# COMPOSITIONS AND METHODS FOR SHORT INTERFERING NUCLEIC ACID INHIBITION OF $Na_v1.8$

## Cross-reference to Related Applications

This application claims the benefit of priority under 35 USC 119(e) of provisional patent application U.S.S.N.: 60/622,484 filed October 27, 2004, the disclosure of which is hereby incorporated by reference in its entirety.

## Background of the Invention

### *Field of the Invention*

The invention provides short interfering nucleic acids, either single-stranded or double-stranded, that cause RNAi-induced degradation of mRNA from the $Na_v1.8$ sodium channel gene; to pharmaceutical compositions comprising such short interfering nucleic acids; recombinant vectors comprising such short interfering nucleic acids; a method for inhibiting translation of an mRNA; a method for inhibiting expression of a polypeptide; a method for blocking the membrane potential in a cell; a method for blocking the sodium current in a cell; and a method for inhibiting chronic pain.

### *Background of the Invention*

Chronic pain is a major symptom of peripheral neuropathies, whether induced by AIDS, cancer chemotherapy, diabetes, or by direct physical trauma to the peripheral nerves. Such neuropathic pain is often highly debilitating and resistant to therapeutic intervention.

Animal models of neuropathic pain have suggested that a prominent feature in the maintenance of the neuropathic state is an abnormal, persistent hyperexcitability of the sensory afferent neurons within the peripheral nerve following injury. In addition, a common clinical finding is that broad-spectrum sodium channel blockers, such as lidocaine, can acutely suppress

neuropathic pain. However, the relative contribution of individual sodium channel subtypes in neuropathic pain remains unclear.

Voltage-gated sodium channels are critical for the initiation and propagation of action potentials in neurons. In addition, these channels are involved in the regulation of neuronal excitability. Therefore, voltage-gated sodium channels play an important role in transmitting nociceptive information throughout both the peripheral and central nervous systems. Peripheral nerve injury causes sodium channels to accumulate in the membranes of primary afferents around the site of injury. This results in repetitive firing and an increase in excitability of both injured afferents and their uninjured neighbors. This increase in excitability appears to be critical for the expression of neuropathic pain.

At least ten different isoforms of sodium channels have been identified in the brain, neurons and striated muscles. The major component of sodium channels is the 260 kDa α-subunit, which forms the pore of the channel. The α-subunit is composed of four homologous domains, DI, DII, DIII and DIV, each of which is composed of six transmembrane segments, S1-S6. Most sodium channels associate with auxiliary β-subunits, β1-β4, which have an average molecular weight of 30 kDa. The β-subunits modulate the level of expression and gating of these channels.

Three sodium channel isoforms, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$, are expressed primarily in the PNS. $Na_v1.7$ is widespread in the peripheral nervous system, such that it is present in all types of dorsal root ganglion neurons, in Schwann cells and in neuroendocrine cells. $Na_v1.7$ is sensitive to nanomolar amounts of tetrodotoxin. $Na_v1.8$ is found only in sensory afferent nerves and neurons of the dorsal root ganglion and trigeminal ganglion. The $Na_v1.8$ channel is highly resistant to tetrodotoxin, with an $IC_{50}$ of greater than 50 μM. $Na_v1.9$ is also expressed in small fibers of the dorsal root ganglion and trigeminal ganglion and is also resistant to nanomolar concentrations of tetrodotoxin, but is half maximally blocked by ~40 μM of tetrodotoxin.

Recent interest in the search for therapeutic targets in the treatment of pain has focused on the tetrodotoxin resistant sodium channels found in adult

dorsal root ganglion neurons, a significant fraction of which are known to be pain-sensing 'nociceptors'. One such sodium channel is $Na_v1.8$, which was formerly known as PN3 or peripheral nerve sodium channel type 3. This channel has been found to be upregulated in the dorsal root ganglion in chronic pain states. In addition, the biophysical properties of $Na_v1.8$ make this channel a likely candidate for maintaining the sustained repetitive firing of the peripheral neuron following injury. Moreover, the expression of $Na_v1.8$ being restricted to the periphery in sensory neurons of the dorsal root ganglion, suggests that blockade of this channel might allow relief from neuropathic pain with minimal side effects. However, this possibility can not be tested pharmacologically because currently available sodium channel blockers do not distinguish between sodium channel subtypes.

