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RHIPICEPHALUS SANGUINEUS

(54) Title: DNA MOLECULES ENCODING L-GLUTAMATE-GATED CHLORIDE CHANNELS FROM RHIPICEPHALUS
SANGUINEUS

(57) **Abrégé/Abstract:**

The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode *Rhipicephalus sanguineus* glutamate gated chloride channels. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding *R. sanguineus* glutamate gated chloride channels, substantially purified forms of associated *R. sanguineus* glutamate gated chloride channels and recombinant membrane fractions comprising these proteins, associated mutant proteins, and methods associated with identifying compounds which modulate associated *Rhipicephalus sanguineus* glutamate gated chloride channels, which will be useful as insecticides.

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ning of each regular issue of the PCT Gazette.*

(54) Title: DNA MOLECULES ENCODING L-GLUTAMATE-GATED CHLORIDE CHANNELS FROM *RHIPICEPHALUS SANGUINEUS*

(57) Abstract: The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode *Rhipicephalus sanguineus* glutamate gated chloride channels. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding *R. sanguineus* glutamate gated chloride channels, substantially purified forms of associated *R. sanguineus* glutamate gated chloride channels and recombinant membrane fractions comprising these proteins, associated mutant proteins, and methods associated with identifying compounds which modulate associated *Rhipicephalus sanguineus* glutamate gated chloride channels, which will be useful as insecticides.



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TITLE OF THE INVENTION

DNA MOLECULES ENCODING L-GLUTAMATE-GATED

5 CHLORIDE CHANNELS FROM *RHIPICEPHALUS SANGUINEUS*

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

15 REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates in part to isolated nucleic acid molecules
20 (polynucleotides) which encode *Rhipicephalus sanguineus* (brown dog tick)
glutamate-gated chloride channels. The present invention also relates to recombinant
vectors and recombinant hosts which contain a DNA fragment encoding
R. sanguineus glutamate-gated chloride channels, substantially purified forms of
associated *R. sanguineus* glutamate-gated chloride channels and recombinant
25 membrane fractions comprising these proteins, associated mutant proteins, and
methods associated with identifying compounds which modulate associated
Rhipicephalus sanguineus glutamate-gated chloride channels, which will be useful as
insecticides.

30 BACKGROUND OF THE INVENTION

Glutamate-gated chloride channels, or H-receptors, have been identified in
arthropod nerve and muscle (Lingle et al, 1981, *Brain Res.* 212: 481-488; Horseman
et al., 1988, *Neurosci. Lett.* 85: 65-70; Wafford and Sattelle, 1989, *J. Exp. Bio.* 144:
449-462; Lea and Usherwood, 1973, *Comp. Gen. Pharmacol.* 4: 333-350; and Cull-

Candy, 1976, *J. Physiol.* 255: 449-464).

Invertebrate glutamate-gated chloride channels are important targets for the widely used avermectin class of anthelmintic and insecticidal compounds. The avermectins are a family of macrocyclic lactones originally isolated from the actinomycete *Streptomyces avermitilis*. The semisynthetic avermectin derivative, ivermectin (22,23-dihydro-avermectin B_{1a}), is used throughout the world to treat parasitic helminths and insect pests of man and animals. The avermectins remain the most potent broad spectrum endectocides exhibiting low toxicity to the host. After many years of use in the field, there remains little resistance to avermectin in the insect population. The combination of good therapeutic index and low resistance strongly suggests that the glutamate-gated chloride (GluCl) channels remain good targets for insecticide development.

Glutamate-gated chloride channels have been cloned from the soil nematode *Caenorhabditis elegans* (Cully et al., 1994, *Nature* 371: 707-711; see also U.S. Patent No. 5,527,703 and Arena et al., 1992, *Molecular Brain Research*. 15: 339-348) and *Ctenocephalides felis* (flea; see WO 99/07828).

In addition, a gene encoding a glutamate-gated chloride channel from *Drosophila melanogaster* was previously identified (Cully et al., 1996, *J. Biol. Chem.* 271: 20187-20191; see also U.S. Patent No. 5,693,492).

Despite the identification of the aforementioned cDNA clones encoding GluCl channels, it would be advantageous to identify additional genes which encode *R. sanguineus* GluCl channels in order to allow for improved screening to identify novel GluCl channel modulators that may have insecticidal, acaricidal and/or nematocidal activity for animal health, especially as related to treatment of tick and mite infestation in dogs, cats, cattle, sheep and other agriculturally important animals. The present invention addresses and meets these needs by disclosing novel genes which express a *R. sanguineus* GluGl1 and *R. sanguineus* GluGl2 channel wherein expression of these *R. sanguineus* GluCl RNAs in *Xenopus* oocytes or other appropriate host cells result in an active GluCl channel. Heterologous expression of a GluCl channel of the present invention will allow the pharmacological analysis of compounds active against parasitic invertebrate species relevant to animal and human health, especially in the treatment of tick infestations in dogs and cats. Heterologous cell lines expressing an active GluCl channel can be used to establish functional or binding assays to identify novel GluCl channel modulators that may be useful in

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control of the aforementioned species groups.

SUMMARY OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule (polynucleotide) which encodes a novel *Rhipicephalus sanguineus* (brown dog tick) invertebrate GluCl1 channel protein. The DNA molecules disclosed herein may be transfected into a host cell of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional single, homomultimeric or heteromultimeric LGIC. Such functional ligand-gated ion channels may possibly respond to other known ligands which will in turn provide for additional screening targets to identify modulators of these channels, modulators which may act as effective insecticidal, mitacidal and/or nematocidal treatment for use in animal and human health and/or crop protection.

According to one aspect of the present invention, there is provided a purified nucleic acid molecule encoding a *Rhipicephalus sanguineus* GluCl channel protein, wherein said nucleic acid molecule comprises: (a) a nucleic acid molecule which encodes an amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8; (b) a nucleic acid molecule which hybridizes under conditions of moderate to high stringency to the complement of (a), and which encodes a protein having the biological activity of the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8; wherein the hybridization conditions include a hybridization step carried out in 5 X SSC, 5 X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours and a washing step carried out in 0.2 X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes; (c) a nucleic acid molecule which hybridizes

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under conditions of moderate stringency to the complement of a second nucleic acid molecule as set forth in SEQ ID NOs: 1, 3, 5 or 7; wherein said nucleic acid molecule has at least about 70% identity determined over the full length of the nucleic acid molecule to at least one of the second nucleic acid molecules as set forth in SEQ ID NOs: 1, 3, 5 or 7, and which encodes a protein having the biological activity of the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8, wherein the hybridization conditions include a hybridization step carried out in 5 X SSC, 5 X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours and a washing step carried out in 0.2 X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

According to another aspect of the present invention, there is provided a purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said protein comprises an amino acid sequence as set forth in SEQ ID NO: 2.

According to still another aspect of the present invention, there is provided a process for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein comprising an amino acid sequence of SEQ ID NO: 2 in a recombinant host cell, comprising: (a) transfecting the expression vector as defined herein into a host cell; and, (b) culturing the host cell of step (a) under conditions which allow expression of said *Rhipicephalus sanguineus* GluCl1 channel protein from said expression vector.

According to yet another aspect of the present invention, there is provided a purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein,

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wherein said purified DNA molecule consists of a nucleotide sequence as set forth in SEQ ID NO: 1.

According to a further aspect of the present invention, there is provided a process for expressing a
5 *Rhipicephalus sanguineus* GluCl1 channel protein comprising an amino acid sequence of SEQ ID NO: 4 in a recombinant host cell, comprising: (a) transfecting the expression vector as defined herein into a host cell; and, (b) culturing the host cell of step (a) under conditions which allow expression of
10 said *Rhipicephalus sanguineus* GluCl1 channel protein from said expression vector.

According to yet a further aspect of the present invention, there is provided a purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein,
15 wherein said purified DNA molecule consists of a nucleotide sequence as set forth in SEQ ID NO: 3.

According to still a further aspect of the present invention, there is provided a process for expressing a
Rhipicephalus sanguineus GluCl1 channel protein comprising
20 an amino acid sequence of SEQ ID NO: 6 in a recombinant host cell, comprising: (a) transfecting the expression vector as defined herein into a host cell; and, (b) culturing the host cell of step (a) under conditions which allow expression of
said *Rhipicephalus sanguineus* GluCl1 channel protein from
25 said expression vector.

According to another aspect of the present invention, there is provided a purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said purified DNA molecule consists of a nucleotide
30 sequence as set forth in SEQ ID NO: 5.

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According to yet another aspect of the present invention, there is provided a process for expressing a *Rhipicephalus sanguineus* GluCl2 channel protein comprising an amino acid sequence of SEQ ID NO: 8 in a recombinant host cell, comprising: (a) transfecting the expression vector as defined herein into a host cell; and, (b) culturing the host cell of step (a) under conditions which allow expression of said *Rhipicephalus sanguineus* GluCl2 channel protein from said expression vector.

According to yet another aspect of the present invention, there is provided a purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl2 channel protein, wherein said purified DNA molecule consists of a nucleotide sequence as set forth in SEQ ID NO: 7.

According to yet another aspect of the present invention, there is provided a method of identifying a modulator of a GluCl channel protein, comprising: (a) contacting a test compound with an isolated host cell that expresses a *Rhipicephalus sanguineus* GluCl channel protein as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8; (b) contacting a test compound with an isolated host cell that does not express a *Rhipicephalus sanguineus* GluCl channel protein as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8; (c) measuring the effect of the test compound on the host cell that expresses the GluCl channel protein; (d) measuring the effect of the test compound on the host cell that does not express the GluCl channel protein; wherein the effect that is measured in (c) and (d) is (i) the expression of DNA encoding the GluCl channel protein; (ii) the expression of RNA encoding the GluCl channel protein; or (iii) a channel activity of

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the GluCl channel protein, and wherein if the effect of the test compound measured in the cell that expresses the GluCl channel protein is greater than or less than the effect measured in the host cell that does not express the GluCl channel protein, the test compound is a modulator of a GluCl channel protein.

According to yet another aspect of the present invention, there is provided an *in vitro* method of identifying a compound that modulates glutamate-gated channel protein activity, which comprises: a) injecting into a host cell solution a population of nucleic acid molecules, at least a portion of which encodes a *Rhipicephalus sanguineus* GluCl channel protein, wherein the *Rhipicephalus sanguineus* GluCl channel protein is as set forth in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 8, such that expression of said portion of nucleic acid molecules results in an active glutamate-gated channel; b) adding a test compound into said solution; and, c) measuring host cell membrane current at a holding potential more positive than the reversal potential for chloride.

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The present invention relates to an isolated or purified nucleic acid molecule (polynucleotide) which encodes a novel *Rhipicephalus sanguineus* invertebrate GluCl2 channel protein.

5 The present invention further relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel *Rhipicephalus sanguineus* GluCl1 channel protein, this DNA molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:5.

10 The present invention further relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel *Rhipicephalus sanguineus* GluCl2 channel protein, this DNA molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:7.

The present invention also relates to biologically active fragments or mutants of SEQ ID NOs:1, 3, 5 and 7 which encodes mRNA expressing a novel *Rhipicephalus sanguineus* invertebrate GluCl1 or GluCl2 channel protein, respectively. Any such
15 biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the pharmacological properties of a *R. sanguineus* GluCl channel protein, including but not limited to the *R. sanguineus* GluCl1 channel proteins as set forth in SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6 as well as the respective GluCl2 channel protein as set forth in SEQ ID NO:8.
20 Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal

truncations such that these mutations encode mRNA which express a functional *R. sanguineus* GluCl channel in a eukaryotic cell, such as *Xenopus* oocytes, so as to be useful for screening for agonists and/or antagonists of *R. sanguineus* GluCl activity.

5 A preferred aspect of this portion of the present invention is disclosed in Figure 1 (SEQ ID NO:1; designated T12), Figure 3 (SEQ ID NO:3; designated T82) and Figure 5 (SEQ ID NO:5; designated T32) encoding novel *Rhipicephalus sanguineus* GluCl1 proteins, and Figure 7 (SEQ ID NO:7, designated B1) encoding a novel *Rhipicephalus sanguineus* GluCl2 protein.

10 The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The present invention also relates to recombinant vectors and recombinant host cells, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification.

20 The present invention also relates to a substantially purified form of an *R. sanguineus* GluCl1 channel protein, which comprises the amino acid sequence disclosed in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4) and Figure 6 (SEQ ID NO:6), as well as to a novel *Rhipicephalus sanguineus* GluCl2 protein, which comprises the amino acid sequence disclosed in Figure 8 (SEQ ID NO:8).

25 A preferred aspect of this portion of the present invention is a *R. sanguineus* GluCl1 channel protein which consists of the amino acid sequence disclosed in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4) and Figure 6 (SEQ ID NO:6).

Another preferred aspect of this portion of the present invention is a *R. sanguineus* GluCl2 channel protein which consists of the amino acid sequence disclosed in Figure 8 (SEQ ID NO:8).

30 Another preferred aspect of the present invention relates to a substantially purified, fully processed (including any proteolytic processing, glycosylation and/or phosphorylation) mature GluCl channel protein obtained from a recombinant host cell containing a DNA expression vector comprises a nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5 and/or 7 and expresses the respective RsGluCl1 or RsGluCl2

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precursor protein. It is especially preferred that the recombinant host cell be a eukaryotic host cell, including but not limited to a mammalian cell line, an insect cell line such as an S2 cell line, or *Xenopus* oocytes.

Another preferred aspect of the present invention relates to a substantially
5 purified membrane preparation, partially purified membrane preparation, or cell lysate which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5 and/or 7, resulting in a functional form of the respective RsGluCl1 or RsGluCl2 channel. The subcellular
10 membrane fractions and/or membrane-containing cell lysates from the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed or transfected cells) contain the functional proteins encoded by the nucleic acids of the present invention. This recombinant-based membrane preparation may comprise a *R. sanguineus* GluCl channel and is essentially free from
15 contaminating proteins, including but not limited to other *R. sanguineus* source proteins or host proteins from a recombinant cell which expresses the T12 (SEQ ID NO:2), T82 (SEQ ID NO:4) T32 (SEQ ID NO:6) GluCl1 channel protein and/or the B1 (SEQ ID NO:8) GluCl2 channel protein. Therefore, a preferred aspect of the invention is a membrane preparation which contains a *R. sanguineus* GluCl channel
20 comprising a GluCl protein comprising the functional form of the full length GluCl1 channel proteins as disclosed in Figure 2 (SEQ ID NO:2; T12), Figure 4 (SEQ ID NO:4; T82), and Figure 6 (SEQ ID NO:6, T32) and/or a functional form of the full length GluCl2 channel protein as disclosed in Figure 8 (SEQ ID NO:8; B1). These subcellular membrane fractions will comprise either wild-type or mutant variations
25 which are biologically functional forms of the *R. sanguineus* GluCl channels, any homomultimeric or heteromultimeric combination thereof (e.g., including but not limited to a T12/T12 GluCl1 homomultimeric channel, a T12/T32 GluCl1 heteromultimeric channel, or a T12/B1 GluCl1/GluCl2 heteromultimeric channel), at levels substantially above endogenous levels and hence will be useful in various
30 assays described throughout this specification. It is also possible that the disclosed channel proteins may, alone or in combination, form functional multimer-based channels with as yet identified channel proteins. A preferred eukaryotic host cell of choice to express the glutamate-gated channels of the present invention is a mammalian cell line, an insect cell line such as an S2 cell line, or *Xenopus* oocytes.

The present invention also relates to biologically active fragments and/or mutants of a *R. sanguineus* GluCl1 channel protein, comprising the amino acid sequence as set forth in SEQ ID NOs:2, 4 and/or 6, as well as biologically active fragments and/or mutants of a *R. sanguineus* GluCl2 channel protein, comprising the amino acid sequence as set forth in SEQ ID NO:8, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators, including but not limited to agonists and/or antagonists for *R. sanguineus* GluCl channel pharmacology.

A preferred aspect of the present invention is disclosed in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6) and Figure 8 (SEQ ID NO:8), respective amino acid sequences which comprise the *R. sanguineus* GluCl1 and GluCl2 proteins of the present invention, respectively. Characterization of one or more of these channel proteins allows for screening methods to identify novel GluCl channel modulators that may have insecticidal, mitacidal and/or nematocidal activity for animal health or crop protection. As noted above, heterologous expression of a *Rhipicephalus sanguineus* GluCl channel will allow the pharmacological analysis of compounds active against parasitic invertebrate species relevant to animal and human health, especially dogs and cats, which are known to suffer from frequent tick infestations. Heterologous cell lines expressing a functional RsGluCl1 channel (e.g., functional forms of SEQ ID NOs:2, 4 and/or 6) or RsGluCl2 channel (e.g., a functional form of SEQ ID NO:8), can be used to establish functional or binding assays to identify novel GluCl channel modulators that may be useful in control of the aforementioned species groups.

The present invention also relates to polyclonal and monoclonal antibodies raised in response to the disclosed forms of RsGluCl1 and/or RsGluCl2, or a biologically active fragment thereof.

The present invention also relates to RsGluCl1 and/or RsGluCl2 fusion constructs, including but not limited to fusion constructs which express a portion of the RsGluCl linked to various markers, including but in no way limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of one or more of the RsGluCl proteins disclosed herein.

The present invention relates to methods of expressing *R. sanguineus* GluCl1 and/or RsGluCl2 channel proteins and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these
5 assays which act as agonists or antagonists of GluCl channel activity.

It is an object of the present invention to provide an isolated nucleic acid molecule (e.g., SEQ ID NOs:1, 3, 5, and 7) which encodes a novel form of *R. sanguineus* GluCl, or fragments, mutants or derivatives RsGluCl1 or RsGluCl2, these proteins as set forth in SEQ ID NOs:2, 4, 6 and 8, respectively. Any such
10 polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators for invertebrate GluCl pharmacology.

15 It is a further object of the present invention to provide the *R. sanguineus* GluCl proteins or protein fragments encoded by the nucleic acid molecules referred to in the preceding paragraph.

It is a further object of the present invention to provide recombinant vectors and recombinant host cells which comprise a nucleic acid sequence encoding
20 *R. sanguineus* GluCl proteins or a biological equivalent thereof.

It is an object of the present invention to provide a substantially purified form of *R. sanguineus* GluCl1 or GluCl2 proteins, respectively, as set forth in SEQ ID NOs:2, 4, 6, and 8.

Is is another object of the present invention to provide a substantially purified
25 recombinant form of a *R. sanguineus* GluCl protein which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5, and 7, resulting in a functional, processed form of the respective RsGluCl channel. It is especially preferred that the recombinant host cell be a
30 eukaryotic host cell, such as a mammalian cell line.

It is an object of the present invention to provide for biologically active fragments and/or mutants of *R. sanguineus* GluCl1 or GluCl2 proteins, respectively, such as set forth in SEQ ID NOs:2, 4, 6, and 8, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and

carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic and/or prophylactic use.

It is further an object of the present invention to provide for substantially purified subcellular membrane preparation, partially purified membrane preparation
5 or crude lysate from recombinant cells which comprise a pharmacologically active *R. sanguineus* GluCl1 or GluCl2-containing single, homomultimeric or heteromultimer channel, respectively, especially subcellular fractions obtained from a host cell transfected or transformed with a DNA vector comprising a nucleotide sequence which encodes a protein which comprises the amino acid as set forth in Figure 2 (SEQ
10 ID NO:2), Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6), and Figure 8 (SEQ ID NO:8).

It is another object of the present invention to provide a substantially purified membrane preparation, partially purified membrane preparation, or crude lysate obtained from a recombinant host cell transformed or transfected with a DNA
15 expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5, and/or 7, resulting in a functional, processed form of the respective RsGluCl channel. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, including but not limited to a mammalian cell line, an insect cell line such as an S2 cell line, or *Xenopus* oocytes.

20 It is also an object of the present invention to use *R. sanguineus* GluCl proteins or membrane preparations containing *R. sanguineus* GluCl proteins or a biological equivalent to screen for modulators, preferably selective modulators, of *R. sanguineus* GluCl channel activity. Any such compound may be useful in screening for and selecting compounds active against parasitic invertebrate species
25 relevant to animal and human health. Such species include but are not limited to worms, fleas, ticks, mites and lice. These membrane preparations may be generated from heterologous cell lines expressing these GluCl proteins and may constitute full length protein, biologically active fragments of the full length protein or may rely on fusion proteins expressed from various fusion constructs which may be constructed with
30 materials available in the art.

As used herein, "substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. As used interchangeably with the terms "substantially free from other nucleic acids" or "substantially purified" or "isolated nucleic acid" or "purified

nucleic acid" also refer to a DNA molecules which comprises a coding region for a *R. sanguineus* GluCl protein that has been purified away from other cellular components. Thus, a *R. sanguineus* GluCl DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-*R. sanguineus* GluCl nucleic acids. Whether a given *R. sanguineus* GluCl DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, e.g., agarose gel electrophoresis combined with appropriate staining methods, e.g., ethidium bromide staining, or by sequencing.

As used herein, "substantially free from other proteins" or "substantially purified" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a *R. sanguineus* GluCl protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-*R. sanguineus* GluCl proteins. Whether a given *R. sanguineus* GluCl protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, e.g., sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, e.g., silver staining or immunoblotting. As used interchangeably with the terms "substantially free from other proteins" or "substantially purified", the terms "isolated *R. sanguineus* GluCl protein" or "purified *R. sanguineus* GluCl protein" also refer to *R. sanguineus* GluCl protein that has been isolated from a natural source. Use of the term "isolated" or "purified" indicates that *R. sanguineus* GluCl protein has been removed from its normal cellular environment. Thus, an isolated *R. sanguineus* GluCl protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated *R. sanguineus* GluCl protein is the only protein present, but instead means that an isolated *R. sanguineus* GluCl protein is substantially free of other proteins and non-amino acid material (e.g., nucleic acids, lipids, carbohydrates) naturally associated with the *R. sanguineus* GluCl protein *in vivo*. Thus, a *R. sanguineus* GluCl protein that is recombinantly expressed in a prokaryotic or eukaryotic cell and substantially purified from this host cell which does not naturally

(i.e., without intervention) express this GluCl protein is of course "isolated *R. sanguineus* GluCl protein" under any circumstances referred to herein. As noted above, a *R. sanguineus* GluCl protein preparation that is an isolated or purified *R. sanguineus* GluCl protein will be substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-*R. sanguineus* GluCl proteins.

As used interchangeably herein, "functional equivalent" or "biologically active equivalent" means a protein which does not have exactly the same amino acid sequence as naturally occurring *R. sanguineus* GluCl, due to alternative splicing, deletions, mutations, substitutions, or additions, but retains substantially the same biological activity as *R. sanguineus* GluCl. Such functional equivalents will have significant amino acid sequence identity with naturally occurring *R. sanguineus* GluCl and genes and cDNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring *R. sanguineus* GluCl. For example, a naturally occurring *R. sanguineus* GluCl1 protein disclosed herein comprises the amino acid sequence shown as SEQ ID NO:2 and is encoded by SEQ ID NO:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ ID NO:1.

