates and vertebrates, which encode amino acid sequence elements unique to this potassium gene family and specifically sourced from *Drosophila melanogaster, Caenorhabditis elegans,* avian libraries, murine and various other mammalian libraries, and libraries from all human tissues including human heart and brain.

In yet another preferred embodiment, the aforementioned channels are mutated so as to confer improved inward potassium flux under acidic conditions. Preferably, these mutations cluster around the second pore-forming domain. In particular, the mutations may arise at one or more of amino acid positions 256, 270, 272, and 274. Such mutations should preferably confer upon selected yeast host cells containing heterologous potassium channel expression plasmids the ability to grow on low pH, low potassium concentration medium. Such yeast host cells are unable to grow in medium containing low potassium concentration in the absence of expression of a heterologous potassium channel (CY 162 for example, see J.A. Anderson et al., *Proc. Natl. Acad Aci. USA* 89:3736-3740 (1992). Potassium channels of any type may be used, with TPCK1 being particularly preferred.

A third aspect of the present invention is a method of controlling nematode and insect pests by inhibiting or activating potassium channels substantially homologous to those encoded by nucleotide sequences as presented herein. Another aspect of the present invention is to influence and alleviate human disease states modulating membrane potential with therapeutic agents that interact with the potassium channels biologically equivalent to those encoded by nucleotide sequences as encoded herein.

Various screening assay embodiments are also presented herein as well as chromosome identification and mapping techniques, diagnostic methodologies and kits therefore, and transgenic animals.

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. CY 162 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 (Ml-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 and SEQ ID NO:4, respectively). 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 DmORFl.

FIGURE 7. Heterologous potassium channel-dependent growth of plasmid bearing CY162 (trkΔ) strains. CY162 bearing pYES2, pKAT1, pDmORFl, 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 KC1.

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 μl of a 1 M solution of potassium channel blocking compound. Clockwise from upper left-hand comer 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 and SEQ ID NO:74). 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.

Figure 10. Depicts a schematic representation of a preferred motif of the potassium channels of the invention.

Figures 11 A- 11 D. Depicts a biophysical analysis of TPKC1 expressed *in Xenopus laevis* oocytes. TPKC1 currents in *Xenopus* oocytes injected with TPKC1 cRNA were

measured by two-electrode clamp. Displayed are current traces measured at voltages adjusted stepwise from the -90 mV resting potential and the corresponding translation to an I/V Plot of current-voltage relationship. Additionally, current-voltage relationships for currents measured in ND96 containing 2, 5, 10, 50, or 96 mM KCl are depicted. Also, the figures indicate that TPKC1 confers potassium selective currents. Finally, current-voltage relationship for currents measured in the presence of 0.5 mM and 1 mM $BaCl_2$ are depicted.

DETAILED DESCRIPTION OF THE INVENTION

Nucleotide bases are abbreviated herein as follows:

Ade; A-Adenine G-Guanine Ura; U-Uracil

C-Cytosine; T-Thymine; Ino; I or N (Inosine -- bonds to any of the others)

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 nucleotide sequences, proteins, and other materials originating from organisms other than the host 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 the host organism.

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 term "channel" and the nucleotide sequences encoding same, is intended to encompass all potassium channels, and mutants, derivatives, homologs, and other variations thereof.

The term "EST" as used herein refers to an expressed sequence tag.

Here we report the cloning and functional expression of a novel family of potassium channels exhibiting a unique topological configuration, and demonstrating particular physiological characteristics. Potassium channels belonging to this family may be derived from a wide variety of animal species, both vertebrate and invertebrate. This family is structurally and functionally novel, as manifested by the presence of two-pore forming domains (2P) in conjunction with a four membrane spanning domain configuration. Nucleotide sequences encoding various representative members of this new family of two-pore K^+ channels were cloned by expression in yeast cells from *Drosophila melanogaster* (dORK or DmORF), and also by degenerate PCR from human brain, heart, and kidney cDNA (TPKC1), and from human and mouse ESTs. Preliminary analyses of expression by a Northern blotting procedure indicates that TPKC1 is present primarily in human brain. Genes encoding structural homologues are present in the genome of *Drosophila melanogaster* (dORK), *Caenorhabditis elegans* (cORK), avian tissue, and various mammalian tissue such as human (TPKC1) and murine.

The potassium channel family of the present invention may be structurally 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 the first helix and the second helix, and the other of which is interposed between the third helix and the fourth helix. While the present inventor does not wish to be bound by theory, it is hypothesized that the 2P channels organize as dimers in the plasma

membrane, consistent with a requirement for four (4P) domains to form a functional channel. 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 more preferred embodiments, the motif comprises GXG, wherein X is an amino acid selected from the group consisting of V, L, Y, F, M, and I, and preferably L or I, such motif generally being found between the first two transmembrane domains. In certain other motif configurations, a second GXG motif, wherein X is an amino acid selected from the aforementioned group, is found between the third and fourth transmembrane domain as well. The channels preferably contain a further peptide motif in the P_1 and/or P_2 pore-forming regions, spanning several amino acids upstream of GXG and particularly for about six (6) amino acids upstream of the first G. Thus, the preferred pore-forming region motif is XXXXXXGXG (SEQ ID NO:65), where X at positions 1, 4, and 5 are preferably the amino acids residues T or S, and X at position 6 is preferably I or V, and X at positions 2, 3, and 8 is an amino acid selected from the group consisting of V, L, Y, F, M, and I, again, with the amino acid residues L or I particularly preferred.

In yet further embodiments, the potassium channels of the invention comprise a second peptide motif, which in terms of the DNA encoding it, is located downstream of the first GXG motif, and within the second transmembrane domain (see Figure 13 for a schematic depiction). This is the XXXXGXPX (SEQ ID NO:66) motif wherein X at position 1is the amino acid residue Y or F, and preferably Y, and X is an amino acid residue wherein X at position 2 is A, S, or G, with A or S preferred, and X at positions 3, 4, 6, and 8 are the amino acid residues M V, L, F, or Y, with L or I particularly preferred. In other embodiments, the preferred XXXXGXPX (SEQ ID NO:66) motif is flanked by the first GXG motif (that is located between the first and second transmembrane domain) and is located in the second transmembrane, and a second pore-forming peptide motif is located downstream of the first pore-forming motif, between the third and fourth transmembrane domains. In preferred

embodiments, the preferred XXXXGXPX (SEQ ID NO:66) motif is located downstream of the first pore-forming peptide motif by about 12-25 amino acids. In other preferred embodiments the first pore-forming peptide motif is within about 16 amino acids. In general, the topological configuration of the potassium channels of the invention is such that one may presume that a regulatory domain of indeterminate length often may be interposed between the second transmembrane domain (TM2) and the third transmembrane domain (TM3). Thus the size and characteristics of this domain may vary with cell type and needs, and is thereby a structure that is conducive to the conveyance of biological flexibility to the requirements and function of a particular cell. In certain embodiments, XXXXGXPX (SEQ ID NO:66) comprise the amino acids YALLGXP (SEQ ID NO:68), where X at position 6 is M, I, V, L, F, or Y, and particularly "YALLGIP" (SEQ ID NO:67).

In yet another preferred embodiment, the aforementioned channels are mutated so as to confer improved inward potassium flux under acidic conditions. Preferably, these mutations cluster around the second pore-forming domain. In particular, the mutations may arise at one or more of amino acid positions 256, 270, 272, and 274. In certain embodiments, the mutation at amino acid position 256 can be a substitution of T for the wild type A (SEQ ID NO:57). In yet another embodiment, the mutation can be at position 272 alone, wherein H is substituted for the wild type Y (SEQ ID NO:58), or that substitution can be coupled with a substitution at position 274 of V for the wild type A (SEQ ID NO:59). And yet a further embodiment is a substitution at position 270 of R for the wild type G (SEQ ID NO:60). Such mutations should preferably confer upon selected yeast host cells containing heterologous potassium channel expression plasmids the ability to grow on low pH, low potassium concentration medium.

In another preferred embodiment, the two pore potassium channels described above are mutated so as to confer improved inward potassium flux under acidic conditions. Preferably, these mutations cluster around the second pore-forming domain at amino acids 256, 270, 272, and 274.

In other embodiments, the potassium channels of the present invention further comprise a glycosylation site. This site may be an amino-terminal glycosylation site and may also be asparagine-linked.

The potassium channels of the present invention possess certain 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. However, a hallmark of the potassium channels of the invention are that they exhibit either outward current rectification or both inward and outward current rectification, in each case affected by potassium concentration.

Potassium channels play an essential role in determining the resting electrical membrane potential by setting the membrane permeability to K^+ ions. The cloned 2P channels confer potassium selective currents when expressed in *Xenopus* oocytes. The dORK channels encode instantaneous open-pore channel activity. Thus, the potassium ions flow either into or out of the cell, depending on the magnitude and direction of the electrochemical driving force. In contrast, the human 2P channel designated herein as TPKC1, is functionally distinguishable from dORK in that the TPKC1 channel permits potassium flow primarily in an outward direction. Even when external potassium concentration is raised to the point where the electrochemical potential will drive potassium flux into oocytes containing dORK, little inward potassium current observed in TPKC1-containing oocytes.

When expressed in yeast host cells that require heterologous potassium channel expression for survival on low potassium medium, the dORK and TPKC1 potassium channels exhibit distinguishable growth promoting properties. Yeast host cells of this type containing dORK are able to grow on low potassium medium, likely as a manifestation of the ability of the dORK potassium channel to promote potassium ion flow into the yeast cell. Lacking the capacity to promote efficient inward potassium ion flux, the TPKC1 channel fails to support the growth of the yeast host cells. This failure certain potassium channels to promote growth of the yeast host cells limits the usefulness of the potassium channels and the expression system for use in high-throughput screening applications. However, if modified potassium channel

proteins that can support the growth of the yeast host cells can be obtained by mutating their genes and phenotypically selecting for growth on low potassium and/or low pH medium, then the modified potassium channels and expression system would be more useful as a drug discovery tool.

It will be understood by those skilled in the art that the invention is not limited to the specific nucleotide and amino acid sequences depicted in the Sequence Listing, but also includes sequences that hybridize to such depicted sequences. Further, the invention also encompasses modifications to the depicted sequences, such as deletions, insertions, or substitutions in the sequence which produce changes in the resulting protein molecule that are not detrimental to the protein's activity. For example alterations in the gene sequence which reflect the degeneracy of the genetic code, or which result in the production of a biologically equivalent amino acid at a given site, are contemplated. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may substituted by a codon encoding another less hydrophobic residue such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product. One skilled in the art will understand that assembly of 2P channel into functional dimers may require disulfide formation, and should take that into consideration when making modifications as taught herein (see *e.g.*, Lesage *et al.*, *EMBO J.* 15:6400-6407 (1996)). In some cases, it may in fact be desirable to make mutants of the sequence in order to study the effect of alteration on the biological activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of the retention of biological activity of the encoded products.

The present invention further provides functional derivatives of the nucleotide sequences encoding the potassium channels of the invention. As used herein, the term "functional derivative" is used to define any DNA sequence which is derived from the original DNA sequence and which still possesses at least one of the biological

activities present in the parent molecule. A functional derivative can be an insertion, deletion, or a substitution of one or more bases in the original DNA sequence.

Functional derivatives of the nucleotide sequences as presented herein, having an altered nucleic acid sequence can be prepared by mutagenesis of the DNA. For example, preparation of functional derivatives may be achieved by random mutagenesis. Random mutagenesis allows the production of functional derivatives through the use of mutator *E. coli* strains (e.g., XL1Red (Stratagene)) which introduce mutations during cloning and amplification of expression plasmids. This can be accomplished using one the mutagenesis procedures known in the art. For example, preparation of functional derivatives may be achieved by site-directed mutagenesis. Site-directed mutagenesis allows the production of functional derivatives through the use of a specific oligonucleotide which contains the desired mutated DNA sequence. Site-directed mutagenesis typically employs a phage vector that exists in both a single-stranded and double-stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M 13 phage, as disclosed by Messing *et al., Third Cleveland Symposium on Macromolecules and Recombinant DNA*, Editor A. Walton, Elsevier, Amsterdam (1981), the disclosure of which is incorporated herein by reference. These phage are commercially available and their use is generally well known to those skilled in the art. Alternatively, plasmid vectors containing a single-stranded phage origin of replication (Veira *et al., Meth. Enzymol.* 153:3 (1987)) may be employed to obtain single-stranded DNA.

While the site for introducing a sequence variation is predetermined, the mutation *per se* need not be predetermined. For example, to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at a target region and the newly generated sequences can be screened for the optimal combination of desired activity.

Biologically equivalent refers to those modified nucleic acid and amino acid sequences in which the modified sequence at least substantially maintains the biological activity of the unmodified sequence; i.e., in the case of a nucleic acid sequence, the protein expressed therefrom at least substantially maintains the biological activity. Thus, the present invention also relates to the biologically

equivalents of the potassium channel proteins whether specifically modified as described above or other isolated proteins. Biologically equivalent as used herein means protein having some homology with the TPCK1 protein, wherein such protein maintains all or substantially all of the biological activity of the TPCK1 protein, and contain the pore-forming peptide motif and, preferably, also the XXXXGXPX (SEQ ID NO:66) motif. The percentage of homology can vary from at least about 20% up to about 99.95%. Certainly percentage homologies of at least about 40%, at least about 70%, at least about 90%, or at least about 95% can be employed based on the retention of biological activity. One skilled in this art will note that forty percent (40%) homology at amino acid level is usually consistent with retention of comparable 2°and 3°structure amongst homologs.

It is difficult to predict the exact effect of the substitution, deletion, insertion, or other modification in advance of making same, or to determine a suspected biological equivalent or functional derivative. However, one skilled in the art will recognize that the functionality of the modified construct or the suspected biological equivalent or functional derivative can be evaluated by routine screening assays. As one example, mRNA encoded by a functional derivative made by site-directed mutagenesis can be injected into an oocyte as described in the EXAMPLES and the oocyte tested for channel activity. Other target constructs may also be tested in this manner.

Any eukaryotic organism can be used as a source for a protein which is a member of the potassium channel family as described herein, or the genes encoding same, so long as the source organism naturally expresses such a protein or contains genes encoding same. As used herein, "source organism" refers to the original organism from which the amino acid or DNA sequence of the protein is derived, regardless of the organism the protein is expressed in and ultimately isolated from. For example, a member of the TPCK1 family of channel proteins expressed in hamster cells, yeast cells, or the like, is of human origin as long as the amino acid sequence is that of a human protein which is a member of this family.

A variety of methodologies known in the art can be utilized to obtain a member of this family of channel proteins. In one method, the protein is purified from

tissues or cells which naturally produce the protein. One skilled in the art can readily follow known methods for isolating proteins in order to obtain a member of the protein family, free of natural contaminants. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immunoaffinity chromatography.

The invention provides further methods of obtaining other members of this novel family of potassium channels, i.e., those sharing significant homology to one or more regions of the proteins described herein. Specifically, by using the sequences disclosed herein as probes or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain other members of the family of potassium channel proteins as well as genomic sequences encoding such additional family members.

Region specific primers or probes derived from any of the sequences in the Sequence Listing can be used to prime DNA synthesis and PCR amplification, as well as to identify colonies containing cloned DNA encoding a member of this family using known methods.

When using primers derived from one of the nucleotide sequences for amplification, one skilled in the art will recognize that by employing high stringency conditions, annealing at 50°-60° C, sequences which are greater than 75% homologous to the primer will be amplified. By employing lower stringency conditions, annealing at 35°-37° C, sequences which are greater than 40-50% homologous to the primer will be amplified.

When using DNA probes derived from one of the nucleotide sequences colony/plaque hybridization, one skilled in the art will recognize that by employing high stringency condition, hybridization at 50°-65° C, 5X SSPC, 0-50% formamide, wash at 50°-65° C, 0.5X SSPC, sequences having regions which are greater than 90% homologous to the probe can be obtained, and by employing lower stringency conditions, hybridization at 35°-37° C, 5X SSPC, 40-45% formamide, wash at 42°C, SSPC, sequences having regions which are greater than 35-45% homologous to the probe will obtained.

Any tissue can be used as the source for the genomic DNA or RNA encoding members of the TPCK1 family of potassium channels. However, with respect RNA, the most preferred source is tissues which express elevated levels of the desired potassium channel family member. However, using the sequences as taught herein, it is now possible to identify such cells using the dORK, cORK, or TPCK1 sequence as a probe in Northern blot or in situ hybridization procedures, thus eliminating the necessity to obtain RNA/DNA from a tissue which expresses elevated levels of such protein.

Genes encoding the potassium channels of the present invention may be expressed in a recombinant host. 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 relate 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 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. The novel nucleic acid sequences of the invention and fragments thereof can be used to express protein in a variety of host cells, both prokaryotic and eukaryotic. Examples of suitable eukaryotic cells include mammalian cells, plant cells, yeast cells, and insect cells. Suitable prokaryotic hosts include *Escherichia coli* and *Bacillus subtilis.* Illustrative of conventional mammalian host cells are chinese hamster ovary (CHO) cell COS cells, human embryonic kidney cells, NIH3T3 fibroblasts, and mouse Ltk cells. Illustrative of insect cells are SP9 cells.

Suitable expression vectors are selected based upon the choice of host cell. Numerous vectors suitable for use in transforming host cells are well known. For example, plasmids and bacteriophages, such as λ phage, are the most commonly used vectors for bacterial hosts, and for *E. coli* in particular. In both mammalian and insect cells, plasmid and virus vectors are frequently used to obtain expression of exogenous DNA. In particular, mammalian cells are commonly transformed with conventional viral vectors, or transfected with plasmids, such as the pcDNAI vector series from Invitrogen Corporation (San Diego, CA) and the pMAM vector series from Clontech, and insect cells in culture may be transformed with baculovirus expression vectors. Yeast vector system include yeast centromere plasmids, yeast episomal plasmids, and yeast integrating plasmids. The invention encompasses any and all host cells transformed or transfected the claimed nucleic acid sequences or fragments thereof, as well as expression vectors used to achieve this.

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. *Saccharoniyces 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 (CY 162, for example, see J.A. Anderson *et al., 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 polyadenylation and transcription termination, and a selectable marker gene. An exemplary plasmid is Yrp7, (Stinchcomb *et al., Nature* 282:39 (1979); Kingsman *et al., Gene* 7:141 (1979); Tschemper *et al., Gene* 10:157 (1980)). 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 (Yep52), 3 -phosphoglycerate kinase (pPGKH, Hitzeman et al., *J. Biol. Chem.* 255:2073 (1980)) or other glycolytic enzymes (pYSKI53, Hess *et al., J. Adv. Enzyme Reg.* 7:149 (1968); and Holland *et al., Biochemistry* 17:4900 (1978)), 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. Hitzeman *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), isocytochrome 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 certain embodiments, the nucleic acid sequences of the invention are used to express proteins in a bacterial host. Protein expressed in bacteria can be used in raising antisera (both polyclonal and monoclonal) by standard methodology. Such antibodies are useful in immunohistochemical studies to determine the level of expression of the channel protein in various tissues and cell lines. The channel can be purified from bacterial cells if found in inclusion bodies, for example, by isolation of inclusion bodies by standard techniques, followed by electrophoresis in SDS-PAGE gels and isolation of the protein band from the gel. Alternately, the potassium channel proteins, or portions thereof, can be expressed as a fusion protein, e.g., with glutathione-s-transferase, or maltose binding protein, and then purified by isolation of the protein to which it is fused. In additional embodiments of the invention, the predicted amino acid sequence can be used to design synthetic peptides unique to the potassium channels as herein described, which peptides can then be used to raise antibodies to the channels.

The present invention further provides methods of identifying cells or tissues which express a member of the family of channel proteins presented herein. For example, a probe comprising a DNA sequence of hORK1, a fragment thereof, or a DNA sequence encoding another member of the TPKC1 family of channel proteins can be used as a probe or amplification primer to detect cells which express a message homologous to the probe or primer. One skilled in the art can readily adapt currently available nucleic acid amplification or detection techniques so that it employs probes or primers based on the sequences encoding a member of this family.

The materials for use in these embodiments are ideally suited for the preparation of a kit. Specifically, a kit is provided, which is compartmentalized to receive in close confinement, one or more containers which comprises: (a) a first container comprising one or more probes or amplification primers based on the

TPCK1 sequence or any of the other sequences, or simply a fragment containing nucleic acids that encode XXXXXXGXG (SEQ ID NO:65) and XXXXGXPX (SEQ ID NO:66); and (b) one or more other containers comprising one or more of the following: a sample reservoir, wash reagents, reagents capable of detecting presence of bound probe from the first container, or reagents capable of amplifying sequences hybridizing to the amplification primers.

A compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris buffers, etc.), and containers which contain the reagents used to detect the bound probe or amplified product.

Types of detection reagents include labeled secondary probes, or in the alternative, if the primary probe is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled probe. One skilled in the art will readily recognize that probes and amplification primers based on the sequence disclosed in the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

The sequences of the present invention are also valuable for chromosome identification. The sequence may be specifically targeted to and hybridize with a particular location on an individual chromosome, for example, the human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNA to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease, or tracking other possible disease pathways.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used rapidly to select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual chromosomes. Only those hybrids containing the gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clones to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 500 or 600 bases; however clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the large clones from which the cDNA was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms

In yet another embodiment of the present invention, a yeast expression system is described, wherein yeast cells bear heterologous potassium channels. Cloning and expression of potassium channels from heterologous species such as those described herein are useful in the 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. In preferred embodiments, these channels expressed in heterologous yeast cells are dORK, RAK (as described below), Shal, Shaw, Eag, cORK, or TPKC1. As noted above, transformed host cells of the present invention express the proteins or protein subunits 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 other preferred screening 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. Thus, in certain preferred screening embodiments of the present invention, a transformed yeast cell is presented, containing a heterologous DNA sequence which codes for a potassium channel, as herein presented, cloned into a suitable expression vector. Various other useful potassium channels may be utilized in the screening assay embodiments of the present invention, such as a delayed rectifier potassium channel referred to as "RAK or RATRAK" (Paulmichl *et al.*, *Proc. Natl. Acad. Sci, USA* 88:7892-7895 (1991), reporting the cloning of this potassium channel from rat cardiac tissue). RAK is capable of complementing the potassium-dependent phenotype of *Saccharomyces cerevisiae* strain CY 162 on medium containing low potassium concentration.

Using the purified proteins, or polypeptide sequences of the invention, the present invention provides methods of obtaining and identifying agents capable of binding to or otherwise interacting with the potassium channels of the invention.

In detail, the method comprises:

(a) contacting a substance with a select member of the family of potassium channels or select channel peptides or proteins; and

(b) determining whether the substance interacts with said channel, peptide, or protein.

The screened substances in the above assay can be, but are not limited to, proteins, peptides, peptidomimetics, carbohydrates, vitamin derivatives, compounds, or other pharmaceutical agents or any mixtures thereof. The substances can be selected and screened at random or rationally selected or designed using protein modeling techniques. As used herein, a substance is said to be "rationally selected or designed" when the substance is chosen based on the configuration of the particular member of the claimed family of channel proteins. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides. For example see Hurby *et al.*,

"Application of Synthetic Peptides: Antisense Peptides," In *Synthetic Peptides, A User's Guide,* W. H. Freeman, N.Y., 289-307 (1992), and Kaspczak *et al., Biochemistry* 28:9230-8 (1989). Pharmaceutical agents and the like may be similarly generated using techniques known to the art.

The present invention further provides methods for modulating the expression of TPCK1, or a member of the TPCK1 family of channel proteins. Specifically, anti-sense RNA expression is used to disrupt the translation of the mRNA encoding the TPCK1 protein.

In detail, a cell is modified using routine procedures such that if expresses an antisense mRNA, an mRNA which is complementary to mRNA encoding the TPCK1 family member. By constitutively or inducibly expressing the antisense RNA, the translation of the TPCK1 family member rnRNA can be regulated.