Antisense oligodeoxynucleotide targeting of $Na_v1.8$ expression in an animal model of neuropathic pain has been employed to test whether a selectively attenuated expression of this channel might allow relief from neuropathic pain. See Porreca *et al.*, "A comparison of the potential role of the tetrodotoxin-insensitive sodium channels, PN3/SNS and NaN/SNS2, in rat models of chronic pain", *Proc. Nat. Acad. Sci.*, vol. 96, pp. 7640-7644 (1999). Inhibition of $Na_v1.8$ expression using antisense deoxyoligonucleotides has also been found to inhibit chronic pain in other animal pain models. See Yoshimura *et al.*, "The involvement of the tetrodotoxin-resistant sodium channel $Na_v1.8$ (PN3/SNS) in a rat model of visceral pain", *J. Neuroscience*, vol. 21, pp. 8690-8696 (2001); and Khasar *et al.*, "A tetrodotoxin-resistant sodium current mediates inflammatory pain in the rat", *Neuroscience Letters*, vol. 256, no. 1, pp. 17-20 (1998). Further data indicate that selective knock-down of $Na_v1.8$ protein in the dorsal root ganglion neurons by specific antisense oligodeoxynucleotides to $Na_v1.8$ prevented the hyperalgesia and allodynia caused by spinal nerve ligation injury. See Kim *et al.*, "An experimental model for peripheral neuopathy produced by segmental spinal nerve ligation in the rat", *Pain*, vol. 50, pp. 355-363 (1992). The above data suggests a pathophysiological role for $Na_v1.8$ in several peripheral neuropathic and inflammatory states.

However, the use of antisense oligodeoxynucleotides as therapeutics is limited by their instability in vivo, by their limited efficacy and by their tendency to produce 'off-target' effects. Since no small molecule has been identified that is capable of specifically blocking $Na_v1.8$, there is a continued need for alternative ways of modulating $Na_v1.8$ in the treatment of pain.

The present invention meets the above need by providing short interfering nucleic acids and siRNAs to specifically knock-down expression of $Na_v1.8$. The use of siRNA is attractive because it has high target specificity, reduced off-target liability and achieves high levels of suppression. siRNA, which stands for short interfering RNA or small interfering RNA, is a form of RNA interference (RNAi). RNAi is a technique used to investigate gene function by degrading a specific mRNA target in a cell, thus knocking-out or knocking-down the level of the encoded protein. The mechanism of action of siRNA is thought to involve a multi-step process. First, double-stranded RNA (dsRNA) is recognized by an RNase III family member and is cleaved into siRNAs of 21 to 23 nucleotides. Next, the siRNAs are incorporated into an RNAi targeting complex called RNA-induced silencing complex (RISC). RISC is a dual function helicase and RNase that recognizes target mRNA. After recognizing a target mRNA, the RISC binds the mRNA and unwinds the siRNA, which allows the antisense strand of the siRNA to bind via complementary base pairing (Watson-Crick base pairing) to the target mRNA. This causes hydrolysis and destruction of the mRNA, which results in decreased protein expression. Furthermore, siRNA is apparently recycled such that one siRNA molecule is capable of inducing cleavage of approximately 1000 mRNA molecules. Therefore, siRNA-mediated RNAi is more effective than other currently available technologies for inhibiting expression of a target gene.

All references, publications, patent applications and patents disclosed herein are hereby incorporated by reference in their entirety.

## Brief Summary of the Invention

The present invention is directed to short interfering nucleic acids that specifically target and cause RNAi-induced degradation of mRNA from the

$Na_v1.8$ sodium channel gene and methods of using such short interfering nucleic acids.

An embodiment of the invention provides an isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof. The isolated or recombinant short interfering nucleic acid may further comprise a 3' overhang. Also provided is a pharmaceutical composition comprising one or more of any of the above short interfering nucleic acids and a pharmaceutically acceptable carrier.

An alternative embodiment of the invention provides an isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof, and further comprising a complementary nucleotide sequence thereto. The isolated or recombinant short interfering nucleic acid may further comprise a 3' overhang. Also provided is a pharmaceutical composition comprising one or more of any of the above short interfering nucleic acids and a pharmaceutically acceptable carrier.