As used herein, "a conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

As used herein, "LGIC" refers to a --ligand-gated ion channel --.

As used herein, "GluCl" refers to -- L-glutamate gated chloride channel --.

As used herein, "RsGluCl" refers to -- *Rhipicephalus sanguineus* L-glutamate gated chloride channel --.

Furthermore, as used herein "RsGluCl" may refer to RsGluCl1 and/or RsGluCl2.

As used herein, the term "mammalian" will refer to any mammal, including a human being.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence of the *R. sanguineus* GluCl1 cDNA clone, T12, set forth in SEQ ID NO:1.

Figure 2 shows the amino acid sequence of the *R. sanguineus* GluCl1 protein, T12, as set forth in SEQ ID NO:2.

Figure 3 shows the nucleotide sequence of the *R. sanguineus* GluCl1 cDNA clone, T82, as set forth in SEQ ID NO:3.

Figure 4 shows the amino acid sequence of the *R. sanguineus* GluCl1 protein, T82, as set forth in SEQ ID NO:4.

Figure 5 shows the nucleotide sequence of the *R. sanguineus* GluCl1 cDNA clone, T32, as set forth in SEQ ID NO:5.

Figure 6 shows the amino acid sequence of the *R. sanguineus* GluCl1 protein, T32, as set forth in SEQ ID NO:6.

Figure 7 shows the nucleotide sequence of the *R. sanguineus* GluCl2 cDNA clone, B1, as set forth in SEQ ID NO:7.

Figure 8 shows the amino acid sequence of the *R. sanguineus* GluCl2 protein, B1, as set forth in SEQ ID NO:8.

Figure 9 shows the amino acid sequence comparison for RsGluCl1 [T12 (SEQ ID NO:2), T82 (SEQ ID NO:4), T32 (SEQ ID NO:6) and RsGluCl2 (B1, SEQ ID NO:8) proteins.

Figure 10 shows the glutamate-activated current in *Xenopus* oocytes injected with RsGluCl1 T12 RNA. Current activation was maximal with 10 μ M glutamate and no current was seen in uninjected oocytes.

Figure 11 shows the activation by ivermectin of RsGluCl2 expressed in *Xenopus* oocytes. Current activation was maximal with $\sim$ 1 μ M ivermectin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes a *Rhipicephalus sanguineus* invertebrate GluCl channel protein. The isolated or purified nucleic acid molecules of the present invention are substantially free from other nucleic acids. For most cloning purposes, DNA is a preferred nucleic acid. As noted above, the DNA molecules disclosed herein may be transfected into a host cell of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional single,

homomultimeric or heteromultimeric GluCl channel. Such functional ligand-gated ion channels may possibly respond to other known ligands which will in turn provide for additional screening targets to identify modulators of these channels, modulators which may act as effective insecticidal, mitacidal and/or nematocidal treatment for use in animal and human health and/or crop protection. It is shown herein that RsGluCl 1 exhibits a current in response to glutamate and that an RsGluCl2 channel protein expressed in *Xenopus* oocytes exhibit a current in response to the addition of ivermectin phosphate. However, it should be noted that a single channel subunit protein might not form a functional channel, such as seen with the GABA-A subunit gamma, which does not express a functional homomultimer. Therefore, the expressed proteins of the present invention may function *in vivo* as a component of a wild type ligand-gated ion channel which contains a number of accessory and/or channel proteins, including the channel proteins disclosed herein. However, the GluCl proteins of the present invention need not directly mimic the wild type channel in order to be useful to the skilled artisan. Instead, the ability to form a functional, single, membrane associated channel within a recombinant host cell renders these proteins amenable to the screening methodology known in the art and described in part within this specification. Therefore, as noted within this specification, the disclosed Rs channel proteins of the present invention are useful as single functional channels, as a homomultimeric channel or as a heteromultimeric channel with various proteins disclosed herein with or without additional Rs channel subunit proteins or accessory proteins which may contribute to the full, functional GluCl channel. As noted above, the DNA molecules disclosed herein may be transfected into a host cell of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional single, homomultimeric or heteromultimeric GluCl. Such functional ligand-gated ion channels may possibly respond to other known ligands which will in turn provide for additional screening targets to identify modulators of these channels, modulators which may act as effective insecticidal, mitacidal and/or nematocidal treatment for use in animal and human health and/or crop protection

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel *Rhipicephalus sanguineus* invertebrate GluCl1 channel protein, this DNA molecule comprising the

nucleotide sequence disclosed herein as SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5.

The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel *Rhipicephalus sanguineus* invertebrate GluCl2 channel protein, this DNA molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:7.

The isolation and characterization of the RsGluCl nucleic acid molecules of the present invention were identified as described in detail in Example Section 1. These cDNA molecules, as discussed herein, are especially useful to establish novel insecticide screens, validate potential lead compounds with insecticidal activity, especially for use in treating cattle, dog and cat tick and mite infestations or that may kill other arachnids, and use these novel cDNA sequences as hybridization probes to isolate related genes from other organisms to establish additional pesticide drug screens. The RsGluCl1 and RsGluCl2 encoding cDNAs of the present invention were isolated from the brown dog tick species *Rhipicephalus sanguineus*. The DNA sequence predicts proteins that share common features with the class of chloride channels sensitive to glutamate and ivermectin. When the RsGluCl1 or RsGluCl2 cDNAs are expressed in *Xenopus* oocytes, a glutamate and ivermectin-sensitive channel is observed. The pharmacology of compounds that act at these channels would likely be different between these species. By screening on the arachnid channel it will be more likely to discover arachnid-specific compounds. Therefore, the cDNAs of the present invention can be expressed in cell lines or other expression systems and used for competition binding experiments or for functional chloride channel assays to screen for compounds that activate, block or modulate the channel.

Invertebrate glutamate-gated chloride channels (GluCls) are related to the glycine- and GABA-gated chloride channels and are distinct from the excitatory glutamate receptors (e.g. NMDA or AMPA receptors). The first two members of the GluCl family were identified in the nematode *C. elegans*, following a functional screen for the receptor of the anthelmintic drug ivermectin. Several additional GluCls have now been cloned in other invertebrate species. However, there is no evidence yet for GluCl counterparts in vertebrates; because of this, GluCls are excellent targets for anthelmintics, insecticides, acaricides, etc. Specific GluCl modulators, such as nodulisporic acid and its derivatives have an ideal safety profile because they lack mechanism-based toxicity in vertebrates. The present invention relates in part to three

novel *R. sanguineus* GluCl1 clones, T12, T82 and T32, and a *R. sanguineus* GluCl2 clone, B1. The RsGluCl1 cDNAs were isolated by low stringency hybridization using a *Drosophila* GluCl probe representing the putative membrane spanning domains, M1, M2 and M3. The RsGluCl2 cDNA was isolated by PCR using degenerate primers
 5 representing conserved regions in amino- and the M2-domains of the GluCl proteins of *Drosophila*, flea (*C.felis*), and *C. elegans*. It appears that RNA editing (A to G transitions) occur in these cDNAs and have resulted in some amino acid changes. RsGluCl1-T12 and T82 are similar except for one amino acid difference while RsGluCl1-T32 contains two additional exons in the coding region.

10 The present invention relates to the isolated or purified DNA molecule described in Figure 1 (T12) and set forth as SEQ ID NO:1, which encodes the *R. sanguineus* GluCl1 protein described in Figure 2 and set forth as SEQ ID NO:2, the nucleotide sequence of T12 is as follows:

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      1 CGCTCCCCCA ATCCTGAGGT TCCTTCTAAC GAGAAGGAGG AGCCACAGCG CCGGCTGCGG
15    61 TACCGCCGCA CGGGCCAACG TGAGACCGCC CGAGCCCGGC GCCCTGACTT AGGCCGCTGA
      121 GCGAAACCCA AGGCGGCGCG CTGGCCACTC CACGGGAACG AGACCGGCCC CCTGGAGACG
      181 ACATCGTCGA CCACAATGAA CTACTTCTCT GACGTGGCGA AGATGGTGGC TTCATCGAAG
      241 AGAGAAATCA TCGAAGCTTT CCACGCGACA TCTGGAGTAC ACGGCGCATG CGAATGAGCG
      301 AACATCGCTG ACCGAGACTC GCCCGTCACC ATGAGCGTAC ATTCATGGCG CTTTTGTGTC
20    361 CCACTGGTGG CTCTAGCGTT TTTCTTGTTG ATTCTTCTGT CGTGTCCATC GGCATGGGGC
      421 AAGGCAAATT TCCGCGCTAT AGAAAAGCGG ATATTGGACA GCATCATTTG CCAGGGTCGT
      481 TATGACTGCA GGATCCGGCC CATGGGAATT AACAAACACAG ACGGGCCGGC TCTTGTACGC
      541 GTTAACATCT TTGTAAGAAG TATCGGCAGA ATTGATGACG TCACCATGGA GTACACAGTG
      601 CAAATGACGT TCAGAGAGCA GTGGCGGGAC GAGAGACTCC AGTACGACGA CTTGGGCGGC
25    661 CAGGTTGCTG ACCTGACGCT CACCGAACCG GACAAGCTTT GGAAGCCGGA CCTGTTTTTC
      721 TCCAACGAGA AAGAGGGACA CTTCCACAAC ATCATCATGC CCAACGTGCT TCTACGCATA
      781 CATCCCAACG GCGACGTTCT CTTCAGCATC AGAATATCCT TGGTGCTTTC ATGTCCGATG
      841 AACCTGAAAT TTTATCCTTT GGATAAACAA ATCTGCTCTA TCGTCATGGT GAGCTATGGG
      901 TATACAACAG AGGACCTGGT GTTTCTATGG AAAGAGGGGG ATCCTGTACA GGTCACAAAA
30    961 AATCTCCACT TGCCACGTTT CACGCTGGAA AGGTTTCAAA CCGACTACTG CACCAGTCGG
      1021 ACCAACACTG GCGAGTACAG CTGCTTGCGC GTGGACCTGG TGTTCAGCG CGAGTTCAGC
      1081 TACTACCTGA TCCAGATCTA CATCCCGTGC TGCATGCTGG TCATCGTGTC CTGGGTGTGCG
      1141 TTCTGGCTCG ACCCCACCTC GATCCCGGCG CGAGTGTCGC TGGGCGTCAC CACCCTGCTC
      1201 ACCATGGCCA CGCAGATATC GGGCATCAAC GCCTCGCTGC CTCCCGTTTC CTACACCAAG
  
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1261 GCCATTGACG TGTGGACCGG CGTCTGTCTG ACCTTCGTAT TCGGCGCGCT CCTCGAGTTC
 1321 GCCCTGGTCA ACTACGCCTC GCGGTCAGAT TCACGCCGGC AGAACATGCA GAAGCAGAAG
 1381 CAGAGGAAAT GGGAGCTCGA GCCGCCCTG GACTCGGACC ACCTGGAGGA CGGCGCCACC
 1441 ACGTTCGCCA TGAGGCCGCT GGTGCACCAC CACGGAGAGC TGCATGCCGA CAAGTTGCGG
 5 1501 CAGTGCGAAG TCCACATGAA GACCCCAAG ACGAACCTTT GCAAGGCCTG GCTTTCCAGG
 1561 TTTCCCACGC GATCCAAACG CATCGACGTC GTCTCGCGGA TCTTCTTTCC GCTCATGTTC
 1621 GCCCTCTTCA ACCTCGTCTA CTGGACAACC TACCTCTTCC GGAAGACGA GGAAGACGAG
 1681 TGACAGAACA CGGACGCCAC GACAGCCGCC ATCCGACACC ATCGTCACTG CAGGCACGCA
 1741 CTCTGTCGCG CGCACACACC ACGAAGACCG GCGCGCCAAC GCACGATGCG CGTTGGCCGC
 10 1801 TGAAAAACCC GGGAGCGGGG CGGTGGGGGA GGCTATGCCC CGGCCCTCG CTCCTCATCC
 1861 TCCGTGCACG CTCGAATCGT CATCGCCACA GCCAGAAAAA AAAAAGATAC CGTGCGAAAA
 1921 GTGGCGGCAA CACAACGTCG ACGCCATCAG CGCCGCCCAG AGCTGCAAGC GGCTCCCACA
 1981 TGGTTGCCAC CGCAGCTTCC TCTACGACCC TTCATCCCCA CCGGCACCAG CTACGAGAAA
 2041 GGGACCTTAT TTCGGGCCAT CCCTACATAG GCGACTGTTG TTTTCGCACG AAAGATCTTT
 15 2101 ACGCAGCTGA TGCTGAAAAA AAAAAAAAAA AAAAAAAAA (SEQ ID NO:1).

The present invention also relates to the isolated or purified DNA molecule described in Figure 3 (T82) and set forth as SEQ ID NO:3, which encodes the *R. sanguineus* GluC11 protein described in Figure 4 and set forth as SEQ ID NO:4, the nucleotide sequence T82 as follows:

20 1 CACACCTCCT GCGTCTCTCC ACTCGATGAA GACCTGTCCC GGAGGCGCGA GCCCAACTGC
 61 GCGCTCTGTC CGCATGTGTC GCCGCCACTG AGAGGCCTCC GGCGTGGCGC GCTTGTCAAC
 121 GCGGCGCGCC GGCCCGCAGC AAATCGCGGG CATTCCACTC AGGGTCTCAT TCGTCCCCC
 181 AATCCTGAGG TTCCTTCTAA CGAGAAGGAG GAGCCACAGC GCCGGCTGCG GTACCGCCGC
 241 ACGGGCCAAC GTGAGACCGC CCGAGCCCGG CGCCCTGACT TAGGCCGCTG AGCGAAACCC
 25 301 AAGGCGGCGC GCTGGCCACT CCACGGGAAC GAGACCGGCC CCCTGGAGAC GACATCGTCG
 361 ACCACAATGA ACTACTTCTC TGACGTGGCG AAGATGGTGG CTTCATCGAA GAGAGAAATC
 421 ATCGAAGCTT TCCACGCGAC ATCTGGAGTA CACGGCGCAT GCGAATGAGC GAACATCGCT
 481 GACCGAGACT CGCCCGTCAC CATGAGCGTA CATTCATGGC GCTTTTGTGT CCCACTGGTG
 541 GCTCTAGCGT TTTTCTTGTT GATTCTTCTG TCGTGTCCAT CGGCATGGGG CAAGGCAAAT
 30 601 TTCCGCGCTA TAGAAAAGCG GATATTGGAC AGCATCATTG GCCAGGGTCG TTATGACTGC
 661 AGGATCCGGC CCATGGGAAT TAACAACACA GACGGGCCGG CTCTTGACG CGTTAACATC
 721 TTTGTAAGAA GTATCGGCAG AATTGATGAC GTCACCATGG AGTACACAGT GCAAATGACG
 781 TTCAGAGAGC AGTGGCGGGA CGAGAGACTC CAGTACGACG ACTTGGGCGG CCAGGTTCGC
 841 TACCTGACGC TCACCGAACC GGACAAGCTT TGGAAGCCGG ACCTGTTTTT CTCCAACGAG

901 AAAGAGGGAC ACTTCCACAA CATCATCATG CCCAACGTGC TTCTACGCAT ACATCCCAAC
 961 GGCGACGTTC TCTTCAGCAT CAGAATATCC TTGGTGCTTT CATGTCCGAT GAACCTGAAA
 1021 TTTTATCCTT TGGATAAACA AATCTGCTCT ATCGTCATGG TGAGCTATGG GTATACAACA
 1081 GAGGACCTGG TGTTCCTATG GAAAGAGGGG GATCCTGTAC AGGTCACAAA AAATCTCCAC
 5 1141 TTGCCACGTT TCACGCTGGA AAGGTTTCAA ACCGACTACT GCACCAGTCG GACCAACACT
 1201 GGCGAGTACA GCTGCTTGCG CGTGGACCTG GTGTTCAAGC GCGAGTTCAG CTACTACCTG
 1261 ATCCAGATCT ACATCCCGTG CTGCATGCTG GTCATCGTGT CCTGGGTGTC GTTCTGGCTC
 1321 GACCCACCT CGATCCCGGC GCGAGTGTCG CTGGGCGTCA CCACCCTGCT CACCATGGCC
 1381 ACGCAGATAT CGGGCATCAA CGCCTCGCTG CCTCCCGTTT CCTACACCAA GGCCATTGAC
 10 1441 GTGTGGACCG GCGTCTGTCT GACCTTCGTA TTCGGCGCGC TCCTCGAGTT CGCCCTGGTC
 1501 AACTACGCCT CGCGGTCAGA TTCACGCCGG CAGAACATGC AGAAGCAGAA GCAGAGGAAA
 1561 TGGGAGCTCG AGCCGCCCTT GGACTCGGAC CACCTGGAGG ACGGCGCCAC CACGTTCGCC
 1621 ATGAGGCCGC TGGTGCACCA CCACGGAGAG CTGCATGCCG ACAAGTTGCG GCAGTGCGAA
 1681 GTCCACATGA AGACCCCAA GACGAACCTT TGCAAGGCCT GGCTTTCCAG GTTTCCACG
 15 1741 CGATCCAAAC GCATCGACGT CGTCTCGCGG ATCTTCTTTC CGCTCATGTT CGCCCTCTTC
 1801 AACCTCGTCT ACTGGACAAC CTACCTCTTC CGGGAAGACA AGGAAGACGA GTGACAGAAC
 1861 ACGAACGCCA CGACAGCCGC CATCCGACAC CATCGTCACT GCAGGCACGC ACTCTGTGCG
 1921 GCGCACACAC CACGAAGACC GGCGCGCCAA CGCACGATGC GCGTTGGCCG CTGAAAAACC
 1981 CGGGAGCGGG GCGGTGGGGG AGGCTATGCC CCGGCCCTC GCTCCTCATC CTCCGTGCAC
 20 2041 GCTCGAATCG TCATCGCCAC AGCCAGAAAA AAAAAAGATA CCGTGCGAAA AGTGGCGGCA
 2101 ACACAACGTC GACGCCATCA GCGCCGCCCA GAGCTGCAAG CGGCTCCAC ATGGTTGCCA
 2161 CCGCAGCTTC CTCTACGACC CTTTCATCCCC ACCGGCACCA GCTACGAGAA AGGGACCTTA
 2221 TTTCGGGCCA TCCCTACATA GGCGACTGTT GTTTTCGCAC GAAAGATCTT TACGCAGCTG
 2281 ATGCTGAAA (SEQ ID NO:3).

25 The present invention also relates to the isolated or purified DNA molecule described in Figure 5 (T32) and set forth as SEQ ID NO:5, which encodes the *R. sanguineus* GluCl1 protein described in Figure 6 and set forth as SEQ ID NO:6, the nucleotide sequence T32 as follows:

30 1 CAGGCTCCGG CGTGA CTGTC GCTCGCTCGG CTCTCGACGC TCGCGGCGGG AACAAACCGCT
 61 ACCCGGACGC TCGATCAGGA GCAGTTCGGG CCACAGAGAA AGGGGCCGAG GAGTGCACAC
 121 CTCCTGCGTC TCTCCACTCG ATGAAGACCT GTCCCGGAGG CGCGAGCCCA ACTGCGCGCT
 181 CTGTCCGCAT GTGTCGCCGC CACTGAGAGG CCTCCGGCGT GGCGCGCTTG TCAACGCGGC
 241 GCGCCGGCCC GCAGCAAATC GCGGGCATTC CACTCAGGGT CTCATTGCT CCCCCAATCC
 301 TGAGGTTCTT TCTAACGAGA AGGAGGAGCC ACAGCGCCGG CTGCGGTACC GCCGCACGGG

361 CCAACGTGAG ACCGCCCCGAG CCCGGCGCCC TGA CT TAGGC CGCTGAGCGA AACCCAAGGC
 421 GCGCGCTGG CCACTCCACG GGAACGAGAC CGGCCCCCTG GAGACGACAT CGTCGACCAC
 481 AATGAACTAC TTCTCTGACG TGGCGAAGAT GGTGGCTTCA TCGAAGAGAG AAATCATCGA
 541 AGCTTTCCAC GCGACATCTG GAGTACACGG CGCATGCGAA TGAGCGAACA TCGCTGACCG
 5 601 AGACTCGCCC GTCACCATGA GCGTACATTC ATGGCGCTTT TGTGTCCCAC TGGTGGCTCT
 661 AGCGTTTTTC TTGTTGATTC TTCTGTCTG TCCATCGGCA TGGGCCGAAA CGCTGCCTAC
 721 GCCACCAACC CGTGGCCAGG GGGGCGTTCC GGTGCGGGCC GCGATGCTCC TGGGGAAACA
 781 GCAAAGTTCC CGCTACCAAG ATAAAGAGGG CAAGGCAAAT TTCCGCGCTA TAGAAAAGCG
 841 GATATTGGAC AGCATCATTC GCCAGGGTCG TTATGACTGC AGGATCCGGC CCATGGGAAT
 10 901 TAACAACACA GACGGGCCGG CTCTTGACG CGTTAACATC TTTGTAAGAA GTATCGGCAG
 961 AATTGATGAC GTCACCATGG AGTACACAGT GCAAATGACG TTCAGAGAGC AGTGGCGGGA
 1021 CGAGAGACTC CAGTACGACG ACTTGGGCGG CCAGGTTCGC TACCTGACGC TCACCGAACC
 1081 GGACAAGCTT TGGAAGCCGG ACCTGTTTTT CTCCAACGAG AAAGAGGGAC ACTTCCACAA
 1141 CATCATCATG CCCAACGTGC TTCTACGCAT ACATCCCAAC GGCGACGTTT TCTTCAGCAT
 15 1201 CAGAATATCC TTGGTGCTTT CATGTCCGAT GAACCTGAAA TTTTATCCTT TGGATAAACA
 1261 AATCTGCTCT ATCGTCATGG TGAGCTATGG GTATACAACA GAGGACCTGG TGTTCCTATG
 1321 GAAAGAGGGG GATCCTGTAC AGGTCACAAA AAATCTCCAC TTGCCACGTT TCACGCTGGA
 1381 AAGGTTTCAA ACCGACTACT GCACCAGTCG GACCAACACT GGCGAGTACA GCTGCTTGCG
 1441 CGTGGACCTG GTGTTCAAGC GCGAGTTCAG CTACTACCTG ATCCAGATCT ACATCCCGTG
 20 1501 CTGCATGCTG GTCATCGTGT CCTGGGTGTC GTTCTGGCTC GACCCACCT CGATCCCGGC
 1561 GCGAGTGTCG CTGGGCGTCA CCACCCTGCT CACCATGGCC ACGCAGATAT CGGGCATCAA
 1621 CGCCTCGCTG CCTCCCGTTT CCTACACCAA GGCCATTGAC GTGTGGACCG GCGTCTGTCT
 1681 GACCTTCGTA TTCGGCGCGC TCCTCGAGTT CGCCCTGGTC AACTACGCCT CGCGGTCAGA
 1741 TTCACGCCGG CAGAACATGC AGAAGCAGAA GCAGAGGAAA TGGGAGCTCG AGCCGCCCT
 25 1801 GGA CT CGGAC CACCTGGAGG ACGGCGCCAC CACGTTGCGC ATGGTGAGCT CCGGCGAGCC
 1861 GGCGGGCCTC ATGGCGCGAA CCTGGCCACC ACCGCCGCTG CCGCCAAACA TGGCGGCCGG
 1921 CTCCGCGCAA GCCGGCGCCA GGCCGCTGGT GCACCACCAC GGAGAGCTGC ATGCCGACAA
 1981 GTTGCGGCAG TCGGAAGTCC ACATGAAGAC CCCCAGACG AACCTTTGCA AGGCCTGGCT
 2041 TTCCAGGTTT CCCACGCGAT CCAAACGCAT CGACGTCGTC TCGCGGATCT TCTTTCCGCT
 30 2101 CGTGTTGCGC CTCTTCAACC TCGTCTACTG GACAACCTAC CTCTTCCGGG AAGACGAGGA
 2161 GGACGAGTGA CAGAACACGA ACGCCACGAC AGCCGCCATC CGACACCATC GTC ACT GCAG
 2221 GCACGCACTC TGTCGCGCGC ACACACCACG AAGACCGGCG CGCCAACGCA CGATGCGCGT
 2281 TGGCCGCTGA AAAACCCGGG AGCGGGGCGG TGGGGGAGGC TATGCCCCGG CCCCTCGCTC