In certain preferred embodiments, the cloning of the members disclosed herein now makes possible the screening capability which enables the identification of agonists (potassium channel openers) and antagonists (potassium channel closers) of this family of channel proteins. The two-pore K^+ channels described herein in humans can be used as targets for novel human therapeutics. The primary target for such therapeutic agents will be conditions related to alterations in the plasma membrane resting potential and/or the duration of the action potential in excitable cells. Potassium channels influence action waveforms and firing frequency of cells and therefore play a role in neuronal integration, muscle contraction, and hormone secretion in excitable cells. Potassium channels play the vital role of determining resting electrical membrane potential by setting membrane permeability to potassium ions in the cell. Inward conductance at membrane potentials below K^+ equilibrium potential (E_k) prevents excessive hyperpolarization which may be caused by the electrogenic Na^+ pump; the slight outward conductance of inward rectifier K^+ channels at membrane potentials just above K^+ equilibrium helps to keep the resting membrane potential close to E_k. Modulation of the conductance level of potassium channels changes the resting potential and alters the excitability of a cell; i.e. the activation of a particular type of inward rectifier K^+ channel has been shown to cause hyperpolarization of the cardiac pacemaker cells and slows the heartbeat. Thus,

modulation of potassium channels can occur when one provides to cells, agents capable of binding to the potassium channel proteins.

In the cardiovascular area, this class of potassium channels may be of use in the discovery of new agents for the treatment of atrial and ventricular arrhythmias, heart failure including associated arrhythmias and cardiac ischemia. The action of such agents would be effected through the modulation of the kinetics duration of the cardiac action potential.

Modulation of cardiac action potential by compounds that effect the behavior of potassium channels may be a useful treatment for serious heart conditions. 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 relationship. 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. Potassium channel openers may also function as smooth muscle relaxants, functioning a vasodilators, vasospasmolytics, and other smooth muscle spasmolytic. As vasodilators, these compounds have use as dilators of peripheral vasculature, coronary arteries, renal vasculature, cerebral vasculature, and mesenteric vasculature. As vasospasmolytics, these compounds have use in the treatment of coronary artery spasm, peripheral vascular spasm, cerebral vascular spasm and impotence. Other smooth muscle spasmolytics have use as bronchodilators, in the control of urinary bladder and gall bladder spasm, and in the control of esophageal, gastric, and intestinal smooth muscle spasm.

Potassium channel closers may function in the pancreas to enhance release of insulin, in the kidney as diuretics and renal epithelial anti-ischemic agents, as hypertensive agents for promoting vasoconstriction for use in hypotensive states as antiarrhythmic agents, and as agents for modifying cardiac muscle contractility.

Other uses for potassium channel agonists or antagonists include anticonfulsants, hair growth promoting agents, and agents effective in preventing or reducing skeletal muscle damage or fatigue.

Thus, in yet further preferred embodiments, methods of modulating cellular activity to provide therapeutic value are provided, by applying to a patient in need of such modulation, a substance capable of interacting with a potassium channel contained in the relevant cells of such patient and modulating the activity of same (a good example of which are cardiac cells, useful for cardiac modulation purposes). These 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 the 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 cell membranes having as an integral part of their structure, one or more potassium channels presented herein, and particularly those comprising the amino acid sequences of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:36, SEQ ID NO:46, 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."

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, among other things, known assays for measuring ion channel current flow. Such compositions may comprise conventional delivery/carrier systems, e.g., liposome or phospholipid encapsulation, water or saline solutions, polymeric compositions, and the like. 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*. The field of insecticides is

instructive for this purpose. For example, *in vivo* tests can be run 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 other 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 biologically equivalent thereto. In the most preferred embodiments, the inhibitor will inhibit the activity of the aforementioned potassium channel without inhibition of other, non-homologous or otherwise non-equivalent 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, said potassium channel comprising the amino acid sequence of SEQ ID NO:4, SEQ ID NO:36, or potassium channels biologically equivalent thereto.

The present invention further provides methods for generating chimeric or transgenic animals 1) in which the animal contains one or more exogenously supplied genes which are expressed in the same temporal and spatial manner as a member of the family of channel proteins as presented herein, or 2) in which such member of this family of channel proteins has been deleted of overexpressed. Such chimeric and transgenic animals are useful in the further elucidation of the mechanisms of potassium channel function as well as their effect an animal physiology. These transgenic and chimeric animals are produced by utilization of techniques which are well known and well described in the technical literature, *e.g.,* see U.S. Patent No. 5,434,340 and scientific references cited therein discussing, among other things, the

introduction of transgenes into the gumline of a non-human animal, herein incorporated by reference.

EXAMPLES

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

Using the yeast expression technology and other teachings as set forth herein, the present inventor has 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 *et al., 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 (Heginbotham *et al., 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 (Wilson *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. DNA sequences encoding a human putative two-pore potassium channel were cloned by polymerase chain reaction (PCR) from human brain cDNA. Degenerate oligonucleotides (5'and 3' oligo) used in the analysis were designed from a compilation of nucleotide sequences encoding the pore-forming domains of putative two pore potassium channels identified in a search of the GENBANK DNA sequence database.

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 (Heginbotham *et al*, *Science* 258:1152-1155, (1992)). These features form the basis of the designation of a new subfamily of potassium channels comprising DmORF1, CORK, CeORF1, TPCK1, and various other homologs. The particulars of this discovery is set forth in more detail below:

Recombinant expression library screening.

Saccharomyces cerevisiae strain CY 162 is described in Anderson, J.A. *el al., Proc. Natl. Acad. Sci. USA* 89:3736-3740 (1992). Growth of bacterial strains and plasmid manipulations are performed by standard methods (Maniatis T., Molecular Cloning, Cold Spring Harbor Laboratory Press, 1982). Media conditions for growth of yeast, isolation of plasmid DNA from yeast, and DNA-mediated transformation of yeast strains are as described in Rose M. D., Methods in yeast genetics, Cold Spring Harbor Laboratory Press, 1990. A multifunctional expression library constructed in pYES2 and containing cDNA made from 3rd instar male *Drosophila mclanogaster* mRNA is used as described in S.J. Elledge *et al., Proc. Natl. Acad. Sci USA* 88:1731-1735 (1991). A multifunctional expression library constructed in pYES2 and

containing cDNA made from mRNA obtained from all life stages of *Caenorhabditis elegans* is custom-made by Invitrogen Corporation.

Isolation of expression plasmids encoding heterologous potassium channels. CY162 cells are transformed with plasmid DNA from each library to give 3 x 10^6 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 M1 and M2, and M3 and M4. Each pore forming H5 domain contains the Y/F-G dipeptide motif required for potassium selectivity (Heginbotham *et al., Science* 258:1152-1155, (1992)).

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 *Coenorhabditis elegans* genome sequencing project (Wilson *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 (Heginbotham *et al, 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-H2- 1:

5' TCCATTTTCTTTGCCGTAACCGTCGTCACTACCATCGGATACGGTAATCCA 3' (SEQ ID NO:5).

F22b7.7-H2-2:

5'TCATTCTACTGGTCCTTCATTACAATGACTACTGTCGGGTTTGGCGACTTG 3' (SEQ ID NO:6).

The oligos were labeled at their 5' ends with ^{32}P using a 5'-end labelling kit according to the manufacturer's instructions (New England Nuclear). The labeled 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 the manufacturer's 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 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' *Hind*III and 3' *Xba*I 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 *Hind*III and *Xba*I 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 $BaCl_2$ but not by CsCl or TEA. KAT-dependent growth is blocked by $BaCl_2$, CsCl, and TEA. RATRAK-dependent growth is blocked by $BaCl_2$, 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 cm^2 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 (2µl 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 pDmORF1 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 DmORF1 was examined by expression in *X. laevis* oocyte. 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:
MPO23: ATAAAGCTTAAAAATGTCGCCGAATCGATGGAT (SEQ ID NO:22)

MPO24: AGCTCTAGACCTCCATCTGGAAGCCCATGT (SEQ ID NO:23)
The full length PCR product was cloned into corresponding sites in pSP64 poly A (Promega), forming pMP 147. Template DNA was linearized with *Eco*RI and RNA transcribed using the Message Machine (Arnbion) in vitro transcription kit according to the manufacturer's 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 MW. 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>NH_4,>Cs>Na>Li. Potassium currents were greatly attenuated by $BaCl_2$.

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 DrnORF1 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 mg) 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 *Xho*I fragment of DmORF1 was isolated from pDmORF1 and labeled with α-^{32}P dCTP using the Ready-to-Go kit (Pharmacia) according to the manufacturer's 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 ORR. Thus, the DmORF is expressed at all developmental stages in the life cycle of *D. melanogaster.*

EXAMPLE 11

Expression of the DrnORF1 acne 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 DmORF1 gene product in vitro using a TnT coupled transcription-translation kit (Promega) according to the manufacturer's 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 counts per minute 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 CY 162 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 DmOPF1, ^{86}Rb uptake studies were performed. Examination of the uptake of this potassium congener revealed important aspects of potassium uptake by DmORFl.

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 (10^8 cells/tube) containing ^{86}RbCl in Ca-MES buffer. The tubes were incubated at room temperature, samples filtered at various time intervals and counted. ^{86}Rb uptake into cells was displayed.

The high-affinity potassium uptake capacity encoded by DmORF1 permits high-affinity uptake of the potassium Congener, ^{86}Rb, as well. Barium inhibited ^{86}Rb uptake. 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' *Hind*III or *Kpn*I, sites and 3' *Xba*I, *Sph*I, or *Xho*I sites:

Shaker 5': AAAAAGCTTAAAATGGCACACATCACG (SEQ ID NO:24)

Shaker 3': AAACTCGAGTCATACCTGTGGACT (SEQ ID NO:25)

Shab 5': AAAAAGCTTAAAATGGTCGGGCAATTG (SEQ ID NO:26)

Shab 3': AAAAGCATGCTCATCTGGATGGGCA (SEQ ID NO:27)

Shal 5':AAAAAGCTTAAAATGGCCTCGGTCGCC (SEQ ID NO:28)
Shal 3':TTTTCTAGACTACATCGTTGTCTT (SEQ ID NO:29)

Shaw 5': AAAAAGCTTAAAATGAATCTGATCAAC (SEQ ID NO:30)
Shaw 3': AAATCTAGATTAGTCGAAACTGAA (SEQ ID NO:31)

Eag 5': AAAAAGCTTAAAATGCCTGGCGGA (SEQ ID NO:32)
Eag 3': AAATCTAGAGGCTACAGGAAGTCC (SEQ ID NO:33)

Slo 5': GGGGGTACCAAAATGTCGGGGTGTGAT (SEQ ID NO:34)
Slo 3': TTTTTCTAGATCAAGAGTTATCATC (SEQ ID NO:35)

Plasmids used as templates for the PCR reactions were: pBSc-DShakerH37, pBSc-dShabl1, pBSc-dShal2+(A)36, pBScMXT-dShaw (A. Wei *et al., Science* 248:599-603 (1990), provided by L. Salkoff), pBScMXT-slo,v4 (Atkinson *el al., Science* 253:551 - 555, (1991), provided by L. Salkoff), and pBIMCH20 Eag (CH20) (Warmke *et al.*, *Science* 252:1560-1564 (1991), Bruggemarin *et al., Nature* 365:445-448 (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 0.1 M 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 anhelmenthic 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 (Inteligenetics) 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 (SEQ ID NO:36). 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. 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 tripeptide motif required for potassium selectivity (Heginbotham *et al*, *Science* 258:1152-1155, (1992)).

EXAMPLE 15

Cloning of the Human Two-Pore Potassium Channel Sequence: TPKC1.

Materials and Methods

DNA sequences encoding a human putative two-pore potassium channel were cloned by polymerase chain reaction (PCR) from human brain cDNA. Degenerate

oligonucleotides (5'and 3' oligo) used in the analysis were designed from a compilation of nucleotide sequences encoding the pore-forming domains of putative two pore potassium channels identified in a search of the GENBANK DNA sequence database.

Oligos used in degenerate PCR cloning approach were:

5' oligo: 5' TIG GAT (AT)(CT)G G(AT)G A(CT)(AT) T (SEQ ID NO:39)

3' oligo: 5' (AG)TC (AT)CC (AG)(AT)A (ACT)CC (AGT)A(CT) (AGT)GT (SEQ ID NO:40)

Clontech QUICK-Clone human brain cDNA was used as template (1 ng cDNA in 20µl reaction) in a reaction mixture containing 1.25 U ArnpliTaq DNA Polymerase (Perkin-Elmer), 1 µM primers, 200 µM dNTPs. PCR was carried out by standard procedures using the cycles given below in a Perkin-Elmer 9600 thermocycler.

PCR:	94° 2'	1 cycle
	94° 30"	
	48° 30"	35 cycles
	60" ramp to 72°	
	72° 30"	
	72°10'	

The resulting PCR fragments were cloned into the Invitrogen TA cloning kit according to the manufacturer's instructions. The cloned DNA fragments were sequenced with ABI Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit on the AB1373 Automated DNA sequencer according to the manufacturer's instructions. One fragment contained a 339 base pair (bp) open reading frame (ORF) with two consensus pore forming domains separated by two putative transmembrane domains. In order to clone the complete DNA sequence encoding TPKC1, fragments

corresponding to 5' and 3' sequences were isolated from fetal brain Marathon Ready cDNA (Clontech) using a rapid analysis of cDNA ends (RACE) procedure according to the manufacturer's instructions. The oligos used to clone 5' and 3' fragments were defined by the DNA sequence encoding the ORF, allowing for a 150 bp overlap between 5' and 3' fragments.

Oligos used in the RACE procedure:

for 5' fragmentCGC AGG CAG AGC CAC AAA GAG TAC ACA G (SEQ ID NO:41)
for 3' fragmentGGA GAT CAG CTA GGC ACC ATA TTT GG (SEQ ID NO:42)

A 1060 bp 5' fragment was isolated which, after DNA sequence analysis, was found to contain a 208 bp 5' untranslated region (UTR) and 852 bp ORF encoding 284 amino acids. Similarly, a 2000 bp 33' fragment was isolated which contained a 432 bp ORF capable of encoding an additional 144 amino acids along with an extensive 3'UTR. A DNA fragment containing the complete TPKC1 ORF sequence was generated by PCR-mediated fusion of the 5' and 3' fragments. The isolated 5' and 3' fragments were added together to a PCR reaction mixture containing oligos corresponding to 14 nucleotides upstream of the ATG and the first 12 nucleotides of the ORF and the complement of the 20 nucleotides after the stop codon.

Oligos used to clone the complete TPKC1 ORF:
5' ATG CTG CAT GCC TCA TGC TTC CCA GC (SEQ ID NO:43)
3' GGT TAT TTA AAG AGA GGG CT (SEQ ID NO:44)

The full length TPKC1 ORF fragment was isolated and cloned into the Invitrogen TA cloning kit according to the manufacturer's instructions. DNA sequence analysis confirmed the presence of a single ORF sufficient to encode a protein of 426 amino acids. The complete amino acid and DNA sequences are as follows:

MLPSASRERPGYRAGVAAPDLLDPKSAAQNSKPRLSFSTKPTVLASRVESDTT
INVMKWKTVSTIFLVVVLYLIIGATVFKALEQPHEISQRTTIVIQKQTFISQHSCV
NSTELDELIQQIVAAINAGIIPLGNTSNQISHWDLGSSFFFAGTVITTIGFGNISPR
TEGGKIFCIIYALLGIPLFGFLLAGVGDQLGTIFGKGIAKVEDTFIKWNVSQTKIR
IISTIIFILFGCVLFVALPAIIFKHIEGWSALDAIYFVVITLTTIGFGDYVAGGSDIE
YLDFYKPVVWFWILVGLAYFAAVLSMIGRLVRVISKKTKEEVGEFRAHAAEW
TANVTAEFKETRRRLSVEIYDKFQRATSIKRKLSAELAGNHNQELTPCRRTLS
VNHLTSERDVLPPLLKTESIYLNGLAPHCAGEEIAVIENIK (SEQ ID NO:45)

ccatcctaatacgactcactatagggctcgagcgnccgcccgggcagtaaaatgcctgcccgtgcagctcggagcgcgc
agcccgtctctgaataagaagtgagtacaatggcgtgtttgtaaaaaaaagcttcaagtccgtctttttcaaaaaacattttgaa
tgctgcatgcctcATGCTTCCCAGCGCCTCGCGGGAGAGACCCGGCTATAGAGCA
GGAGTGGCGGCACCTGACTTGCTGGATCCTAAATCTGCCGCTCAGAACTC
CAAACCGAGGCTCTCATTTTCCACGAAACCCACAGTGCTTGCTTCCCGGGT
GGAGAGTGACACGACCATTAATGTTATGAAATGGAAGACGGTCTCCACGA
TATTCCTGGTGGTTGTCCTCTATCTGATCATCGGAGCCACCGTGTTCAAAG
CATTGGAGCAGCCTCATGAGATTTCACAGAGGACCACCATTGTGATCCAG
AAGCAAACATTCATATCCCAACATTCCTGTGTCAATTCGACGGAGCTGGA
TGAACTCATTCAGCAAATAGTGGCAGCAATAAATGCAGGGATTATACCGT
TAGGAAACACCTCCAATCAAATCAGTCACTGGGATTTGGGAAGTTCCTTCT
TCTTTGCTGGCACTGTTATTACAACCATAGGATTTGGAAACATCTCACCAC
GCACAGAAGGCGGCAAAATATTCTGTATCATCTATGCCTTACTGGGAATT
CCCCTCTTTGGTTTTCTCTTGGCTGGAGTTGGAGATCAGCTAGGCACCATA
TTTGGAAAAGGAATTGCCAAAGTGGAAGATACGTTTATTAAGTGGAATGT
TAGTCAGACCAAGATTCGCATCATCTCAACAATCATATTTATACTATTTGG
CTGTGTACTCTTTGTGGCTCTGCCTGCGATCATATTCAAACACATAGAAGG
CTGGAGTGCCCTGGACGCCATTTATTTTGTGGTTATCACTCTAACAACTAT
TGGATTTGGTGACTACGTTGCAGGTGGATCCGATATTGAATATCTGGACTT
CTATAAGCCTGTCGTGTGGTTCTGGATCCTTGTAGGGCTTGCTTACTTTGCT
GCTGTCCTGAGCATGATTGGGAGATTGGTCCGAGTGATATCTAAAAAGAC
AAAAGAAGAGGTGGGAGAGTTCAGAGCACACGCTGCTGAGTGGACAGCC

AACGTCACAGCCGAATTCAAAGAAACCAGGAGGCGACTGAGTGTGGAGA
TTTATGACAAGTTCCAGCGGGCCACCTCCATCAAGCGGAAGCTCTCGGCA
GAACTGGCTGGAAACCACAATCAGGAGCTGACTCCTTGTAGGAGGACCCT
GTCAGTGAACCACCTGACCAGCGAGAGGGATGTCTTGCCTCCCTTACTGA
AGACTGAGAGTATCTATCTGAATGGTTTGGCGCCACACTGTGCTGGTGAA
GAGATTGCTGTGATTGAGAACATCAAATAGccctctctttaaataaccttaggcatagccatag
gtgaggacttctctatgctctttatgactgttgctggtagcatttttaaattgtgcatgagctcaaaggggaacaaaatagata
cacccatcatggtcatctatcatcaagagaatttggaattctgagccagcactttctttctgatgatgcttgttgaacggcccact
ttctttgatgagtggaatgacaagcaatgtctgatgcctttgtgtgcccagactgttttcctctctctttccctaatgtgccataag
gcctcagaatgaattgagaattgtttctggtaacaatgtagctttgagggatcagttcttaacttttcagggtctacctaactgag
cctagatatggaccatttatggatgacaacaatttttttttttgtaaatgacaagaaattcttatgcagccttttacctaagaaatttct
gtcagtgccttatcttatgaagaaacagaacctctctagctaatgtgtggtttctccttccctgccccccacccctaggctcacct
ctgcagtcttttaccccagttctcccatttgaataccataccttgntggaaacagngtgtaaaatgactgaagtgatgatgccg
aagatgaaatagatgncaaattagntggacattga (SEQ ID NO:46)

The TPKC1 ORF was amplified using oligos that added restriction endonuclease cleavage sites appropriate for insertion into the yeast expression vectors pLP100 and pYES2 (Invitrogen). The corresponding TPKC1 expression plasmids, pLP155 and pLP156, were constructed using standard molecular biological methodology and used to transform *S. cerevisiae* CY162 cells using the lithium acetate method. The resulting yeast strains were examined for their ability to grow on standard synthetic agar media containing a low concentration of KCl. Expression of TPKC1 in CY162 cells supports their growth on low (2-3 mM KCl) potassium media. Growth was observed to be more extensive when TPKC1 was expressed under control of the ADH1 promoter (pLP155) than with the GAL1/10 promoter (pLP156). The growth of TPKC1-containing CY162 cells was inhibited by the known potassium channel blockers $Ba2^+$, $Ca2^+$, Cs^+, and quinine, but not by TEA. The oligos used for the cloning of 5'and 3' RACE fragments were used in this analysis as well.

Oligos used to clone the TPKC1 ORF into pLP 100:

5' AAA AGA TCT AAA ATG CTT CCC AGC GCC (SEQ ID NO:47)

3' AAA GTC GAC CTA TTT GAT GTT CTC AAT (SEQ ID NO:48)

Oligos used to clone the TPKC1 ORF into pYES2:

5' AAA AAG CTT AAA ATG CTT CCC AGC GCC (SEQ ID NO:49)

3' AAA TCT AGA CTA TTT GAT GTT CTC AAT (SEQ ID NO:50)

A fragment corresponding to the coding region of the TPCK1 gene was generated by PCR with the 5' primer (5'-AAT GCT GCA TGC CTC ATG CTT CCC AGC-3') (SEQ ID NO:70) and the 3'-primer (5'-GGT TAT TTA AAG AGA GGG CT-3') (SEQ ID NO:44) and used to probe the Human Multiple Tissue Northern Blots I and II (Clontech). A fragment corresponding to nucleotide bases 900-1300 was generated by PCR with the 5' primer (5'-TAA GAG CAT CGG ACC ATC AG-3') (SEQ ID NO:71) and the 3' primer (5'-GGT TAT TTA AAG AGA GGG CT-3') (SEQ ID NO:44) and used to probe Human Brain Blot II and III (Clontech). For both fragments, 50 ng of DNA was labeled with Ready-To-Go DNA Labeling Beads (Pharmacia Biotech) with ^{32}P-dCTP (Amersham). Probes were purified over a NICK™ column (Pharmacia Biotech). Probes were hybridized with blots for 1 hour in the presence of ExpressHyb Hybridization Solution (Clontech) at 68°C. Membranes were washed at room temperature in 2X SSC, 0.05% SDS for 20 minutes, and then at 50°C in O.1X SSC, 0.1% SDS for40 minutes. The blots were exposed to Kodak Biomax MS X-ray film at -70°C for 24 hours with two Biomax MS intensifying screens.

This Northern blotting analysis of TPKC1 expression in human tissues indicates that a 3.5 kb mRNA is expressed predominately in brain. The TPKC1 transcript was not detected in heart, placenta, lung, liver, kidney or pancreas. Analysis of blots containing RNA from separate regions of the brain was examined and further localized high levels of TPKC1 expression in the caudate nucleus, amygdala, putamen, frontal lobe, hippocampus, and spinal cord. The TPKC1 transcript is present at significantly lower levels in other regions of the brain; cerebellum, cerebral

cortex, medulla, occipital lobe, temporal lobe, corpus callosum, substantia nigra, subthalamic nucleus, and thalamus.

EXAMPLE 16

TPCK1-induced currents in *X laevis* oocytes assayed by two-electrode voltage clamp.