Another embodiment provides an isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof, and further comprising a complementary nucleotide sequence thereto, wherein the nucleotide sequence and the complementary nucleotide sequence hybridize to form a duplex. The nucleotide sequence and the complementary nucleotide sequence may each further comprise a 3' overhang. Also provided is a pharmaceutical composition comprising one or more of any of the above duplexes and a pharmaceutically acceptable carrier.

An additional embodiment provides an isolated or recombinant short interfering nucleic acid comprising a sense strand and an antisense strand, wherein the sense strand and the antisense strand hybridize to form a duplex, wherein the sense strand comprises a nucleotide sequence substantially identical to a target sequence, and wherein the target sequence is selected from the group consisting of SEQ ID NOs: 12-577. The sense strand and the

antisense strand may each further comprise a 3' overhang. Also provided is a pharmaceutical composition comprising one or more of any of the above duplexes and a pharmaceutically acceptable carrier.

An embodiment of the invention provides a recombinant vector comprising one or more of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof.

Another embodiment provides a method for inhibiting translation of an mRNA to a polypeptide comprising contacting a cell capable of expressing a $Na_v1.8$ mRNA with one or more isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof.

An alternative embodiment provides a method for inhibiting expression of a polypeptide comprising contacting a cell capable of expressing a $Na_v1.8$ polypeptide with one or more isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof.

Another alternative embodiment provides a method for blocking the $Na_v1.8$ derived membrane potential in a cell comprising contacting a cell expressing a $Na_v1.8$ polypeptide, with one or more isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof.

An additional embodiment provides a method for blocking the $Na_v1.8$ derived sodium ion flux in a cell comprising contacting a cell expressing a $Na_v1.8$ polypeptide with one or more isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof.

A further embodiment provides a method for inhibiting chronic pain comprising administering to a subject in need thereof an effective amount of a

pharmaceutical composition comprising one or more isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof, and a pharmaceutically acceptable carrier. The above isolated or recombinant short interfering nucleic acid may further comprise a 3' overhang.

## Detailed Description of the Invention

All publications cited herein are incorporated by reference in their entirety.

Definitions

The term "antisense strand", as used in this application, means a nucleic acid sequence that is complementary to a sense strand.

The term "chronic pain", as used in this application, is defined as pain that lasts for a long period of time

The term "complementary", as defined in this application, means a nucleotide sequence that exhibits Watson-Crick base pairing with another nucleotide sequence, *i.e.*, a purine binds to a pyrimidine. For example, a nucleotide sequence may represent a sense strand while the complementary nucleotide sequence thereto may represent the antisense strand.

The term "duplex", as used herein, means two complementary nucleic acid sequences that have hybridized, such as a sense strand and an antisense strand.

The terms "express", "expresses" and "expression", as used herein, mean the molecular biological process by which transcription and translation of a nucleic acid produce a polypeptide, *i.e.*, the process by which genetic information flows from genes to proteins, and by which the effects of genes are manifested.

The terms "homology" and "homologous" refer to a comparison between two nucleic acid sequences, such that when the sequences are aligned and compared, they exhibit similarities. Homology between two

nucleic acid sequences can be determined by sequence comparison or based upon hybridization conditions. Nucleotide sequence homology is observed when there is identity in nucleotide residues in two sequences (or in their complementary strands) when optimally aligned to account for nucleotide insertions or deletions. Homology also exists when one nucleotide sequence will hybridize to another sequence under selective hybridization conditions. Stringency of conditions employed in hybridizations to establish homology are dependent upon such factors as salt concentration, temperature, the presence of organic solvents, and other parameters.

The term "knock-down", as used in this application, means to decrease the level of expression of mRNA, such that translation of mRNA to protein is diminished.

The term "knock-out", as defined in this application, means to prevent expression of mRNA, such that translation of mRNA to protein does not occur.

The term "mRNA", as used herein, means messenger RNA.

The term "membrane potential", as used herein, means the difference in electrical charge across both sides of a cell membrane.

The term "pain", as used in this application, means physical pain, such as an uncomfortable sensation in the body, ranging from slight uneasiness to extreme distress or torture, that is usually the result of disease, injury or stress; or mental pain, such as uneasiness of the mind, mental distress, anguish, anxiety or grief.

The term "RNAi", as used in this application, means RNA interference, which is a technique used to investigate gene function by degrading a specific mRNA target in a cell or organism, thus knocking-out or knocking-down the level of the encoded protein. This is also referred to as RNA silencing.