2341 CTCATCCTCC GTGCACGCTC GAATCGTCAT CGCCACAGCC AGAAAAAAAA AAAAAAAAAA
(SEQ ID NO:5) .

The present invention also relates to an isolated or purified DNA molecule which encodes a *R. sanguineus* GluCl2 protein. One such nucleic acid is described in Figure 7 (B1) and set forth as SEQ ID NO:7, which encodes the *R. sanguineus* GluCl2 protein described in Figure 8 and set forth as SEQ ID NO:8, the nucleotide sequence B1 as follows:

1 CGCCGCTCAA TCGCGGGCTA CGGACTCGTC GTTCCCGGAG GGGCTTGGAC
51 CACAGCTCGC TCGTCACCGT GGTGGCTGGC CGCTTCGCCT GGCGGTCCTG
10 101 CACGCACGCT GTAACGAACG TCGCCACGCG ATGTTTGGTG TGCCATGCTC
151 CCGCGCCTGC CGCCTTGTGG TGGTGATAGC TGC GTTCTGC TGGCCGCCCCG
201 CTCTGCCGCT CGTACCCGGG GGAGTTTCCT CCAGAGCAAA CGATCTGGAC
251 ATTCTGGACG AGCTCCTCAA AAAC TACGAT CGAAGGGCCC TGCCGAGCAG
301 TCACCTCGGA AATGCAACTA TTGTGTCATG CGAAATTTAC ATACGAAGTT
15 351 TTGGATCAAT AAATCCTTCG AACATGGACT ACGAAGTCGA CCTCTACTTC
401 CGGCAGTCGT GGCTCGACGA GCGGTACGC AAATCCACGC TATCTCGTCC
451 GCTCGACCTT AATGACCCAA AGCTGGTACA AATGATATGG AAGCCAGAAG
501 TTTTCTTTGC GAACGCGAAA CACGCCGAGT TCCAATATGT GACTGTACCT
551 AACGTCCTCG TTAGGATCAA CCCGACTGGA ATAATCTTGT ACATGTTGCG
20 601 GTTAAAACTG AGGTTCCTCT GCATGATGGA CCTGTACCGG TACCCCATGG
651 ATTCCCAAGT CTGCAGCATC GAAATTGCCT CTTTTTCCAA AACCACCGAA
701 GAGCTGCTGC TGAAATGGTC CGAGAGTCAG CCTGTCTGTT TCTTCGATAA
751 CCTCAAGTTG CCCCAGTTTG AAATAGAGAA GGTGAACACG TCCTTATGCA
801 AAGAAAAGTT TCACATAGGG GAATACAGTT GCCTGAAAGC CGACTTCTAT
25 851 CTGCAGCGTT CCCTCGGTTA TCACATGGTG CAGACCTATC TTCCGACCAC
901 GCTTATCGTG GTCATCTCAT GGGTGTCAAT CTGGCTCGAC GTAGACGCCA
951 TACCCGCCCCG TGTCACCCTG GCGGTAACCA CGCTGCTCAC CATCTCATCC
1001 AAGGGTGCCG GTATCCAGGG AAACCTGCCT CCCGTCTCGT ACATCAAGGC
1051 CATGGACGTC TGGATAGGAT CCTGTACTTC GTTTGTCTTT GCGGCCCTTC
30 1101 TAGAGTTCAC ATTCGTCAAC TATCTCTGGA GCGGCTGCC CAATAAGCGC
1151 CCATCTTCTG ACGTACCGGT GACGGATATA CCAAGCGACG GCTCAAAGCA
1201 TGACATTGCG GCACAGCTCG TACTCGACAA GAATGGACAC ACCGAAGTTC
1251 GCACGTTGGT CCAAGCGATG CCACGCAGCG TCGGAAAAGT GAAGGCCAAG
1301 CAGATTGATC AACTCAGCCG AGTCGCCTTT CCCGCTCTTT TTCTCCTCTT

1351 CAACCTCGTG TACTGGCCGT ACTACATTAA GTCATAAAGA ACGTAGTTTT

1401 CT (SEQ ID NO:7) .

The above-exemplified isolated DNA molecules, shown in Figure 1, 3 5, and 7, respectively, comprise the following characteristics:

5 T12 (SEQ ID NO:1):

2138 nuc.:initiating Met (nuc. 331-333) and "TGA" term. codon (nuc.1681-1683), the open reading frame resulting in an expressed protein of 450 amino acids, as set forth in SEQ ID NO:2.

T82 (SEQ ID NO:3):

10 2289 nuc.:initiating Met (nuc. 502-504) and "TGA" term. codon (nuc. 1852-1854), the open reading frame resulting in an expressed protein of 450 amino acids, as set forth in SEQ ID NO:4.

T32 (SEQ ID NO:5):

15 2400 nuc.:initiating Met (nuc. 617-619) and "TGA" term. codon (nuc. 2168-2170), the open reading frame resulting in an expressed protein of 517 amino acids, as set forth in SEQ ID NO:6.

B1 (SEQ ID NO:7):

20 1402 nuc.:initiating Met (nuc. 131-133) and "TAA" term. codon (nuc. 1385-1387), the open reading frame resulting in an expressed protein of 418 amino acids, as set forth in SEQ ID NO:8.

The present invention also relates to biologically active fragments or mutants of SEQ ID NOs:1, 3, 5 and 7 which encodes mRNA expressing a novel *Rhipicephalus sanguineus* invertebrate GluCl1 or GluCl2 channel protein, respectively. Any such biologically active fragment and/or mutant will encode either a protein or protein
25 fragment which at least substantially mimics the pharmacological properties of a *R. sanguineus* GluCl channel protein, including but not limited to the *R. sanguineus* GluCl1 channel proteins as set forth in SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6 as well as the respective GluCl2 channel protein as set forth in SEQ ID NO:8. Any such polynucleotide includes but is not necessarily limited to nucleotide
30 substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a functional *R. sanguineus* GluCl channel in a eukaryotic cell, such as *Xenopus* oocytes, so as to be useful for screening for agonists and/or antagonists of *R. sanguineus* GluCl activity.

A preferred aspect of this portion of the present invention is disclosed in Figure 1 (SEQ ID NO:1; designated T12), Figure 3 (SEQ ID NO:3; designated T82) and Figure 5 (SEQ ID NO:5; designated T32) encoding novel *Rhipicephalus sanguineus* GluCl1 proteins, and Figure 7 (SEQ ID NO:7, designated B1) encoding a novel *Rhipicephalus sanguineus* GluCl2 protein.

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the RsGluCl1 or RsGluCl2 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NOs:1, 3, 5, and 7 but still encodes the same RsGluCl protein as SEQ ID NO:1, 3, 5 and 7. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of the RsGluCl channel protein in the host. In other words, this redundancy in the various codons which code for specific amino acids is within the scope of the present invention. Therefore, this invention is also directed to those DNA sequences which encode RNA comprising alternative codons which code for the eventual translation of the identical amino acid, as shown below:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
 C=Cys=Cysteine: codons UGC, UGU
 D=Asp=Aspartic acid: codons GAC, GAU
 E=Glu=Glutamic acid: codons GAA, GAG
 F=Phe=Phenylalanine: codons UUC, UUU
 G=Gly=Glycine: codons GGA, GGC, GGG, GGU
 H=His =Histidine: codons CAC, CAU
 I=Ile =Isoleucine: codons AUA, AUC, AUU

K=Lys=Lysine: codons AAA, AAG

L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU

M=Met=Methionine: codon AUG

N=Asp=Asparagine: codons AAC, AAU

5 P=Pro=Proline: codons CCA, CCC, CCG, CCU

Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

10 V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Therefore, the present invention discloses codon redundancy which may result in differing DNA molecules expressing an identical protein. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a
 15 degenerate variation. Another source of sequence variation may occur through RNA editing, as discussed *infra*. Such RNA editing may result in another form of codon redundancy, wherein a change in the open reading frame does not result in an altered amino acid residue in the expressed protein. Also included within the
 20 scope of this invention are mutations either in the DNA sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

25 It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide having properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or a receptor
 30 for a ligand.

Included in the present invention are DNA sequences that hybridize to SEQ ID NOs:1, 3, 5 and 7 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer

composed of 6X SSC, 5X Denhardt's solution, and 100 $\mu\text{g/ml}$ denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 $\mu\text{g/ml}$ denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing
5 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes. Reagents
10 mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. In addition to the foregoing, other conditions of high stringency which may be used are well known in
15 the art.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, e.g.,: (Computational Molecular Biology, Lesk, A. M., ed.
20 Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M
25 Stockton Press, New York, 1991). While there exists a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo and Lipton, 1988, *SIAM J Applied Math* 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to
30 Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo and Lipton, 1988, *SIAM J Applied Math* 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, et al, 1984, *Nucleic Acids*

Research 12(1):387), BLASTN, and FASTA (Altschul, et al., 1990, *J Mol. Biol.* 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO:1
5 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations or alternative nucleotides per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO:1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide
10 sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations or alternative nucleotide substitutions of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or
15 anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. One source of such a "mutation" or change which results in a less than 100% identity may occur through RNA editing. The process of RNA editing results in modification of an mRNA molecule such that use of that modified mRNA
20 as a template to generate a cloned cDNA may result in one or more nucleotide changes, which may or may not result in a codon change. This RNA editing is known to be catalyzed by an RNA editase. Such an RNA editase is RNA adenosine deaminase, which converts an adenosine residue to an inosine residue, which tends to mimic a cytosine residue. To this end, conversion of an mRNA residue from A to I
25 will result in A to G transitions in the coding and noncoding regions of a cloned cDNA (e.g., see Hanrahan et al, 1999, *Annals New York Acad. Sci.* 868:51-66; for a review see Bass, 1997, *TIBS* 22: 157-162). Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the
30 polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO:2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be

deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence of
5 anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. Again, as noted above, RNA editing may result in a codon change which will result in an expressed protein which differs in "identity" from other proteins expressed from "non-RNA edited" transcripts, which correspond directly to
10 the open reading frame of the genomic sequence. The open reading frame of the T12 and T82 clones are identical, save for a single nucleotide change which results in a single amino acid change (T12 - "gag"/Glu v. T82 - "aag"/Lys at amino acid residue 447 of SEQ ID NOs: 2 and 4). The T12/T82 clone shows about a 57% identity with the B1 clone at the nucleotide level whereas the T32 clone shows about
15 a 57% identity with the B1 clone at the nucleotide level.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification. The nucleic acid molecules of the present invention encoding a RsGluCl channel protein, in whole or
20 in part, can be linked with other DNA molecules, i.e, DNA molecules to which the RsGluCl coding sequence are not naturally linked, to form "recombinant DNA molecules" which encode a respective RsGluCl channel protein. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding RsGluCl or a functional equivalent. These vectors may be
25 comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a RsGluCl channel protein. It is well within the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

30 The present invention also relates to a substantially purified form of a respective RsGluCl channel protein, which comprise the amino acid sequence disclosed in Figure 2, Figure 4, Figure 6 and Figure 8, and as set forth in SEQ ID NOs :2, 4, 6, and 8, respectively. The disclosed RsGluCl proteins contain an open reading frame of 450 amino acids (T12 and T82, SEQ ID NOs: 2 and 4, respectively),

517 amino acids (T32, SEQ ID NO: 6) and 418 amino acids (SEQ ID NO:8) in length, as shown in Figures 2, 4, 6, and 8, and as follows:

T12:

MSVHSWRFCV PLVALAFFLL ILLSCPSAWG KANFRAIEKR ILDSIIGQGR YDCRIRPMGI
 5 NNTDGPALVR VNIFVRSIGR IDDTVMEYTV QMTFREQWRD ERLQYDDLGG QVRYLTLTEP
 DKLWKPDLEF SNEKEGHFHN IIMPNVLLRI HPNGDVLFISI RISLVLSCPM NLKFYPLDKQ
 ICSIVMVSYG YTTEDLVFLW KEGDPVQVTK NLHLPRFTLE RFQTDYCTSR TNTGEYSCLR
 VDLVFKREFS YYLIQIYIPC CMLVIVSWVS FWLDPTSIPA RVSLGVTTLL TMATQISGIN
 ASLPPVSYTK AIDVWTGVCL TFVFGALLEF ALVNYASRSD SRRQNMQKQK QRKWELEPPL
 10 DSDHLEDGAT TFAMRPLVHH HGELHADKLR QCEVHMKTPK TNLCKAWLSR FPTRSKRIDV
 VSRIFPLMF ALFNLVYWTT YLFREDEEDE* (SEQ ID NO:2);

T82:

MSVHSWRFCV PLVALAFFLL ILLSCPSAWG KANFRAIEKR ILDSIIGQGR YDCRIRPMGI
 NNTDGPALVR VNIFVRSIGR IDDTVMEYTV QMTFREQWRD ERLQYDDLGG QVRYLTLTEP
 15 DKLWKPDLEF SNEKEGHFHN IIMPNVLLRI HPNGDVLFISI RISLVLSCPM NLKFYPLDKQ
 ICSIVMVSYG YTTEDLVFLW KEGDPVQVTK NLHLPRFTLE RFQTDYCTSR TNTGEYSCLR
 VDLVFKREFS YYLIQIYIPC CMLVIVSWVS FWLDPTSIPA RVSLGVTTLL TMATQISGIN
 ASLPPVSYTK AIDVWTGVCL TFVFGALLEF ALVNYASRSD SRRQNMQKQK QRKWELEPPL
 DSDHLEDGAT TFAMRPLVHH HGELHADKLR QCEVHMKTPK TNLCKAWLSR FPTRSKRIDV
 20 VSRIFPLMF ALFNLVYWTT YLFREDKEDE* (SEQ ID NO:4);

T32:

MSVHSWRFCV PLVALAFFLL ILLSCPSAWA ETLPTPPTRG QGGVPVAAAM LLGKQQSSRY
 QDKEGKANFR AIEKRILDSI IGQGRYDCRI RPMGINNTDG PALVRVNIFV RSIGRIDDTV
 MEYTVQMTFR EQWRDERLQY DDLGGQVRYL TLTEPDKLWK PDLFFSNEKE GHFHNIIMPN
 25 VLLRIHPNGD VLFSIRISLV LSCPMNLKFY PLDKQICSIV MVSYGYTTED LVFLWKEGDP
 VQVTKNLHLP RFTLERFQTD YCTSRNTNTGE YSCLRVDLVF KREFSYyliQ IYIPCCMLVI
 VSWVSFWLDP TSIPARVSLG VTTLLTMATQ ISGINASLPP VSYTKAIDVW TGVCLTFVFG
 ALLEFALVNY ASRSDSRRQN MQKQKQRKWE LEPPLSDHL EDGATTFAMV SSGEPAGLMA
 RTWPPPPLPP NMAAGSAQAG ARPLVHHHGE LHADKLRQCE VHMKTPKTNL CKAWLSRFPT
 30 RSKRIDVVSRIFFPLVFALF NLVYWTTYLEF REDEEDE* (SEQ ID NO:6); and,

B1:

MFGVPCSRAC RLVVVIAAFC WPPALPLVPG GVSSRANDLD ILDELLKNYD RRALPSSHG
 NATIVSCEIY IRSFGSINPS NMDYEVDLYF RQSWLDERLR KSTLSRPLDL NDPKLVQMIW
 KPEVFFANAK HAEFQYVTVP NVLVRINPTG IILYMLRLKL RFSCMMDLYR YPMDSQVCSI

EIASFSKTTE ELLLKWSESQ PVVLFNKLK PQFEIEKVNT SLCKEKFHIG EYSCLKADFY
 LQRSLGYHVM QTYLPTTLIV VISWVSFWLD VDAIPARVTL GVTLLTISS KGAGIQGNLP
 PVSYIKAMDV WIGSCTSFVF AALLEFTFVN YLWRRLPNKR PSSDVPVTDI PSDGSKHDIA
 AQLVLDKNGH TEVRTLVQAM PRSVGKVKAK QIDQLSRVAF PALFLLFNLV YWPYYIKS (SEQ
 5 ID NO:8).

The open reading frames of the T12 and T82 clones are identical, save for a
 single nucleotide change which results in a single amino acid change at residue 447 of
 SEQ ID NOs: 2 and 4. The T32 open reading frame contains two addition exons
 when compared to the T12/T82 reading frame, which result in a 35 amino acid
 10 insertion in the amino terminal region of the T32 protein (amino acid residue 30-64 of
 SEQ ID NO:6) and another 32 amino acid insertion within the COOH-terminal region
 (amino acid residue 410-441). The T12/T82 clones show about a 57% identity with
 the B1 clone at the nucleotide level whereas the T32 clone shows about a 57%
 identity with the B1 clone at the nucleotide level.

15 The present invention also relates to biologically active fragments and/or
 mutants of the RsGluCl1 and RsGluCl2 proteins comprising the amino acid sequence
 as set forth in SEQ ID NOs:2, 4, 6, and 8, including but not necessarily limited to
 amino acid substitutions, deletions, additions, amino terminal truncations and
 carboxy-terminal truncations such that these mutations provide for proteins or protein
 20 fragments of diagnostic, therapeutic or prophylactic use and would be useful for
 screening for agonists and/or antagonists of RsGluCl function.

To this end, a preferred aspect of the present invention is a functional RsGluCl
 channel receptor, comprised of either a single channel protein or a channel comprising
 multiple subunits, referred to herein as a homomultimeric channel or a
 25 heteromultimeric channel. Therefore, a single channel may be comprised of a protein
 as disclosed in SEQ ID NOs: 2, 4, 6 or 8, or a biologically active equivalent thereof
 (i.e., an altered channel protein which still functions in a similar fashion to that of a
 wild-type channel receptor). A homomultimeric channel receptor complex will
 comprise more than one polypeptide selected from the disclosed group of SEQ ID
 30 NOs: 2, 4, 6 and 8, as well as biologically active equivalents. A heteromultimeric
 channel receptor complex will comprise multiple subunits wherein at least 2 of the 3
 proteins disclosed herein contribute to channel formation, or where at least one of the
 proteins associates with additional proteins or channel components to provide for an
 active channel receptor complex. Therefore, the present invention additionally relates

to substantially purified channels as described herein, as well as substantially purified membrane preparations, partially purified membrane preparations, or cell lysates which contain the functional single, homomultimeric or heteromultimeric channels described herein. These substantially purified, fully processed GluCl channel proteins
5 may be obtained from a recombinant host cell containing a DNA expression vector comprises a nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5, and/or 7, and expresses the respective RsGluCl precursor protein. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, including but not limited to a mammalian cell line, an insect cell line such as an S2 cell line, or *Xenopus* oocytes,
10 as noted above.

As with many proteins, it is possible to modify many of the amino acids of RsGluCl channel protein and still retain substantially the same biological activity as the wild type protein. Thus this invention includes modified RsGluCl polypeptides which have amino acid deletions, additions, or substitutions but that still retain
15 substantially the same biological activity as a respective, corresponding RsGluCl. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., *Molecular Biology of the Gene*, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, *Science* 244:1081-1085). Accordingly, the
20 present invention includes polypeptides where one amino acid substitution has been made in SEQ ID NO:2, 4, 6, and/or 8, wherein the polypeptides still retain substantially the same biological activity as a corresponding RsGluCl protein. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ ID NO:2, 4, 6, or 8, wherein the polypeptides
25 still retain substantially the same biological activity as a corresponding RsGluCl protein. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions.

One skilled in the art would also recognize that polypeptides that are functional equivalents of RsGluCl and have changes from the RsGluCl amino acid
30 sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, (i.e., minimizing the differences in amino acid sequence between RsGluCl and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions or insertions on the biological activity of the modified RsGluCl polypeptide can easily

be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding RsGluCl and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression.

The present invention also includes truncated forms of RsGluCl which contain
5 the region comprising the active site of the enzyme. Such truncated proteins are useful in various assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also relates to membrane-containing crude lysates or substantially purified subcellular membrane fractions from the recombinant host cells
10 (both prokaryotic and eukaryotic as well as both stably and transiently transformed or transfected cells) which contain the nucleic acid molecules of the present invention. These recombinant host cells express RsGluCl or a functional equivalent, which becomes post translationally associated with the cell membrane in a biologically active fashion. These subcellular membrane fractions will comprise either wild-type
15 or mutant forms of RsGluCl at levels substantially above endogenous levels and hence will be useful in assays to select modulators of RsGluCl proteins or channels. In other words, a specific use for such subcellular membranes involves expression of RsGluCl within the recombinant cell followed by isolation and substantial purification of the membranes away from other cellular components and subsequent use in assays
20 to select for modulators, such as agonist or antagonists of the protein or biologically active channel comprising one or more of the proteins disclosed herein. Alternatively, the lysed cells, containing the membranes, may be used directly in assays to select for modulators of the recombinantly expressed protein(s) disclosed herein. Therefore, another preferred aspect of the present invention relates to a substantially purified
25 membrane preparation or lysed recombinant cell components which include membranes, which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5, and/or 7, resulting in a functional form of the respective RsGluCl channel. It is
30 especially preferred is that the recombinant host cell be a eukaryotic host cell, , including but not limited to a mammalian cell line, an insect cell line such as an S2 cell line, .

The present invention also relates to isolated nucleic acid molecules which are fusion constructions expressing fusion proteins useful in assays to identify compounds

which modulate wild-type RsGluCl activity, as well as generating antibodies against RsGluCl. One aspect of this portion of the invention includes, but is not limited to, glutathione S-transferase (GST)-RsGluCl fusion constructs. Recombinant GST-RsGluCl fusion proteins may be expressed in various expression systems, including *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen). Another aspect involves RsGluCl fusion constructs linked to various markers, including but not limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Again, any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of one or more of the RsGluCl proteins disclosed herein.