The expression vector pLP160 was generated by inserting a poly $(A)_n$ tract (n=30) followed by a *Bgl*II site between the *Not*I and *Xba*I sites of pBluescript SK (+/-) (Stratagene). The final vector contains a deletion from the poly(A) tract through the *Bam*HI site. The TPKC1 ORF was amplified by PCR with 5' primer (5'-AAA AAG CTT GCC ACC ATG CTT CCC AGC GCC-3') (SEQ ID NO:72) and 3' primer (5'-CTA TTT GAT GTT CTC-3') (SEQ ID NO:73) digested with *Hind*III and inserted into the vector pLP160 digested with *Hind*III and *Sma*I to give pLP163. This construct was linearized with *Bgl*II for *in vitro* cRNA transcription with T7 RNA polymerase (Ambion). The cRNA was quantified by gel electrophoresis using RNA standards (Gibco BRL). One ng of TPKC1 cRNA (23 nl of 40 ng/μl solution) was microinjected into defolliculated oocytes from *Xenopus laevis*. Oocytes were incubated at 17°C with gentle shaking in ND96 medium. Whole cell electrophysiological recordings were taken 1-3 days post-injection at room temperature in a constantly-perfusing bath using a two-electrode voltage clamp protocol of 300 ms pulses from -150 to +60 mV from a holding potential of -90 mV. The interval between pulses was one second. Electrodes (3) MΩ current injection, 30 MΩ voltage recording) contained 4 M potassium acetate.

Injection of TPCK1 cRNA results in a substantial outward current not present in the uninjected or water injected oocyte. Currents corresponding to the channel are rapidly responsive to changes in applied transmembrane membrane voltage, rising to their highest level with little apparent delay. Currents are non-inactivating and outwardly rectifying (FIGURE 11A). TPKC1 expression supplies a potassium selective pore, permitting movement of potassium ions in preference to sodium. Currents obtained after isotonic substitution of NaCl for KCl in the bath solution were in agreement with values predicted by the Nernst equation indicating a high degree of

selectivity over both sodium and chloride ions (FIGURE 11B). Replacement of aspartate for chloride had no demonstrable effect (data not shown).

When external potassium concentration is raised by isotonic replacement for NaCl, the reversal potential shifts, in agreement with the Nernst equation for a potassium electrode (FIGURE 11C). At high potassium concentrations, a modest inward current is observed at negative voltages, a condition under which little inward current was detected at physiological potassium concentration. Potassium currents expressed in oocytes were sensitive to potassium channel blocking compounds. The potassium channel blocking ion blockers Ba^{2+} inhibited 50 % of the current when applied at 1 mM (FIGURE 11D), while Ca^{2+}, quinine and 4-AP block to a lesser degree (data not shown). Cesium and TEA failed to block the TPKC1 current (data not shown).

EXAMPLE 17

Yeast expression in strains deficient in the transport of potassium.

The following yeast molecular biological and genetic manipulations were performed by standard procedures, such as described by Rose, M. et al. in *Methods in Yeast Genetics,* Cold Spring Harbor Press,(1990).

LY890 (*MAT trkl:: LYS2 trk2::TRP1 ura3-52 lys*2-801, *ade*2-101, *trp*1-Δ63, *his*3-Δ200, *leu*2-Δ1*)* was constructed by deletion of the *TRKI* and *TRK2* genes from the parent yeast strain YPH500 (Stratagene). The full length TPKC1 ORF fragment was generated by PCR; 5' primer (5'-AAA AGA TCT AAA ATG CTT CCC AGC GCC-3') (SEQ ID NO:47), 3' primer (AAA GTC GAC CTA TTT GAT GTT CTC AAT-3') (SEQ ID NO:48) and inserted into the yeast expression vector pLP100 (18) to yield pLP155. pLP155 was used to transform S. *cerevisiae* LY890 cells using standard methods. CY162 was constructed as described in J.A. Anderson et al., *Proc. Natl Acad. Sci USA* 89:3736-3740 (1992). Yeast cells (10^5) containing the indicated plasmids were plated in RPD (arginine phosphate glucose) low potassium (2 mM) agar media and compounds applied to the surface of the agar.

A randomly mutagenized library of TPKC1 sequences was obtained by passage of pLP 155 through the mutator bacterial strain XL1-Red according to the manufacturer's instructions (Stratagene). Yeast cells lacking potassium uptake capacity were transformed with mutagenized pMP 155 and plated on synthetic complete medium lacking uracil and containing 0.1 M KCl in order to maximize transformation efficiency. After three days incubation at 30°C, viable colonies were replica plated on synthetic complete medium lacking uracil and containing 7 mM KCl (SCD-ura). Under the foregoing conditions, wild type TPKC1 protein is incapable of supporting yeast cell growth. Thus, the inability of trk1 trk2 cells containing TPKC1 to grow on medium at pH 4.5 provided a useful phenotype for genetic selection of TPKC1 mutants able to support growth at low pH and low potassium.

The plates were incubated for several days at 30°C. Colonies surviving the selection were reassayed on SCD-ura. Plasmids present in surviving colonies were isolated, retransformed back into LY890 or CY 162, and the resulting strains assayed on selective medium. The TPKC1 ORF fragment from plasmids capable of conferring growth under selective conditions were subcloned into unmutagenized pLP155, retransformed back into LY890 or Cy162 and the resulting strains assayed on selective medium. The DNA sequences of TPKC1 ORFs from these positive plasmids were determined. After DNA sequencing of the plasmids (SEQ ID NOS:61 - 64) several mutations that conferred the ability to grow on low pH medium were identified. The mutations cluster around the second putative pore-forming domain (A256T, Y272H, Y272H + A274V, G270R; SEQ ID NOS:57, 58, 59, and 60, respectively), suggesting that this domain plays a role in the regulation of this 2P potassium channel by pH.

EXAMPLE 18

2P channels obtained by searching the EST database.

The GENBANK expressed sequence tag database (dbEST) was searched for putative 2P channel coding sequences using the program TBLASTN to compare all open reading frames to the amino acid sequence of TPKC1. Several sequences corresponding, to TWIK were identified. In addition, one human and five murine

cDNA sequences different than TWIK were identified. The five cDNAs were purchased (ATCC, Genome Systems Inc.) and subjected to automated DNA sequence analysis.

A predicted open reading frame found in partial human cDNA sequence (GENBANK accession # n39619) apparently encodes a portion of a unique putative 2P channel. DNA sequence analysis of the purchased cDNA clone (277113, SEQ ID NO:51) revealed the presence of a single long open reading frame:

AACAAAAACCTTTTTTGTTTTGAATGGCCTAGAGAGGGTAAGGGATCCCCT
GACGAACAGGAGCAGAGCCAGCTAGAACCTGGGCCTGGCCAGTTCAAGG
CCACCAGAGGGCAGCCTTCTGCGGAAGGCAGTATTGGGGTAGGCAGGGA
CCCCAGCAGACATGGCACTCAGAGCTCTCACTGTCCACTGACTCTCTCTTC
TCCAGGTTATGGCCACATGGCCCCACTATCGCCAGGCGGAAAGGCCTTCT
GCATGGTCTTANTAGCCCTTGGGCTGCCAGCCTCCTTAGCTCTCGTGGCCA
CCCTGCGCCATTGCCTGCTGCCTGTGCTCAGCCGCCCACGTGCCTGGGTAG
CGGTCCACTGGCAGCTGTCACCGGCCAGGGCTGCGCTGCTGCAGGCAGTT
GCACTGGGACTGCTGGTGGCCAGCAGCTTTGTGCTGCTGCCAGCGCTGGT
GCTGTGGGGCCTTCAGGGCGACTGCAGCCTGCTGGGGGCCGTCTACTTCT
GCTTCAGCTCGCTCAGCACCATTGGCCTGGGG

The predicted translation product contains amino acid motifs corresponding to pore forming domains, transmembrane domains, and XXXXGXPX (SEQ ID NO:66) consensus sequences:

asn lys asn leu phe cys phe glu trp pro arg glu gly lys gly ser pro asp glu gln glu gln ser gln leu glu pro gly pro gly gln phe lys ala thr arg gly gln pro ser ala glu gly ser ile gly val gly arg asp pro ser arg his gly thr gln ser ser his cys pro leu thr leu ser ser pro gly tyr gly his met ala pro leu ser pro gly gly lys ala phe cys met val leu xxx ala leu gly leu pro ala ser leu ala leu val ala thr leu arg his cys leu leu pro val leu ser arg pro arg ala trp val ala val his trp gln leu ser pro ala arg ala ala leu leu gln ala val ala leu gly leu leu

val ala ser ser phe val leu leu pro ala leu val leu trp gly leu gln gly asp cys ser leu leu gly ala val tyr phe cys phe ser ser leu ser thr ile gly leu gly (SEQ ID NO:54).

NKNLFCFEWPREGKGSPDEQEQSQLEPGPGQFKATRGQPSAEGSIGVGRDPSR HGTQSSHCPLTLSSPGYGHMAPLSPGGKAFCMVLXALGLPASLALVATLRHC LLPVLSRPRAWVAVHWQLSPARAALLQAVALGLLVASSFVLLPALVLWGLQ GDCSLLGAVYFCFSSLSTIGLG (SEQ ID NO:54).

Four overlapping murine cDNA sequences (w09160, w36852, w36914, w99136) contain a predicted open reading frame sufficient to encode a portion of a unique putative 2P channel. DNA sequence analysis of the purchased cDNA clones (303895, 421453, 334194, 421453) revealed the presence of amino acid motifs corresponding to pore forming domains, transmembrane domains, and XXXXGXPX (SEQ ID NO:66) consensus sequences:

ATGATACGATTTAATACGACTCACTATAGGGAATTTGGCCCTCGAGGCCA
AGAATTCGGCACGAGGAGAATGTGCGCACGTTGGCTCTCATCGTGTGCAC
CTTCACCTACCTGCTGGTGGGCGCCGCGGTGTTCGACGCACTGGAGTCGG
AGCCGGAGATGATCGAGCGGCAGCGGCTGGAGCTGCGGCAGCTGGAGCT
GCGGGCGCGCTACAACCTCAGCGAGGGCGGCTACGAGGAGCTGGAGCGC
GTCGTGCTGCGCCTCAAGCCGCACAAGGCCGGCGTGCAGTGGCGCTTCGC
CGGCTCCTTCTACTTCGCCATCACCGTCATCACCACCATCGGCTATGGTCA
TGCGGCGCCCAGCACGGACGGAGGCAAGGTGTTCTGCATGTTCTACGCGC
TGCTGGGCATCCCGCTCACACTAGTCATGTTCCAGAGCCTGGGTGAACGC
ATCAACACCTCCGTGAGGTACCTGCTGCACCGTGCCAAGAGGGGGCTGGG
CATGCGGCACGCCGAAGTGTCCATGGCCAACATGGTGCTCATCGGTTTCG
TGTCGTGCATCAGCACGCTGTGCATCGGCGCAGCTGCCTTCTCCTACTACG
AGCGCTGGACTTTCTTCCAGGCCTATTACTACTGCTTCATCACCCTCACCA
CCATCGGCTTCGGCGACTATGTGGCGCTGCAGAAGGACCAGGCGCTGCAG
ACGCAGCCGCAGTATGTGGCTTCAGCTTCGTGTACATCCTCACGGGCTCAC
GGTCATCGGCGCTTCCTCAACCTCGTGGTGCTGCGATTCATGACCATGAAC

GCCGAGGACGAGAAGCGTGATGCGGAGCACCGCGCCCTGCTCACGCACA
ACGGCCAGGCTGTCGGCCTGGGTGGCCTGAGCTGCCTGAGCGGTAGCCTG
GGCGACGGCGTGCGTCCCCGCGACCCAGTCACATGCGCTGCGGCCGCAAG
CTTA (SEQ ID NO:52).

gly ile trp pro ser arg pro arg ile arg his glu glu asn val arg thr leu ala leu ile val cys thr phe thr tyr leu leu val gly ala ala val phe asp ala leu glu ser glu pro glu met ile glu arg gln arg leu glu leu arg gln leu glu leu arg ala arg tyr asn leu ser glu gly gly tyr glu glu leu glu arg val val leu arg leu lys pro his lys ala gly val gln trp arg phe ala gly ser phe tyr phe ala ile thr val ile thr thr ile gly tyr gly his ala ala pro ser thr asp gly gly lys val phe cys met phe tyr ala leu leu gly ile pro leu thr leu val met phe gln ser
leu gly glu arg ile asn thr ser val arg tyr leu leu his arg ala lys arg gly leu gly met arg his ala glu val ser met ala asn met val leu ile gly phe val ser cys ile ser thr leu cys ile gly ala ala ala phe ser tyr tyr glu arg trp thr phe phe gln ala tyr tyr tyr cys phe ile thr leu thr thr ile gly phe gly asp tyr val ala leu gln lys asp gln ala leu gln thr gln pro gln tyr val ala ser ala ser cys thr ser ser arg ala his gly his arg arg phe leu asn leu val val leu arg phe met thr met asn ala glu asp glu lys arg asp ala glu his arg ala leu leu thr his asn gly gln ala val gly leu gly gly leu ser cys leu ser gly ser leu gly asp gly val arg pro arg asp pro val thr cys ala ala ala ala ser leu (SEQ ID NO:55)

GIWPSRPRIRHEENVRTLALIVCTFTYLLVGAAVFDALESEPEMIERQRLELRQ
LELRARYNLSEGGYEELERVVLRLKPHKAGVQWRFAGSFYFAITVITTIGYGH
AAPSTDGGKVFCMFYALLGIPLTLVMFQSLGERINTSVRYLLHRAKRGLGMR
HAEVSMANMVLIGFVSCISTLCIGAAAFSYYERWTFFQAYYYCFITLTTIGFGD
YVALQKDQALQTQPQYVASASCTSSRAHGHRRFLNLVVLRFMTMNAEDEKR
DAEHRALLTHNGQAVGLGGLSCLSGSLGDGVRPRDPVTCAAAASL (SEQ ID
NO:55).

Tissue distribution of mRNA expression determined by Northern blotting analysis using a probe constituting a fragment of the open reading frame indicated high level expression in heart tissue.

A predicted open reading frame found in partial murine cDNA sequence (GENBANK accession # w18545) apparently encodes a portion of a unique putative 2P channel. DNA sequence analysis of the purchased cDNA clone (333546) revealed the presence of a single long open reading frame:

CTGAAACCATGGGCCCGATACCTGCTCCTGCTTATGGCCCACCTGCTGGCC
ATGGGCCTTGGGGCTGTGGTGCTTCAGGCCCTGGAGGGCCCTCCAGCTCG
CCACCTCCAGGCCCAGGTCCAGGCTGAACTGGCTAGCTTCCAGGCAGAGC
ACAGGGCCTGCTTGCCACCTGAGGCCCTGGAGGAGCTGCTAGGTGCGGTC
CTGAGAGCACAGGCCCATGGAGTTTCCAGCCTGGGCAACAGCTCANAGAC
AAGCAACTGGGATCTGCCCTCAGCTCTGCTGTTCACTGCCAGCATCCTCAC
CACCACCGGTTATGGCCACATGGCCCCACTCTCCTCAGGTGGAAAGGCCT
TCTGTGTGGTCTATGCAGCCCTTGGGCTGCCAGCCTCTCTAGCACTTGTGG
CTGCCCTGCGCCACTGCTTGCTGCCTGTGTTCAGTCGCCCAGGTGACTGGG
TAGCCATTCGCTGGCAGCTGGCACCAGCTCAGGCTGCTCTGCTACAGGCA
GCAGGACTGGGCCTCCTGGTGGCCTGTGTCTTCATGCTGCTGCCAGCACTG
GTGCTGTGGGGTGTACAGGGTGACTGGCAGCCTGCTANAACCATCTACTT
CTGTTTCGGCTCACTCAGCACGATCGGCCTAGGAGACTTGCTGCCTGCCCA
TGGACGTGGCCTGCACCCAGCCATTTACCACCTTGGGCAGTTTGCACTTCT
TGGTTACTTGCTCCTGGGGCTCCTGGCCATGTTGTTAGCAGTAGAGACCTT
CTCAGAGCTGCCTCAGGTCCGTGCCATGGTGAAATTCTTTGGGCCCAGTGG
CTCTAGAACCGATGAAGATCAAGATGGCATCCTAGGCCAAGATGAGCTGG
CTCTGAGCACTGTGCTGCCTGACGCCCCAGTCTTGGGACCAACCACCCCA
GCCTGAGCGGGAGGCACCAAGGAGTGCTTGAAGAACATAGCANGAAGGG
TTATGGGAATGAATATGTCATGGGATAATGTTAATTTTAAAAATTAAATGG
GCTGCTTAGCATGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
(SEQ ID NO:53).

The predicted translation product contains amino acid motifs corresponding to pore forming domains, transmembrane domains, and XXXXGXPX (SEQ ID NO:66) consensus sequences:

leu lys pro trp ala arg tyr leu leu leu leu met ala his leu leu ala met gly leu gly ala val val leu gln ala leu glu gly pro pro ala arg his leu gln ala gln val gln ala glu leu ala ser phe gln ala glu his arg ala cys leu pro pro glu ala leu glu glu leu leu gly ala val leu arg ala gln ala his gly val ser ser leu gly asn ser ser xxx thr ser asn trp asp leu pro ser ala leu leu phe thr ala ser ile leu thr thr thr gly tyr gly his met ala pro leu ser ser gly gly lys ala phe cys val val tyr ala ala leu gly leu pro ala ser leu ala leu val ala ala leu arg his cys leu leu pro val phe ser arg pro gly asp trp val ala ile arg trp gln leu ala pro ala gln ala ala leu leu gln ala ala gly leu gly leu leu val ala cys val phe met leu leu pro ala leu val leu trp gly val gln gly asp trp gln pro ala xxx thr ile tyr phe cys phe gly ser leu ser thr ile gly leu gly asp leu leu pro ala his gly arg gly leu his pro ala ile tyr his leu gly gln phe ala leu leu gly tyr leu leu leu gly leu leu ala met leu leu ala val glu thr phe ser glu leu pro gln val arg ala met val lys phe phe gly pro ser gly ser arg thr asp glu asp gln asp gly ile leu gly gln asp glu leu ala leu ser thr val leu pro asp ala pro val leu gly pro thr thr pro ala (SEQ ID NO:56).

LKPWARYLLLLMAHLLAMGLGAVVLQALEGPPARHLQAQVQAELASFQAE HRACLPPEALEELLGAVLRAQAHGVSSLGNSSXTSNWDLPSALLFTASILTTT GYGHMAPLSSGGKAFCVVYAALGLPASLALVAALRHCLLPVFSRPGDWVAI RWQLAPAQAALLQAAGLGLLVACVFMLLPALVLWGVQGDWQPAXTIYFCF GSLSTIGLGDLLPAHGRGLHPAIYHLGQFALLGYLLLGLLAMLLAVETFSELP QVRAMVKFFGPSGSRTDEDQDGILGQDELALSTVLPDAPVLGPTTPA (SEQ ID NO:56).

What is claimed is:

1. A purified or isolated nucleic acid encoding a mutant potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the nucleic acid in a cell confers on the cell the ability to grow in the presence of 7mM potassium.

2. The nucleic acid of claim 1, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 3mM potassium.

3. The nucleic acid of claim 1, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 2mM potassium.

4. The nucleic acid of claim 1, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 2-7 mM potassium.

5. The nucleic acid of claim 1, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow under acidic conditions.

6. The nucleic acid of claim 2, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow at a pH of 4.5.

7. The nucleic acid of claim 1, wherein the nucleic acid is of human, murine, avian, insect, or nematode origin.

8. The nucleic acid of claim 7, wherein the nucleic acid is of human origin.

9. The nucleic acid of claim 7, wherein the nucleic acid is of insect origin, and wherein the insect is *Drosophila melanogaster.*

10. The nucleic acid of claim 7, wherein the nucleic acid is of nematode origin, and wherein the nematode is *Caenorhabditis elegans.*

11. The nucleic acid of claim 1, wherein the nucleic acid encodes a protein of the TPKC1 family.

12. The nucleic acid of claim 1, wherein the nucleic acid comprises a nucleotide sequence represented by SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, or SEQ ID NO:64.

13. The nucleic acid of claim 1, wherein the nucleic acid encodes a polypeptide comprising an amino acid sequence represented by SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, or SEQ ID NO:60.

14. A vector comprising the nucleic acid of claim 1.

15. A cell comprising a nucleic acid encoding a mutant potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 7mM potassium.

16. The cell of claim 15, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 3mM potassium.

17. The cell of claim 15, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 2mM potassium.

18. The cell of claim 15, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 2-7 mM potassium.

19. The cell of claim 15, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow under acidic conditions.

20. The cell of claim 19, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow at a pH of 4.5.

21. The cell of claim 15, wherein the cell is a mammalian cell, an avian cell, a yeast cell, an insect cell, a nematode cell, a frog cell, or a plant cell.

22. The cell of claim 21, wherein the cell is a plant cell.

23. The cell of claim 21, wherein the cell is a yeast cell, which is *Saccharomyces cerevisiae* strain CY162.

24. The cell of claim 15, wherein the cell is a recombinant cell.

25. The recombinant cell of claim 24, wherein the cell is a mammalian cell, an avian cell, a yeast cell, an insect cell, a nematode cell, a frog cell, or a plant cell.

26. The recombinant cell of claim 25, which is a plant cell.

27. An isolated or purified polypeptide, wherein the polypeptide is a mutant potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the mutant potassium ion channel in a cell confers on the cell the ability to grow in the presence of 7mM potassium.

28. The polypeptide of claim 27, wherein expression of the polypeptide in the cell confers on the cell the ability to grow in the presence of 3mM potassium.

29. The polypeptide of claim 27, wherein expression of the polypeptide in the cell confers on the cell the ability to grow in the presence of 2mM potassium.

30. The polypeptide of claim 27, wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 2-7 mM potassium.

31. The polypeptide of claim 27, wherein expression of the polypeptide in the cell confers on the cell the ability to grow under acidic conditions.

32. The polypeptide of claim 31, wherein expression of the polypeptide in the cell confers on the cell the ability to grow at a pH of 4.5.

33. The polypeptide of claim 27, wherein the polypeptide is of human, murine, avian, insect, or nematode origin.

34. The polypeptide of claim 33, wherein the polypeptide is of human origin.

35. The polypeptide of claim 33, wherein the polypeptide is of insect origin, and wherein the insect is *Drosophila melanogaster.*

36. The polypeptide of claim 33, wherein the polypeptide is of nematode origin, and wherein the nematode is *Caenorhabditis elegans.*

37. The polypeptide of claim 27, wherein the polypeptide comprises an amino acid sequence represented by SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, or SEQ ID NO:60.

38. The polypeptide of claim 27, which is a polypeptide of the TPKC1 family.

39. The polypeptide of claim 38, wherein the polypeptide is mutated at one or more of positions 256, 270, 272, and 274.

40. The polypeptide of claim 39, wherein the polypeptide is mutated at position 256, and wherein the amino acid at position 256 is Thr.

41. The polypeptide of claim 39, wherein the polypeptide is mutated at position 270, and wherein the amino acid at position 270 is Arg.

42. The polypeptide of claim 39, wherein the polypeptide is mutated at position 272, and wherein the amino acid at position 272 is His.

43. The polypeptide of claim 39, wherein the polypeptide is mutated at position 274, and wherein the amino acid at position 274 is Val.

44. The polypeptide of claim 39, wherein the polypeptide is mutated at positions 272 and 274, and wherein the amino acid at position 272 is His and the amino acid at position 274 is Val.

45. A cell comprising a mutant potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the mutant potassium ion channel protein in the cell confers on the cell the ability to grow in the presence of 7mM potassium.

46. The cell of claim 45, wherein expression of the mutant potassium ion channel protein in the cell confers on the cell the ability to grow in the presence of 3mM potassium.

47. The cell of claim 45, wherein expression of the mutant potassium ion channel protein in the cell confers on the cell the ability to grow in the presence of 2mM potassium.

48. The cell of claim 45, wherein expression of the mutant potassium ion channel protein in the cell confers on the cell the ability to grow in the presence of 2-7 mM potassium.

49. The cell of claim 45, wherein expression of the mutant potassium ion channel protein in the cell confers on the cell the ability to grow under acidic conditions.

50. The cell of claim 49, wherein expression of the mutant potassium ion channel protein in the cell confers on the cell the ability to grow at a pH of 4.5.

51. The cell of claim 45, wherein the mutant potassium ion channel protein is of human, murine, avian, insect, or nematode origin.