The term "sense strand", as used in this application, means the coding strand of a nucleic acid.

The term "siRNA", as used in this application, means either short or small interfering ribonucleic acid, which is one of the types of RNA silencing mechanisms of RNA interference.

The term "short interfering nucleic acid", as defined in this application, means short interfering stretches of either deoxyribonucleic acids (DNA), ribonucleic acids (RNA) or combinations of both. The term also includes modifications to the nucleic acids and non-traditional bases. Preferably, the short interfering nucleic acid is an siRNA.

The term "sodium current", as used herein, means the part of a cell's membrane potential that is due to the effects of sodium ions..,

The term "subject" means both human and non-human animals.

The term "transfect", as used in this application, means the introduction of a nucleic acid into a cell, such as through the use of a virus.

The term "transcription", as defined in this application, means the molecular biological process by which a single-stranded RNA is synthesized from a double-stranded DNA.

The term "translation", as used in this application, means the molecular biological process by which a polypeptide is synthesized from a mRNA.

The term "treatment", as defined herein, means therapeutic, prophylactic or suppressive measures for a disease or disorder leading to any clinically desirable or beneficial effect, including, but not limited to, alleviation of one or more symptoms, regression, slowing or cessation of progression of the disease or disorder.

$Na_v1.8$ Characterization

The $Na_v1.8$ sodium channel comprises an alpha subunit and at least one beta subunit. The nucleotide sequence of the complete open reading frame and the corresponding amino acid sequence of $Na_v1.8$ are known in the art. For example, both the nucleic acid and the amino acid sequence for rat $Na_v1.8$ may be found in SEQ ID NOs: 1 and 2, respectively, of United States Patent No. 6,451,554. The nucleic acid sequence and amino acid sequence for $Na_v1.8$ and its subunits may also be found in the GenBank® database, as shown in Table 1 below.

Table 1

| species | GenBank® No. for Nucleic Acid Sequence | GenBank® No. for Amino Acid Sequence |
|---|---|---|
| rat | NM 017247 | NP 058943 |
| human | AF 117907 | AAD 30863 |

The human $Na_v1.8$ gene has a high degree of homology, approximately 82%, with the rat $Na_v1.8$ gene. Therefore, human $Na_v1.8$ short interfering nucleic acids corresponding to rat $Na_v1.8$ short interfering nucleic acids that are capable of knock-down of the rat $Na_v1.8$ sodium channel are likely to be effective in the knock-down of the human $Na_v1.8$ sodium channel.

Nucleic Acids

Compositions and methods comprising short interfering nucleic acids targeted to $Na_v1.8$ mRNA are advantageously used to knock-down or knock-out expression of the $Na_v1.8$ sodium channel for the treatment of chronic pain. Specifically, the short interfering nucleic acids of the invention cause RNAi-mediated destruction of the $Na_v1.8$ mRNA.

The $Na_v1.8$ sodium channel is upregulated in the dorsal root ganglion in chronic pain states. Therefore, short interfering nucleic acid sequences capable of knocking-down or knocking-out the expression of $Na_v1.8$ mRNA, as well as $Na_v1.8$ function should be useful in blocking or treating chronic pain.

The present invention, therefore, provides isolated or recombinant short interfering nucleic acids. As used herein, the term "isolated" means a nucleic acid, such as an RNA or DNA molecule, or a mixed polymer, which is substantially separated from other components that are normally found in cells or in recombinant expression systems. These components include, but are not limited to, ribosomes, polymerases, serum components, and flanking genomic sequences. The term thus embraces a short interfering nucleic acid that has been removed from its naturally occurring environment, and includes recombinant or cloned short interfering nucleic acid isolates and chemically

synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule.

An isolated nucleic acid will generally be a homogeneous composition of molecules but may, in some embodiments, contain minor heterogeneity. Such heterogeneity is typically found at the ends of nucleic acid coding sequences or in regions not critical to a desired biological function or activity.

A "recombinant" short interfering nucleic acid is defined either by its method of production or structure. Some recombinant nucleic acids are thus made by the use of recombinant DNA techniques that involve human intervention, either in manipulation or selection. Others are made by fusing two fragments that are not naturally contiguous to each other. Engineered vectors are encompassed, as well as nucleic acids comprising sequences derived using any synthetic oligonucleotide process.