A preferred aspect for screening for modulators of RsGluCl channel activity is an expression system for the electrophysiological-based assays for measuring glutamate-gated chloride channel activity comprising injecting the DNA molecules of the present invention into *Xenopus laevis* oocytes. The general use of *Xenopus* oocytes in the study of ion channel activity is known in the art (Dascal, 1987, *Crit. Rev. Biochem.* 22: 317-317; Lester, 1988, *Science* 241: 1057-1063; see also *Methods of Enzymology*, Vol. 207, 1992, Ch. 14-25, Rudy and Iverson, ed., Academic Press, Inc., New York). An improved method exists for measuring channel activity and modulation by agonists and/or antagonists which is several-fold more sensitive than previous techniques. The *Xenopus* oocytes are injected with nucleic acid material, including but not limited to DNA, mRNA or cRNA which encode a gated-channel, wherein channel activity may be measured as well as response of the channel to various modulators. Ion channel activity is measured by utilizing a holding potential more positive than the reversal potential for chloride (i.e, greater than -30 mV), preferably about 0 mV. This alteration in assay measurement conditions results in a 10-fold increase in sensitivity of the assay to modulation by ivermectin phosphate. Therefore, this improved assay allows screening and selecting for compounds which modulate GluCl activity at levels which were previously thought to be undetectable.

Any of a variety of procedures may be used to clone RsGluCl. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85: 8998-9002). 5' and/or 3' RACE may be performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of RsGluCl cDNA. These gene-specific primers are designed through identification of an expressed

sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the RsGluCl cDNA following the construction of a RsGluCl-containing cDNA library in an appropriate expression vector system; (3) screening a RsGluCl-
5 containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the RsGluCl protein; (4) screening a RsGluCl-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the RsGluCl protein. This partial cDNA is obtained by the specific PCR
10 amplification of RsGluCl DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other GluCl channels which are related to the RsGluCl protein; (5) screening a RsGluCl-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA or oligonucleotide with homology to a RsGluCl protein. This
15 strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of RsGluCl cDNA identified as an EST as described above; or (6) designing 5' and 3' gene specific oligonucleotides using SEQ ID NO: 1, 3, and 5 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known
20 RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding RsGluCl.

Alternatively, the RsGluCl1 and RsGluCl2 cDNAs of the present invention may be cloned as described in Example Section 1. For RsGluCl1 cDNA clones, adult brown
25 dog tick polyA⁺ RNA was isolated using the Poly(A)PureTM mRNA Isolation Kit (Ambion). Tick cDNA was synthesized using oligo-dT primers and the ZAP cDNA[®] Synthesis Kit (Stratagene), and cDNA >1kb was selected using cDNA Size Fractionation Columns (BRL). A tick cDNA library was constructed in the Lambda ZAP[®] II vector using the GIGAPACK[®] III Gold Cloning Kit (Stratagene). A
30 *Drosophila* GluCl cDNA fragment spanning the M1 to M3 region was used in a low-stringency screen of the tick cDNA library. Filters were exposed for eleven days and six positives were isolated for sequence analysis. Three of the clones (T12, T82 and T32) encode GluCl-related proteins and were sequenced on both ends. For isolation of the RsGluCl2 cDNAs, most molecular procedures were again performed

following standard procedures available in references such as Ausubel et. al. (1992. Short protocols in molecular biology. F.M. Ausubel et al., -2nd. ed. (John Wiley & Sons), and Sambrook et al. (1989. Molecular cloning. A laboratory manual. J. Sambrook, E.F. Fritsch, and T. Maniatis - 2nd ed. (Cold Spring Harbor Laboratory Press). Poly (A)+ RNA was isolated from Tick heads. First strand cDNA was synthesized from 50 ng RNA using a SUPERScript preamplification System (Life Technologies). A tenth of the first strand reaction was used for PCR. The degenerate oligos utilized were designed based on sequences obtained from *C. elegans*, *Drosophila*, and Flea (*C. felis*) GluCl₁: Two PCR rounds, using the combinations “27F2 + 3AF1, then 27F2 + 3BF2” were performed. One tenth of the PCR reaction products was tested by Southern blot analysis, in order to identify and prevent the PCR-cloning of contaminating sequences. Novel PCR products of the appropriate size were cloned into the pCR2.1 plasmid vector using a “TA” cloning kit (Invitrogen, Inc.). Following sequence analysis (ABI Prism, PE Applied Biosystems), selected PCR clone inserts were radiolabelled and used as probes to screen a cDNA library generated into the Uni-ZAP[®] vector (Stratagene, Inc.) from using the RNA preparation mentioned above. Sequences from full-length cDNA clones were analysed using the GCG Inc. package. Subcloning of RsGluCl₂ into a mammalian expression vector was done by excision of an 1.85 kb coding-region-containing fragment (XhoI-EcoRI digest) from the original insert of clone RsGluCl₂ B1 from the UniZap[®] pBS plasmid, followed by ligation into the TetSplice[®] vector (Life Technologies Inc.).

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types-or species types, may be useful for isolating a RsGluCl-encoding DNA or a RsGluCl homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other brown dog tick cell types.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have RsGluCl activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding RsGluCl may be done by first measuring cell-associated RsGluCl activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for

example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

5 It is also readily apparent to those skilled in the art that DNA encoding RsGluCl may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in Sambrook, et al., *supra*. One may prepare genomic libraries, especially in P1 artificial
10 chromosome vectors, from which genomic clones containing the RsGluCl can be isolated, using probes based upon the RsGluCl nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou et al., 1994, *Nature Genet.* 6:84-89).

In order to clone a RsGluCl gene by one of the preferred methods, the amino
15 acid sequence or DNA sequence of a RsGluCl or a homologous protein may be necessary. To accomplish this, a respective RsGluCl channel protein may be purified and the partial amino acid sequence determined by automated sequenators. It is not necessary to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids can be determined for the PCR amplification of a partial
20 RsGluCl DNA fragment. Once suitable amino acid sequences have been identified, the DNA sequences capable of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the RsGluCl
25 sequence but others in the set will be capable of hybridizing to RsGluCl DNA even in the presence of DNA oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still sufficiently hybridize to the RsGluCl DNA to permit identification and isolation of RsGluCl encoding DNA. Alternatively, the nucleotide sequence of a region of an expressed sequence may be identified by searching one or
30 more available genomic databases. Gene-specific primers may be used to perform PCR amplification of a cDNA of interest from either a cDNA library or a population of cDNAs. As noted above, the appropriate nucleotide sequence for use in a PCR-based method may be obtained from SEQ ID NO: 1, 3, 5, or 7 either for the purpose of isolating overlapping 5' and 3' RACE products for generation of a full-length

sequence coding for RsGluCl, or to isolate a portion of the nucleotide sequence coding for RsGluCl for use as a probe to screen one or more cDNA- or genomic-based libraries to isolate a full-length sequence encoding RsGluCl or RsGluCl-like proteins.

5 This invention also includes vectors containing a RsGluCl gene, host cells containing the vectors, and methods of making substantially pure RsGluCl protein comprising the steps of introducing the RsGluCl gene into a host cell, and cultivating the host cell under appropriate conditions such that RsGluCl is produced. The RsGluCl so produced may be harvested from the host cells in conventional ways.
10 Therefore, the present invention also relates to methods of expressing the RsGluCl protein and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of RsGluCl activity.

15 The cloned RsGluCl cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector (such as pcDNA3.neo, pcDNA3.1, pCR2.1, pBlueBacHis2 or pLITMUS28, as well as other examples, listed *infra*) containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host
20 cells to produce recombinant RsGluCl. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned DNA and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Specifically designed vectors allow the shuttling
25 of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase
30 to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. To determine the RsGluCl cDNA sequence(s) that yields optimal levels of RsGluCl, cDNA molecules including but not limited to the following can be constructed: a cDNA fragment containing the full-length open reading frame for RsGluCl as well as various constructs containing

portions of the cDNA encoding only specific domains of the protein or rearranged domains of the protein. All constructs can be designed to contain none, all or portions of the 5' and/or 3' untranslated region of a RsGluCl cDNA. The expression levels and activity of RsGluCl can be determined following the introduction, both singly and in
5 combination, of these constructs into appropriate host cells. Following determination of the RsGluCl cDNA cassette yielding optimal expression in transient assays, this RsGluCl cDNA construct is transferred to a variety of expression vectors (including recombinant viruses), including but not limited to those for mammalian cells, plant cells, insect cells, oocytes, bacteria, and yeast cells. Techniques for such
10 manipulations can be found described in Sambrook, et al., *supra*, are well known and available to the artisan of ordinary skill in the art. Therefore, another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding the RsGluCl. An expression vector containing DNA encoding a RsGluCl-like protein may be used for expression of RsGluCl in a
15 recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce RsGluCl or a biologically equivalent form. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be suitable for recombinant RsGluCl expression,
20 include but are not limited to, pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Biolabs), pcDNA1, pcDNA1amp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC
25 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565). Also, a variety of bacterial expression vectors may be used to express recombinant RsGluCl in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant RsGluCl expression include, but are not limited to pCR2.1 (Invitrogen),
30 pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia). In addition, a variety of fungal cell expression vectors may be used to express recombinant RsGluCl in fungal cells. Commercially available fungal cell expression vectors which may be suitable for recombinant RsGluCl expression include but are not limited to pYES2 (Invitrogen) and *Pichia* expression vector (Invitrogen). Also, a

variety of insect cell expression vectors may be used to express recombinant protein in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of RsGluCl include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharmingen).

5 Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin; and insect cells including but not limited to *R. sanguineus* and silkworm derived cell lines. For instance, one insect expression system utilizes *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharmingen). Also, mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650),
10 COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

 The specificity of binding of compounds showing affinity for RsGluCl is shown by measuring the affinity of the compounds for recombinant cells expressing
20 the cloned receptor or for membranes from these cells, which form a functional single, homomultimeric or heteromultimeric membrane channel. Expression of the cloned receptor and screening for compounds that bind to RsGluCl or that inhibit the binding of a known, radiolabeled ligand of RsGluCl to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of
25 compounds with high affinity for RsGluCl. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of RsGluCl and may be peptides, proteins, or non-proteinaceous organic or inorganic
30 molecules.

 A preferred aspect for screening for modulators of RsGluCl channel activity is an expression system for electrophysiologically-based assays for measuring ligand gated channel activity (such as GluCl channel activity) comprising injecting the DNA or RNA molecules of the present invention into *Xenopus laevis* oocytes. The general

use of *Xenopus* oocytes in the study of ion channel activity is known in the art (Dascal, 1987, *Crit. Rev. Biochem.* 22: 317-317; Lester, 1988, *Science* 241: 1057-1063; see also *Methods of Enzymology*, Vol. 207, 1992, Ch. 14-25, Rudy and Iverson, ed., Academic Press, Inc., New York). The *Xenopus* oocytes are injected with nucleic acid material, including but not limited to DNA, mRNA or cRNA which encode a
5 ligand gated-channel, whereafter channel activity may be measured as well as response of the channel to various modulators.

Accordingly, the present invention is directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a RsGluCl
10 protein as well as compounds which effect the function of the RsGluCl protein. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of a RsGluCl channel. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for identifying substances that inhibit agonist binding to rat neurokinin
15 receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled
20 agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential ligand of the neurokinin receptor. Where
25 binding of the substance such as an agonist or antagonist to RsGluCl is measured, such binding can be measured by employing a labeled ligand. The ligand can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

Therefore, the present invention is directed to methods for screening for
30 compounds which modulate the expression of DNA or RNA encoding a RsGluCl protein. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic or inorganic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding RsGluCl, or the function of the RsGluCl-based channels. Compounds that modulate

the expression of DNA or RNA encoding RsGluCl or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the
5 levels of expression or function in a standard sample. Kits containing RsGluCl, antibodies to RsGluCl, or modified RsGluCl may be prepared by known methods for such uses.

To this end, the present invention relates in part to methods of identifying a substance which modulates RsGluCl receptor activity, which involves:

10 (a) adding a test substance in the presence and absence of a RsGluCl receptor protein wherein said RsGluCl receptor protein comprises the amino acid sequence as set forth in SEQ ID NOs: 2, 6 and/or 8; and,

(b) measuring and comparing the effect of the test substance in the presence and absence of the RsGluCl receptor protein or respective functional channel.

15 In addition, several specific embodiments are disclosed herein to show the diverse types of screening or selection assays which the skilled artisan may utilize in tandem with an expression vector directing the expression of the RsGluCl receptor protein. Methods for identifying ligands of other receptors are well known in the art and can be adapted to ligands of RsGluCl. Therefore, these embodiments are
20 presented as examples and not as limitations. To this end, the present invention includes assays by which RsGluCl modulators (such as agonists and antagonists) may be identified. Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of RsGluCl that comprises:

(a) transfecting or transforming cells with an expression vector that directs
25 expression of RsGluCl in the cells, resulting in test cells;

(b) allowing the test cells to grow for a time sufficient to allow RsGluCl to be expressed and for a functional channel to be generated;

(c) exposing the cells to a labeled ligand of RsGluCl in the presence and in the absence of the substance;

30 (d) measuring the binding of the labeled ligand to the RsGluCl channel; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential ligand of RsGluCl.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

5 The test cells may be harvested and resuspended in the presence of the substance and the labeled ligand. In a modification of the above-described method, step (c) is modified in that the cells are not harvested and resuspended but rather the radioactively labeled known agonist and the substance are contacted with the cells while the cells are attached to a substratum, e.g., tissue culture plates.

10 The present invention also includes a method for determining whether a substance is capable of binding to RsGluCl, i.e., whether the substance is a potential modulator of RsGluCl channel activation, where the method comprises:

- (a) transfecting or transforming cells with an expression vector that directs the expression of RsGluCl in the cells, resulting in test cells;
- 15 (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to RsGluCl;
- (d) comparing the amount of binding of the substance to RsGluCl in the test cells with the amount of binding of the substance to control cells that have not been transfected with RsGluCl;

20 wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to RsGluCl. Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays, such as an electrophysiological assay described herein.

25 The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C. The test cells are harvested and resuspended in the presence of the substance.

30 The above described assays may be functional assays, where electrophysiological assays (e.g., see Example 2) may be carried out in transfected mammalian cell lines, an insect cell line, or *Xenopus* oocytes to measure the various effects test compounds may have on the ability of a known ligand (such as glutamate) to activate the channel, or for a test compound to modulate activity in and of itself

(similar to the effect of ivermectin on known GluCl channels). Therefore, the skilled artisan will be comfortable adapting the cDNA clones of the present invention to known methodology for both initial and secondary screens to select for compounds that bind and/or activate the functional RsGluCl channels of the present invention.

5 A preferred method of identifying a modulator of a RsGluCl channel protein comprise firstly contacting a test compound with a *R. sanguineus* RsGluCl channel protein selected from the group consisting of SEQ ID NOs:2,4, 6 and 8; and, secondly measuring the effect of the test compound on the RsGluCl channel protein. A preferred aspect involves using a *R. sanguineus* RsGluCl protein which is a product of
10 a DNA expression vector contained within a recombinant host cell.

Another preferred method of identifying a compound that modulates RsGluCl glutamate-gated channel protein activity comprises firstly injecting into a host cell a population of nucleic acid molecules, at least a portion of which encodes a *R. sanguineus* GluCl channel protein selected from the group consisting of SEQ ID
15 NOs:2,4, 6 and 8, such that expression of said portion of nucleic acid molecules results in an active ligand-gated channel, secondly measuring host cell membrane current in the presence and absence of a test compound. Numerous templates may be used, including but not limited to complementary DNA, poly A⁺ messenger RNA and complementary RNA.

20 The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of RsGluCl. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of RsGluCl. Such a kit would comprise a compartmentalized carrier suitable to hold in close
25 confinement at least one container. The carrier would further comprise reagents such as recombinant RsGluCl or anti-RsGluCl antibodies suitable for detecting RsGluCl. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

The assays described herein can be carried out with cells that have been
30 transiently or stably transfected with RsGluCl. The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. Transfection is meant to include any method known in the art for introducing RsGluCl into the test cells. For example, transfection includes calcium phosphate or

calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing RsGluCl, and electroporation. The expression vector-containing cells are individually analyzed to determine whether they produce RsGluCl protein. Identification of RsGluCl expressing cells may be done by several means, including
5 but not limited to immunological reactivity with anti-RsGluCl antibodies, labeled ligand binding, or the presence of functional, non-endogenous RsGluCl activity.

The specificity of binding of compounds showing affinity for RsGluCl is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned
10 receptor and screening for compounds that bind to RsGluCl or that inhibit the binding of a known, ligand of RsGluCl to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for RsGluCl. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radioactively,
15 fluorescently or enzymatically labeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of RsGluCl.

Therefore, the specificity of binding of compounds having affinity for RsGluCl is shown by measuring the affinity of the compounds for recombinant cells
20 expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to RsGluCl or that inhibit the binding of a known, radiolabeled ligand of RsGluCl (such as glutamate, ivermectin or nodulisporic acid) to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for RsGluCl.
25 Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radioactively, fluorescently or enzymatically labeled compounds or that can be used as activators in functional assays. Compounds identified by the above method again are likely to be agonists or antagonists of RsGluCl. As noted elsewhere in this specification, compounds may
30 modulate by increasing or attenuating the expression of DNA or RNA encoding RsGluCl, or by acting as an agonist or antagonist of the RsGluCl receptor protein. Again, these compounds that modulate the expression of DNA or RNA encoding RsGluCl or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in

expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

RsGluCl 1 and/or 2 gated chloride channel functional assays measure one or more ligand-gated chloride channel activities where the channel is made up in whole, or in part, by the RsGluCl channel. RsGluCl channel activity can be measured using the channel described herein by itself; or as a subunit in combination with one or more additional ligand-gated chloride channel subunits (preferably one or more RsGluCl), where the subunits combine together to provide functional channel activity.

Assays measuring RsGluCl-gated chloride channel activity include functional screening using ^{36}Cl , functional screening using patch clamp electrophysiology and functional screening using fluorescent dyes. Techniques for carrying out such assays in general are well known in the art. (See, for example, Smith et al., 1998, *European Journal of Pharmacology* 159:261-269; González and Tsien, 1997, *Chemistry & Biology* 4:269-277; Millar et al., 1994, *Proc. R. Soc. Lond. B.* 258:307-314; Rauh et al., 1990 *TiPS* 11:325-329, and Tsien et al., U.S. Patent No. 5,661,035.) Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect RsGluCl channel activity can be divided into smaller groups of compounds to identify the compound(s) affecting RsGluCl channel activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay. Recombinantly produced RsGluCl channels present in different environments can be used in a functional assay. Suitable environments include live cells and purified cell extracts containing the RsGluCl channel and an appropriate membrane for activity; and the use of a purified RsGluCl channel produced by recombinant means that is introduced into a different environment suitable for measuring RsGluCl channel activity. RsGluCl derivatives can be used to assay for compounds active at the channel and to obtain information concerning different regions of the channel. For example, RsGluCl channel derivatives can be produced where amino acid regions in the native channel are altered and the effect of the alteration on channel activity can be measured to obtain information regarding different channel regions.

Expression of RsGluCl DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free

systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

5 Following expression of RsGluCl in a host cell, RsGluCl protein may be recovered to provide RsGluCl protein in active form. Several RsGluCl protein purification procedures are available and suitable for use. Recombinant RsGluCl protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size
10 exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant RsGluCl protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length RsGluCl protein, or polypeptide fragments of RsGluCl protein.

15 Expression of RsGluCl DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being
20 preferred.

 Following expression of RsGluCl in a host cell, RsGluCl protein may be recovered to provide RsGluCl protein in active form. Several RsGluCl protein purification procedures are available and suitable for use. Recombinant RsGluCl protein may be purified from cell lysates and extracts by various combinations of, or
25 individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant RsGluCl protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length RsGluCl
30 protein, or polypeptide fragments of RsGluCl protein.

 Polyclonal or monoclonal antibodies may be raised against RsGluCl1 or RsGluCl2 or a synthetic peptide (usually from about 9 to about 25 amino acids in length) from a portion of RsGluCl or RsGluCl2 as disclosed in SEQ ID NOs:2, 4, 6 and/or 8. Monospecific antibodies to RsGluCl are purified from mammalian antisera

containing antibodies reactive against RsGluCl or are prepared as monoclonal antibodies reactive with RsGluCl using the technique of Kohler and Milstein (1975, *Nature* 256: 495-497). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for RsGluCl. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with RsGluCl, as described above. Human RsGluCl-specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of RsGluCl protein or a synthetic peptide generated from a portion of RsGluCl with or without an immune adjuvant.

Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of RsGluCl protein associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of RsGluCl protein or peptide fragment thereof in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections are generally given an equal amount of RsGluCl in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with RsGluCl are prepared by immunizing inbred mice, preferably Balb/c, with RsGluCl protein. The mice are immunized by the IP or SC route with about 1 mg to about 100 mg, preferably about 10 mg, of RsGluCl protein in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 1 to about 100 mg of RsGluCl in a buffer solution such as phosphate

buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 mol. wt., at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using RsGluCl as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, 1973, Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press.

Monoclonal antibodies are produced *in vivo* by injection of pristine primed Balb/c mice, approximately 0.5 ml per mouse, with about 2×10^6 to about 6×10^6 hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

In vitro production of anti-RsGluCl mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of RsGluCl in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for RsGluCl peptide fragments, or a respective full-length RsGluCl.

RsGluCl antibody affinity columns are made, for example, by adding the antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell culture supernatants or cell extracts containing full-length RsGluCl or RsGluCl protein fragments are slowly passed through the column. The column is then washed with phosphate buffered saline until the optical density (A_{280}) falls to background, then the protein is eluted with 0.23 M glycine-HCl (pH 2.6). The purified RsGluCl protein is then dialyzed against phosphate buffered saline.

The present invention also relates to a non-human transgenic animal which is useful for studying the ability of a variety of compounds to act as modulators of RsGluCl, or any alternative functional RsGluCl channel *in vivo* by providing cells for culture, *in vitro*. In reference to the transgenic animals of this invention, reference is made to transgenes and genes. As used herein, a transgene is a genetic construct including a gene. The transgene is integrated into one or more chromosomes in the cells in an animal by methods known in the art. Once integrated, the transgene is carried in at least one place in the chromosomes of a transgenic animal. Of course, a gene is a nucleotide sequence that encodes a protein, such as one or a combination of the cDNA clones described herein. The gene and/or transgene may also include genetic regulatory elements and/or structural elements known in the art. A type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells can be obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature* 292:154-156; Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:9065-9069; and Robertson et al., 1986 *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by a variety of standard techniques such as DNA transfection, microinjection, or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal (Jaenisch, 1988, *Science* 240: 1468-1474). It will also be within the

purview of the skilled artisan to produce transgenic or knock-out invertebrate animals (e.g., *C. elegans*) which express the RsGluCl transgene in a wild type *C. elegans* GluCl background as well in *C. elegans* mutants knocked out for one or both of the *C. elegans* GluCl subunits.

5 Pharmaceutically useful compositions comprising modulators of RsGluCl may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such
10 compositions will contain an effective amount of the protein, DNA, RNA, modified RsGluCl, or either RsGluCl agonists or antagonists including tyrosine kinase activators or inhibitors.

 Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose disorders. The effective amount
15 may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

 The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.

 The term "chemical derivative" describes a molecule that contains additional
20 chemical moieties which are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

25 Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

 The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of
30 treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations),

5 pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or
10 via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

For combination treatment with more than one active agent, where the active
15 agents are in separate dosage formulations, the active agents can be administered concurrently, or they each can be administered at separately staggered times.

The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated;
20 the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without
25 toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

30

EXAMPLE 1

Isolation and Characterization of DNA Molecules Encoding RsGluCl and RsGluCl2

Most molecular procedures were performed following standard procedures available in references such as Ausubel et. al. (1992. Short protocols in molecular

biology. F.M. Ausubel et al., -2nd. ed. (John Wiley & Sons), and Sambrook et al. (1989. Molecular cloning. A laboratory manual. J. Sambrook, E.F. Fritsch, and T. Maniatis -2nd ed. (Cold Spring Harbor Laboratory Press).

RsGluCl1 - Adult brown dog tick polyA⁺ RNA was isolated using the Poly(A)PureTM mRNA Isolation Kit (Ambion). Tick cDNA was synthesized using oligo-dT primers and the ZAP cDNA[®] Synthesis Kit (Stratagene), and cDNA >1kb was selected using cDNA Size Fractionation Columns (BRL). A tick cDNA library was constructed in the Lambda ZAP[®] II vector using the GIGAPACK[®] III Gold Cloning Kit (Stratagene). A *Drosophila* GluCl cDNA fragment spanning the M1 to M3 region was used in a low-stringency screen [25% v/v formamide/5X SSCP (1XSSCP = 120mM NaCl / 15mM sodium citrate / 20mM sodium phosphate, pH 6.8)/0.1% SDS/10X Denhardt's solution/salmon sperm DNA (250 µg/ml) at 42°C; wash, 0.2X SSC/0.1%SDS at 42°C] of the tick cDNA library. The nucleotide sequence of the probe is as follows:

15 5' ATTACTTAATACAAATTTATATACCATGCTGTATGTTGGTCATTGTATCATGGGTATCATTCTGGCT
GGATCAAGGAGCAGTACCGGCGCGAGTGTCACCTGGGTGTCACCACCCTGCTGACCATGGCCACCCAGAC
GTCGGGCATAAACGCCTCCCTGCCGCCCGTTTCCTATACGAAGGCCATCGATGTGTGGACAGGCGTGTG
TCTGACGTTCGTGTTCGGGGCCCTGCTCGAGTTCGCCCTGGTG-3' (SEQ ID NO:12).

Filters were exposed for eleven days and six positives were isolated for sequence analysis. Three of the clones (T12, T82 and T32) encode GluCl-related proteins and were sequenced on both strands.

RsGluCl2 - Poly (A)⁺ RNA was isolated from brown dog tick heads. First strand cDNA was synthesized from 50 ng RNA using a SUPERScript preamplification System (Life Technologies). A tenth of the first strand reaction was used for PCR. The degenerate oligos utilized were designed based on sequences obtained from *C. elegans*, *Drosophila*, and flea (*C. felis*) GluCls:

Forward (27F2):

GGAT (G/T) CCNGA (C/T) N (C/T) NTT (C/T) TTNN (A/C) NA (A/C) (C/T) G (SEQ ID NO:9);

Reverse 1 (3AF1):

30 CNA (A/G) (A/C) A (A/G) NGCNC (A/C) GAANA (C/T) (A/G) AA (C/T) G (SEQ ID NO:10);

Reverse 2 (3AF2):

CAN (A/G) CNCCN (A/G) (G/T) CCANAC (A/G) TCNA (C/T) N (A/G) C (SEQ ID NO:11).

Two PCR rounds, using the combinations "27F2 + 3AF1, then 27F2 + 3BF2" were performed. The cycles were as follow: 1 X (95°C for 120 sec.), then 30 X (95°C for 45 sec.; 50°C for 90 sec.; and 72°C for 120 sec.), then 1 X (72°C for 120 sec.).

Reagents were from Life Technology Inc. The oligonucleotide concentration was

5 5 µM. One tenth of the PCR reaction products was tested by Southern blot analysis, in order to identify and prevent the PCR-cloning of contaminating sequences. Novel PCR products of the appropriate size were cloned into the PCR2.1 plasmid vector using a "TA" cloning kit (Invitrogen, Inc.). Following sequence analysis (ABI Prism, PE Applied Biosystems), selected PCR clone inserts were radiolabelled and used as
10 probes to screen a cDNA library generated into the Uni-ZAP[®] vector (Stratagene, Inc.) from using the RNA preparation mentioned above. Sequences from full-length cDNA clones were analysed using the GCG Inc. package. Subcloning of RsGluCl2 into a mammalian expression vector was done by excision of an 1.85 kb coding-region-containing fragment (XhoI-EcoRI digest) from the original insert of clone
15 RsGluCl2 B1 from the UniZap[®] pBS plasmid, followed by ligation into the TetSplice[®] vector (Life Technologies Inc.). cDNA clones T12 and T82 are identical in the coding region except for a single nucleotide difference resulting in a single amino acid substitution which is probably a naturally occurring polymorphism. The T32 clone has 2 additional exons not present in the T12 and T82 cDNAs, one is near the 5'
20 end of the coding region (135bp exon) and the other is in the M3-M4 intracellular linker (96bp exon). Additionally, these optional exons are not included in DrosGluCl-1 ORF. These cDNA clones are also denoted as RsGluCl-1L (T32-2.48kb) and RsGluCl-1S (T12 and T82-2.126kb). The predicted RsGluCl-1S protein is approximately 71% identical to the DrosGluCl1 protein.

25

EXAMPLE 2

Functional expression of RsGluCl1 and RsGluCl2 clones in *Xenopus* oocytes

Xenopus laevis oocytes were prepared and injected using standard methods previously described [Arena, J.P., Liu, K.K., Paress, P.S. & Cully, D.F. Mol.
30 Pharmacol. 40, 368-374 (1991); Arena, J.P., Liu, K.K., Paress, P.S., Schaeffer, J.M. & Cully, D.F., Mol. Brain Res. 15, 339-348 (1992)]. Adult female *Xenopus laevis* were anesthetized with 0.17% tricaine methanesulfonate and the ovaries were surgically removed and placed in a solution consisting of (mM): NaCl 82.5, KCl 2, MgCl₂ 1, HEPES 5, NaPyruvate 2.5, Penicillin G. 100,000 units/L, Streptomycin Sulfate 1000

mg/L, pH 7.5 (Mod. OR-2). Ovarian lobes were broken open, rinsed several times in Mod. OR-2, and incubated in 0.2% collagenase (Sigma, Type1) in Mod. OR-2 at room temperature with gentle shaking. After 1 hour the collagenase solution was renewed and the oocytes were incubated for an additional 30-90 min until
5 approximately 50% of the oocytes were released from the ovaries. Stage V and VI oocytes were selected and placed in media containing (mM): NaCl 96, KCl 2, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, NaPyruvate 2.5, theophylline 0.5, gentamicin 50 mg/ml, pH 7.5 (ND-96) for 16-24 hours before injection. Oocytes were injected with 50 nl of Dv8, Dv9, RsGluCl1 or RsGluCl2 RNA at a concentration of 0.2 mg/ml.
10 Oocytes were incubated at 18°C for 1-6 days in ND-96 before recording.

Recordings were made at room temperature in modified ND-96 consisting of (mM): NaCl 96, MgCl₂ 1, CaCl₂ 0.1, BaCl₂ 3.5, HEPES 5, pH 7.5. Oocytes were voltage clamped using a Dagan CA1 two microelectrode amplifier (Dagan Corporation, Minneapolis, MN) interfaced to a Macintosh 7100/80 computer. The
15 current passing electrode was filled with 0.7 M KCl, 1.7 M KCitrate, and the voltage recording electrode was filled with 1 M KCl. Throughout the experiment oocytes were superfused with modified ND-96 (control solution) or with ND-96 containing potential channel activators and blockers at a rate of approximately 3 ml/min. Data were acquired at 100 Hz and filtered at 33.3 Hz using Pulse software from HEKA
20 Elektronik (Lambrecht, Germany). All recordings were performed from a holding potential of either 0 or -30 mV.

cRNA was synthesized from the RsGluCl 1S clone T12 and expressed in *Xenopus* oocytes. The channel encoded by RsGluCl-1 is a glutamate-gated chloride channel activated by IVM-PO₄.

25 Figure 10 shows the glutamate-activated current in oocytes injected with RsGluCl1 T12 RNA. Current activation was maximal with 10 µM glutamate and no current was seen in uninjected oocytes. Application of 100 nM ivermectin produces a similar although non-inactivating current.

Figure 11 shows the activation by ivermectin of RsGluCl2 expressed in
30 *Xenopus* oocytes. Current activation was maximal with ~1 µM ivermectin and glutamate failed to activate a current when expressed as a single functional channel.

EXAMPLE 3

Functional expression of RsGluCl Clones in Mammalian Cells

A RsGluCl may be subcloned into a mammalian expression vector and used to transfect the mammalian cell line of choice. Stable cell clones are selected by growth in the presence of G418. Single G418 resistant clones are isolated and tested to confirm the presence of an intact RsGluCl gene. Clones containing the RsGluCl are then analyzed for expression using immunological techniques, such as immunoprecipitation, Western blot, and immunofluorescence using antibodies specific to the RsGluCl proteins. Antibody is obtained from rabbits inoculated with peptides that are synthesized from the amino acid sequence predicted from the RsGluCl sequences. Expression is also analyzed using patch clamp electrophysiological techniques and an anion flux assay.

Cells that are expressing RsGluCl stably or transiently, are used to test for expression of active channel proteins. These cells are used to identify and examine other compounds for their ability to modulate, inhibit or activate the respective channel.

Cassettes containing the RsGluCl cDNA in the positive orientation with respect to the promoter are ligated into appropriate restriction sites 3' of the promoter and identified by restriction site mapping and/or sequencing. These cDNA expression vectors may be introduced into fibroblastic host cells, for example, COS-7 (ATCC# CRL1651), and CV-1 tat [Sackevitz et al., 1987, *Science* 238: 1575], 293, L (ATCC# CRL6362) by standard methods including but not limited to electroporation, or chemical procedures (cationic liposomes, DEAE dextran, calcium phosphate). Transfected cells and cell culture supernatants can be harvested and analyzed for RsGluCl expression as described herein.

All of the vectors used for mammalian transient expression can be used to establish stable cell lines expressing RsGluCl. Unaltered RsGluCl cDNA constructs cloned into expression vectors are expected to program host cells to make RsGluCl protein. In addition, RsGluCl is expressed extracellularly as a secreted protein by ligating RsGluCl cDNA constructs to DNA encoding the signal sequence of a secreted protein. The transfection host cells include, but are not limited to, CV-1-P [Sackevitz et al., 1987, *Science* 238: 1575], tk-L [Wigler, et al., 1977, *Cell* 11: 223], NS/0, and dHFr- CHO [Kaufman and Sharp, 1982, *J. Mol. Biol.* 159: 601].

Co-transfection of any vector containing a RsGluCl cDNA with a drug selection plasmid including, but not limited to G418, aminoglycoside phosphotransferase; hygromycin, hygromycin-B phosphotransferase; APRT, xanthine-guanine phosphoribosyl-transferase, will allow for the selection of stably transfected clones. Levels of RsGluCl are quantitated by the assays described herein. RsGluCl cDNA constructs may also be ligated into vectors containing amplifiable drug-resistance markers for the production of mammalian cell clones synthesizing the highest possible levels of RsGluCl. Following introduction of these constructs into cells, clones containing the plasmid are selected with the appropriate agent, and isolation of an over-expressing clone with a high copy number of plasmids is accomplished by selection with increasing doses of the agent. The expression of recombinant RsGluCl is achieved by transfection of full-length RsGluCl cDNA into a mammalian host cell.

15

EXAMPLE 4

Cloning of RsGluCl cDNA into a Baculovirus Expression Vector
for Expression in Insect Cells

Baculovirus vectors, which are derived from the genome of the AcNPV virus, are designed to provide high level expression of cDNA in the Sf9 line of insect cells (ATCC CRL# 1711). A recombinant baculovirus expressing RsGluCl cDNA is produced by the following standard methods (In Vitrogen Maxbac Manual): The RsGluCl cDNA constructs are ligated into the polyhedrin gene in a variety of baculovirus transfer vectors, including the pAC360 and the BlueBac vector (In Vitrogen). Recombinant baculoviruses are generated by homologous recombination following co-transfection of the baculovirus transfer vector and linearized AcNPV genomic DNA [Kitts, 1990, *Nuc. Acid. Res.* 18: 5667] into Sf9-cells. Recombinant pAC360 viruses are identified by the absence of inclusion bodies in infected cells and recombinant pBlueBac viruses are identified on the basis of b-galactosidase expression (Summers, M. D. and Smith, G. E., Texas Agriculture Exp. Station Bulletin No. 1555). Following plaque purification, RsGluCl expression is measured by the assays described herein.

The cDNA encoding the entire open reading frame for RsGluCl GluCl is inserted into the BamHI site of pBlueBacII. Constructs in the positive orientation are identified by sequence analysis and used to transfect Sf9 cells in the presence of linear AcNPV mild type DNA.

5

EXAMPLE 5

Cloning of RsGluCl cDNA into a Yeast Expression Vector

Recombinant RsGluCl is produced in the yeast *S. cerevisiae* following the insertion of the optimal RsGluCl cDNA cistron into expression vectors designed to direct the intracellular or extracellular expression of heterologous proteins. In the case of intracellular expression, vectors such as EmBLyex4 or the like are ligated to the RsGluCl cistron [Rinas, et al., 1990, *Biotechnology* 8: 543-545; Horowitz B. et al., 1989, *J. Biol. Chem.* 265: 4189-4192]. For extracellular expression, the RsGluCl GluCl cistron is ligated into yeast expression vectors which fuse a secretion signal (a yeast or mammalian peptide) to the NH₂ terminus of the RsGluCl protein [Jacobson, 1989, *Gene* 85: 511-516; Riett and Bellon, 1989, *Biochem.* 28: 2941-2949].

These vectors include, but are not limited to pAVE1-6, which fuses the human serum albumin signal to the expressed cDNA [Steep, 1990, *Biotechnology* 8: 42-46], and the vector pL8PL which fuses the human lysozyme signal to the expressed cDNA [Yamamoto, *Biochem.* 28: 2728-2732]. In addition, RsGluCl is expressed in yeast as a fusion protein conjugated to ubiquitin utilizing the vector pVEP [Ecker, 1989, *J. Biol. Chem.* 264: 7715-7719, Sabin, 1989 *Biotechnology* 7: 705-709, McDonnell, 1989, *Mol. Cell Biol.* 9: 5517-5523 (1989)]. The levels of expressed RsGluCl are determined by the assays described herein.

EXAMPLE 6

Purification of Recombinant RsGluCl

Recombinantly produced RsGluCl may be purified by antibody affinity chromatography. RsGluCl GluCl antibody affinity columns are made by adding the anti-RsGluCl GluCl antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then

30

coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in
5 phosphate buffered saline (pH 7.3) together with appropriate membrane solubilizing agents such as detergents and the cell culture supernatants or cell extracts containing solubilized RsGluCl are slowly passed through the column. The column is then washed with phosphate- buffered saline together with detergents until the optical density (A280) falls to background, then the protein is
10 eluted with 0.23 M glycine-HCl (pH 2.6) together with detergents. The purified RsGluCl protein is then dialyzed against phosphate buffered saline.

54a

SEQUENCE LISTING

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acatcgctga ccacaatgaa ctacttctct gacgtggcga agatgggtggc ttcacggaag	240
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Met Ser Val His Ser Trp Arg Phe
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Cys Val Pro Leu Val Ala Leu Ala Phe Phe Leu Leu Ile Leu Leu Ser	
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ata ttg gac agc atc att ggc cag ggt cgt tat gac tgc agg atc cgg	498
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Pro Met Gly Ile Asn Asn Thr Asp Gly Pro Ala Leu Val Arg Val Asn	
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---	-----

54b

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Thr	Val	Gln	Met	Thr	Phe	Arg	Glu	Gln	Trp	Arg	Asp	Glu	Arg	Leu	Gln	
		90				95					100					
tac	gac	gac	ttg	ggc	ggc	cag	gtt	cgc	tac	ctg	acg	ctc	acc	gaa	ccg	690
Tyr	Asp	Asp	Leu	Gly	Gly	Gln	Val	Arg	Tyr	Leu	Thr	Leu	Thr	Glu	Pro	
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Asp	Lys	Leu	Trp	Lys	Pro	Asp	Leu	Phe	Phe	Ser	Asn	Glu	Lys	Glu	Gly	
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His	Phe	His	Asn	Ile	Ile	Met	Pro	Asn	Val	Leu	Leu	Arg	Ile	His	Pro	
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Asn	Gly	Asp	Val	Leu	Phe	Ser	Ile	Arg	Ile	Ser	Leu	Val	Leu	Ser	Cys	
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Pro	Met	Asn	Leu	Lys	Phe	Tyr	Pro	Leu	Asp	Lys	Gln	Ile	Cys	Ser	Ile	
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Val	Met	Val	Ser	Tyr	Gly	Tyr	Thr	Thr	Glu	Asp	Leu	Val	Phe	Leu	Trp	
185					190					195					200	
aaa	gag	ggg	gat	cct	gta	cag	gtc	aca	aaa	aat	ctc	cac	ttg	cca	cgt	978
Lys	Glu	Gly	Asp	Pro	Val	Gln	Val	Thr	Lys	Asn	Leu	His	Leu	Pro	Arg	
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ttc	acg	ctg	gaa	agg	ttt	caa	acc	gac	tac	tgc	acc	agt	cgg	acc	aac	1026
Phe	Thr	Leu	Glu	Arg	Phe	Gln	Thr	Asp	Tyr	Cys	Thr	Ser	Arg	Thr	Asn	
			220					225					230			
act	ggc	gag	tac	agc	tgc	ttg	cgc	gtg	gac	ctg	gtg	ttc	aag	cgc	gag	1074
Thr	Gly	Glu	Tyr	Ser	Cys	Leu	Arg	Val	Asp	Leu	Val	Phe	Lys	Arg	Glu	
		235					240					245				
ttc	agc	tac	tac	ctg	atc	cag	atc	tac	atc	ccg	tgc	tgc	atg	ctg	gtc	1122
Phe	Ser	Tyr	Tyr	Leu	Ile	Gln	Ile	Tyr	Ile	Pro	Cys	Cys	Met	Leu	Val	
		250				255					260					
atc	gtg	tcc	tgg	gtg	tcg	ttc	tgg	ctc	gac	ccc	acc	tcg	atc	ccg	gcg	1170
Ile	Val	Ser	Trp	Val	Ser	Phe	Trp	Leu	Asp	Pro	Thr	Ser	Ile	Pro	Ala	
265					270					275					280	
cga	gtg	tcg	ctg	ggc	gtc	acc	acc	ctg	ctc	acc	atg	gcc	acg	cag	ata	1218
Arg	Val	Ser	Leu	Gly	Val	Thr	Thr	Leu	Leu	Thr	Met	Ala	Thr	Gln	Ile	
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tcg	ggc	atc	aac	gcc	tcg	ctg	cct	ccc	gtt	tcc	tac	acc	aag	gcc	att	1266
Ser	Gly	Ile	Asn	Ala	Ser	Leu	Pro	Pro	Val	Ser	Tyr	Thr	Lys	Ala	Ile	

54c

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315	320	325	
gag ttc gcc ctg gtc aac tac gcc tcg cgg tca gat tca cgc cgg cag			1362
Glu Phe Ala Leu Val Asn Tyr Ala Ser Arg Ser Asp Ser Arg Arg Gln			
330	335	340	
aac atg cag aag cag aag cag agg aaa tgg gag ctc gag ccg ccc ctg			1410
Asn Met Gln Lys Gln Lys Gln Arg Lys Trp Glu Leu Glu Pro Pro Leu			
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gac tcg gac cac ctg gag gac ggc gcc acc acg ttc gcc atg agg ccg			1458
Asp Ser Asp His Leu Glu Asp Gly Ala Thr Thr Phe Ala Met Arg Pro			
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ctg gtg cac cac cac gga gag ctg cat gcc gac aag ttg cgg cag tgc			1506
Leu Val His His His Gly Glu Leu His Ala Asp Lys Leu Arg Gln Cys			
380	385	390	
gaa gtc cac atg aag acc ccc aag acg aac ctt tgc aag gcc tgg ctt			1554
Glu Val His Met Lys Thr Pro Lys Thr Asn Leu Cys Lys Ala Trp Leu			
395	400	405	
tcc agg ttt ccc acg cga tcc aaa cgc atc gac gtc gtc tcg cgg atc			1602
Ser Arg Phe Pro Thr Arg Ser Lys Arg Ile Asp Val Val Ser Arg Ile			
410	415	420	
ttc ttt ccg ctc atg ttc gcc ctc ttc aac ctc gtc tac tgg aca acc			1650
Phe Phe Pro Leu Met Phe Ala Leu Phe Asn Leu Val Tyr Trp Thr Thr			
425	430	435	440
tac ctc ttc cgg gaa gac gag gaa gac gag tga cagaacacgg acgccacgac			1703
Tyr Leu Phe Arg Glu Asp Glu Glu Asp Glu *			
445	450		
agccgccatc cgacaccatc gtcactgcag gcacgcactc tgtcgcgcgc acacaccacg			1763
aagaccggcg cgccaacgca cgatgcgcgt tggccgctga aaaaccggg agcggggcgg			1823
tgggggagcg tatgccccgg cccctcgcct ctcatectcc gtgcacgctc gaatcgatc			1883
cgccacagcc agaaaaaaaa aagataccgt gcgaaaagtg gcggcaacac aacgtcgacg			1943
ccatcagcgc cgcccagagc tgcaagcggc tcccacatgg ttgccaccgc agcttcctct			2003
acgacccttc atccccaccg gcaccagcta cgagaaaggg accttatttc gggccatccc			2063
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<210> 2