52. The cell of claim 51, wherein the mutant potassium ion channel protein is of human origin.

53. The cell of claim 51, wherein the mutant potassium ion channel protein is of insect origin, and wherein the insect is *Drosophila melanogaster.*

54. The cell of claim 51, wherein the mutant potassium ion channel protein is of nematode origin, and wherein the nematode is *Caenorhabditis elegans.*

55. The cell of claim 45, wherein the cell is *Saccharomyces cerevisiae* strain CY162.

56. The cell of claim 45, wherein the cell is a recombinant cell.

57. The recombinant cell of claim 56, wherein the cell is a mammalian cell, an avian cell, a yeast cell, an insect cell, a nematode cell, a frog cell, or a plant cell.

58. The recombinant cell of claim 57, which is a plant cell.

59. The cell of claim 45, wherein the cell is a mammalian cell, an avian cell, a yeast cell, an insect cell, a nematode cell, a frog cell, or a plant cell.

60. The cell of claim 59, which is a plant cell.

61. The cell of claim 45, wherein the mutant potassium ion channel protein is a member of the TPKC1 family.

62. The cell of claim 61, wherein the mutant potassium ion channel protein is mutated at one or more of positions 256, 270, 272, and 274.

63. The cell of claim 62, wherein the mutant potassium ion channel protein is mutated at position 256, and wherein the amino acid at position 256 is Thr.

64. The cell of claim 62, wherein the mutant potassium ion channel protein is mutated at position 270, and wherein the amino acid at position 270 is Arg.

65. The cell of claim 62, wherein the mutant potassium ion channel protein is mutated at position 272, and wherein the amino acid at position 272 is His.

66. The cell of claim 62, wherein the mutant potassium ion channel protein is mutated at position 274, and wherein the amino acid at position 274 is Val.

67. The cell of claim 62, wherein the mutant potassium ion channel protein is mutated at positions 272 and 274, and wherein the amino acid at position 272 is His and the amino acid at position 274 is Val.

68. A method of identifying a substance that affects the potassium ion transporting activity of a potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, said method comprising

a) contacting the potassium ion channel protein with a substance, and

b) determining whether the potassium ion transporting activity of the potassium ion channel protein is altered,

wherein the potassium ion channel protein is a mutant that is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the mutant protein in a cell confers on the cell the ability to grow in the presence of 7mM potassium.

69. The method of claim 68, wherein the substance is an agonist of the potassium ion channel protein.

70. The method of claim 68, wherein the substance is an antagonist of the potassium ion channel protein.

71. The method of claim 68, which is a high throughput method of screening.

72. The method of claim 68, which results in identification of a drug.

73. The method of claim 68, which results in identification of an insecticide.

74. The method of claim 68, wherein the potassium ion channel protein is present on the surface of a cell.

75. The method of claim 74, wherein the cell is a recombinant cell.

76. The method of claim 74, wherein the cell is a mammalian cell, a yeast cell, a frog cell, a nematode cell, or a plant cell.

77. The method of claim 75, wherein the cell is a yeast cell.

78. A substance identified by the method of claim 68.

79. A method of controlling pests, said method comprising applying an agonist or antagonist of a potassium ion channel protein having four membrane-spanning domains and two pore-forming domains to the pests, wherein the agonist or antagonist is identified using the method of claim 68.

80. The method of claim 79, wherein the pests are nematodes.

81. The method of claim 79, wherein the pests are insects.

82. A method of altering the requirement of plant cells for potassium, said method comprising:

expressing a mutant potassium ion channel protein on the surface of the plant cells,

wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at an amino acid located in a pore-forming domain, and

wherein expression of the mutant potassium ion channel protein confers on the cell the ability to grow in the presence of 7mM potassium.

83. The method of claim 82, wherein the requirement of the plant cells for potassium is reduced.

84. The method of claim 82, wherein the mutant potassium ion channel protein is expressed from a mutant potassium ion channel gene that was mutated by random mutagenesis.

85. The method of claim 82, wherein the plant cells comprise a plant.

86. The method of claim 82, wherein the plant cells are recombinant cells.

87. The method of claim 86, wherein the mutant potassium ion channel protein is expressed from a nucleic acid that is heterologous to the plant cells.

88. The method of claim 86, wherein the mutant potassium ion channel protein is of human, murine, avian, insect, or nematode origin.

89. The method of claim 88, wherein the mutant potassium ion channel protein is of human origin.

90. The method of claim 88, wherein the mutant potassium ion channel protein is of the TPKC1 family.

91. The method of claim 82, wherein the mutant potassium ion channel protein comprises four membrane-spanning domains and two pore-forming domains.

92. A method of growing plant cells in the presence of low levels of potassium, said method comprising

genetically modifying a plant cell such that it expresses a mutant potassium ion channel protein on its surface,

wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at an amino acid located in a pore-forming domain, and

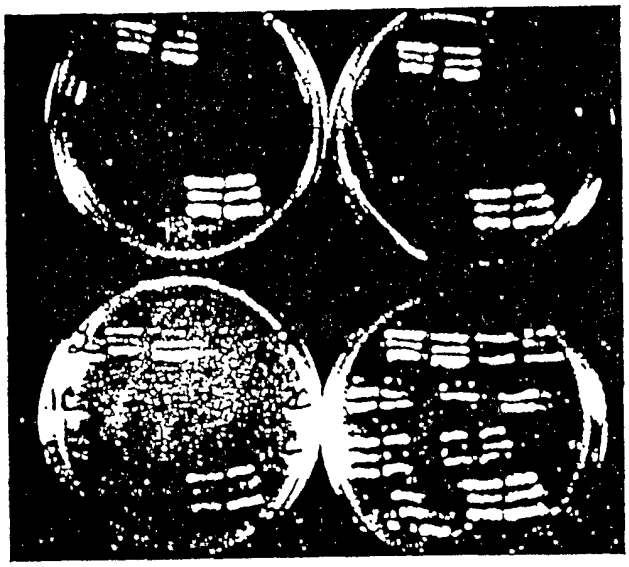
wherein expression of the mutant potassium ion channel protein confers on the cell the ability to grow in the presence of 7mM potassium,

culturing the plant cells under conditions that permit growth, with the proviso that the plant cells are exposed to no more than 7 mM potassium.

93. The method of claim 92, wherein the plant cells are exposed to no more than between 2 and 7 mM potassium.

94. A kit comprising a purified or isolated nucleic acid encoding a mutant potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the nucleic acid in a cell confers on the cell the ability to grow in the presence of 7mM potassium.

95. A kit comprising a cell comprising a nucleic acid encoding a mutant potassium ion channel protein having four membrane-spanning domains and two pore-forming domains, wherein the mutant potassium ion channel protein is mutated, with respect to the wild-type amino acid sequence, at the second pore-forming domain, and wherein expression of the nucleic acid in the cell confers on the cell the ability to grow in the presence of 7mM potassium.



SC galactose, 100 mM KCl

SC glucose, 0mM KCl

SC galactose, 0 mM KCl

SC glucose, 100 mM KCl

FIG. 1

```
5'...ACGCGATCGCCGCGAGTGTATATTTTTTTTTTAGCTCAGTCTTCAGTGTTTCGCGATTCTCTTTAAAAGAAAAAAAAAAATAATAAGTCAA
AACTACAAACCACACAGCGAAAGGCGAAAGCAACGGTTCCTGCGAGTGTTTATTTTTTTTTTCAACAATTTTTGATCGTAGTGCGACAATCCGTCGAGC      -1
                                        10                                  M1              20
Met Ser Pro Asn Arg Trp Ile Leu Leu Leu Ile Phe Tyr Ile Ser Tyr Leu Met Phe Gly Ala Ala Ile Tyr Tyr
ATG TCG CCG AAT CGA TGG ATC CTG CTG CTC ATC TTC TAC ATA TCC TAC CTG ATG TTC GGG GCG GCA ATC TAT TAC      75
                    30                                                  40                              50
His Ile Glu His Gly Glu Glu Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Gln Ile Ala Ile Asn Glu Tyr Leu
CAT ATT GAG CAC GGC GAG GAG AAG ATA TCG CGC GCC GAA CAG CGC AAG GCG CAA ATT GCA ATC AAC GAA TAT CTG     150
                          G     60                                              70
Leu Glu Glu Leu Gly Asp Lys Asn Thr Thr Thr Gln Asp Glu Ile Leu Gln Arg Ile Ser Asp Tyr Cys Asp Lys
CTG GAG GAG CTG GGC GAC AAG AAT ACG ACC ACA CAG GAT GAG ATT CTT CAA CGG ATC TCG GAT TAC TGT GAC AAA     225
                    80                                                  90                              100
Pro Val Thr Leu Pro Pro Thr Tyr Asp Asp Thr Pro Tyr Thr Trp Thr Phe Tyr His Ala Phe Phe Phe Ala Phe
CCG GTT ACA TTG CCG CCG ACA TAT GAT GAT ACG CCC TAC ACG TGG ACC TTC TAC CAT GCC TTC TTC TTC GCC TTC     300
          H5-1                              110                                 120
Thr Val Cys Ser Thr Val Gly Tyr Gly Asn Ile Ser Pro Thr Thr Phe Ala Gly Arg Met Ile Met Ile Ala Tyr
ACC GTT TGC TCC ACG GTG GGA TAT GGG AAT ATA TCG CCA ACC ACC TTC GCC GGA CGG ATG ATC ATG ATC GCG TAT     375
                    130   M2                                            140                             150
Ser Val Ile Gly Ile Pro Val Asn Gly Ile Leu Phe Ala Gly Leu Gly Glu Tyr Phe Gly Arg Thr Phe Glu Ala
TCG GTG ATT GGC ATC CCC GTC AAT GGT ATC CTC TTT GCC GGC CTC GGC GAA TAC TTT GGA CGT ACG TTT GAA GCG     450
                                    160                                 170
Ile Tyr Arg Arg Tyr Lys Lys Tyr Lys Met Ser Thr Asp Met His Tyr Val Pro Pro Gln Leu Gly Leu Ile Thr
ATC TAC AGA CGC TAC AAA AAG TAC AAG ATG TCC ACG GAT ATG CAC TAT GTC CCG CCG CAG CTG GGA TTG ATC ACC     525
                    180       M3                                        190                             200
Thr Val Val Ile Ala Leu Ile Pro Gly Ile Ala Leu Phe Leu Val Leu Pro Cys Val Gly Val His Leu Leu Arg
ACG GTG GTG ATT GCC CTG ATT CCG GGA ATA GCT CTC TTC CTG GTG CTG CCC TGC GTG GGT GTT CAC CTA CTT CGA     600
                                    210                 H5-2            220
Glu Leu Gly Leu Ser Ser Ile Ser Leu Tyr Tyr Ser Tyr Val Thr Thr Thr Thr Ile Gly Phe Gly Asp Tyr Val
GAA CTG GGC CTA TCT TCC ATC TCG CTG TAC TAC AGC TAT GTG ACC ACC ACA ACA ATT GGA TTC GGT GAC TAT GTG     675
                    230                                                 240                             250
Pro Thr Phe Gly Ala Asn Gln Pro Lys Glu Phe Gly Gly Trp Phe Val Val Tyr Gln Ile Phe Val Ile Val Trp
CCC ACA TTT GGA GCC AAC CAG CCC AAG GAG TTC GGC GGC TGG TTC GTG GTC TAT CAG ATC TTT GTG ATC GTG TGG     750
      M4                                260                                 270
Phe Ile Phe Ser Leu Gly Tyr Leu Val Met Ile Met Thr Phe Ile Thr Arg Gly Leu Gln Ser Lys Lys Leu Ala
TTC ATC TTC TCG CTG GGA TAT CTT GTG ATG ATC ATG ACA TTT ATC ACT CGG GGC CTC CAG AGC AAG AAG CTG GCA     825
                    280                                                 290                             300
Tyr Leu Glu Gln Gln Leu Ser Ser Asn Leu Lys Ala Thr Gln Asn Arg Ile Trp Ser Gly Val Thr Lys Asp Val
TAC CTG GAG CAG CAG TTG TCC TCC AAC CTG AAG GCC ACA CAG AAT CGC ATC TGG TCT GGC GTC ACC AAG GAT GTG     900
                                    310                                 320
Gly Tyr Leu Arg Arg Met Leu Asn Glu Leu Tyr Ile Leu Lys Val Lys Pro Val Tyr Thr Asp Val Asp Ile Ala
GGC TAC CTC CGG CGA ATG CTC AAC GAG CTG TAC ATC CTC AAA GTG AAG CCT GTG TAC ACC GAT GTA GAT ATC GCC     975
```

FIG. 2A

```
                330                                                 340                                                 350
Tyr Thr Leu Pro Arg Ser Asn Ser Cys Pro Asp Leu Ser Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser Arg
TAC ACA CTG CCA CGT TCC AAT TCG TGT CCG GAT CTG AGC ATG TAC CGC GTG GAG CCG GCT CCC ATT CCC AGC CGG   1050
                                360                                                 370
Lys Arg Ala Phe Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg Glu Ala Gly Met Val His Ala Asn Ser Asp
AAG AGG GCA TTC TCC GTG TGC GCC GAC ATG GTT GGC GCC CAA AGG GAG GCG GGC ATG GTA CAC GCC AAT TCC GAT   1125
                380                                                 390                                                 400
Thr Asp Leu Thr Lys Leu Asp Arg Glu Lys Thr Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr Asp Leu Leu
ACG GAT CTA ACC AAA CTG GAT CGC GAG AAG ACA TTC GAG ACG GCG GAG GCG TAC CAC CAG ACC ACC GAT TTG CTG   1200
                                410                                                 420
Ala Lys Val Val Asn Ala Leu Ala Thr Val Lys Pro Pro Pro Ala Glu Gln Glu Asp Ala Ala Leu Tyr Gly Gly
GCC AAG GTG GTC AAC GCA CTG GCC ACG GTG AAG CCA CCG CCG GCG GAA CAG GAA GAT GCG GCT CTC TAT GGT GGC   1275
                430                                                 440                                                 450
Tyr His Gly Phe Ser Asp Ser Gln Ile Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn Glu Phe Thr Ser Pro
TAT CAT GGC TTC TCC GAC TCC CAG ATC CTG GCC AGC GAA TGG TCG TTC TCG ACG GTC AAC GAG TTC ACA TCA CCG   1350
                                460                                                 470
Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe Asn Leu Glu Ala Pro Arg Trp Gln Ser Glu Arg Pro Leu Arg
CGA CGT CCA AGA GCA CGT GCC TGC TCC GAT TTC AAT CTG GAG GCA CCT CGC TGG CAG AGC GAG AGG CCA CTG CGT   1425
                480                                                 490                                                 500
Ser Ser His Asn Glu Trp Thr Trp Ser Gly Asp Asn Gln Gln Ile Gln Glu Ala Phe Asn Gln Arg Tyr Lys Gly
TCG AGC CAC AAC GAA TGG ACA TGG AGC GGC GAC AAC CAG CAG ATC CAG GAG GCA TTC AAC CAG CGC TAC AAG GGA   1500
                                510                                                 520
Gln Gln Arg Ala Asn Gly Ala Ala Asn Ser Thr Met Val His Leu Glu Pro Asp Ala Leu Glu Glu Gln Leu Arg
CAG CAG CGT GCC AAC GGA GCA GCC AAC TCG ACC ATG GTC CAT CTG GAG CCG GAT GCT TTG GAG GAG CAG CTG AGA   1575
                530                                                 540                                                 550
Asn Asn His Arg Val Pro Val Ala Ser Arg Ser Ser Pro Cys Arg Met Val Cys Asp Val Cys Phe Pro Ser Arg
AAC AAT CAC CGG GTG CCG GTC GCG TCA AGA AGT TCT CCA TGC CGG ATG GTC TGC GAC GTC TGT TTC CCT TCC AGA   1650
                                560                                                 570
Arg Ser Thr Pro Arg Arg Ile Trp Ser Ala Ser Cys Pro Trp Ser Arg Tyr Pro Arg Val Ser Ser Arg Arg Lys
AGA AGC ACC CCT CGC AGG ATC TGG AGC GCA AGT TGT CCG TGG TCT CGG TAC CCG AGG GTG TCA TCT CGC AGG AAG   1725
                580                                                 590                                                 600
Pro Asp Pro Arg Trp Thr Thr Thr Ser Thr Arg Ser Arg Arg Pro Pro Val Asn Pro Ile Cys Ala Thr Asp Ala
CCA GAT CCC CGC TGG ACT ACT ACA TCA ACA CGG TCA CGG CGG CCT CCA GTC AAT CCT ATT TGC GCA ACG GAC GCG   1800
                                610                                 618
Val Arg His Arg Pro Ser Asn Arg Met Ala Ala Trp Pro Ala Ala Ala Ala Gly
GTC CGC CAC CGC CCT TCG AAT CGA ATG GCA GCT TGG CCA GCG GCG GCG GCG GGC TAA CGAACATGGGCTTCCAGATGGAG   1880

GATGGAGCAACCCCGCCATCGGCATTGGGCGGTGGAGCCTATCAACGCAAGGCGGCTGCTGGCAAGCGCCGACGCGAGAGCATCTACACCCAGAATCAA
GCCCCATCCGCTCGCCGGGGCAGCATGTATCCGCCGACCGCGCACGCCTTGGCCCAGATGCAGATGCGACGCGGCAGCTTGGCAACCAGTGGCTCTGGA
TCGGCGGCCATGGCGGCAGTGGCCGCGCGTCGTGGCAGCCTCTTCCCAGCTACAGCATCGGCATCATCGCTGACCTCTGCTCCGCGCCGAAGCAGCATA
TTCTCGGTTACCTCCGAAAAGGATATGAATGTGCTGGAGCAGACGACCATTGCGGATCTGATTCGTGCGCTCGAG . . .3'
```

FIG. 2B

```
                                        10                                  20
Met Ser Asp Gln Leu Phe Val Ala Phe Glu Lys Tyr Phe Leu Thr Ser Asn Glu Val Lys
ATG TCC GAT CAG CTG TTT GTC GCA TTT GAG AAG TAT TTC TTG ACG AGT AAC GAG GTC AAG    60
                                        30                                  40
Lys Asn Ala Ala thr Glu Thr Trp Thr Phe Ser Ser Ser Ile Phe Phe Ala Val Thr Val
AAG AAT GCA GCA ACG GAG ACA TGG ACA TTT TCA TCG TCC ATT TTC TTT GCC GTA ACC GTC   120
   H5-1                                 50                                  60
Val Thr Thr Ile Gly Tyr Gly Asn Pro Val Pro Val Thr Asn Ile Gly Arg Ile Trp Cys
GTC ACT ACC ATC GGA TAC GGT AAT CCA GTT CCA GTG ACA AAC ATT GGA CGG ATA TGG TGT   180
                        M2              70                                  80
Ile Leu Phe Ser Leu Leu Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Asp Leu Ala Gly
ATA TTG TTC TCC TTG CTT GGA ATA CCT CTA ACA CTG GTT ACC ATC GCT GAC TTG GCA GGT   240
                                        90                                 100
Lys Phe Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys Leu Lys Tyr Leu
AAA TTC CTA TCT GAA CAT CTT GTT TGG TTG TAT GGA AAC TAT TTG AAA TTA AAA TAT CTC   300
                                       110                                 120
Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His Val Cys Glu His Cys His Ser His
ATA TTG TCA CGA CAT CGA AAA GAA CGG AGA GAG CAC GTT TGT GAG CAC TGT CAC AGT CAT   360
                                       130                                 140
Gly Met Gly His Asp Met Asn Ile Glu Glu Lys Arg Ile Pro Ala Phe Leu Val Leu Ala
GGA ATG GGG CAT GAT ATG AAT ATC GAG GAG AAA AGA ATT CCT GCA TTC CTG GTA TTA GCT   420
          M3                            150                                 160
Ile Leu Ile Val Tyr Thr Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser
ATT CTG ATA GTA TAT ACA GCG TTT GGC GGT GTC CTA ATG TCA AAA TTA GAG CCG TGG TCT   480
```

FIG. 3A

```
                                                170       H5-2                                  180
Phe Phe Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly Asp Leu
TTC TTC ACT TCA TTC TAC TGG TCC TTC ATT ACA ATG ACT ACT GTC GGG TTT GGC GAC TTG    540
                                                190                                   200
Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu Leu Tyr Ile Ile Leu Gly Lys Phe
ATG CCC AGA AGG GAC GGA TAC ATG TAT ATC ATA TTG CTC TAT ATC ATT TTA GGT AAA TTT    600
                                                210                        M4         220
Ser Met Lys Lys Lys Gln Lys Phe Lys Ile Phe Leu Gly Leu Ala Ile Thr Thr Met Cys
TCA ATG AAA AAA AAA CAA AAA TTC AAA ATA TTT TTA GGT CTT GCA ATA ACT ACA ATG TGC    660
                                                230                                   240
Ile Asp Leu Val Gly Val Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln
ATT GAT TTG GTA GGA GTA CAG TAT ATT CGA AAG ATT CAT TAT TTC GGA AGA AAA ATT CAA    720
                                                250                                   260
Asp Ala Arg Ser Ala Leu Ala Val Val Gly Gly Lys Val Val Leu Val Ser Glu Leu Tyr
GAC GCT AGA TCT GCA TTG GCG GTT GTA GGA GGA AAG GTA GTC CTT GTA TCA GAA CTC TAC    780
                                                270                                   280
Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser Arg Glu Ala Phe Ile Val Glu Asn
GCA AAT TTA ATG CAA AAG CGA GCT CGT AAC ATG TCC CGA GAA GCT TTT ATA GTG GAG AAT    840
                                                290                                   300
Leu Tyr Val Ser Lys His Ile Ile Pro Phe Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr
CTC TAT GTT TCC AAA CAC ATC ATA CCA TTC ATA CCA ACT GAT ATC CGA TGT ATT CGA TAT    900
                                                310                                   320
Ile Asp Gln Thr Ala Asp Ala Ala Thr Ile Ser Thr Ser Ser Ser Ala Ile Asp Met Gln
ATT GAT CAA ACT GCC GAT GCT GCT ACC ATT TCC ACG TCA TCG TCT GCA ATT GAT ATG CAA    960
                                                330                          336
Ser Cys Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys
AGT TGT AGA TTT TGT CAT TCA AGA TAT TCT CTC AAT CGT GCA TTC AAA TAG                1011
```

FIG. 3B

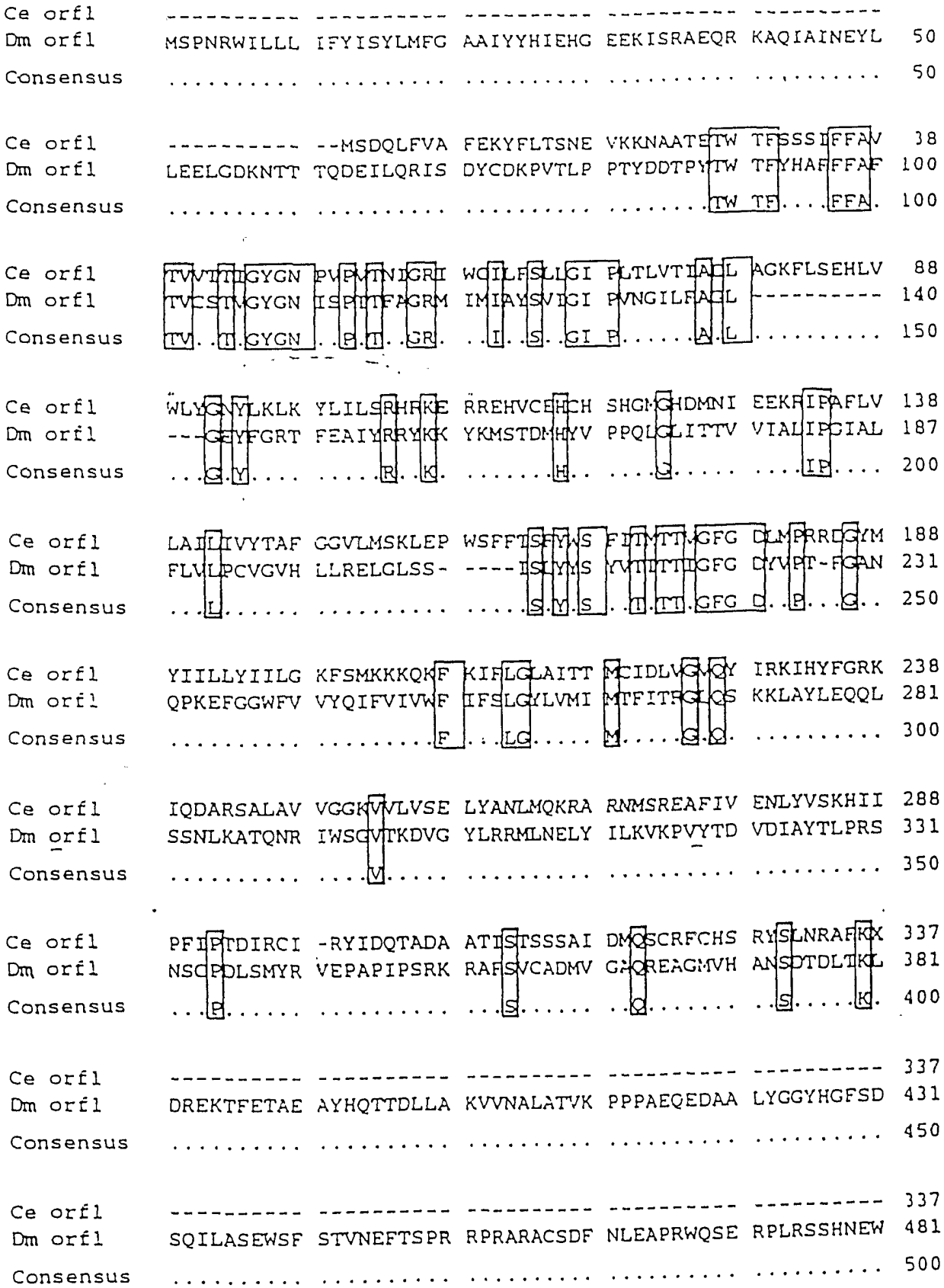

Ce orf1 ---------- ---------- ---------- ---------- ----------
Dm orf1 MSPNRWILLL IFYISYLMFG AAIYYHIEHG EEKISRAEQR KAQIAINEYL 50
Consensus 50
Ce orf1 ---------- --MSDQLFVA FEKYFLTSNE VKKNAATETW TFSSSIFFAV 38
Dm orf1 LEELGDKNTT TQDEILQRIS DYCDKPVTLP PTYDDTPYTW TFYHAFFFAF 100
Consensus ....TW TF....FFA. 100
Ce orf1 TVVTITIGYGN PVPVTNIGRI WCILFSLLGI PLTLVTIALL AGKFLSEHLV 88
Dm orf1 TVCSTNGYGN ISPITFAGRM IMIAYSVIGI PVNGILFALL ---------- 140
Consensus TV..T.GYGN..P.T..GR. ..I..S..GI P......A.L.......... 150
Ce orf1 WLYGVYLKLK YLILSRHFKE RREHVCEHCH SHGMGHDMNI EEKFIPAFLV 138
Dm orf1 ---GEYFGRT FEAIYRRYKK YKMSTDMHYV PPQLGLITTV VIALIPGIAL 187
Consensus ...G.Y.... .....R..K. ....H.. ....G..... ....IP.... 200
Ce orf1 LAILIVYTAF GGVLMSKLEP WSFFTSFYWS FITMTTVGFG DLMPRRDGYM 188
Dm orf1 FLVLPCVGVH LLRELGLSS- ----ISLYMS YVTITTIGFG DYVPT-FGAN 231
Consensus ...L..... ....S.Y.S..I.TT.GFG D..P...G.. 250
Ce orf1 YIILLYIILG KFSMKKKQKF KIFLGLAITT MCIDLVGMQY IRKIHYFGRK 238
Dm orf1 QPKEFGGWFV VYQIFVIVWF IFSLGYLVMI MTFITRGLQS KKLAYLEQQL 281
Consensus ....F...LG..... M.....G.Q. 300
Ce orf1 IQDARSALAV VGGKVVLVSE LYANLMQKRA RNMSREAFIV ENLYVSKHII 288
Dm orf1 SSNLKATQNR IWSGVTKDVG YLRRMLNELY ILKVKPVYTD VDIAYTLPRS 331
Consensus ....V..... 350
Ce orf1 PFIPTDIRCI -RYIDQTADA ATISTSSSAI DMQSCRFCHS RYSLNRAFKX 337
Dm orf1 NSCPDLSMYR VEPAPIPSRK RAFSVCADMV GAQREAGMVH ANSDTDLTKL 381
Consensus ...P..... ....S..... ..Q..... ..S.....K. 400
Ce orf1 ---------- ---------- ---------- ---------- ---------- 337
Dm orf1 DREKTFETAE AYHQTTDLLA KVVNALATVK PPPAEQEDAA LYGGYHGFSD 431
Consensus 450
Ce orf1 ---------- ---------- ---------- ---------- ---------- 337
Dm orf1 SQILASEWSF STVNEFTSPR RPRARACSDF NLEAPRWQSE RPLRSSHNEW 481
Consensus 500

FIG. 4

```
mIRK        AFLFSIETQTTIGYGFRCVTDECP    {G,A,S,T},   {D,E}
hROMK1      AFLFSLETQVTIGYGFRCVTEQCA    {N,Q},   {K,R,H}
rGIRK1      AFLFFIETEATIGYGYRYITDHCP    {F,Y,W}={I,L,M,V}
            ||.|..   .|.|||    |   .
Dm H5-1     AFFFAFTVCSTVGYGNISPTTFAG
            ||.||.|  ||||||||.||||||
Shak        AFWWAVVTMTTVGYGDMTPVGFWG
Shal        AFWYTIVTMTTLGYGDMVPETIAG
Shab        AFWWAGITMTTVGYGDICPTTALG
Shaw        GLWWALVTMTTVGYGDMAPKTYIG
Eag         ALYFTMTCMTSVGFGNVAAETDNE
Slo         CVYFLIVTMSTVGYGDVYCETVLG
            .|| |.|| ||||||||.||| .|
Dm H5-2     SLYTSYVTTTTIGFGDYVPTFGAN


Dm H5-1     AFFFAFTVCSTVGYGNISPTTFAG
Ce 5-1      SIFFAVTVVTTIGYGNPVPVTNTG
Dm H5-2     SLYTSYVTTTTIGFGDYVPTFGAN
Ce H5-2     SFYWSFITMTTVGFGDLMPRRDGY
```

FIG. 5A

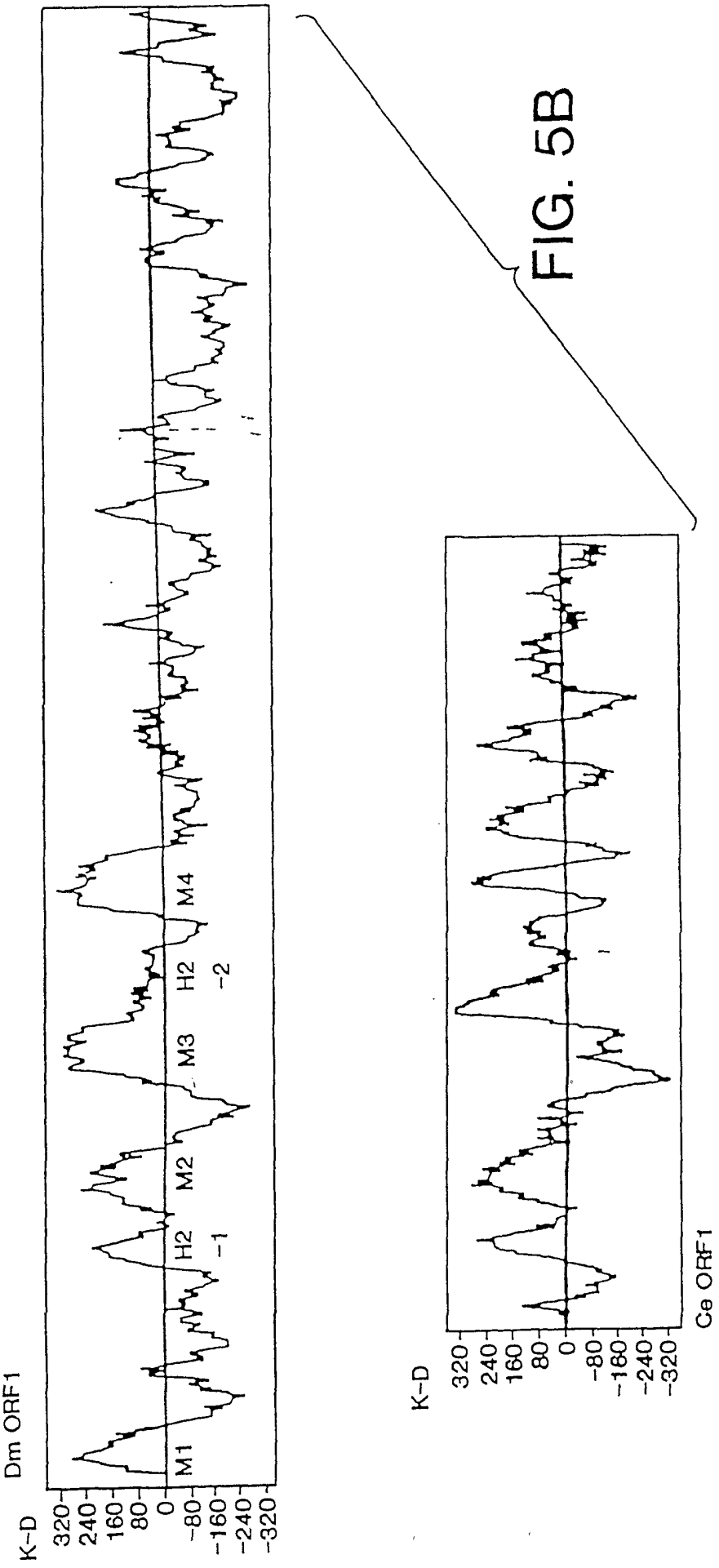

Dm ORF1
K-D
320
240
160
80
0
-80
-160
-240
-320
M1
H2
-1
M2
M3
H2
-2
M4
Ce ORF1
K-D
320
240
160
80
0
-80
-160
-240
-320

FIG. 5B

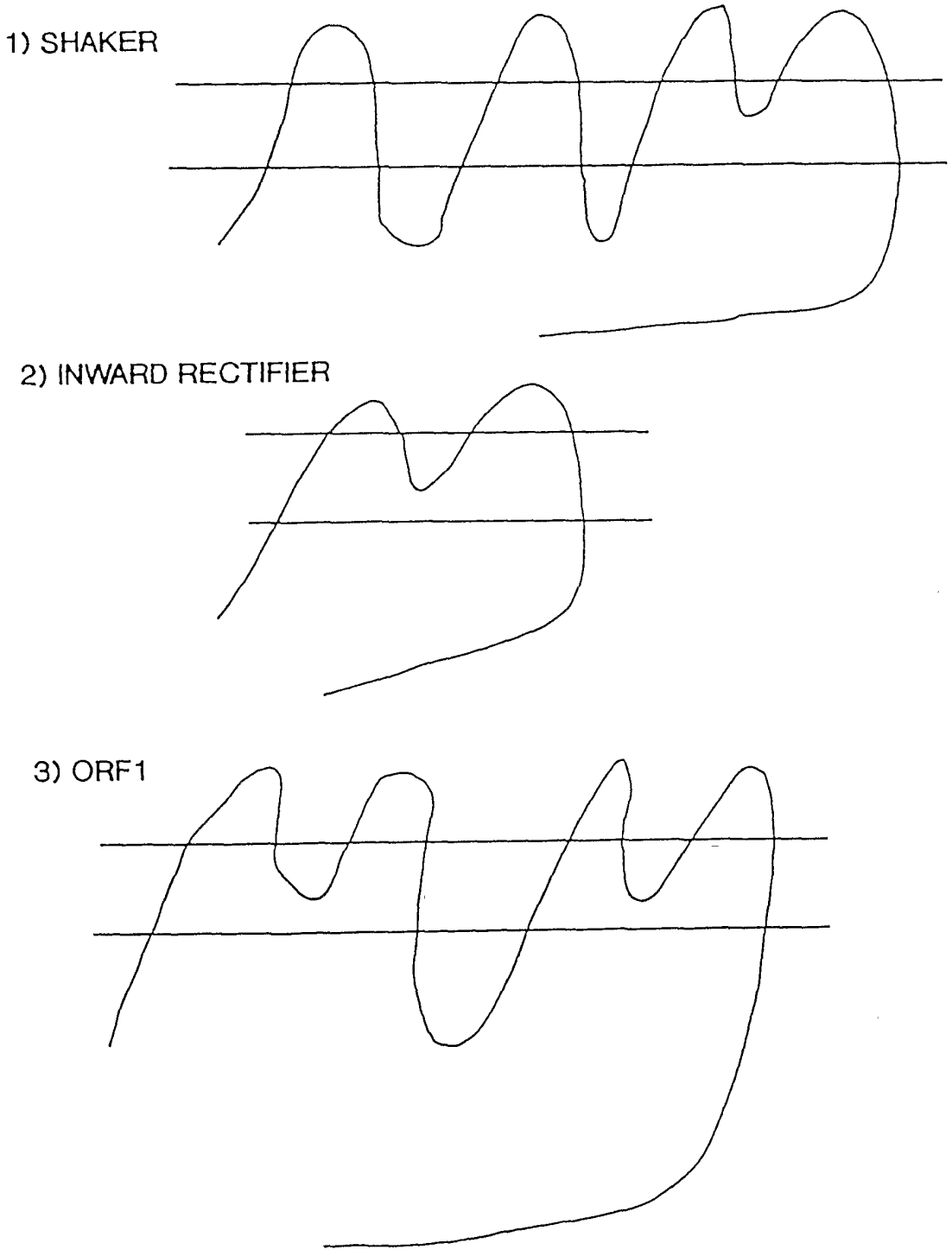


FIG. 6

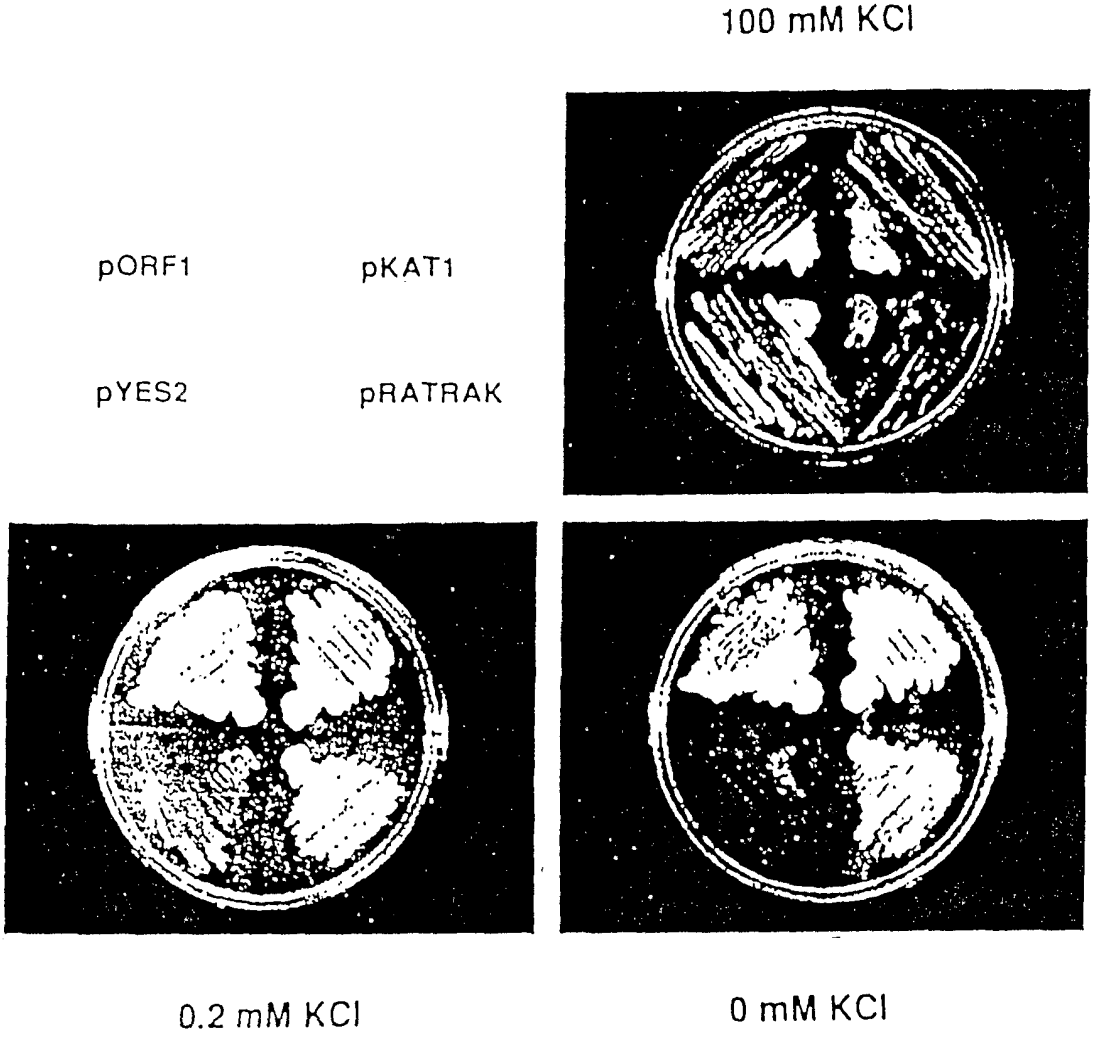


FIG. 7

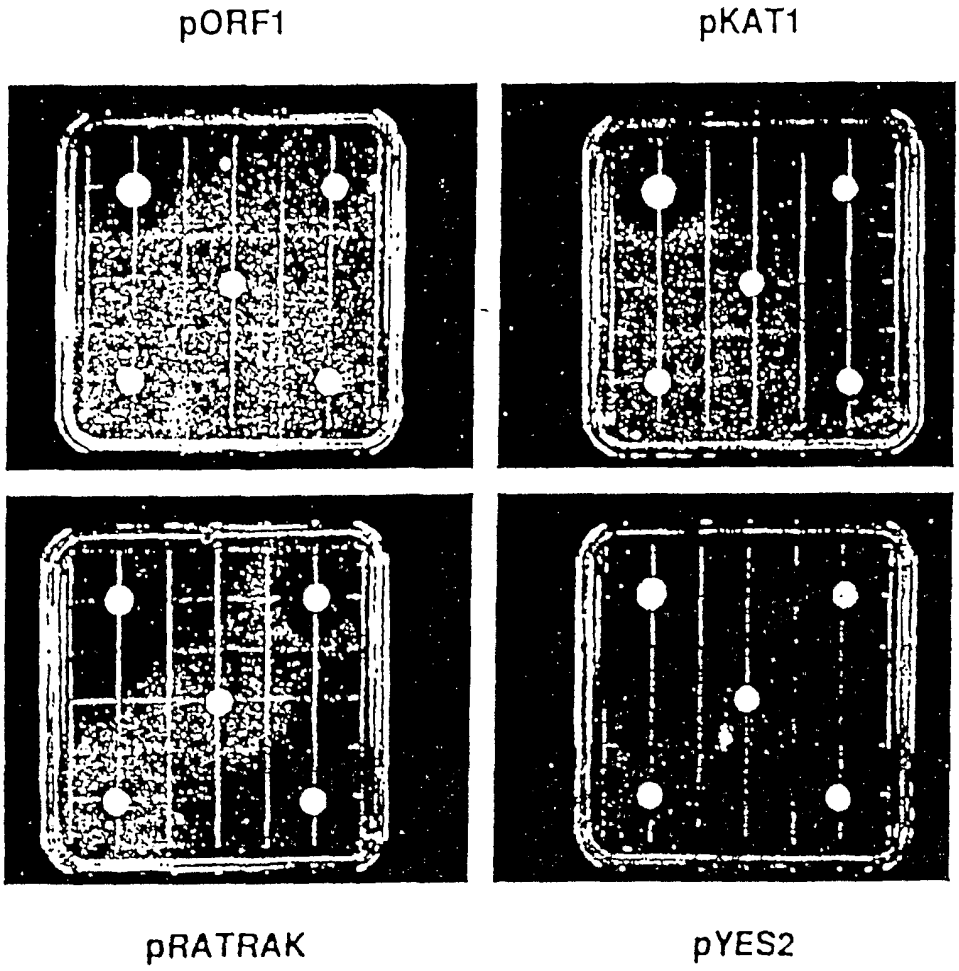


FIG. 8

```
                          G          10                                  20
Met Val Ile Ile Asn Arg Ser Asn Thr Tyr Ala Val Glu Gln Glu Ala Phe Pro Arg Asp Lys Tyr Asn Ile Val
ATG GTA ATA ATC AAC CGA TCG AAC ACC TAT GCC GTT GAG CAG GAA GCA TTT CCA AGA GAC AAG TAC AAT ATT GTC    75

                  30                                  40                                  50
Tyr Trp Leu Val Ile Leu Val Gly Phe Gly Val Leu Leu Pro Trp Asn Met Phe Ile Thr Ile Ala Pro Glu Tyr
TAC TGG CTC GTC ATT CTT GTT GGA TTC GGA GTT CTT CTG CCA TGG AAT ATG TTC ATT ACT ATC GCC CCT GAG TAT   150

                              60                                  70
Tyr Val Asn Tyr Trp Phe Lys Pro Asp Gly Val Glu Thr Trp Tyr Ser Lys Glu Phe Met Gly Ser Leu Thr Ile
TAT GTG AAT TAT TGG TTC AAA CCG GAT GGC GTG GAG ACA TGG TAT TCG AAA GAA TTC ATG GGA TCT TTG ACG ATT   225

                  80          G                       90                                  100
Gly Ser Gln Leu Pro Asn Ala Ser Ile Asn Val Phe Asn Leu Phe Leu Ile Ile Ala Gly Pro Leu Ile Tyr Arg
GGC TCA CAA CTT CCA AAC GCA AGC ATT AAT GTT TTC AAC CTG TTC CTC ATT ATT GCT GGT CCC CTG ATC TAC CGC   300

                              110                                 120
Val Phe Ala Pro Val Cys Phe Asn Ile Val Asn Leu Thr Ile Ile Leu Ile Leu Val Ile Val Leu Glu Pro Thr
GTC TTT GCT CCG GTT TGC TTC AAC ATC GTC AAC CTG ACA ATC ATT CTC ATC CTC GTC ATT GTT CTG GAG CCC ACT   375

                  130                                 140                 G               150
Glu Asp Ser Met Ser Trp Phe Phe Trp Val Thr Leu Gly Met Ala Thr Ser Ile Asn Phe Ser Asn Gly Leu Tyr
GAA GAT TCC ATG TCC TGG TTT TTC TGG GTA ACT CTT GGA ATG GCG ACT TCA ATC AAT TTT AGC AAT GGG CTA TAT   450

                              160                                 170
Glu Asn Ser Val Tyr Gly Val Gly Gly Asp Phe Pro His Thr Tyr Ile Gly Ala Leu Leu Ile Gly Asn Asn Ile
GAA AAC TCG GTT TAT GGA GTT GGT GGC GAT TTT CCG CAC ACC TAC ATT GGC GCT CTC TTG ATT GGA AAC AAC ATT   525

                  180                                 190                                 200
Cys Gly Leu Leu Ile Thr Val Val Lys Ile Gly Val Thr Tyr Phe Leu Asn Asp Glu Pro Lys Leu Val Ala Ile
TGC GGA TTG CTG ATA ACG GTT GTG AAA ATC GGA GTG ACC TAT TTT CTG AAT GAT GAG CCT AAA CTT GTT GCA ATC   600

                              210                                 220
Val Tyr Phe Gly Ile Ser Leu Val Ile Leu Leu Val Cys Ala Ile Ala Leu Phe Phe Ile Thr Lys Gln Asp Phe
GTC TAT TTC GGC ATA TCG TTG GTG ATC CTT CTG GTG TGT GCA ATT GCA CTT TTC TTT ATC ACA AAG CAA GAT TTC   675
```