<211> 450

<212> PRT

<213> Rhipicephalus sanguineus

<400> 2

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Phe	Phe	Leu	Leu	Ile	Leu	Leu	Ser	Cys	Pro	Ser	Ala	Trp	Gly	Lys	Ala
		20				25						30			
Asn	Phe	Arg	Ala	Ile	Glu	Lys	Arg	Ile	Leu	Asp	Ser	Ile	Ile	Gly	Gln
		35				40						45			
Gly	Arg	Tyr	Asp	Cys	Arg	Ile	Arg	Pro	Met	Gly	Ile	Asn	Asn	Thr	Asp
	50					55					60				
Gly	Pro	Ala	Leu	Val	Arg	Val	Asn	Ile	Phe	Val	Arg	Ser	Ile	Gly	Arg
65					70					75					80
Ile	Asp	Asp	Val	Thr	Met	Glu	Tyr	Thr	Val	Gln	Met	Thr	Phe	Arg	Glu
				85					90					95	
Gln	Trp	Arg	Asp	Glu	Arg	Leu	Gln	Tyr	Asp	Asp	Leu	Gly	Gly	Gln	Val
			100					105					110		
Arg	Tyr	Leu	Thr	Leu	Thr	Glu	Pro	Asp	Lys	Leu	Trp	Lys	Pro	Asp	Leu
		115					120					125			
Phe	Phe	Ser	Asn	Glu	Lys	Glu	Gly	His	Phe	His	Asn	Ile	Ile	Met	Pro
	130					135					140				
Asn	Val	Leu	Leu	Arg	Ile	His	Pro	Asn	Gly	Asp	Val	Leu	Phe	Ser	Ile
145					150					155					160
Arg	Ile	Ser	Leu	Val	Leu	Ser	Cys	Pro	Met	Asn	Leu	Lys	Phe	Tyr	Pro
				165					170					175	
Leu	Asp	Lys	Gln	Ile	Cys	Ser	Ile	Val	Met	Val	Ser	Tyr	Gly	Tyr	Thr
			180					185					190		
Thr	Glu	Asp	Leu	Val	Phe	Leu	Trp	Lys	Glu	Gly	Asp	Pro	Val	Gln	Val
		195					200					205			
Thr	Lys	Asn	Leu	His	Leu	Pro	Arg	Phe	Thr	Leu	Glu	Arg	Phe	Gln	Thr
	210					215					220				
Asp	Tyr	Cys	Thr	Ser	Arg	Thr	Asn	Thr	Gly	Glu	Tyr	Ser	Cys	Leu	Arg
225					230					235					240
Val	Asp	Leu	Val	Phe	Lys	Arg	Glu	Phe	Ser	Tyr	Tyr	Leu	Ile	Gln	Ile
				245					250					255	
Tyr	Ile	Pro	Cys	Cys	Met	Leu	Val	Ile	Val	Ser	Trp	Val	Ser	Phe	Trp
			260					265					270		
Leu	Asp	Pro	Thr	Ser	Ile	Pro	Ala	Arg	Val	Ser	Leu	Gly	Val	Thr	Thr
		275					280					285			
Leu	Leu	Thr	Met	Ala	Thr	Gln	Ile	Ser	Gly	Ile	Asn	Ala	Ser	Leu	Pro
	290					295					300				
Pro	Val	Ser	Tyr	Thr	Lys	Ala	Ile	Asp	Val	Trp	Thr	Gly	Val	Cys	Leu
305					310					315					320
Thr	Phe	Val	Phe	Gly	Ala	Leu	Leu	Glu	Phe	Ala	Leu	Val	Asn	Tyr	Ala
				325					330					335	
Ser	Arg	Ser	Asp	Ser	Arg	Arg	Gln	Asn	Met	Gln	Lys	Gln	Lys	Gln	Arg
			340					345					350		
Lys	Trp	Glu	Leu	Glu	Pro	Pro	Leu	Asp	Ser	Asp	His	Leu	Glu	Asp	Gly
		355					360					365			
Ala	Thr	Thr	Phe	Ala	Met										

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<222> (502) ... (1854)
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gcggcgcgcc	ggcccgcagc	aaatcgcggg	cattccactc	aggggtctcat	tcgctccccc		180									
aatcctgagg	ttcctttctaa	cgagaaggag	gagccacagc	gccggctgcg	gtaccgcgcg		240									
acggggccaac	gtgagaccgc	ccgagcccgg	cgccttgact	tagggcgctg	agcgaaaccc		300									
aaggcggcgc	gctggccact	ccacgggaac	gagaccggcc	ccctggagac	gacatcgtcg		360									
accacaatga	actactttctc	tgacgtggcg	aagatgggtg	cttcacgcga	gagagaaatc		420									
atcgaagctt	tccacgcgac	atctggagta	caaggcgcat	gcgaatgagc	gaacatcgct		480									
gaccgagact	cgcccgtcac	c atg agc gta cat tca	tgg cgc ttt tgt gtc				531									
		Met Ser Val His Ser	Trp Arg Phe Cys Val													
		1	5		10											
cca ctg gtg gct cta gcg ttt ttc ttg ttg att ctt ctg tcg tgt cca							579									
Pro Leu Val Ala Leu Ala Phe Phe Leu Leu Ile Leu Leu Ser Cys Pro																
	15		20		25											
tcg gca tgg ggc aag gca aat ttc cgc gct ata gaa aag cgg ata ttg							627									
Ser Ala Trp Gly Lys Ala Asn Phe Arg Ala Ile Glu Lys Arg Ile Leu																
	30		35		40											
gac agc atc att ggc cag ggt cgt tat gac tgc agg atc cgg ccc atg							675									
Asp Ser Ile Ile Gly Gln Gly Arg Tyr Asp Cys Arg Ile Arg Pro Met																
	45		50		55											
gga att aac aac aca gac ggg ccg gct ctt gta cgc gtt aac atc ttt							723									
Gly Ile Asn Asn Thr Asp Gly Pro Ala Leu Val Arg Val Asn Ile Phe																
	60		65		70											
gta aga agt atc ggc aga att gat gac gtc acc atg gag tac aca gtg							771									
Val Arg Ser Ile Gly Arg Ile Asp Asp Val Thr Met Glu Tyr Thr Val																
	75		80		85		90									
caa atg acg ttc aga gag cag tgg cgg gac gag aga ctc cag tac gac							819									
Gln Met Thr Phe Arg Glu Gln Trp Arg Asp Glu Arg Leu Gln Tyr Asp																
	95		100		105											
gac ttg ggc ggc cag gtt cgc tac ctg acg ctc acc gaa ccg gac aag							867									
Asp Leu Gly Gly Gln Val Arg Tyr Leu Thr Leu Thr Glu Pro Asp Lys																
	110		115		120											
ctt tgg aag ccg gac ctg ttt ttc tcc aac gag aaa gag gga cac ttc							915									
Leu Trp Lys Pro Asp Leu Phe Phe Ser Asn Glu Lys Glu Gly His Phe																
	125		130		135											
cac aac atc atc atg ccc aac gtg ctt cta cgc ata cat ccc aac ggc							963									

54f

His	Asn	Ile	Ile	Met	Pro	Asn	Val	Leu	Leu	Arg	Ile	His	Pro	Asn	Gly		
140						145					150						
gac	gtt	ctc	ttc	agc	atc	aga	ata	tcc	ttg	gtg	ctt	tca	tgt	ccg	atg	1011	
Asp	Val	Leu	Phe	Ser	Ile	Arg	Ile	Ser	Leu	Val	Leu	Ser	Cys	Pro	Met		
155					160					165					170		
aac	ctg	aaa	ttt	tat	cct	ttg	gat	aaa	caa	atc	tgc	tct	atc	gtc	atg	1059	
Asn	Leu	Lys	Phe	Tyr	Pro	Leu	Asp	Lys	Gln	Ile	Cys	Ser	Ile	Val	Met		
				175					180					185			
gtg	agc	tat	ggg	tat	aca	aca	gag	gac	ctg	gtg	ttt	cta	tgg	aaa	gag	1107	
Val	Ser	Tyr	Gly	Tyr	Thr	Thr	Glu	Asp	Leu	Val	Phe	Leu	Trp	Lys	Glu		
			190					195					200				
ggg	gat	cct	gta	cag	gtc	aca	aaa	aat	ctc	cac	ttg	cca	cgt	ttc	acg	1155	
Gly	Asp	Pro	Val	Gln	Val	Thr	Lys	Asn	Leu	His	Leu	Pro	Arg	Phe	Thr		
		205					210					215					
ctg	gaa	agg	ttt	caa	acc	gac	tac	tgc	acc	agt	cgg	acc	aac	act	ggc	1203	
Leu	Glu	Arg	Phe	Gln	Thr	Asp	Tyr	Cys	Thr	Ser	Arg	Thr	Asn	Thr	Gly		
	220					225					230						
gag	tac	agc	tgc	ttg	cgc	gtg	gac	ctg	gtg	ttc	aag	cgc	gag	ttc	agc	1251	
Glu	Tyr	Ser	Cys	Leu	Arg	Val	Asp	Leu	Val	Phe	Lys	Arg	Glu	Phe	Ser		
235					240					245					250		
tac	tac	ctg	atc	cag	atc	tac	atc	ccg	tgc	tgc	atg	ctg	gtc	atc	gtg	1299	
Tyr	Tyr	Leu	Ile	Gln	Ile	Tyr	Ile	Pro	Cys	Cys	Met	Leu	Val	Ile	Val		
				255					260					265			
tcc	tgg	gtg	tgc	ttc	tgg	ctc	gac	ccc	acc	tgc	atc	ccg	gcg	cga	gtg	1347	
Ser	Trp	Val	Ser	Phe	Trp	Leu	Asp	Pro	Thr	Ser	Ile	Pro	Ala	Arg	Val		
			270					275					280				
tgc	ctg	ggc	gtc	acc	acc	ctg	ctc	acc	atg	gcc	acg	cag	ata	tgc	ggc	1395	
Ser	Leu	Gly	Val	Thr	Thr	Leu	Leu	Thr	Met	Ala	Thr	Gln	Ile	Ser	Gly		
		285					290					295					
atc	aac	gcc	tgc	ctg	cct	ccc	gtt	tcc	tac	acc	aag	gcc	att	gac	gtg	1443	
Ile	Asn	Ala	Ser	Leu	Pro	Pro	Val	Ser	Tyr	Thr	Lys	Ala	Ile	Asp	Val		
	300					305					310						
tgg	acc	ggc	gtc	tgt	ctg	acc	ttc	gta	ttc	ggc	gcg	ctc	ctc	gag	ttc	1491	
Trp	Thr	Gly	Val	Cys	Leu	Thr	Phe	Val	Phe	Gly	Ala	Leu	Leu	Glu	Phe		
315					320					325					330		
gcc	ctg	gtc	aac	tac	gcc	tgc	cgg	tca	gat	tca	cgc	cgg	cag	aac	atg	1539	
Ala	Leu	Val	Asn	Tyr	Ala	Ser	Arg	Ser	Asp	Ser	Arg	Arg	Gln	Asn	Met		
				335				340					345				
cag	aag	cag	aag	cag	agg	aaa	tgg	gag	ctc	gag	ccg	ccc	ctg	gac	tgc	1587	
Gln	Lys	Gln	Lys	Gln	Arg	Lys	Trp	Glu	Leu	Glu	Pro	Pro	Leu	Asp	Ser		
			350				355						360				
gac	cac	ctg	gag	gac	ggc	gcc	acc	acg	ttc	gcc	atg	agg	ccg	ctg	gtg	1635	
Asp	His	Leu	Glu	Asp	Gly	Ala	Thr	Thr	Phe	Ala	Met	Arg	Pro	Leu	Val		

54g

365	370	375	
cac cac cac gga gag ctg cat gcc gac aag ttg cgg cag tgc gaa gtc			1683
His His His Gly Glu Leu His Ala Asp Lys Leu Arg Gln Cys Glu Val			
380	385	390	
cac atg aag acc ccc aag acg aac ctt tgc aag gcc tgg ctt tcc agg			1731
His Met Lys Thr Pro Lys Thr Asn Leu Cys Lys Ala Trp Leu Ser Arg			
395	400	405	410
ttt ccc acg cga tcc aaa cgc atc gac gtc gtc tgc cgg atc ttc ttt			1779
Phe Pro Thr Arg Ser Lys Arg Ile Asp Val Val Ser Arg Ile Phe Phe			
	415	420	425
ccg ctc atg ttc gcc ctc ttc aac ctc gtc tac tgg aca acc tac ctc			1827
Pro Leu Met Phe Ala Leu Phe Asn Leu Val Tyr Trp Thr Thr Tyr Leu			
	430	435	440
ttc cgg gaa gac aag gaa gac gag tga cagaacacga acgccacgac			1874
Phe Arg Glu Asp Lys Glu Asp Glu *			
445	450		
agccgccatc cgacaccatc gtcactgcag gcaagcactc tgtegcgcgc acacaccacg			1934
aagaccggcg cgccaacgca cgatgcgcgt tggecgctga aaaaccggg agcggggcg			1994
tgggggaggg tatgccccgg cccctcgctc ctcatectcc gtgcacgctc gaatcgatc			2054
cgccacagcc agaaaaaaa aagataccgt gcgaaaagtg gcggcaacac aacgtcgacg			2114
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acgacccttc atccccaccg gcaccagcta cgagaaaggg accttatttc gggccatccc			2234
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Asn Phe Arg Ala Ile Glu Lys Arg Ile Leu Asp Ser Ile Ile Gly Gln			
	35	40	45
Gly Arg Tyr Asp Cys Arg Ile Arg Pro Met Gly Ile Asn Asn Thr Asp			
	50	55	60
Gly Pro Ala Leu Val Arg Val Asn Ile Phe Val Arg Ser Ile Gly Arg			
	65	70	75
Ile Asp Asp Val Thr Met Glu Tyr Thr Val Gln Met Thr Phe Arg Glu			
	85	90	95
Gln Trp Arg Asp Glu Arg Leu Gln Tyr Asp Asp Leu Gly Gly Gln Val			
	100	105	110
Arg Tyr Leu Thr Leu Thr Glu Pro Asp Lys Leu Trp Lys Pro Asp Leu			
	115	120	125
Phe Phe Ser Asn Glu Lys Glu Gly His Phe His Asn Ile Ile Met Pro			
	130	135	140
Asn Val Leu Leu Arg Ile His Pro Asn Gly Asp Val Leu Phe Ser Ile			
145	150	155	160

54h

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Arg Ile Ser Leu Val Leu Ser Cys Pro Met Asn Leu Lys Phe Tyr Pro
      165                      170                      175
Leu Asp Lys Gln Ile Cys Ser Ile Val Met Val Ser Tyr Gly Tyr Thr
      180                      185                      190
Thr Glu Asp Leu Val Phe Leu Trp Lys Glu Gly Asp Pro Val Gln Val
      195                      200                      205
Thr Lys Asn Leu His Leu Pro Arg Phe Thr Leu Glu Arg Phe Gln Thr
      210                      215                      220
Asp Tyr Cys Thr Ser Arg Thr Asn Thr Gly Glu Tyr Ser Cys Leu Arg
225                      230                      235                      240
Val Asp Leu Val Phe Lys Arg Glu Phe Ser Tyr Tyr Leu Ile Gln Ile
      245                      250                      255
Tyr Ile Pro Cys Cys Met Leu Val Ile Val Ser Trp Val Ser Phe Trp
      260                      265                      270
Leu Asp Pro Thr Ser Ile Pro Ala Arg Val Ser Leu Gly Val Thr Thr
      275                      280                      285
Leu Leu Thr Met Ala Thr Gln Ile Ser Gly Ile Asn Ala Ser Leu Pro
      290                      295                      300
Pro Val Ser Tyr Thr Lys Ala Ile Asp Val Trp Thr Gly Val Cys Leu
305                      310                      315                      320
Thr Phe Val Phe Gly Ala Leu Leu Glu Phe Ala Leu Val Asn Tyr Ala
      325                      330                      335
Ser Arg Ser Asp Ser Arg Arg Gln Asn Met Gln Lys Gln Lys Gln Arg
      340                      345                      350
Lys Trp Glu Leu Glu Pro Pro Leu Asp Ser Asp His Leu Glu Asp Gly
      355                      360                      365
Ala Thr Thr Phe Ala Met Arg Pro Leu Val His His His Gly Glu Leu
      370                      375                      380
His Ala Asp Lys Leu Arg Gln Cys Glu Val His Met Lys Thr Pro Lys
385                      390                      395                      400
Thr Asn Leu Cys Lys Ala Trp Leu Ser Arg Phe Pro Thr Arg Ser Lys
      405                      410                      415
Arg Ile Asp Val Val Ser Arg Ile Phe Phe Pro Leu Met Phe Ala Leu
      420                      425                      430
Phe Asn Leu Val Tyr Trp Thr Thr Tyr Leu Phe Arg Glu Asp Lys Glu
      435                      440                      445
Asp Glu
      450

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<210> 5
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<213> Rhipicephalus sanguineus

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<222> (617)...(2170)

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ctcctgcgtc tctccactcg atgaagacct gtcctggagg cgcgagccca actgcgcgct      180
ctgtccgcat gtgtcgccgc cactgagagg cctcggcgct ggcgcgcttg tcaacgcggc      240
gcgcgggcc gcagcaaata gcgggcattc cactcagggt ctattcgct cccccaatcc      300
tgaggttcct tctaacgaga aggaggagcc acagcgccgg ctgcggtacc gccgcacggg      360

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54i

ccaacgtgag accgcccagag cccggcgccc tgaacttaggc cgctgagcga aacccaaggc	420
ggcgcgctgg ccactccaag ggaacgagac cggcccctg gagacgacat cgtcgaccac	480
aatgaactac ttctctgacg tggcgaagat ggtggcttca tcgaagagag aaatcatcga	540
agctttccac gcgacatctg gactacacgg cgcattgcgaa tgagcgaaca tcgctgaccg	600
agaactcgccc gtcacc atg agc gta cat tca tgg cgc ttt tgt gtc cca ctg	652
Met Ser Val His Ser Trp Arg Phe Cys Val Pro Leu	
1 5 10	
gtg gct cta gcg ttt ttc ttg ttg att ctt ctg tgc tgt cca tgc gca	700
Val Ala Leu Ala Phe Phe Leu Leu Ile Leu Leu Ser Cys Pro Ser Ala	
15 20 25	
tgg gcc gaa acg ctg cct acg cca cca acc cgt ggc cag ggg ggc gtt	748
Trp Ala Glu Thr Leu Pro Thr Pro Pro Thr Arg Gly Gln Gly Gly Val	
30 35 40	
ccg gtc gcg gcc gcg atg ctc ctg ggg aaa cag caa agt tcc cgc tac	796
Pro Val Ala Ala Ala Met Leu Leu Gly Lys Gln Gln Ser Ser Arg Tyr	
45 50 55 60	
caa gat aaa gag ggc aag gca aat ttc cgc gct ata gaa aag cgg ata	844
Gln Asp Lys Glu Gly Lys Ala Asn Phe Arg Ala Ile Glu Lys Arg Ile	
65 70 75	
ttg gac agc atc att ggc cag ggt cgt tat gac tgc agg atc cgg ccc	892
Leu Asp Ser Ile Ile Gly Gln Gly Arg Tyr Asp Cys Arg Ile Arg Pro	
80 85 90	
atg gga att aac aac aca gac ggg ccg gct ctt gta cgc gtt aac atc	940
Met Gly Ile Asn Asn Thr Asp Gly Pro Ala Leu Val Arg Val Asn Ile	
95 100 105	
ttt gta aga agt atc ggc aga att gat gac gtc acc atg gag tac aca	988
Phe Val Arg Ser Ile Gly Arg Ile Asp Asp Val Thr Met Glu Tyr Thr	
110 115 120	
gtg caa atg acg ttc aga gag cag tgg cgg gac gag aga ctc cag tac	1036
Val Gln Met Thr Phe Arg Glu Gln Trp Arg Asp Glu Arg Leu Gln Tyr	
125 130 135 140	
gac gac ttg ggc ggc cag gtt cgc tac ctg acg ctc acc gaa ccg gac	1084
Asp Asp Leu Gly Gly Gln Val Arg Tyr Leu Thr Leu Thr Glu Pro Asp	
145 150 155	
aag ctt tgg aag ccg gac ctg ttt ttc tcc aac gag aaa gag gga cac	1132
Lys Leu Trp Lys Pro Asp Leu Phe Phe Ser Asn Glu Lys Glu Gly His	
160 165 170	
ttc cac aac atc atc atg ccc aac gtg ctt cta cgc ata cat ccc aac	1180
Phe His Asn Ile Ile Met Pro Asn Val Leu Leu Arg Ile His Pro Asn	
175 180 185	
ggc gac gtt ctc ttc agc atc aga ata tcc ttg gtg ctt tca tgt ccg	1228
Gly Asp Val Leu Phe Ser Ile Arg Ile Ser Leu Val Leu Ser Cys Pro	
190 195 200	
atg aac ctg aaa ttt tat cct ttg gat aaa caa atc tgc tct atc gtc	1276

54j

Met	Asn	Leu	Lys	Phe	Tyr	Pro	Leu	Asp	Lys	Gln	Ile	Cys	Ser	Ile	Val	
205					210					215					220	
atg	gtg	agc	tat	ggg	tat	aca	aca	gag	gac	ctg	gtg	ttt	cta	tgg	aaa	1324
Met	Val	Ser	Tyr	Gly	Tyr	Thr	Thr	Glu	Asp	Leu	Val	Phe	Leu	Trp	Lys	
				225					230					235		
gag	ggg	gat	cct	gta	cag	gtc	aca	aaa	aat	ctc	cac	ttg	cca	cgt	ttc	1372
Glu	Gly	Asp	Pro	Val	Gln	Val	Thr	Lys	Asn	Leu	His	Leu	Pro	Arg	Phe	
			240					245					250			
acg	ctg	gaa	agg	ttt	caa	acc	gac	tac	tgc	acc	agt	cgg	acc	aac	act	1420
Thr	Leu	Glu	Arg	Phe	Gln	Thr	Asp	Tyr	Cys	Thr	Ser	Arg	Thr	Asn	Thr	
		255					260					265				
ggc	gag	tac	agc	tgc	ttg	cgc	gtg	gac	ctg	gtg	ttc	aag	cgc	gag	ttc	1468
Gly	Glu	Tyr	Ser	Cys	Leu	Arg	Val	Asp	Leu	Val	Phe	Lys	Arg	Glu	Phe	
	270					275					280					
agc	tac	tac	ctg	atc	cag	atc	tac	atc	ccg	tgc	tgc	atg	ctg	gtc	atc	1516
Ser	Tyr	Tyr	Leu	Ile	Gln	Ile	Tyr	Ile	Pro	Cys	Cys	Met	Leu	Val	Ile	
285					290					295					300	
gtg	tcc	tgg	gtg	tcg	ttc	tgg	ctc	gac	ccc	acc	tcg	atc	ccg	gcg	cga	1564
Val	Ser	Trp	Val	Ser	Phe	Trp	Leu	Asp	Pro	Thr	Ser	Ile	Pro	Ala	Arg	
				305					310					315		
gtg	tcg	ctg	ggc	gtc	acc	acc	ctg	ctc	acc	atg	gcc	acg	cag	ata	tcg	1612
Val	Ser	Leu	Gly	Val	Thr	Thr	Leu	Leu	Thr	Met	Ala	Thr	Gln	Ile	Ser	
			320				325						330			
ggc	atc	aac	gcc	tcg	ctg	cct	ccc	gtt	tcc	tac	acc	aag	gcc	att	gac	1660
Gly	Ile	Asn	Ala	Ser	Leu	Pro	Pro	Val	Ser	Tyr	Thr	Lys	Ala	Ile	Asp	
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Val	Trp	Thr	Gly	Val	Cys	Leu	Thr	Phe	Val	Phe	Gly	Ala	Leu	Leu	Glu	
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Ser	Asp	His	Leu	Glu	Asp	Gly	Ala	Thr	Thr	Phe	Ala	Met	Val	Ser	Ser	
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Gly	Glu	Pro	Ala	Gly	Leu	Met	Ala	Arg	Thr	Trp	Pro	Pro	Pro	Pro	Leu	
		415					420					425				
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54k

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Val His His His Gly Glu Leu His Ala Asp Lys Leu Arg Gln Cys Glu			
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 100 105 110
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 115 120 125
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 130 135 140
 Gly Gln Val Arg Tyr Leu Thr Leu Thr Glu Pro Asp Lys Leu Trp Lys
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541

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Gly Tyr Thr Thr Glu Asp Leu Val Phe Leu Trp Lys Glu Gly Asp Pro
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Val Gln Val Thr Lys Asn Leu His Leu Pro Arg Phe Thr Leu Glu Arg
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Phe Gln Thr Asp Tyr Cys Thr Ser Arg Thr Asn Thr Gly Glu Tyr Ser
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Cys Leu Arg Val Asp Leu Val Phe Lys Arg Glu Phe Ser Tyr Tyr Leu
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	Met Phe Gly Val	Pro Cys Ser Arg	Ala Cys Arg	Leu Val		
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Asn Pro Ser	Asn Met Asp	Tyr Glu Val	Asp Leu Tyr	Phe Arg Gln	Ser	
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ccctggtg						248

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CLAIMS:

1. A purified nucleic acid molecule encoding a *Rhipicephalus sanguineus* GluCl channel protein, wherein said nucleic acid molecule comprises:

5 (a) a nucleic acid molecule which encodes an amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) a nucleic acid molecule which hybridizes under conditions of moderate to high stringency to the complement of (a), and which encodes a protein having the biological
10 activity of the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8; wherein the hybridization conditions include a hybridization step carried out in 5 X SSC, 5 X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours and a washing step carried out in 0.2 X SSPE, 0.2% SDS at 65°C for
15 30 to 60 minutes;

(c) a nucleic acid molecule which hybridizes under conditions of moderate stringency to the complement of a second nucleic acid molecule as set forth in SEQ ID NOs: 1, 3, 5 or 7; wherein said nucleic acid molecule has at least
20 about 70% identity determined over the full length of the nucleic acid molecule to at least one of the second nucleic acid molecules as set forth in SEQ ID NOs: 1, 3, 5 or 7, and which encodes a protein having the biological activity of the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8; wherein
25 the hybridization conditions include a hybridization step carried out in 5 X SSC, 5 X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours and a washing step carried out in 0.2 X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

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2. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said protein comprises an amino acid sequence as set forth in SEQ ID NO: 2.