FIG. 9A

```
                230                                                 240                                                 250
Tyr His Tyr His His Gln 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   750

                                260                                                 270
Trp Thr Thr Phe Thr Asn Cys Tyr Gly Gln 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   825

                280                                                 290                                                 300
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 GGC TTC CTA AAC AAA ATT ATG TCT GAA AAC GAT GAA ATC   900

                                310                                                 320
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 GCG ATT GGA TCC ATA GTT GCT TCC AAG ATT CAC   975

                330                                                 340                                                 350
Trp Pro Thr Pro Arg Tyr Leu Lys Phe Ala Ile Ile Leu Arg Ala Leu Phe Ile Pro Phe Phe Phe Phe Cys Asn
TGG CCG ACA CCC CGT TAC CTC AAA TTT GCC ATA ATC TTG CGT GCT CTT TTC ATT CCA TTC TTC TTC TTC TGC AAC  1050

                                360                                                 370
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 CGT GTC CAG ACG CGT GCT TAT CCT GTT TTC TTT GAG TCT ACT GAC ATT TTT GTG ATT GGT GGA ATT GCC ATG  1125

                380                                                 390                                                 400
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 GGA TAC ACT CCA AAC GTC GTG CCA TCT CAC TAC TCA AGA  1200

                                410                                                 420
Phe Ala Ala Gln 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 GTT TGC ACT CTT ATG GTT GGC CTT CTC ACC GGT GGC CTG TGG CCC GTT GTT ATT GAG  1275

                430             434
His Phe Val Asp Lys Pro Ser Ile Leu
CAC TTC GTG GAC AAG CCA AGT ATC TTA TAA ATATTTATAGCATTAGAGTATACTTGTTATATGTTGTTTTTATTAAGCTGTGGAATAAA  1364

ATAATTATTAAAAAAAAAAAAAAA  1388
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FIG. 9B

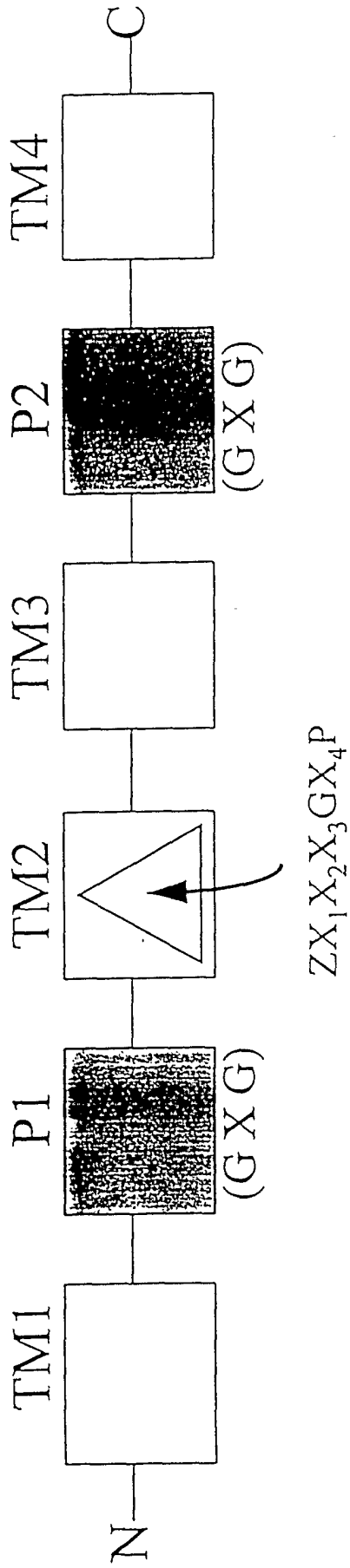


FIG. 10

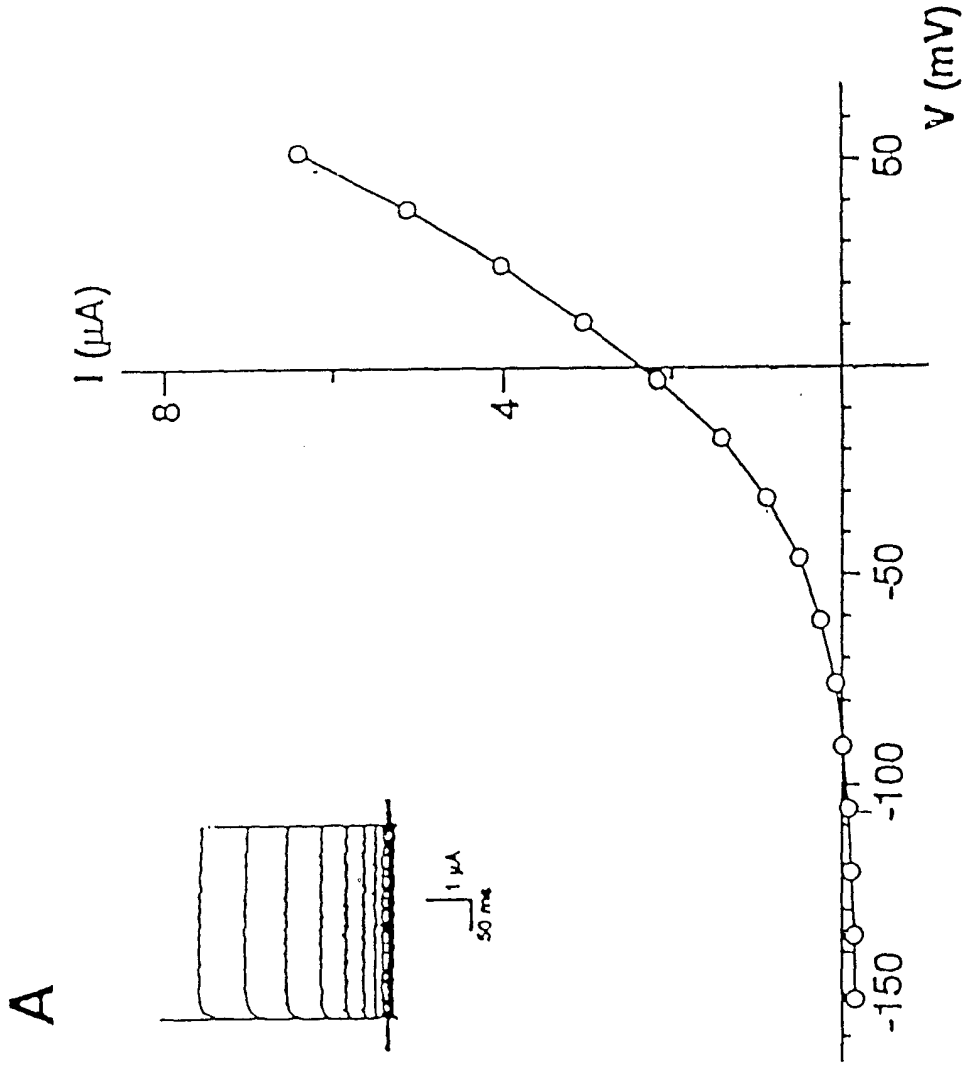


FIG. 11A

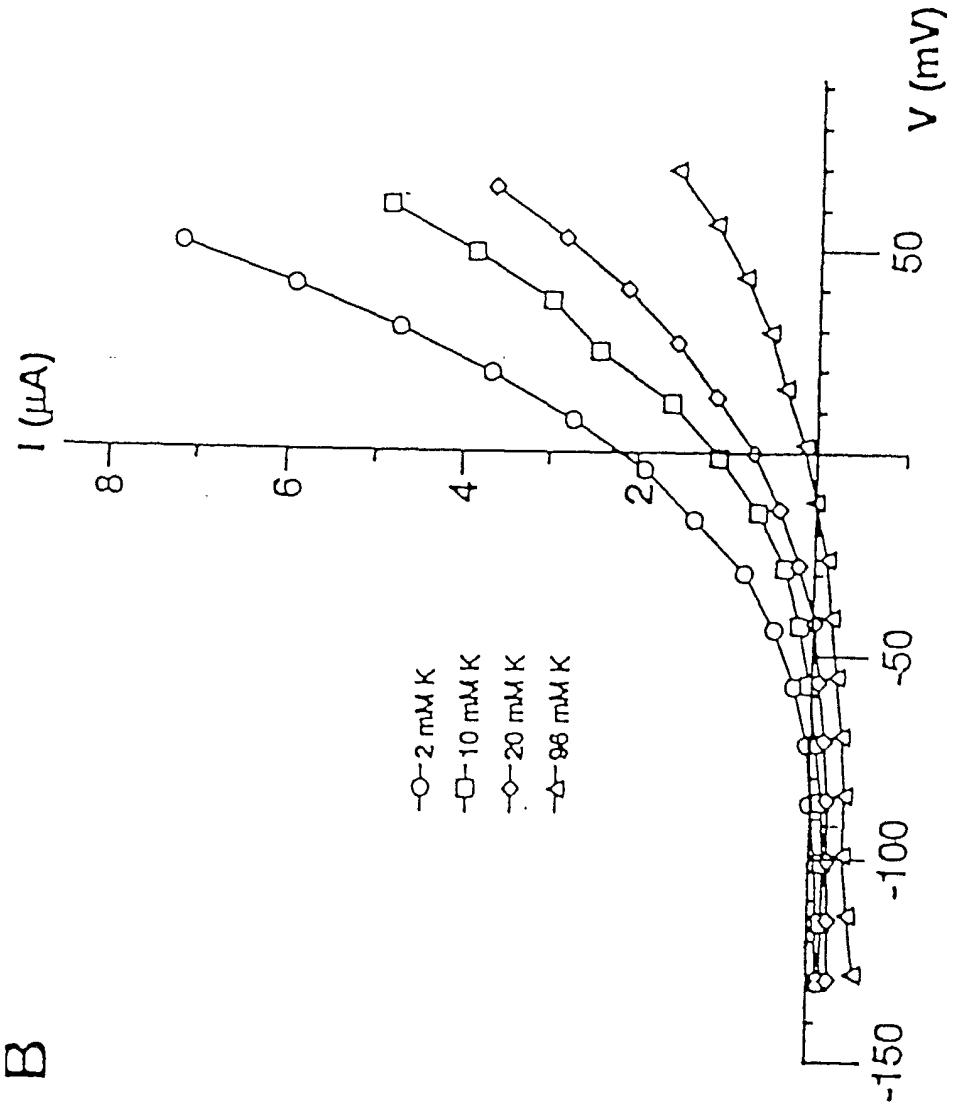
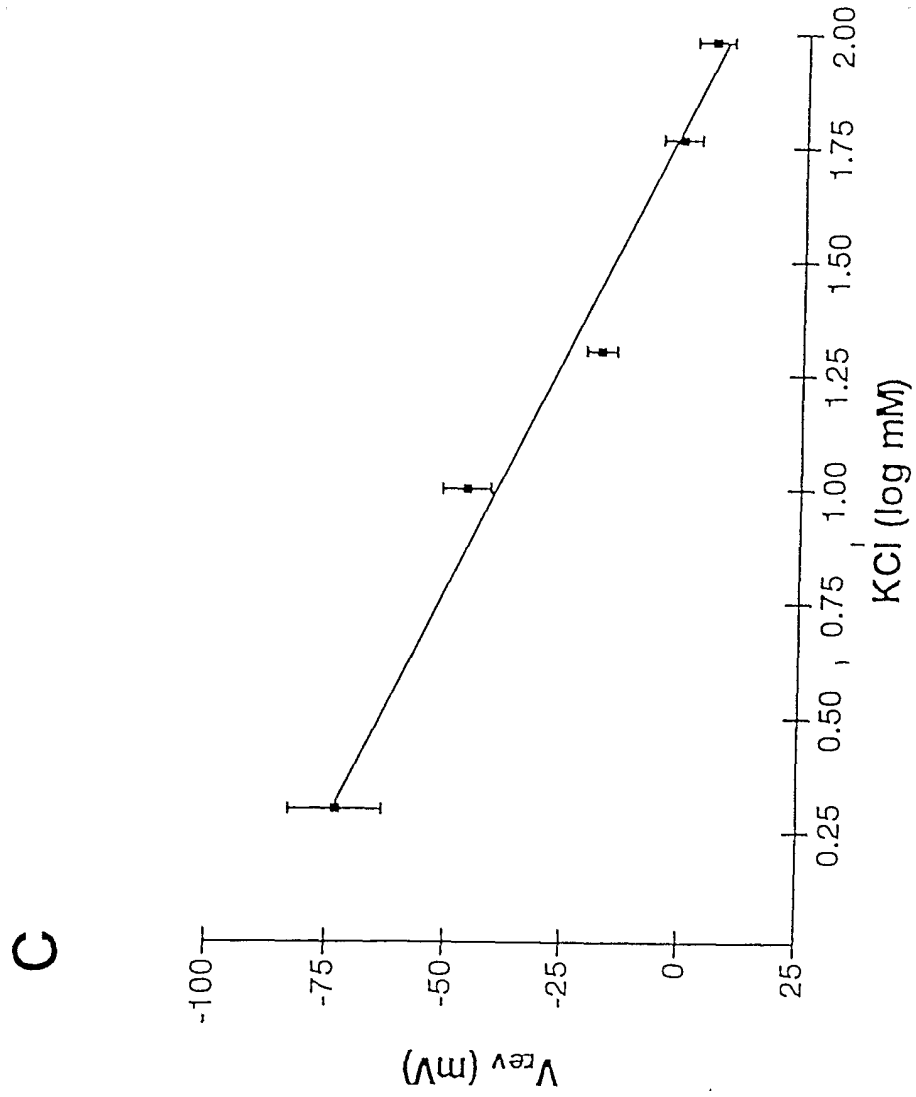


FIG. 11B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-100
-75
-50
-25
0
25
V_{rev} (mV)
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0.50
0.75
1.00
1.25
1.50
1.75
2.00
KCl (log mM)

FIG. 11C

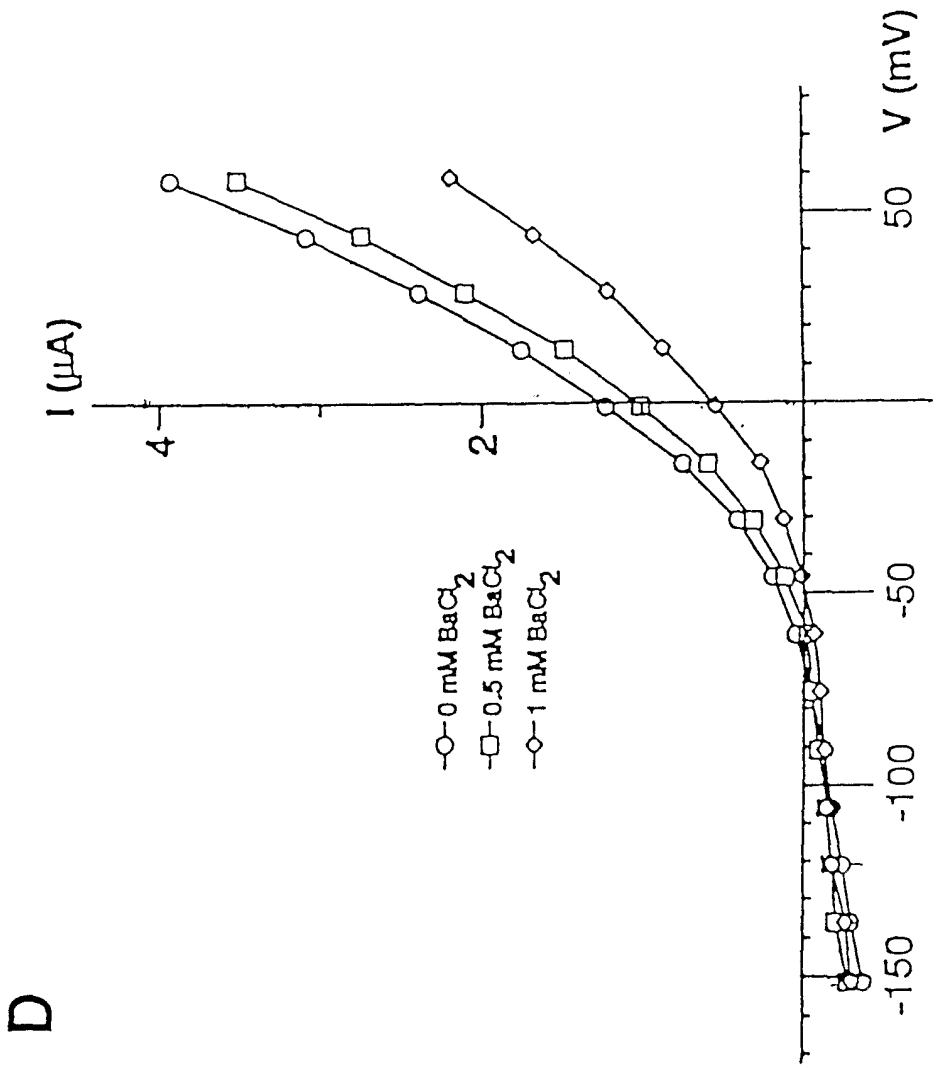

FIG. 11D

SEQUENCE LISTING

<110> PAUSCH, MARK H.

<120> POTASSIUM CHANNELS, NUCLEOTIDE SEQUENCES ENCODING THEM, AND METHODS OF USING SAME

<130> 01142.0114 SEQUENCE LISTING

<140> 09/503,849
<141> 2000-02-15

<150> 08/816,011
<151> 1997-03-11

<150> PCT/US95/14364
<151> 1995-10-25

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Leu Met Phe Gly Ala Ala Ile Tyr Tyr His Ile Glu His Gly Glu Glu
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Lys Ile Ser Arg Ala Glu Gln Arg Lys Ala Gln 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 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
130 135 140

Gly Arg Thr Phe Glu Ala Ile 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

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 Thr 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 Cys Pro Asp
325 330 335

Leu Ser Met Tyr Arg Val Glu Pro Ala Pro Ile Pro Ser Arg Lys Arg

340 345 350

Ala Phe Ser Val Cys Ala Asp Met Val Gly Ala Gln Arg Glu Ala Gly
355 360 365

Met Val His Ala Asn Ser Asp Thr Asp Leu Thr Lys Leu Asp Arg Glu
370 375 380

Lys Thr Phe Glu Thr Ala Glu Ala Tyr His Gln Thr Thr Asp Leu Leu
385 390 395 400

Ala Lys Val Val Asn Ala Leu Ala Thr Val Lys Pro Pro Pro Ala Glu
405 410 415

Gln Glu Asp Ala Ala Leu Tyr Gly Gly Tyr His Gly Phe Ser Asp Ser
420 425 430

Gln Ile Leu Ala Ser Glu Trp Ser Phe Ser Thr Val Asn Glu Phe Thr
435 440 445

Ser Pro Arg Arg Pro Arg Ala Arg Ala Cys Ser Asp Phe Asn Leu Glu
450 455 460

Ala Pro Arg Trp Gln Ser Glu Arg Pro Leu Arg Ser Ser His Asn Glu
465 470 475 480

Trp Thr Trp Ser Gly Asp Asn Gln Gln Ile Gln Glu Ala Phe Asn Gln
485 490 495

Arg Tyr Lys Gly Gln Gln Arg Ala Asn Gly Ala Ala Asn Ser Thr Met
500 505 510

Val His Leu Glu Pro Asp Ala Leu Glu Glu Gln Leu Arg Asn Asn His
515 520 525

Arg Val Pro Val Ala Ser Arg Ser Ser Pro Cys Arg Met Val Cys Asp
530 535 540

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 Arg Pro Pro Val
580 585 590

Asn Pro Ile Cys Ala Thr Asp Ala Val Arg His Arg Pro Ser Asn Arg

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Met Ala Ala Trp Pro Ala Ala Ala Ala Gly
610 615

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<212> DNA
<213> Caenorhabditis elegans

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ggaatggggc atgatatgaa tatcgaggag aaaagaattc ctgcattcct ggtattagct 420
attctgatag tatatacagc gtttggcggt gtcctaatgt caaaattaga gccgtggtct 480
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tcaatgaaaa aaaaacaaaa attcaaaata tttttaggtc ttgcaataac tacaatgtgc 660
attgatttgg taggagtaca gtatattcga aagattcatt atttcggaag aaaaattcaa 720
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<213> Drosophila melanogaster

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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 Cys Ile Leu Phe Ser
50 55 60

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Leu Leu Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Asp Leu Ala Gly
 65                  70                  75                  80

Lys Phe Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys
                 85                  90                  95

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
    130                 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 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 Ser Thr Ser Ser Ser Ala Ile Asp Met Gln
305                 310                 315                 320
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<210> 6
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<213> Drosophila melanogaster

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Arg Cys Val Thr Asp Glu Cys Pro
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<211> 24
<212> PRT
<213> Drosophila melanogaster

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Ile Ser Pro Thr Thr Phe Ala Gly
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<210> 19
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Leu Met Pro Arg Arg Asp Gly Tyr
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<210> 36
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cccgttgtta ttgagcactt cgtggacaag ccaagtatct tataaatatt tatagcatta 1320
gagtatactt gttatatgtt gtttttatta agctgtggaa taaaataatt attaaaaaaa 1380
aaaaaaaaaa aaaa 1394

<210> 37
<211> 479
<212> PRT
<213> Drosophila melanogaster

<400> 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 Gln 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 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                 140

Gly Arg Thr Phe Glu Ala Ile 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

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
```

<210> 38
<211> 334
<212> PRT
<213> Drosophila melanogaster

<400> 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
     50                  55                  60

Ile Gly Ile Pro Leu Thr Leu Val Thr Ile Ala Leu Ala Gly Lys Phe
 65                  70                  75                  80

Leu Ser Glu His Leu Val Trp Leu Tyr Gly Asn Tyr Leu Lys Leu Lys
                 85                  90                  95

Tyr Leu Ile Leu Ser Arg His Arg Lys Glu Arg Arg Glu His Val Cys
            100                 105                 110

Glu His Cys His Ser His Gly Met Gly His Asp Met Asn Ile Glu Glu
        115                 120                 125

Lys Arg Ile Pro Ala Phe Leu Val Leu Ala Ile Leu Ile Val Tyr Thr
    130                 135                 140

Ala Phe Gly Gly Val Leu Met Ser Lys Leu Glu Pro Trp Ser Phe Phe
145                 150                 155                 160

Thr Ser Phe Tyr Trp Ser Phe Ile Thr Met Thr Thr Val Gly Phe Gly
                165                 170                 175

Asp Leu Met Pro Arg Arg Asp Gly Tyr Met Tyr Ile Ile Leu Leu Tyr
            180                 185                 190

Ile Ile Leu Gly Lys Phe Ser Met Lys Lys Lys Gln Lys Phe Lys Ile
        195                 200                 205

Phe Leu Gly Leu Ala Ile Thr Thr Met Cys Ile Asp Leu Val Gly Val
    210                 215                 220

Gln Tyr Ile Arg Lys Ile His Tyr Phe Gly Arg Lys Ile Gln Asp Ala
225                 230                 235                 240

Arg Ser Ala Leu Ala Val Val Gly Gly Lys Val Val Leu Val Ser Glu
```

245 250 255

Leu Tyr Ala Asn Leu Met Gln Lys Arg Ala Arg Asn Met Ser Arg Glu
260 265 270

Ala Phe Ile Val Glu Asn Leu Tyr Val Ser Lys His Ile Ile Pro Phe
275 280 285

Ile Pro Thr Asp Ile Arg Cys Ile Arg Tyr Ile Asp Gln Thr Ala Asp
290 295 300

Ala Ala Thr Ile Ser Thr Ser Ser Ser Ala Ile Asp Met Gln Ser Cys
305 310 315 320

Arg Phe Cys His Ser Arg Tyr Ser Leu Asn Arg Ala Phe Lys
325 330

<210> 39
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<222> (2)
<223> n at position 2 is inosine

<220>
<223> Description of Artificial Sequence: Oligo used in degenerate PCR cloning approach

<400> 39
tnggatwygg wgaywt 16

<210> 40
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: oligo used in degenrate PCR cloning approach

<400> 40
rtcwccrwah ccdaydgt 18

<210> 41
<211> 28
<212> DNA
<213> Homo sapiens .

<400> 41
cgcaggcaga gccacaaaga gtacacag 28

<210> 42
<211> 26
<212> DNA
<213> Homo sapiens

<400> 42
ggagatcagc taggcaccat atttgg 26

<210> 43
<211> 26
<212> DNA
<213> Homo sapiens

<400> 43
atgctgcatg cctcatgctt cccagc 26

<210> 44
<211> 20
<212> DNA
<213> Homo sapiens

<400> 44
ggttatttaa agagagggct 20

<210> 45
<211> 426
<212> PRT
<213> Homo sapiens

<400> 45
Met Leu Pro Ser Ala Ser Arg Glu Arg Pro Gly Tyr Arg Ala Gly Val
1 5 10 15

Ala Ala Pro Asp Leu Leu Asp Pro Lys Ser Ala Ala Gln Asn Ser Lys
20 25 30

```
Pro Arg Leu Ser Phe Ser Thr Lys Pro Thr Val Leu Ala Ser Arg Val
         35                  40                  45

Glu Ser Asp Thr Thr Ile Asn Val Met Lys Trp Lys Thr Val Ser Thr
     50                  55                  60

Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Thr Val Phe
 65                  70                  75                  80

Lys Ala Leu Glu Gln Pro His Glu Ile Ser Gln Arg Thr Thr Ile Val
                 85                  90                  95

Ile Gln Lys Gln Thr Phe Ile Ser Gln His Ser Cys Val Asn Ser Thr
            100                 105                 110

Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn Ala Gly
        115                 120                 125

Ile Ile Pro Leu Gly Asn Thr Ser Asn Gln Ile Ser His Trp Asp Leu
    130                 135                 140

Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile Gly Phe
145                 150                 155                 160

Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys Ile Ile
                165                 170                 175

Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala Gly Val
            180                 185                 190

Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys Val Glu
        195                 200                 205

Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg Ile Ile
    210                 215                 220

Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val Ala Leu
225                 230                 235                 240

Pro Ala Ile Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu Asp Ala
                245                 250                 255

Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Gly Asp Tyr
            260                 265                 270

Val Ala Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys Pro Val
        275                 280                 285
```

```
Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala Val Leu
    290                 295                 300

Ser Met Ile Gly Arg Leu Val Arg Val Ile Ser Lys Lys Thr Lys Glu
305                 310                 315                 320

Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala Asn Val
                325                 330                 335

Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu Ile Tyr
            340                 345                 350

Asp Lys Phe Gln Arg Ala Thr Ser Ile Lys Arg Lys Leu Ser Ala Glu
        355                 360                 365

Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg Thr Leu
    370                 375                 380

Ser Val Asn His Leu Thr Ser Glu Arg Asp Val Leu Pro Pro Leu Leu
385                 390                 395                 400

Lys Thr Glu Ser Ile Tyr Leu Asn Gly Leu Ala Pro His Cys Ala Gly
                405                 410                 415

Glu Glu Ile Ala Val Ile Glu Asn Ile Lys
            420                 425
```

<210> 46
<211> 2130
<212> DNA
<213> Homo sapiens

<400> 46

```
ccatcctaat acgactcact atagggctcg agcgnccgcc cgggcagtaa aatgcctgcc 60
cgtgcagctc ggagcgcgca gcccgtctct gaataagaag tgagtacaat ggcgtgtttg 120
taaaaaaaag cttcaagtcc gtctttttca aaaaacattt tgaatgctgc atgcctcatg 180
cttcccagcg cctcgcggga gagacccggc tatagagcag gagtggcggc acctgacttg 240
ctggatccta aatctgccgc tcagaactcc aaaccgaggc tctcattttc cacgaaaccc 300
acagtgcttg cttcccgggt ggagagtgac acgaccatta atgttatgaa atggaagacg 360
gtctccacga tattcctggt ggttgtcctc tatctgatca tcggagccac cgtgttcaaa 420
gcattggagc agcctcatga gatttcacag aggaccacca ttgtgatcca gaagcaaaca 480
ttcatatccc aacattcctg tgtcaattcg acggagctgg atgaactcat tcagcaaata 540
gtggcagcaa taaatgcagg gattataccg ttaggaaaca cctccaatca aatcagtcac 600
tgggatttgg gaagttcctt cttctttgct ggcactgtta ttacaaccat aggatttgga 660
aacatctcac cacgcacaga aggcggcaaa atattctgta tcatctatgc cttactggga 720
attcccctct ttggttttct cttggctgga gttggagatc agctaggcac catatttgga 780
```

aaaggaattg ccaaagtgga agatacgttt attaagtgga atgttagtca gaccaagatt 840
cgcatcatct caacaatcat atttatacta tttggctgtg tactctttgt ggctctgcct 900
gcgatcatat tcaaacacat agaaggctgg agtgccctgg acgccattta ttttgtggtt 960
atcactctaa caactattgg atttggtgac tacgttgcag gtggatccga tattgaatat 1020
ctggacttct ataagcctgt cgtgtggttc tggatccttg tagggcttgc ttactttgct 1080
gctgtcctga gcatgattgg gagattggtc cgagtgatat ctaaaaagac aaaagaagag 1140
gtgggagagt tcagagcaca cgctgctgag tggacagcca acgtcacagc cgaattcaaa 1200
gaaaccagga ggcgactgag tgtggagatt tatgacaagt tccagcgggc cacctccatc 1260
aagcggaagc tctcggcaga actggctgga aaccacaatc aggagctgac tccttgtagg 1320
aggaccctgt cagtgaacca cctgaccagc gagagggatg tcttgcctcc cttactgaag 1380
actgagagta tctatctgaa tggtttggcg ccacactgtg ctggtgaaga gattgctgtg 1440
attgagaaca tcaaatagcc ctctctttaa ataaccttag gcatagccat aggtgaggac 1500
ttctctatgc tctttatgac tgttgctggt agcatttttt aaattgtgca tgagctcaaa 1560
gggggaacaa aatagataca cccatcatgg tcatctatca tcaagagaat ttggaattct 1620
gagccagcac tttctttctg atgatgcttg ttgaacggcc cactttcttt gatgagtgga 1680
atgacaagca atgtctgatg cctttgtgtg cccagactgt tttcctctct ctttccctaa 1740
tgtgccataa ggcctcagaa tgaattgaga attgtttctg gtaacaatgt agctttgagg 1800
gatcagttct taacttttca gggtctacct aactgagcct agatatggac catttatgga 1860
tgacaacaat tttttttttg taaatgacaa gaaattctta tgcagccttt tacctaagaa 1920
atttctgtca gtgccttatc ttatgaagaa acagaacctc tctagctaat gtgtggtttc 1980
tccttccctg cccccacccc taggctcacc tctgcagtct tttaccccag ttctcccatt 2040
tgaataccat accttgntgg aaacagngtg taaaatgact gaagtgatga tgccgaagat 2100
gaaatagatg ncaaattagn tggacattga 2130

<210> 47
<211> 27
<212> DNA
<213> Homo sapiens

<400> 47
aaaagatcta aaatgcttcc cagcgcc 27

<210> 48
<211> 27
<212> DNA
<213> Homo sapiens

<400> 48
aaagtcgacc tatttgatgt tctcaat 27

<210> 49
<211> 27
<212> DNA
<213> Homo sapiens

<400> 49
aaaaagctta aaatgcttcc cagcgcc 27

<210> 50
<211> 27
<212> DNA
<213> Homo sapiens

<400> 50
aaatctagac tatttgatgt tctcaat 27

<210> 51
<211> 534
<212> DNA
<213> Homo sapiens

<400> 51
aacaaaaacc ttttttgttt tgaatggcct agagagggta agggatcccc tgacgaacag 60
gagcagagcc agctagaacc tgggcctggc cagttcaagg ccaccagagg gcagccttct 120
gcggaaggca gtattggggt aggcagggac cccagcagac atggcactca gagctctcac 180
tgtccactga ctctctcttc tccaggttat ggccacatgg ccccactatc gccaggcgga 240
aaggccttct gcatggtctt atagcccttg ggctgccagc ctccttagct ctcgtggcca 300
ccctgcgcca ttgcctgctg cctgtgctca gccgcccacg tgcctgggta gcggtccact 360
ggcagctgtc accggccagg gctgcgctgc tgcaggcagt tgcactggga ctgctggtgg 420
ccagcagctt tgtgctgctg ccagcgctgg tgctgtgggg ccttcagggc gactgcagcc 480
tgctgggggc cgtctacttc tgcttcagct cgctcagcac cattggcctg gggg 534

<210> 52
<211> 956
<212> DNA
<213> Mus musculus

<400> 52
atgatacgat ttaatacgac tcactatagg gaatttggcc ctcgaggcca agaattcggc 60
acgaggagaa tgtgcgcacg ttggctctca tcgtgtgcac cttcacctac ctgctggtgg 120
gcgccgcggt gttcgacgca ctggagtcgg agccggagat gatcgagcgg cagcggctgg 180
agctgcggca gctggagctg cgggcgcgct acaacctcag cgagggcggc tacgaggagc 240
tggagcgcgt cgtgctgcgc ctcaagccgc acaaggccgg cgtgcagtgg cgcttcgccg 300
gctccttcta cttcgccatc accgtcatca ccaccatcgg ctatggtcat gcggcgccca 360
gcacggacgg aggcaaggtg ttctgcatgt tctacgcgct gctgggcatc ccgctcacac 420
tagtcatgtt ccagagcctg ggtgaacgca tcaacacctc cgtgaggtac ctgctgcacc 480
gtgccaagag ggggctgggc atgcggcacg ccgaagtgtc catggccaac atggtgctca 540
tcggtttcgt gtcgtgcatc agcacgctgt gcatcggcgc agctgccttc tcctactacg 600
agcgctggac tttcttccag gcctattact actgcttcat caccctcacc accatcggct 660
tcggcgacta tgtggcgctg cagaaggacc aggcgctgca gacgcagccg cagtatgtgg 720

cttcagcttc gtgtacatcc tcacgggctc acggtcatcg gcgcttcctc aacctcgtgg 780
tgctgcgatt catgaccatg aacgccgagg acgagaagcg tgatgcggag caccgcgccc 840
tgctcacgca caacggccag gctgtcggcc tgggtggcct gagctgcctg agcggtagcc 900
tgggcgacgg cgtgcgtccc cgcgacccag tcacatgcgc tgcggccgca agctta 956

<210> 53
<211> 1052
<212> DNA
<213> Mus musculus

<400> 53
ctgaaaccat gggcccgata cctgctcctg cttatggccc acctgctggc catgggcctt 60
ggggctgtgg tgcttcaggc cctggagggc cctccagctc gccacctcca ggcccaggtc 120
caggctgaac tggctagctt ccaggcagag cacagggcct gcttgccacc tgaggccctg 180
gaggagctgc taggtgcggt cctgagagca caggcccatg gagtttccag cctgggcaac 240
agctcaagac aagcaactgg gatctgccct cagctctgct gttcactgcc agcatcctca 300
ccaccaccgg ttatggccac atggccccac tctcctcagg tggaaaggcc ttctgtgtgg 360
tctatgcagc ccttgggctg ccagcctctc tagcacttgt ggctgccctg cgccactgct 420
tgctgcctgt gttcagtcgc ccaggtgact gggtagccat tcgctggcag ctggcaccag 480
ctcaggctgc tctgctacag gcagcaggac tgggcctcct ggtggcctgt gtcttcatgc 540
tgctgccagc actggtgctg tggggtgtac agggtgactg gcagcctgct aaaccatcta 600
cttctgtttc ggctcactca gcacgatcgg cctaggagac ttgctgcctg cccatggacg 660
tggcctgcac ccagccattt accaccttgg gcagtttgca cttcttggtt acttgctcct 720
ggggctcctg gccatgttgt tagcagtaga gaccttctca gagctgcctc aggtccgtgc 780
catggtgaaa ttctttgggc ccagtggctc tagaaccgat gaagatcaag atggcatcct 840
aggccaagat gagctggctc tgagcactgt gctgcctgac gccccagtct tgggaccaac 900
caccccagcc tgagcgggag gcaccaagga gtgcttgaag aacatagcag aagggttatg 960
ggaatgaata tgtcatggga taatgttaat tttaaaaatt aaatgggctg cttagcatgc 1020
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 1052

<210> 54
<211> 178
<212> PRT
<213> Homo sapiens

<220>
<221> UNSURE
<222> (88)
<223> Xaa at position 88 indicates an undetermined
residue

<400> 54
Asn Lys Asn Leu Phe Cys Phe Glu Trp Pro Arg Glu Gly Lys Gly Ser
1 5 10 15

Pro Asp Gln Glu Glu Gln Ser Gln Leu Glu Pro Gly Pro Gly Gln Phe

20 25 30

Lys Ala Thr Arg Gly Gln Pro Ser Ala Glu Gly Ser Ile Gly Val Gly
35 40 45

Arg Asp Pro Ser Arg His Gly Thr Gln Ser Ser His Cys Pro Leu Thr
50 55 60

Leu Ser Ser Pro Gly Tyr Gly His Met Ala Pro Leu Ser Pro Gly Gly
65 70 75 80

Lys Ala Phe Cys Met Val Leu Xaa Ala Leu Gly Leu Pro Ala Ser Leu
85 90 95

Ala Leu Val Ala Thr Leu Arg His Cys Leu Leu Pro Val Leu Ser Arg
100 105 110

Pro Arg Ala Trp Val Ala Val His Trp Gln Leu Ser Pro Ala Arg Ala
115 120 125

Ala Leu Leu Gln Ala Val Ala Leu Gly Leu Leu Val Ala Ser Ser Phe
130 135 140

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

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

Leu Gly

<210> 55
<211> 312
<212> PRT
<213> Mus musculus

<400> 55
Gly Ile Trp Pro Ser Arg Pro Arg Ile Arg His Glu Glu Asn Val Arg
1 5 10 15

Thr Leu Ala Leu Ile Val Cys Thr Phe Thr Tyr Leu Leu Val Gly Ala
20 25 30

Ala Val Phe Asp Ala Leu Glu Ser Glu Pro Glu Met Ile Glu Arg Gln
35 40 45

```
Arg Leu Glu Leu Arg Gln Leu Glu Leu Arg Ala Arg Tyr Asn Leu Ser
     50                  55                  60

Glu Gly Gly Tyr Glu Glu Leu Glu Arg Val Val Leu Arg Leu Lys Pro
 65                  70                  75                  80

His Lys Ala Gly Val Gln Trp Arg Phe Ala Gly Ser Phe Tyr Phe Ala
                 85                  90                  95

Ile Thr Val Ile Thr Thr Ile Gly Tyr Gly His Ala Ala Pro Ser Thr
            100                 105                 110

Asp Gly Gly Lys Val Phe Cys Met Phe Cys Met Phe Tyr Ala Leu Leu
        115                 120                 125

Gly Ile Pro Leu Thr Leu Val Met Phe Gln Ser Leu Gly Glu Arg Ile
    130                 135                 140

Asn Thr Ser Val Arg Tyr Leu Leu His Arg Ala Lys Arg Gly Leu Gly
145                 150                 155                 160

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

Val Ser Cys Ile Ser Thr Leu Cys Ile Gly Ala Ala Ala Phe Ser Tyr
            180                 185                 190

Tyr Glu Arg Trp Thr Phe Phe Gln Ala Tyr Tyr Tyr Cys Phe Ile Thr
        195                 200                 205

Leu Thr Thr Ile Gly Phe Gly Asp Tyr Val Ala Leu Gln Lys Asp Gln
    210                 215                 220

Ala Leu Gln Thr Gln Pro Gln Tyr Val Ala Ser Ala Ser Cys Thr Ser
225                 230                 235                 240

Ser Arg Ala His Gly His Arg Arg Phe Leu Asn Leu Val Val Leu Arg
                245                 250                 255

Phe Met Thr Met Asn Ala Glu Asp Glu Lys Arg Asp Ala Glu His Arg
            260                 265                 270

Ala Leu Leu Thr His Asn Gly Gln Ala Val Gly Leu Gly Gly Leu Ser
        275                 280                 285

Cys Leu Ser Gly Ser Leu Gly Asp Gly Val Arg Pro Arg Asp Pro Val
    290                 295                 300
```

Thr Cys Ala Ala Ala Ala Ser Leu
305 310

<210> 56
<211> 304
<212> PRT
<213> Mus musculus

<220>
<221> UNSURE
<222> (83)
<223> Xaa at position 83 indicates an undetermined residue

<220>
<221> UNSURE
<222> (198)
<223> Xaa at position 198 indicates an undetermined residue

<400> 56
Leu Lys Pro Trp Ala Arg Tyr Leu Leu Leu Leu Met Ala His Leu Leu
1 5 10 15

Ala Met Gly Leu Gly Ala Val Val Leu Gln Ala Leu Glu Gly Pro Pro
20 25 30

Ala Arg His Leu Gln Ala Gln Val Gln Ala Glu Leu Ala Ser Phe Gln
35 40 45

Ala Glu His Arg Ala Cys Leu Pro Pro Glu Ala Leu Glu Glu Leu Leu
50 55 60

Gly Ala Val Leu Arg Ala Gln Ala His Gly Val Ser Ser Leu Gly Asn
65 70 75 80

Ser Ser Xaa Thr Ser Asn Trp Asp Leu Pro Ser Ala Leu Leu Phe Thr
85 90 95

Ala Ser Ile Leu Thr Thr Thr Gly Tyr Gly His Met Ala Pro Leu Ser
100 105 110

Ser Gly Gly Lys Ala Phe Cys Val Val Tyr Ala Ala Leu Gly Leu Pro
115 120 125

Ala Ser Leu Ala Leu Val Ala Ala Leu Arg His Cys Leu Leu Pro Val
130 135 140

```
Phe Ser Arg Pro Gly Asp Trp Val Ala Ile Arg Trp Gln Leu Ala Pro
145                 150                 155                 160

Ala Gln Ala Ala Leu Leu Gln Ala Ala Gly Leu Gly Leu Leu Val Ala
                165                 170                 175

Cys Val Phe Met Leu Leu Pro Ala Leu Val Leu Trp Gly Val Gln Gly
            180                 185                 190

Asp Trp Gln Pro Ala Xaa Thr Ile Tyr Phe Cys Phe Gly Ser Leu Ser
        195                 200                 205

Thr Ile Gly Leu Gly Asp Leu Leu Pro Ala His Gly Arg Gly Leu His
    210                 215                 220

Pro Ala Ile Tyr His Leu Gly Gln Phe Ala Leu Leu Gly Tyr Leu Leu
225                 230                 235                 240

Leu Gly Leu Leu Ala Met Leu Leu Ala Val Glu Thr Phe Ser Glu Leu
                245                 250                 255

Pro Gln Val Arg Ala Met Val Lys Phe Phe Gly Pro Ser Gly Ser Arg
            260                 265                 270

Thr Asp Glu Asp Gln Asp Gly Ile Leu Gly Gln Asp Glu Leu Ala Leu
        275                 280                 285

Ser Thr Val Leu Pro Asp Ala Pro Val Leu Gly Pro Thr Thr Pro Ala
    290                 295                 300
```

<210> 57
<211> 426
<212> PRT
<213> Homo sapiens

```
<400> 57
Met Leu Pro Ser Ala Ser Arg Glu Arg Pro Gly Tyr Arg Ala Gly Val
  1               5                  10                  15

Ala Ala Pro Asp Leu Leu Asp Pro Lys Ser Ala Ala Gln Asn Ser Lys
            20                  25                  30

Pro Arg Leu Ser Phe Ser Thr Lys Pro Thr Val Leu Ala Ser Arg Val
```

```
         35                  40                  45

Glu Ser Asp Thr Thr Ile Asn Val Met Lys Trp Lys Thr Val Ser Thr
     50                  55                  60

Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Thr Val Phe
 65                  70                  75                  80

Lys Ala Leu Glu Gln Pro His Glu Ile Ser Gln Arg Thr Thr Ile Val
                 85                  90                  95

Ile Gln Lys Gln Thr Phe Ile Ser Gln His Ser Cys Val Asn Ser Thr
            100                 105                 110

Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn Ala Gly
        115                 120                 125

Ile Ile Pro Leu Gly Asn Thr Ser Asn Gln Ile Ser His Trp Asp Leu
    130                 135                 140

Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile Gly Phe
145                 150                 155                 160

Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys Ile Ile
                165                 170                 175

Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala Gly Val
            180                 185                 190

Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys Val Glu
        195                 200                 205

Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg Ile Ile
    210                 215                 220

Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val Ala Leu
225                 230                 235                 240

Pro Ala Ile Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu Asp Thr
                245                 250                 255

Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Gly Asp Tyr
            260                 265                 270

Val Ala Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys Pro Val
        275                 280                 285

Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala Val Leu
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290 295 300

Ser Met Ile Gly Arg Leu Val Arg Val Ile Ser Lys Lys Thr Lys Glu
305 310 315 320

Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala Asn Val
325 330 335

Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu Ile Tyr
340 345 350

Asp Lys Phe Gln Arg Ala Thr Ser Ile Lys Arg Lys Leu Ser Ala Glu
355 360 365

Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg Thr Leu
370 375 380

Ser Val Asn His Leu Thr Ser Glu Arg Asp Val Leu Pro Pro Leu Leu
385 390 395 400

Lys Thr Glu Ser Ile Tyr Leu Asn Gly Leu Ala Pro His Cys Ala Gly
405 410 415

Glu Glu Ile Ala Val Ile Glu Asn Ile Lys
420 425

<210> 58
<211> 426
<212> PRT
<213> Homo sapiens

<400> 58
Met Leu Pro Ser Ala Ser Arg Glu Arg Pro Gly Tyr Arg Ala Gly Val
1 5 10 15

Ala Ala Pro Asp Leu Leu Asp Pro Lys Ser Ala Ala Gln Asn Ser Lys
20 25 30

Pro Arg Leu Ser Phe Ser Thr Lys Pro Thr Val Leu Ala Ser Arg Val
35 40 45

Glu Ser Asp Thr Thr Ile Asn Val Met Lys Trp Lys Thr Val Ser Thr
50 55 60

Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Thr Val Phe
65 70 75 80

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Lys Ala Leu Glu Gln Pro His Glu Ile Ser Gln Arg Thr Thr Ile Val
                85                  90                  95

Ile Gln Lys Gln Thr Phe Ile Ser Gln His Ser Cys Val Asn Ser Thr
            100                 105                 110

Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn Ala Gly
        115                 120                 125

Ile Ile Pro Leu Gly Asn Thr Ser Asn Gln Ile Ser His Trp Asp Leu
    130                 135                 140

Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile Gly Phe
145                 150                 155                 160

Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys Ile Ile
                165                 170                 175

Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala Gly Val
            180                 185                 190

Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys Val Glu
        195                 200                 205

Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg Ile Ile
    210                 215                 220

Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val Ala Leu
225                 230                 235                 240

Pro Ala Ile Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu Asp Ala
                245                 250                 255

Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Gly Asp His
            260                 265                 270

Val Ala Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys Pro Val
        275                 280                 285

Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala Val Leu
    290                 295                 300

Ser Met Ile Gly Arg Leu Val Arg Val Ile Ser Lys Lys Thr Lys Glu
305                 310                 315                 320

Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala Asn Val
                325                 330                 335
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Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu Ile Tyr
            340                 345                 350

Asp Lys Phe Gln Arg Ala Thr Ser Ile Lys Arg Lys Leu Ser Ala Glu
        355                 360                 365

Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg Thr Leu
    370                 375                 380

Ser Val Asn His Leu Thr Ser Glu Arg Asp Val Leu Pro Pro Leu Leu
385                 390                 395                 400

Lys Thr Glu Ser Ile Tyr Leu Asn Gly Leu Ala Pro His Cys Ala Gly
                405                 410                 415

Glu Glu Ile Ala Val Ile Glu Asn Ile Lys
            420                 425
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<210> 59
<211> 426
<212> PRT
<213> Homo sapiens

<400> 59

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Met Leu Pro Ser Ala Ser Arg Glu Arg Pro Gly Tyr Arg Ala Gly Val
  1               5                  10                  15

Ala Ala Pro Asp Leu Leu Asp Pro Lys Ser Ala Ala Gln Asn Ser Lys
             20                  25                  30

Pro Arg Leu Ser Phe Ser Thr Lys Pro Thr Val Leu Ala Ser Arg Val
         35                  40                  45