5 3. An expression vector for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein in a recombinant host cell, wherein said expression vector comprises the DNA molecule of claim 2.

4. A host cell which expresses a recombinant
10 *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said host cell contains the expression vector of claim 3.

5. A process for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein comprising an amino acid sequence of SEQ ID NO: 2 in a recombinant host cell,
15 comprising:

(a) transfecting the expression vector of claim 3 into a host cell; and,

(b) culturing the host cell of step (a) under conditions which allow expression of said *Rhipicephalus*
20 *sanguineus* GluCl1 channel protein from said expression vector.

6. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said purified DNA molecule consists of a nucleotide sequence as set forth in
25 SEQ ID NO: 1.

7. A purified DNA molecule consisting of nucleotides 331 to 1683 of SEQ ID NO: 1.

8. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said protein

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comprises an amino acid sequence as set forth in SEQ ID NO: 4.

9. An expression vector for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein in a recombinant host cell, wherein said expression vector comprises the DNA molecule of claim 8.

10. A host cell which expresses a recombinant *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said host cell contains the expression vector of claim 9.

10 11. A process for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein comprising an amino acid sequence of SEQ ID NO: 4 in a recombinant host cell, comprising:

(a) transfecting the expression vector of claim 9 into a host cell; and,

(b) culturing the host cell of step (a) under conditions which allow expression of said *Rhipicephalus sanguineus* GluCl1 channel protein from said expression vector.

20 12. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said purified DNA molecule consists of a nucleotide sequence as set forth in SEQ ID NO: 3.

25 13. A purified DNA molecule consisting of nucleotides 502 to 1854 of SEQ ID NO: 3.

14. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said protein comprises an amino acid sequence as set forth in SEQ ID NO: 6.

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15. An expression vector for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein in a recombinant host cell, wherein said expression vector comprises the DNA molecule of claim 14.

5 16. A host cell which expresses a recombinant *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said host cell contains the expression vector of claim 15.

17. A process for expressing a *Rhipicephalus sanguineus* GluCl1 channel protein comprising an amino acid
10 sequence of SEQ ID NO: 6 in a recombinant host cell, comprising:

(a) transfecting the expression vector of claim 15 into a host cell; and,

(b) culturing the host cell of step (a) under
15 conditions which allow expression of said *Rhipicephalus sanguineus* GluCl1 channel protein from said expression vector.

18. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl1 channel protein, wherein said purified DNA
20 molecule consists of a nucleotide sequence as set forth in SEQ ID NO: 5.

19. A purified DNA molecule consisting of nucleotides 617 to 2170 of SEQ ID NO: 5.

20. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl2 channel protein, wherein said protein
25 comprises an amino acid sequence as set forth in SEQ ID NO: 8.

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21. An expression vector for expressing a *Rhipicephalus sanguineus* GluCl2 channel protein in a recombinant host cell, wherein said expression vector comprises the DNA molecule of claim 20.

22. A host cell which expresses a recombinant *Rhipicephalus sanguineus* GluCl2 channel protein, wherein said host cell contains the expression vector of claim 21.

23. A process for expressing a *Rhipicephalus sanguineus* GluCl2 channel protein comprising an amino acid sequence of SEQ ID NO: 8 in a recombinant host cell, comprising:

10 (a) transfecting the expression vector of claim 21 into a host cell; and,
(b) culturing the host cell of step (a) under conditions which allow expression of said *Rhipicephalus sanguineus* GluCl2 channel protein from said expression vector.

24. A purified DNA molecule encoding a *Rhipicephalus sanguineus* GluCl2 channel protein, wherein said purified DNA molecule consists of a nucleotide sequence as set forth in SEQ ID NO: 7.

25. A purified DNA molecule consisting of nucleotides 131 to 1387 of SEQ ID NO: 7.

26. A *Rhipicephalus sanguineus* GluCl1 channel protein at least 90% free from non-*R. sanguineus* GluCl channel proteins, which comprises an amino acid sequence as set forth in SEQ ID NO: 2.

27. The *Rhipicephalus sanguineus* GluCl1 channel protein of claim 26, which is a product of a DNA expression vector contained within a recombinant host cell.

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28. A membrane preparation comprising a *Rhipicephalus sanguineus* GluCl1 channel protein purified from the recombinant host cell of claim 27, wherein the membrane preparation is at least 90% free from non-*R. sanguineus* GluCl channel proteins.

5 29. A *Rhipicephalus sanguineus* GluCl1 channel protein at least 90% free from non-*R. sanguineus* GluCl channel proteins which comprises an amino acid sequence as set forth in SEQ ID NO: 4.

30. The *Rhipicephalus sanguineus* GluCl1 channel protein of claim 29,
10 which is a product of a DNA expression vector contained within a recombinant host cell.

31. A membrane preparation comprising a *Rhipicephalus sanguineus* GluCl1 channel protein purified from the recombinant host cell of claim 30, wherein the membrane preparation is at least 90% free from non-*R. sanguineus* GluCl channel proteins.

15 32. A *Rhipicephalus sanguineus* GluCl1 channel protein at least 90% free from non-*R. sanguineus* GluCl channel proteins which comprises an amino acid sequence as set forth in SEQ ID NO: 6.

33. The *Rhipicephalus sanguineus* GluCl1 channel protein of claim 32,
20 which is a product of a DNA expression vector contained within a recombinant host cell.

34. A membrane preparation comprising a *Rhipicephalus sanguineus* GluCl1 channel protein purified from the recombinant host cell of claim 33, wherein the membrane preparation is at least 90% free from non-*R. sanguineus* GluCl channel proteins.

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35. A *Rhipicephalus sanguineus* GluCl2 channel protein at least 90% free from non-*R. sanguineus* GluCl channel proteins which comprises an amino acid sequence as set forth in SEQ ID NO: 8.

36. The *Rhipicephalus sanguineus* GluCl2 channel protein of claim 35,
5 which is a product of a DNA expression vector contained within a recombinant host cell.

37. A membrane preparation comprising a *Rhipicephalus sanguineus* GluCl2 channel protein purified from the recombinant host cell of claim 36, wherein the membrane preparation is at least 90% free from non-*R. sanguineus* GluCl
10 channel proteins.

38. A *Rhipicephalus sanguineus* GluCl1 channel protein which consists of an amino acid sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6.

39. A *Rhipicephalus sanguineus* GluCl2 channel protein which consists of
15 an amino acid sequence as set forth in SEQ ID NO: 8.

40. A method of identifying a modulator of a GluCl channel protein, comprising:

(a) contacting a test compound with an isolated host cell that expresses a *Rhipicephalus sanguineus* GluCl channel protein as set forth in SEQ ID NO:2, SEQ
20 ID NO:4, SEQ ID NO:6 or SEQ ID NO:8;

(b) contacting a test compound with an isolated host cell that does not express a *Rhipicephalus sanguineus* GluCl channel protein as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8;

(c) measuring the effect of the test compound on the host cell that
25 expresses the GluCl channel protein;

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(d) measuring the effect of the test compound on the host cell that does not express the GluCl channel protein;

wherein the effect that is measured in (c) and (d) is (i) the expression of DNA encoding the GluCl channel protein; (ii) the expression of RNA encoding the GluCl channel protein; or (iii) a channel activity of the GluCl channel protein, and wherein if the effect of the test compound measured in the cell that expresses the GluCl channel protein is greater than or less than the effect measured in the host cell that does not express the GluCl channel protein, the test compound is a modulator of a GluCl channel protein.

41. The method of claim 40, wherein the *Rhipicephalus sanguineus* GluCl protein of step (a) is a product of a DNA expression vector contained within a recombinant host cell.

42. An *in vitro* method of identifying a compound that modulates glutamate-gated channel protein activity, which comprises:

a) injecting into a host cell solution a population of nucleic acid molecules, at least a portion of which encodes a *Rhipicephalus sanguineus* GluCl channel protein, wherein the *Rhipicephalus sanguineus* GluCl channel protein is as set forth in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 8, such that expression of said portion of nucleic acid molecules results in an active glutamate-gated channel;

b) adding a test compound into said solution; and,

c) measuring host cell membrane current at a holding potential more positive than the reversal potential for chloride.

43. The method of claim 42, wherein said nucleic acid molecule is a complementary DNA, a poly A⁺ messenger RNA or a complementary RNA.

1/1.1

>clone T12

```
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541 GTTAACATCT TTGTAAGAAG TATCGGCAGA ATTGATGACG TCACCATGGA GTACACAGTG
601 CAAATGACGT TCAGAGAGCA GTGGCGGGAC GAGAGACTCC AGTACGACGA CTTGGGCGGC
661 CAGGTTCGCT ACCTGACGCT CACCGAACCG GACAAGCTTT GGAAGCCGGA CCTGTTTTTC
721 TCCAACGAGA AAGAGGGACA CTTCCACAAC ATCATCATGC CCAACGTGCT TCTACGCATA
781 CATCCCAACG GCGACGTTCT CTTCAGCATC AGAATATCCT TGGTGCTTTC ATGTCCGATG
841 AACCTGAAAT TTTATCCTTT GGATAAACAA ATCTGCTCTA TCGTCATGGT GAGCTATGGG
901 TATACAACAG AGGACCTGGT GTTTCTATGG AAAGAGGGGG ATCCTGTACA GGTCACAAAA
961 AATCTCCACT TGCCACGTTT CACGCTGGAA AGGTTTCAAA CCGACTACTG CACCAGTCGG
1021 ACCAACACTG GCGAGTACAG CTGCTTGCGC GTGGACCTGG TGTTC AAGCG CGAGTTCAGC
1081 TACTACCTGA TCCAGATCTA CATCCCGTGC TGCATGCTGG TCATCGTGTC CTGGGTGTCTG
1141 TTCTGGCTCG ACCCCACCTC GATCCCGGCG CGAGTGTCGC TGGGCGTCAC CACCCTGCTC
1201 ACCATGGCCA CGCAGATATC GGGCATCAAC GCCTCGCTGC CTCCCGTTTC CTACACCAAG
1261 GCCATTGACG TGTGGACCGG CGTCTGTCTG ACCTTCGTAT TCGGCGCGCT CCTCGAGTTC
1321 GCCCTGGTCA ACTACGCCTC GCGGTCAGAT TCACGCCGGC AGAACATGCA GAAGCAGAAG
1381 CAGAGGAAAT GGGAGCTCGA GCCGCCCTG GACTCGGACC ACCTGGAGGA CGGCGCCACC
1441 ACGTTCGCCA TGAGGCCGCT GGTGCACCAC CACGGAGAGC TGCATGCCGA CAAGTTGCGG
1501 CAGTGCGAAG TCCACATGAA GACCCCAAG ACGAACCTTT GCAAGGCCTG GCTTTCCAGG
1561 TTTCCACGC GATCCAAACG CATCGACGTC GTCTCGCGGA TCTTCTTTCC GCTCATGTTC
1621 GCCCTCTTCA ACCTCGTCTA CTGGACAACC TACCTCTTCC GGAAGACGA GGAAGACGAG
1681 TGACAGAACA CGGACGCCAC GACAGCCGCC ATCCGACACC ATCGTCACTG CAGGCACGCA
1741 CTCTGTCGCG CGCACACACC ACGAAGACCG GCGCGCCAAC GCACGATGCG CGTTGGCCGC
1801 TGAAAAACCC GGGAGCGGGG CGGTGGGGGA GGCTATGCCC CGGCCCTCG CTCCTCATCC
1861 TCCGTGCACG CTCGAATCGT CATCGCCACA GCCAGAAAAA AAAAAGATAC CGTGCGAAAA
1921 GTGGCGGCAA CACAACGTCG ACGCCATCAG CGCCGCCCAG AGCTGCAAGC GGCTCCCACA
1981 TGGTTGCCAC CGCAGCTTCC TCTACGACCC TTCATCCCCA CCGGCACCAG CTACGAGAAA
2041 GGGACCTTAT TTCGGGCCAT CCCTACATAG GCGACTGTTG TTTTCGCACG AAAGATCTTT
2101 ACGCAGCTGA TGCTGAAAAA AAAAAAAAAA AAAAAAAA (SEQ ID NO:1)
```

FIG. 1

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>T12

MSVHSWRFCVPLVALAFFLLILLSCPSAWGKANFRAIEKRILDSIIGQGRYDCRIRPMGINNTDGPALV
RVNIFVRSIGRIDDVMEYTVQMTFREQWRDERLQYDDLGGQVRYLTLTEPDKLWKPDFFSNEKEGHF
HNIIMPNVLLRIHPNGDVLFSIRISLVLSCPMNLKFYPLDKQICSIVMVSYGTTEDLVFLWKEGDPVQ
VTKNLHLPRFTLERFQTDYCTSRNTGEYSCLRVDLVFKREFSYyliQIYIPCCMLVIVSWVSWLDPT
SIPARVSLGVTLLTMTATQISGINASLPPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSDSRRQN
MQKQKQRKWELEPPLDSDHLEDGATTFAMRPLVHHHGELHADKLQRQCEVHMKTPKTNLCKAWLSRFPTR
SKRIDVVSRIFFPLMFALFNLVYWTTYLFREDEEDE* (SEQ ID NO:2)

FIG. 2

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>clone T82

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1 CACACCTCCT GCGTCTCTCC ACTCGATGAA GACCTGTCCC GGAGGCGCGA GCCCAACTGC
61 GCGCTCTGTC CGCATGTGTC GCCGCCACTG AGAGGCCTCC GCGGTGGCGC GCTTGTCAAC
121 GCGGCGCGCC GGCCCGCAGC AAATCGCGGG CATTCCACTC AGGGTCTCAT TCGTCCCCC
181 AATCCTGAGG TTCCTTCTAA CGAGAAGGAG GAGCCACAGC GCCGGCTGCG GTACCGCCGC
241 ACGGGCCAAC GTGAGACCGC CCGAGCCCGG CGCCCTGACT TAGGCCGCTG AGCGAAACCC
301 AAGGCGGCGC GCTGGCCACT CCACGGGAAC GAGACCGGCC CCCTGGAGAC GACATCGTCG
361 ACCACAATGA ACTACTTCTC TGACGTGGCG AAGATGGTGG CTTTCATCGAA GAGAGAAATC
421 ATCGAAGCTT TCCACGCGAC ATCTGGAGTA CACGGCGCAT GCGAATGAGC GAACATCGCT
481 GACCGAGACT CGCCCGTCAC CATGAGCGTA CATTTCATGGC GCTTTTGTGT CCCACTGGTG
541 GCTCTAGCGT TTTTCTTGTT GATTCTTCTG TCGTGTCCAT CGGCATGGGG CAAGGCAAAT
601 TTCCGCGCTA TAGAAAAGCG GATATTGGAC AGCATCATTG GCCAGGGTCG TTATGACTGC
661 AGGATCCGGC CCATGGGAAT TAACAACACA GACGGGCCGG CTCTTGACG CGTTAACATC
721 TTTGTAAGAA GTATCGGCAG AATTGATGAC GTCACCATGG AGTACACAGT GCAAATGACG
781 TTCAGAGAGC AGTGGCGGGA CGAGAGACTC CAGTACGACG ACTTGGGCGG CCAGGTTCGC
841 TACCTGACGC TCACCGAACC GGACAAGCTT TGGAAGCCGG ACCTGTTTTT CTCCAACGAG
901 AAAGAGGGAC ACTTCCACAA CATCATCATG CCCAACGTGC TTCTACGCAT ACATCCCAAC
961 GGCGACGTTT TCTTCAGCAT CAGAATATCC TTGGTGCTTT CATGTCCGAT GAACCTGAAA
1021 TTTTATCCTT TGGATAAACA AATCTGCTCT ATCGTCATGG TGAGCTATGG GTATACAACA
1081 GAGGACCTGG TGTTTCTATG GAAAGAGGGG GATCCTGTAC AGGTCACAAA AAATCTCCAC
1141 TTGCCACGTT TCACGCTGGA AAGGTTTCAA ACCGACTACT GCACCAGTCG GACCAACACT
1201 GGCGAGTACA GCTGCTTGCG CGTGGACCTG GTGTTCAAGC GCGAGTTCAG CTACTACCTG
1261 ATCCAGATCT ACATCCCGTG CTGCATGCTG GTCATCGTGT CCTGGGTGTC GTTCTGGCTC
1321 GACCCACCT CGATCCCGGC GCGAGTGTG CTGGGCGTCA CCACCCTGCT CACCATGGCC
1381 ACGCAGATAT CGGGCATCAA CGCCTCGCTG CCTCCCGTTT CCTACACCAA GGCCATTGAC
1441 GTGTGGACCG GCGTCTGTCT GACCTTCGTA TTCGGCGCGC TCCTCGAGTT CGCCCTGGTC
1501 AACTACGCCT CGCGGTCAGA TTCACGCCGG CAGAACATGC AGAAGCAGAA GCAGAGGAAA
1561 TGGGAGCTCG AGCCGCCCTT GGACTCGGAC CACCTGGAGG ACGGCGCCAC CACGTTGCGC
1621 ATGAGGCCGC TGGTGCACCA CCACGGAGAG CTGCATGCCG ACAAGTTGCG GCAGTGCGAA
1681 GTCCACATGA AGACCCCCAA GACGAACCTT TGCAAGGCCT GGCTTTCCAG GTTTCCACG
1741 CGATCCAAAC GCATCGACGT CGTCTCGCGG ATCTTCTTTC CGCTCATGTT CGCCCTCTTC
1801 AACCTCGTCT ACTGGACAAC CTACCTCTTC CGGGAAGACA AGGAAGACGA GTGACAGAAC
1861 ACGAACGCCA CGACAGCCGC CATCCGACAC CATCGTCACT GCAGGCACGC ACTCTGTCGC
1921 GCGCACACAC CACGAAGACC GGCGCGCCAA CGCACGATGC GCGTTGGCCG CTGAAAACC
1981 CGGGAGCGGG GCGGTGGGGG AGGCTATGCC CCGGCCCTC GTCCTCATC CTCCGTGCAC
2041 GCTCGAATCG TCATCGCCAC AGCCAGAAAA AAAAAAGATA CCGTGCGAAA AGTGGCGGCA
2101 ACACAACGTC GACGCCATCA GCGCCGCCCA GAGCTGCAAG CGGCTCCAC ATGGTTGCCA
2161 CCGCAGCTTC CTCTACGACC CTTTCATCCC ACCGGCACCA GCTACGAGAA AGGGACCTTA
2221 TTTCGGGCCA TCCCTACATA GGCGACTGTT GTTTTCGCAC GAAAGATCTT TACGCAGCTG
2281 ATGCTGAAA (SEQ ID NO:3)
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FIG.3

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>T82

MSVHSWRFCVPLVALAFFLLILLSCPSAWGKANFRAIEKRILDSIIGQGRYDCRIRPMGINNTDGPALV
RVNIFVRSIGRIDDVMEYTVQMTFREQWRDERLQYDDLGGQVRYLTLTEPDKLWKPDFFSNEKEGHF
HNIIMPVNLLRIHPNGDVLFSIRISLVLSCPMNLKFYPLDKQICSIVMVSYGYTTEDLVFLWKEGDPVQ
VTKNLHLPRFTLERFQTDYCTSRNTNGEYSCLRVDLVFKREFSYyliQIYIPCCMLVIVSWVSFWLDPT
SIPARVSLGVTTLLTMATQISGINASLPPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSDSRRQN
MQKQKQRKWELEPPLDSDHLEDGATTfamRPLVHHHGELHADKLRQCEVHMKTPKTNLCKAWLSRFPTR
SKRIDVVSRIFFPLMFALFNLVYWTTYLFREDKEDE* (SEQ ID NO:4)