Glu Ser Asp Thr Thr Ile Asn Val Met Lys Trp Lys Thr Val Ser Thr
     50                  55                  60

Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Thr Val Phe
 65                  70                  75                  80

Lys Ala Leu Glu Gln Pro His Glu Ile Ser Gln Arg Thr Thr Ile Val
                 85                  90                  95

Ile Gln Lys Gln Thr Phe Ile Ser Gln His Ser Cys Val Asn Ser Thr
            100                 105                 110

Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn Ala Gly
        115                 120                 125
```

Ile Ile Pro Leu Gly Asn Thr Ser Asn Gln Ile Ser His Trp Asp Leu
130 135 140

Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile Gly Phe
145 150 155 160

Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys Ile Ile
165 170 175

Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala Gly Val
180 185 190

Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys Val Glu
195 200 205

Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg Ile Ile
210 215 220

Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val Ala Leu
225 230 235 240

Pro Ala Ile Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu Asp Ala
245 250 255

Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Gly Asp His
260 265 270

Val Val Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys Pro Val
275 280 285

Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala Val Leu
290 295 300

Ser Met Ile Gly Arg Leu Val Arg Val Ile Ser Lys Lys Thr Lys Glu
305 310 315 320

Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala Asn Val
325 330 335

Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu Ile Tyr
340 345 350

Asp Lys Phe Gln Arg Ala Thr Ser Ile Lys Arg Lys Leu Ser Ala Glu
355 360 365

Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg Thr Leu
370 375 380

```
Ser Val Asn His Leu Thr Ser Glu Arg Asp Val Leu Pro Pro Leu Leu
385                 390                 395                 400

Lys Thr Glu Ser Ile Tyr Leu Asn Gly Leu Ala Pro His Cys Ala Gly
                405                 410                 415

Glu Glu Ile Ala Val Ile Glu Asn Ile Lys
            420                 425
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<210> 60
<211> 426
<212> PRT
<213> Homo sapiens

<400> 60

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Met Leu Pro Ser Ala Ser Arg Glu Arg Pro Gly Tyr Arg Ala Gly Val
  1               5                  10                  15

Ala Ala Pro Asp Leu Leu Asp Pro Lys Ser Ala Ala Gln Asn Ser Lys
             20                  25                  30

Pro Arg Leu Ser Phe Ser Thr Lys Pro Thr Val Leu Ala Ser Arg Val
         35                  40                  45

Glu Ser Asp Thr Thr Ile Asn Val Met Lys Trp Lys Thr Val Ser Thr
     50                  55                  60

Ile Phe Leu Val Val Val Leu Tyr Leu Ile Ile Gly Ala Thr Val Phe
 65                  70                  75                  80

Lys Ala Leu Glu Gln Pro His Glu Ile Ser Gln Arg Thr Thr Ile Val
                85                  90                  95

Ile Gln Lys Gln Thr Phe Ile Ser Gln His Ser Cys Val Asn Ser Thr
            100                 105                 110

Glu Leu Asp Glu Leu Ile Gln Gln Ile Val Ala Ala Ile Asn Ala Gly
        115                 120                 125

Ile Ile Pro Leu Gly Asn Thr Ser Asn Gln Ile Ser His Trp Asp Leu
    130                 135                 140

Gly Ser Ser Phe Phe Phe Ala Gly Thr Val Ile Thr Thr Ile Gly Phe
145                 150                 155                 160

Gly Asn Ile Ser Pro Arg Thr Glu Gly Gly Lys Ile Phe Cys Ile Ile
```

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                165                 170                 175

Tyr Ala Leu Leu Gly Ile Pro Leu Phe Gly Phe Leu Leu Ala Gly Val
            180                 185                 190

Gly Asp Gln Leu Gly Thr Ile Phe Gly Lys Gly Ile Ala Lys Val Glu
        195                 200                 205

Asp Thr Phe Ile Lys Trp Asn Val Ser Gln Thr Lys Ile Arg Ile Ile
    210                 215                 220

Ser Thr Ile Ile Phe Ile Leu Phe Gly Cys Val Leu Phe Val Ala Leu
225                 230                 235                 240

Pro Ala Ile Ile Phe Lys His Ile Glu Gly Trp Ser Ala Leu Asp Ala
                245                 250                 255

Ile Tyr Phe Val Val Ile Thr Leu Thr Thr Ile Gly Phe Arg Asp Tyr
            260                 265                 270

Val Ala Gly Gly Ser Asp Ile Glu Tyr Leu Asp Phe Tyr Lys Pro Val
        275                 280                 285

Val Trp Phe Trp Ile Leu Val Gly Leu Ala Tyr Phe Ala Ala Val Leu
    290                 295                 300

Ser Met Ile Gly Arg Leu Val Arg Val Ile Ser Lys Lys Thr Lys Glu
305                 310                 315                 320

Glu Val Gly Glu Phe Arg Ala His Ala Ala Glu Trp Thr Ala Asn Val
                325                 330                 335

Thr Ala Glu Phe Lys Glu Thr Arg Arg Arg Leu Ser Val Glu Ile Tyr
            340                 345                 350

Asp Lys Phe Gln Arg Ala Thr Ser Ile Lys Arg Lys Leu Ser Ala Glu
        355                 360                 365

Leu Ala Gly Asn His Asn Gln Glu Leu Thr Pro Cys Arg Arg Thr Leu
    370                 375                 380

Ser Val Asn His Leu Thr Ser Glu Arg Asp Val Leu Pro Pro Leu Leu
385                 390                 395                 400

Lys Thr Glu Ser Ile Tyr Leu Asn Gly Leu Ala Pro His Cys Ala Gly
                405                 410                 415

Glu Glu Ile Ala Val Ile Glu Asn Ile Lys
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420 425

<210> 61
<211> 2130
<212> DNA
<213> Homo sapiens

<220>
<221> unsure
<222> (35)
<223> n at position 35 indicates undetermined nucleotide

<220>
<221> unsure
<222> (2057)
<223> n at position 2057 indicates undetermined nucleotide

<220>
<221> unsure
<222> (2067)
<223> n at position 2067 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2111)
<223> n at position 2111 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2120)
<223> n at position 2120 indicates an undetermined nucleotide

<400> 61
ccatcctaat acgactcact atagggctcg agcgnccgcc cgggcagtaa aatgcctgcc 60
cgtgcagctc ggagcgcgca gcccgtctct gaataagaag tgagtacaat ggcgtgtttg 120
taaaaaaaag cttcaagtcc gtctttttca aaaaacattt tgaatgctgc atgcctcatg 180
cttcccagcg cctcgcggga gagacccggc tatagagcag gagtggcggc acctgacttg 240
ctggatccta aatctgccgc tcagaactcc aaaccgaggc tctcattttc cacgaaaccc 300
acagtgcttg cttcccgggt ggagagtgac acgaccatta atgttatgaa atggaagacg 360
gtctccacga tattcctggt ggttgtcctc tatctgatca tcggagccac cgtgttcaaa 420
gcattggagc agcctcatga gatttcacag aggaccacca ttgtgatcca gaagcaaaca 480
ttcatatccc aacattcctg tgtcaattcg acggagctgg atgaactcat tcagcaaata 540
gtggcagcaa taaatgcagg gattataccg ttaggaaaca cctccaatca aatcagtcac 600

tgggatttgg gaagttcctt cttctttgct ggcactgtta ttacaaccat aggatttgga 660
aacatctcac cacgcacaga aggcggcaaa atattctgta tcatctatgc cttactggga 720
attcccctct ttggttttct cttggctgga gttggagatc agctaggcac catatttgga 780
aaaggaattg ccaaagtgga agatacgttt attaagtgga atgttagtca gaccaagatt 840
cgcatcatct caacaatcat atttatacta tttggctgtg tactctttgt ggctctgcct 900
gcgatcatat tcaaacacat agaaggctgg agtgccctgg acaccattta ttttgtggtt 960
atcactctaa caactattgg atttggtgac tacgttgcag gtggatccga tattgaatat 1020
ctggacttct ataagcctgt cgtgtggttc tggatccttg tagggcttgc ttactttgct 1080
gctgtcctga gcatgattgg gagattggtc cgagtgatat ctaaaaagac aaaagaagag 1140
gtgggagagt tcagagcaca cgctgctgag tggacagcca acgtcacagc cgaattcaaa 1200
gaaaccagga ggcgactgag tgtggagatt tatgacaagt tccagcgggc cacctccatc 1260
aagcggaagc tctcggcaga actggctgga aaccacaatc aggagctgac tccttgtagg 1320
aggaccctgt cagtgaacca cctgaccagc gagagggatg tcttgcctcc cttactgaag 1380
actgagagta tctatctgaa tggtttggcg ccacactgtg ctggtgaaga gattgctgtg 1440
attgagaaca tcaaatagcc ctctctttaa ataaccttag gcatagccat aggtgaggac 1500
ttctctatgc tctttatgac tgttgctggt agcatttttt aaattgtgca tgagctcaaa 1560
gggggaacaa aatagataca cccatcatgg tcatctatca tcaagagaat ttggaattct 1620
gagccagcac tttctttctg atgatgcttg ttgaacggcc cactttcttt gatgagtgga 1680
atgacaagca atgtctgatg cctttgtgtg cccagactgt tttcctctct ctttccctaa 1740
tgtgccataa ggcctcagaa tgaattgaga attgtttctg gtaacaatgt agctttgagg 1800
gatcagttct taacttttca gggtctacct aactgagcct agatatggac catttatgga 1860
tgacaacaat tttttttttg taaatgacaa gaaattctta tgcagccttt tacctaagaa 1920
atttctgtca gtgccttatc ttatgaagaa acagaacctc tctagctaat gtgtggtttc 1980
tccttccctg cccccacccc taggctcacc tctgcagtct tttaccccag ttctcccatt 2040
tgaataccat accttgntgg aaacagngtg taaaatgact gaagtgatga tgccgaagat 2100
gaaatagatg ncaaattagn tggacattga 2130

<210> 62
<211> 2130
<212> DNA
<213> Homo sapiens

<220>
<221> unsure
<222> (35)
<223> n at position 35 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2057)
<223> n at position 2057 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2067)

<223> n at position 2067 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2120)
<223> n at position 2120 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2111)
<223> n at position 2111 indicates an undetermined nucleotide

<400> 62
ccatcctaat acgactcact atagggctcg agcgnccgcc cgggcagtaa aatgcctgcc 60
cgtgcagctc ggagcgcgca gcccgtctct gaataagaag tgagtacaat ggcgtgtttg 120
taaaaaaaag cttcaagtcc gtctttttca aaaaacattt tgaatgctgc atgcctcatg 180
cttcccagcg cctcgcggga gagacccggc tatagagcag gagtggcggc acctgacttg 240
ctggatccta aatctgccgc tcagaactcc aaaccgaggc tctcattttc cacgaaaccc 300
acagtgcttg cttcccgggt ggagagtgac acgaccatta atgttatgaa atggaagacg 360
gtctccacga tattcctggt ggttgtcctc tatctgatca tcggagccac cgtgttcaaa 420
gcattggagc agcctcatga gatttcacag aggaccacca ttgtgatcca gaagcaaaca 480
ttcatatccc aacattcctg tgtcaattcg acggagctgg atgaactcat tcagcaaata 540
gtggcagcaa taaatgcagg gattataccg ttaggaaaca cctccaatca aatcagtcac 600
tgggatttgg gaagttcctt cttctttgct ggcactgtta ttacaaccat aggatttgga 660
aacatctcac cacgcacaga aggcggcaaa atattctgta tcatctatgc cttactggga 720
attcccctct ttggttttct cttggctgga gttggagatc agctaggcac catatttgga 780
aaaggaattg ccaaagtgga agatacgttt attaagtgga atgttagtca gaccaagatt 840
cgcatcatct caacaatcat atttatacta tttggctgtg tactctttgt ggctctgcct 900
gcgatcatat tcaaacacat agaaggctgg agtgccctgg acgccattta ttttgtggtt 960
atcactctaa caactattgg atttggtgac cacgttgcag gtggatccga tattgaatat 1020
ctggacttct ataagcctgt cgtgtggttc tggatccttg tagggcttgc ttactttgct 1080
gctgtcctga gcatgattgg gagattggtc cgagtgatat ctaaaaagac aaaagaagag 1140
gtgggagagt tcagagcaca cgctgctgag tggacagcca acgtcacagc cgaattcaaa 1200
gaaaccagga ggcgactgag tgtggagatt tatgacaagt tccagcgggc cacctccatc 1260
aagcggaagc tctcggcaga actggctgga aaccacaatc aggagctgac tccttgtagg 1320
aggaccctgt cagtgaacca cctgaccagc gagagggatg tcttgcctcc cttactgaag 1380
actgagagta tctatctgaa tggtttggcg ccacactgtg ctggtgaaga gattgctgtg 1440
attgagaaca tcaaatagcc ctctctttaa ataaccttag gcatagccat aggtgaggac 1500
ttctctatgc tctttatgac tgttgctggt agcatttttt aaattgtgca tgagctcaaa 1560
gggggaacaa aatagataca cccatcatgg tcatctatca tcaagagaat ttggaattct 1620
gagccagcac tttctttctg atgatgcttg ttgaacggcc cactttcttt gatgagtgga 1680
atgacaagca atgtctgatg cctttgtgtg cccagactgt tttcctctct ctttccctaa 1740
tgtgccataa ggcctcagaa tgaattgaga attgtttctg gtaacaatgt agctttgagg 1800
gatcagttct taacttttca gggtctacct aactgagcct agatatggac catttatgga 1860
tgacaacaat tttttttttg taaatgacaa gaaattctta tgcagccttt tacctaagaa 1920

atttctgtca gtgccttatc ttatgaagaa acagaacctc tctagctaat gtgtggtttc 1980
tccttccctg cccccacccc taggctcacc tctgcagtct tttaccccag ttctcccatt 2040
tgaataccat accttgntgg aaacagngtg taaaatgact gaagtgatga tgccgaagat 2100
gaaatagatg ncaaattagn tggacattga 2130

<210> 63
<211> 2130
<212> DNA
<213> Homo sapiens

<220>
<221> unsure
<222> (35)
<223> n at position 35 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2057)
<223> n at position 2057 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2067)
<223> n at position 2067 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2111)
<223> n at position 2111 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2120)
<223> n at position 2120 indicates an undetermined nucleotide

<400> 63
ccatcctaat acgactcact atagggctcg agcgnccgcc cgggcagtaa aatgcctgcc 60
cgtgcagctc ggagcgcgca gcccgtctct gaataagaag tgagtacaat ggcgtgtttg 120
taaaaaaaag cttcaagtcc gtctttttca aaaaacattt tgaatgctgc atgcctcatg 180
cttcccagcg cctcgcggga gagacccggc tatagagcag gagtggcggc acctgacttg 240
ctggatccta aatctgccgc tcagaactcc aaaccgaggc tctcattttc cacgaaaccc 300
acagtgcttg cttcccgggt ggagagtgac acgaccatta atgttatgaa atggaagacg 360

gtctccacga tattcctggt ggttgtcctc tatctgatca tcggagccac cgtgttcaaa 420
gcattggagc agcctcatga gatttcacag aggaccacca ttgtgatcca gaagcaaaca 480
ttcatatccc aacattcctg tgtcaattcg acggagctgg atgaactcat tcagcaaata 540
gtggcagcaa taaatgcagg gattataccg ttaggaaaca cctccaatca aatcagtcac 600
tgggatttgg gaagttcctt cttctttgct ggcactgtta ttacaaccat aggatttgga 660
aacatctcac cacgcacaga aggcggcaaa atattctgta tcatctatgc cttactggga 720
attcccctct ttggttttct cttggctgga gttggagatc agctaggcac catatttgga 780
aaaggaattg ccaaagtgga agatacgttt attaagtgga atgttagtca gaccaagatt 840
cgcatcatct caacaatcat atttatacta tttggctgtg tactctttgt ggctctgcct 900
gcgatcatat tcaaacacat agaaggctgg agtgccctgg acgccattta ttttgtggtt 960
atcactctaa caactattgg atttggtgac cacgttgtag gtggatccga tattgaatat 1020
ctggacttct ataagcctgt cgtgtggttc tggatccttg tagggcttgc ttactttgct 1080
gctgtcctga gcatgattgg gagattggtc cgagtgatat ctaaaaagac aaaagaagag 1140
gtgggagagt tcagagcaca cgctgctgag tggacagcca acgtcacagc cgaattcaaa 1200
gaaaccagga ggcgactgag tgtggagatt tatgacaagt tccagcgggc cacctccatc 1260
aagcggaagc tctcggcaga actggctgga aaccacaatc aggagctgac tccttgtagg 1320
aggaccctgt cagtgaacca cctgaccagc gagagggatg tcttgcctcc cttactgaag 1380
actgagagta tctatctgaa tggtttggcg ccacactgtg ctggtgaaga gattgctgtg 1440
attgagaaca tcaaatagcc ctctctttaa ataaccttag gcatagccat aggtgaggac 1500
ttctctatgc tctttatgac tgttgctggt agcatttttt aaattgtgca tgagctcaaa 1560
gggggaacaa aatagataca cccatcatgg tcatctatca tcaagagaat ttggaattct 1620
gagccagcac tttctttctg atgatgcttg ttgaacggcc cactttcttt gatgagtgga 1680
atgacaagca atgtctgatg cctttgtgtg cccagactgt tttcctctct ctttccctaa 1740
tgtgccataa ggcctcagaa tgaattgaga attgtttctg gtaacaatgt agctttgagg 1800
gatcagttct taacttttca gggtctacct aactgagcct agatatggac catttatgga 1860
tgacaacaat tttttttttg taaatgacaa gaaattctta tgcagccttt tacctaagaa 1920
atttctgtca gtgccttatc ttatgaagaa acagaacctc tctagctaat gtgtggtttc 1980
tccttccctg cccccacccc taggctcacc tctgcagtct tttaccccag ttctcccatt 2040
tgaataccat accttgntgg aaacagngtg taaaatgact gaagtgatga tgccgaagat 2100
gaaatagatg ncaaattagn tggacattga 2130

<210> 64
<211> 2130
<212> DNA
<213> Homo sapiens

<220>
<221> unsure
<222> (35)
<223> n at position 35 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2057)
<223> n at position 2057 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2067)
<223> n at position 2067 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2111)
<223> n at position 2111 indicates an undetermined nucleotide

<220>
<221> unsure
<222> (2120)
<223> n at position 2120 indicates an undetermined nucleotide

<400> 64
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cgtgcagctc ggagcgcgca gcccgtctct gaataagaag tgagtacaat ggcgtgtttg 120
taaaaaaaag cttcaagtcc gtctttttca aaaaacattt tgaatgctgc atgcctcatg 180
cttcccagcg cctcgcggga gagacccggc tatagagcag gagtggcggc acctgacttg 240
ctggatccta aatctgccgc tcagaactcc aaaccgaggc tctcattttc cacgaaaccc 300
acagtgcttg cttcccgggt ggagagtgac acgaccatta atgttatgaa atggaagacg 360
gtctccacga tattcctggt ggttgtcctc tatctgatca tcggagccac cgtgttcaaa 420
gcattggagc agcctcatga gatttcacag aggaccacca ttgtgatcca gaagcaaaca 480
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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: generalized motif for potassium channel

<220>
<221> VARIANT
<222> (1)..(9)
<223> X at positions 1, 4, and 5 are T or S; X at position 6 is I or V; X at positions 2, 3, and 8 areY, F, V, I, M, or L

<400> 65
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1 5

<210> 66
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: consensus motif for potassium channel

<220>
<221> VARIANT
<222> (1)..(8)
<223> X at position 1 is Y or F; X at position 2 is A, S, or G; X at positions 3, 4, 6, and 8 is M, I, V, L, F, or Y

<400> 66

Xaa Xaa Xaa Xaa Gly Xaa Pro Xaa
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<210> 67
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: consensus sequence of potassium channel motif

<400> 67
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1 5

<210> 68
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: consensus sequence of potassium ion channel motif

<220>
<221> VARIANT
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1 5

<210> 69
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: consensus sequence of potassium ion channel motif

<220>
<221> VARIANT

<222> (1)..(7)
<223> X at position 1 is Y or F; X at position 2 is A, S, or G; X at positions 3, 4, and 6 are M, I, V, L, F, or Y

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1 5

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<212> DNA
<213> Homo sapiens

<400> 70
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<210> 71
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<213> Homo sapiens

<400> 71
taagagcatc ggaccatcag 20

<210> 72
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer used to amplify the TPKC1 ORF

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<210> 73
<211> 15
<212> DNA
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<220>
<223> Description of Artificial Sequence: primer used

to amplify the TPKC1 ORF

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<210> 74
<211> 434
<212> PRT
<213> Caenorhabditis elegans

<400> 74
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Phe Pro Arg Asp Lys Tyr Asn Ile Val Tyr Trp Leu Val Ile Leu Val
20 25 30

Gly Phe Gly Val Leu Leu Pro Trp Asn Met Phe Ile Thr Ile Ala Pro
35 40 45

Glu Tyr Tyr Val Asn Tyr Trp Phe Lys Pro Asp Gly Val Glu Thr Trp
50 55 60

Tyr Ser Lys Glu Phe Met Gly Ser Leu Thr Ile Gly Ser Gln Leu Pro
65 70 75 80

Asn Ala Ser Ile Asn Val Phe Asn Leu Phe Leu Ile Ile Ala Gly Pro
85 90 95

Leu Ile Tyr Arg Val Phe Ala Pro Val Cys Phe Asn Ile Val Asn Leu
100 105 110

Thr Ile Ile Leu Ile Leu Val Ile Val Leu Glu Pro Thr Glu Asp Ser
115 120 125

Met Ser Trp Phe Phe Trp Val Thr Leu Gly Met Ala Thr Ser Ile Asn
130 135 140

Phe Ser Asn Gly Leu Tyr Glu Asn Ser Val Tyr Gly Val Gly Gly Asp
145 150 155 160

Phe Pro His Thr Tyr Ile Gly Ala Leu Leu Ile Gly Asn Asn Ile Cys
165 170 175

Gly Leu Leu Ile Thr Val Val Lys Ile Gly Val Thr Tyr Phe Leu Asn
180 185 190

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

Ile Leu Leu Val Cys Ala Ile Ala Leu Phe Phe Ile Thr Lys Gln Asp
    210                 215                 220

Phe Tyr His Tyr His His Gln Lys Gly Met Glu Ile Arg Glu Lys Ala
225                 230                 235                 240

Glu Thr Asp Arg Pro Ser Pro Ser Ile Leu Trp Thr Thr Phe Thr Asn
                245                 250                 255

Cys Tyr Gly Gln Leu Phe Asn Val Trp Phe Cys Phe Ala Val Thr Leu
            260                 265                 270

Thr Ile Phe Pro Val Met Met Thr Val Thr Thr Arg Gly Asp Ser Gly
        275                 280                 285

Phe Leu Asn Lys Ile Met Ser Glu Asn Asp Glu Ile Tyr Thr Leu Leu
    290                 295                 300

Thr Ser Phe Leu Val Phe Asn Leu Phe Ala Ala Ile Gly Ser Ile Val
305                 310                 315                 320

Ala Ser Lys Ile His Trp Pro Thr Pro Arg Tyr Leu Lys Phe Ala Ile
                325                 330                 335

Ile Leu Arg Ala Leu Phe Ile Pro Phe Phe Phe Phe Cys Asn Tyr Arg
            340                 345                 350

Val Gln Thr Arg Ala Tyr Pro Val Phe Phe Glu Ser Thr Asp Ile Phe
        355                 360                 365

Val Ile Gly Gly Ile Ala Met Ser Phe Ser His Gly Tyr Leu Ser Ala
    370                 375                 380

Leu Ala Met Gly Tyr Thr Pro Asn Val Val Pro Ser His Tyr Ser Arg
385                 390                 395                 400

Phe Ala Ala Gln Leu Ser Val Cys Thr Leu Met Val Gly Leu Leu Thr
                405                 410                 415

Gly Gly Leu Trp Pro Val Val Ile Glu His Phe Val Asp Lys Pro Ser
            420                 425                 430

Ile Leu
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