FIG.4

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>clone T32

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1  CAGGCTCCGG CGTGACTGTC GCTCGCTCGG CTCTCGACGC TCGCGGCGGG AACAACCGCT
61  ACCCGGACGC TCGATCAGGA GCAGTTCGGG CCACAGAGAA AGGGGCGGAG GAGTGCACAC
121 CTCCTGCGTC TCTCCACTCG ATGAAGACCT GTCCCGGAGG CGCGAGCCCA ACTGCGCGCT
181 CTGTCCGCAT GTGTCGCCGC CACTGAGAGG CCTCCGGCGT GGCGCGCTTG TCAACGCGGC
241 GCGCCGGCCC GCAGCAAATC GCGGGCATTG CACTCAGGGT CTCATTGCTT CCCCCAATCC
301 TGAGGTTCCT TCTAACGAGA AGGAGGAGCC ACAGCGCCGG CTGCGGTACC GCCGCACGGG
361 CCAACGTGAG ACCGCCCGAG CCCGGCGCCC TGACTTAGGC CGCTGAGCGA AACCCAAGGC
421 GGCGCGCTGG CCACTCCACG GGAACGAGAC CGGCCCCCTG GAGACGACAT CGTCGACCAC
481 AATGAACTAC TTCTCTGACG TGGCGAAGAT GGTGGCTTCA TCGAAGAGAG AAATCATCGA
541 AGCTTTCCAC GCGACATCTG GAGTACACGG CGCATGCGAA TGAGCGAACA TCGCTGACCG
601 AGACTCGCCC GTCACCATGA GCGTACATTC ATGGCGCTTT TGTGTCCAC TGGTGGCTCT
661 AGCGTTTTTC TTGTTGATTC TTCTGTCGTG TCCATCGGCA TGGGCCGAAA CGCTGCCTAC
721 GCCACCAACC CGTGGCCAGG GGGGCGTTCC GGTGCGGGCC GCGATGCTCC TGGGGAAACA
781 GCAAAGTTCC CGCTACCAAG ATAAAGAGGG CAAGGCAAAT TTCCGCGCTA TAGAAAAGCG
841 GATATTGGAC AGCATCATTG GCCAGGGTCG TTATGACTGC AGGATCCGGC CCATGGGAAT
901 TAACAACACA GACGGGCCGG CTCTTGTAAG CGTTAACATC TTTGTAAGAA GTATCGGCAG
961 AATTGATGAC GTCACCATGG AGTACACAGT GCAAATGACG TTCAGAGAGC AGTGGCGGGA
1021 CGAGAGACTC CAGTACGACG ACTTGGGCGG CCAGGTTCGC TACCTGACGC TCACCGAACC
1081 GGACAAGCTT TGGAAGCCGG ACCTGTTTTT CTCCAACGAG AAAGAGGGAC ACTTCCACAA
1141 CATCATCATG CCCAACGTGC TTCTACGCAT ACATCCCAAC GGCGACGTTT TCTTCAGCAT
1201 CAGAATATCC TTGGTGCTTT CATGTCCGAT GAACCTGAAA TTTTATCCTT TGGATAAACA
1261 AATCTGCTCT ATCGTCATGG TGAGCTATGG GTATAACAAC GAGGACCTGG TGTCTCTATG
1321 GAAAGAGGGG GATCCTGTAC AGGTCACAAA AAATCTCCAC TTGCCACGTT TCACGCTGGA
1381 AAGGTTTCAA ACCGACTACT GCACCACTCG GACCAACACT GGCGAGTACA GCTGCTTGCG
1441 CGTGGACCTG GTGTTCAAGC GCGAGTTCAG CTACTACCTG ATCCAGATCT ACATCCCGTG
1501 CTGCATGCTG GTCATCGTGT CCTGGGTGTC GTTCTGGCTC GACCCACCT CGATCCCGGC
1561 GCGAGTGTCG CTGGGCGTCA CCACCCTGCT CACCATGGCC ACGCAGATAT CGGGCATCAA
1621 CGCCTCGCTG CCTCCCGTTT CCTACACCAA GGCCATTGAC GTGTGGACCG GCGTCTGTCT
1681 GACCTTCGTA TTCGGCGCGC TCCTCGAGTT CGCCCTGGTC AACTACGCCT CGCGGTCAGA
1741 TTCACGCCGG CAGAACATGC AGAAGCAGAA GCAGAGGAAA TGGGAGCTCG AGCCGCCCTT
1801 GGACTCGGAC CACCTGGAGG ACGGCGCCAC CACGTTTCGC ATGGTGAGCT CCGGCGAGCC
1861 GGCGGGCCTC ATGGCGCGAA CCTGGCCACC ACCGCCGCTG CCGCCAAACA TGGCGGCCGG
1921 CTCCGCGCAA GCCGGCGCCA GGCCGCTGGT GCACCACCAC GGAGAGCTGC ATGCCGACAA
1981 GTTGCGGCAG TGCGAAGTCC ACATGAAGAC CCCCAAGACG AACCTTTGCA AGGCCTGGCT
2041 TTCCAGGTTT CCCACGCGAT CCAAACGCAT CGACGTCGTC TCGCGGATCT TCTTTCCGCT
2101 CGTGTTCCGC CTCTTCAACC TCGTCTACTG GACAACCTAC CTCTTCCGGG AAGACGAGGA
2161 GGACGAGTGA CAGAACACGA ACGCCACGAC AGCCGCCATC CGACACCATC GTCACTGCAG
2221 GCACGCACTC TGTCGCGCGC ACACACCACG AAGACCGGCG CGCCAACGCA CGATGCGCGT
2281 TGGCCGCTGA AAAACCCGGG AGCGGGGCGG TGGGGGAGGC TATGCCCGGG CCCCTCGCTC
2341 CTCATCCTCC GTGCACGCTC GAATCGTCAT CGCCACAGCC AGAAAAAAAA AAAAAAAAAA
(SEQ ID NO:5)
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FIG. 5

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>T32

MSVHSWRFCVPLVALAFFLLILLSCPSAWAETLPTPTRGQGGVPVAAAMLLGKQQSSRYQDKEGKANF
RAIEKRILDSIIGQGGRYDCRIRPMGINNTDGPALVRVNI FVRSIGRIDDTVMEYTVQMTFREQWRDERL
QYDDLGGQVRYLTLTEPDKLWKPD LFFSNEKEGHFHNIIMP NVLLRIHPNGDVLFSIRISLVLSCPMNL
KFYPLDKQICSIVMVS YGYTTEDLVFLWKEGDPVQVTKNLHLPRFTLERFQTDYCTSR TNGEYSCLR V
DLVFKREFSYYLIQIYIPCCMLVIVSWVSFWLDPTSIPARVSLGVTTLLT MATQISGINASLPPVSYTK
AIDVWTGVCLTFVFGALLEFALVNYASRSDSRRQNMQKQKQRKWELEPPLDSDHLEDGATT FAMVSSGE
PAGLMARTWPPPPLPPNMAAGSAQAGARPLVHHHGELHADKLRQCEVHMKTPKTNLCKAWLSRFPTRSK
RIDVVSRIFFPLVFALFNLVYWTTYLFREDEEDE*(SEQ ID NO:6)

FIG. 6

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B1

1 CGCCGCTCAA TCGCGGGCTA CGGACTCGTC GTTCCCGGAG GGGCTTGGAC
51 CACAGCTCGC TCGTCACCGT GGTGGCTGGC CGCTTCGCCT GGCGGTCCTG
101 CACGCACGCT GTAACGAACG TCGCCACGCG ATGTTTGGTG TGCCATGCTC
151 CCGCGCCTGC CGCCTTGTGG TGGTGATAGC TGC GTTCTGC TGGCCGCCCG
201 CTCTGCCGCT CGTACCCGGG GGAGTTTCCT CCAGAGCAAA CGATCTGGAC
251 ATTCTGGACG AGCTCCTCAA AACTACGAT CGAAGGGCCC TGCCGAGCAG
301 TCACCTCGGA AATGCAACTA TTGTGTCATG CGAAATTTAC ATACGAAGTT
351 TTGGATCAAT AAATCCTTCG AACATGGACT ACGAAGTCGA CCTCTACTTC
401 CGGCAGTCGT GGCTCGACGA GCGGTTACGC AAATCCACGC TATCTCGTCC
451 GCTCGACCTT AATGACCCAA AGCTGGTACA AATGATATGG AAGCCAGAAG
501 TTTTCTTTGC GAACGCGAAA CACGCCGAGT TCCAATATGT GACTGTACCT
551 AACGTCCTCG TTAGGATCAA CCCGACTGGA ATAATCTTGT ACATGTTGCG
601 GTTAAAACTG AGGTTCTCCT GCATGATGGA CCTGTACCGG TACCCCATGG
651 ATTCCCAAGT CTGCAGCATC GAAATTGCCT CTTTTTCCAA AACCACCGAA
701 GAGCTGCTGC TGAAATGGTC CGAGAGTCAG CCTGTCGTTC TCTTCGATAA
751 CCTCAAGTTG CCCAGTTTG AAATAGAGAA GGTGAACACG TCCTTATGCA
801 AAGAAAAGTT TCACATAGGG GAATACAGTT GCCTGAAAGC CGACTTCTAT
851 CTGCAGCGTT CCCTCGGTTA TCACATGGTG CAGACCTATC TTCCGACCAC
901 GCTTATCGTG GTCATCTCAT GGGTGTCATT CTGGCTCGAC GTAGACGCCA
951 TACCCGCCCG TGTCACCCTG GGC GTAACCA CGCTGCTCAC CATCTCATCC
1001 AAGGGTGCCG GTATCCAGGG AAACCTGCCT CCCGTCTCGT ACATCAAGGC
1051 CATGGACGTC TGGATAGGAT CCTGTACTTC GTTTGTCTTT GCGGCCCTTC
1101 TAGAGTTCAC ATTCGTCAAC TATCTCTGGA GCGGGCTGCC CAATAAGCGC
1151 CCATCTTCTG ACGTACCGGT GACGGATATA CCAAGCGACG GCTCAAAGCA
1201 TGACATTGCG GCACAGCTCG TACTCGACAA GAATGGACAC ACCGAAGTTC
1251 GCACGTTGGT CCAAGCGATG CCACGCAGCG TCGGAAAAGT GAAGGCCAAG
1301 CAGATTGATC AACTCAGCCG AGTCGCCTTT CCCGCTCTTT TTCTCCTCTT
1351 CAACCTCGTG TACTGGCCGT ACTACATTAA GTCATAAAGA ACGTAGTTTT
1401 CT (SEQ ID NO:7)

FIG. 7

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B1

MFGVPCSRACRLVVVIAAFCWPPALPLVPGGVSSRANDLDILDEL
LKNYDRRALPSSHLGNATIVSCEIYIRSFGSINPSNMDYEVDLYF
RQSWLDERLRKSTLSRPLDLNDPKLVQMIWKPEVFFANAKHAEFQ
YVTVPNVLVRINPTGIILYMLRLKLRFSMMMDLYRYPMDSQVCSI
EIASFSKTTEELLLKWESEQPVVLFNLKLPQFEIEKVNTSLCKE
KFHIGEYSCLKADFYLRSLGYHMQTYLPTTLIVVISWVSFWLD
VDAIPARVTLGVTTLLTISSKGAGIQGNLPPVSYIKAMDVWIGSC
TSFVFAALLEFTFVNYLWRRLPNKRPSDVPVTDIPSDGSKHDIA
AQLVLDKNGHTEVRTLVQAMPRSVGKVKAKQIDQLSRVAFPALFL
LFNLVYWPYYIKS (SEQ ID NO:8)

FIG.8

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T32 MS.VHSWRFVPLVALAFFLLILLSCPSAWAETLPTPPTRGQGGVPVAAAMLLGKQQSSR
 T12 MS.VHSWRFVPLVALAFFLLILLSCPSAW.....
 T82 MS.VHSWRFVPLVALAFFLLILLSCPSAW.....
 B1 MFGVPCSRACRLVVVIAAFC.....WPPALPLVP.....

T32 YQDKEGKANFRAIEKRILDSIIGQGRYDCRIRP...MGINNTDGPALVRVNIIFVRSIGRI
 T12 ...GKANFRAIEKRILDSIIGQGRYDCRIRP...MGINNTDGPALVRVNIIFVRSIGRI
 T82 GKANFRAIEKRILDSIIGQGRYDCRIRP...MGINNTDGPALVRVNIIFVRSIGRI
 B1 GGVSSRANDLDILDELLKN..YDRRALPSSHG.NAT....IVSCEIYIRSFGSI

T32 DDVTMEYTVQMTFREQWRDERLQYDDLGGQVRYLTLTEPDKL...WKPDFFSNEKEGH
 T12 DDVTMEYTVQMTFREQWRDERLQYDDLGGQVRYLTLTEPDKL...WKPDFFSNEKEGH
 T82 DDVTMEYTVQMTFREQWRDERLQYDDLGGQVRYLTLTEPDKL...WKPDFFSNEKEGH
 B1 NPSNMDYEVDLYFRQSWLDERLRKSTLS...RPLDLNDP.KLVQMIWKPEVFFANAKHAE

T32 FHNIIMPVLLRIHPNGDVLFSIRISLVLSCPMNLKFYPLDKQICSIVMVSYGTTEDLV
 T12 FHNIIMPVLLRIHPNGDVLFSIRISLVLSCPMNLKFYPLDKQICSIVMVSYGTTEDLV
 T82 FHNIIMPVLLRIHPNGDVLFSIRISLVLSCPMNLKFYPLDKQICSIVMVSYGTTEDLV
 B1 FQYVTPVNLVRINPTGIILYMLRLKLRFSMMDLYRYPMDSQVCSIEIASFSKTTEELL

T32 FLWKEGDPVQVTKNLHLPRFTLERFQTDYCTSRTNTGEYSCLRVDLVFKREFSYLIQIY
 T12 FLWKEGDPVQVTKNLHLPRFTLERFQTDYCTSRTNTGEYSCLRVDLVFKREFSYLIQIY
 T82 FLWKEGDPVQVTKNLHLPRFTLERFQTDYCTSRTNTGEYSCLRVDLVFKREFSYLIQIY
 B1 LKWSSESQPVVLF DN LKLPQFEIEKVNTSLCKEKFHIGEYSCLKADFYLRSLGYHVMVQTY

T32 IPCCMLVIVSWVSFWLDPTSIPARVSLGVTTLLT MATQIS....GINASLPPVSYTKAID
 T12 IPCCMLVIVSWVSFWLDPTSIPARVSLGVTTLLT MATQIS....GINASLPPVSYTKAID
 T82 IPCCMLVIVSWVSFWLDPTSIPARVSLGVTTLLT MATQIS....GINASLPPVSYTKAID
 B1 LPTTLIVVISWVSFWLDVDAIPARVTLGVTTLLT....ISSKGAGIQGNLPPVSYIKAMD

T32 VWTGVCLTFVFGALLEFALVNYASRSDSRRQNMQKQKQKWELEPPLDS...DHLEDG.A
 T12 VWTGVCLTFVFGALLEFALVNYASRSDSRRQNMQKQKQKWELEPPLDS...DHLEDG.A
 T82 VWTGVCLTFVFGALLEFALVNYASRSDSRRQNMQKQKQKWELEPPLDS...DHLEDG.A
 B1 VWIGSCTSFVFAALLEFTFVNYLWR...RLPNK.....R.....PSSDVPVTDIPSDGSK

T32 TTFAMVSSGEPAGLMARTWPPPPLPPNMAAGSAQAGARPLVHHHGELHADKL RQCEVHMK
 T12 TTFAM.....RPLVHHHGELHADKL RQCEVHMK
 T82 TTFAM.....RPLVHHHGELHADKL RQCEVHMK
 B1 HDIAAQ.....LVLDKNGHTEVR..

T32 TPKTNLCKAWLSRFPTRS.....KRIDVVSRIFFPLVFALFNLVYWT TYLFREDEEDE (SEQ ID NO:6)
 T12 TPKTNLCKAWLSRFPTRS.....KRIDVVSRIFFPLMFALFNLVYWT TYLFREDEEDE (SEQ ID NO:2)
 T82 TPKTNLCKAWLSRFPTRS.....KRIDVVSRIFFPLMFALFNLVYWT TYLFREDKEDE (SEQ ID NO:4)
 B1 T.LVQAMP.....RSVGKVKAKQIDQLSRVAFPALFLLFNLVYWPYYIKS... (SEQ ID NO:8)

FIG.9

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EXPRESSION OF RsGluC11 IN OOCYTES

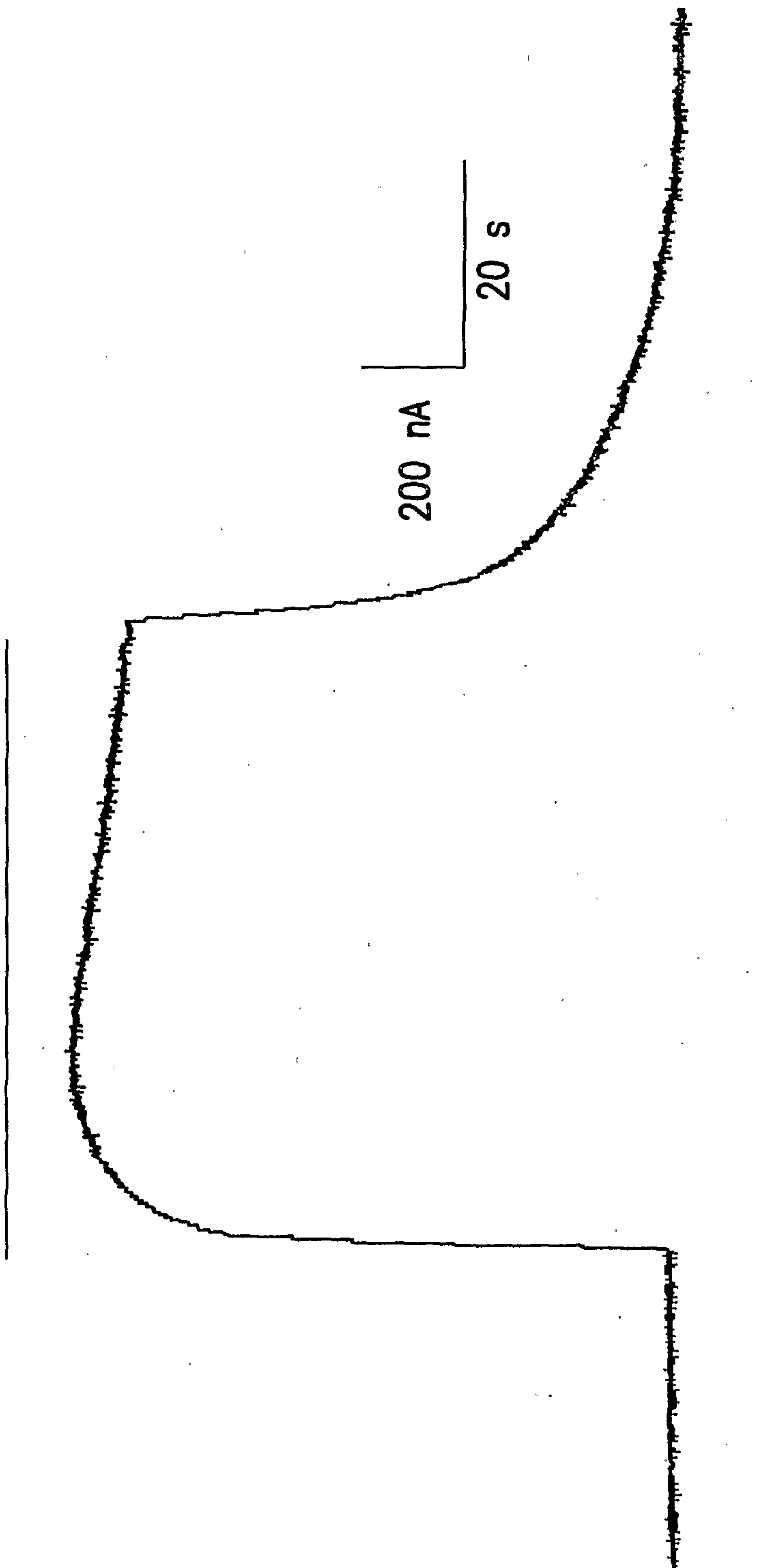
10 μ M GLUTAMATE

FIG.10

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RsGluC12 EXPRESSION IN OOCYTES

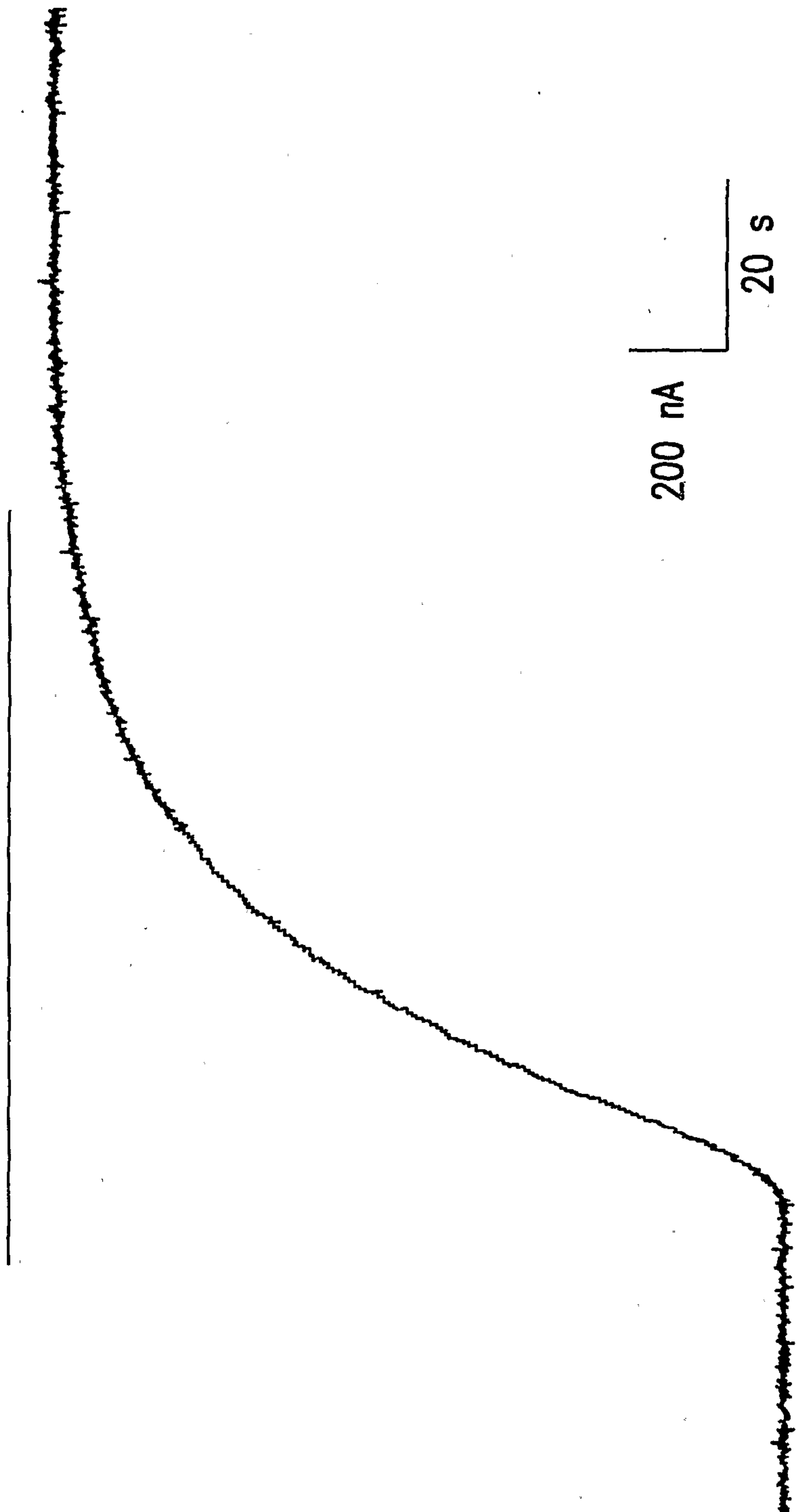
1 μ M IVERMECTIN

FIG. 11