The short interfering nucleic acids may be either single-stranded or double-stranded. A single-stranded short interfering nucleic acid comprises a sense strand while a double-stranded short interfering nucleic acid comprises both a sense strand and an antisense strand. Preferably, the sense and antisense strands in the double-stranded short interfering nucleic acids hybridize by standard Watson-Crick base-pairing interactions to form a duplex or are connected by a single-stranded hairpin area. It is believed that the hairpin area of the latter type of siRNA molecule is cleaved intracellularly by the "Dicer" protein, or its equivalent, to form an siRNA of two individual base-paired RNA molecules.

The short interfering nucleic acids may range in length from 17 to 29 nucleotides, preferably 19 to 25 nucleotides, more preferably 21-23 nucleotides, and most preferably 21 nucleotides.

Preferably, the short interfering nucleic acid is an siRNA. That is, all of the nucleotides in the sequence are ribonucleotide bases.

However, the present invention also encompasses analogues of the small interfering nucleic acids. Analogues of short interfering nucleic acids contain additions, deletions, alterations, substitutions or modifications of one

or more nucleotide bases. For example, the short interfering nucleic acids can be altered, substituted or modified to contain one or more deoxyribonucleotide bases or non-traditional bases or any combination of these.

Preferably, one or both strands of a short interfering nucleic acid of the invention comprises a 3' overhang. As used herein, a "3' overhang" refers to at least one unpaired nucleotide extending from the 3'-end of a short interfering nucleic acid strand. The 3' overhang may range from 1 to 6 nucleotides (which includes ribonucleotides or deoxyribonucleotides), preferably from 1 to 5 nucleotides, more preferably from 1 to 4 nucleotides, particularly preferably from 2 to 4 nucleotides, and most preferably 2 nucleotides.

In another embodiment of the invention, both the sense and antisense strands of the duplex comprise 3' overhangs. The length of the overhangs can be the same or different for each strand of the duplex. Most preferably, a 3' overhang is present on both strands of the duplex, and the overhang for each strand is 2 nucleotides in length. For example, each strand of the duplex can comprise 3' overhangs of dithymidylic acid ("tt") or diuridylic acid ("uu").

In order to enhance the stability of the short interfering nucleic acids, the
3' overhangs can also be stabilized against degradation. In one embodiment, the 3' overhangs are stabilized by including purine nucleotides, such as adenosine or guanosine nucleotides. Alternatively, substitution of pyrimidine nucleotides by modified analogues, e.g., substitution of uridine nucleotides in the 3' overhangs with 2'-deoxythymidine, is tolerated and does not affect the efficiency of RNAi degradation. In particular, the absence of a 2' hydroxyl in the 2'-deoxythymidine significantly enhances the nuclease resistance of the 3' overhang in tissue culture medium.

The short interfering nucleic acids are targeted to a $Na_v1.8$ target mRNA. As used herein, short interfering nucleic acids that are "targeted to a $Na_v1.8$ target mRNA" means either a single-stranded or double-stranded short

interfering nucleic acid in which the sense strand has a nucleotide sequence that is either identical or substantially identical to that of a target mRNA and is capable of inducing RNAi-mediated degradation of the mRNA. Of course, the antisense strand of a double-stranded siRNA will have a sequence that is complementary to both the sense strand of the siRNA and the target mRNA.

As used herein, a short interfering nucleic acid that is "substantially identical" to a target sequence is a nucleic acid sequence that differs from the target sequence by 1-4 nucleotides. For example, a short interfering nucleic acid may comprise a sense strand that differs from a target sequence by one, two, three or four nucleotides, as long as RNAi-mediated degradation of the target mRNA is induced by the short interfering nucleic acid.

As used herein, "target mRNA" or "target sequence" means human $Na_v1.8$ mRNA, mutant or alternative splice forms of $Na_v1.8$ mRNA, or mRNA from cognate $Na_v1.8$ genes. The term "mutant", as used herein, means a short interfering nucleic acid that differs from the target mRNA by having a nucleotide insertion, nucleotide deletion, nucleotide substitution or nucleotide modification. Such alterations can include, for example, the: addition of non-nucleotide material, such as to the end(s) of the short interfering nucleic acids or to one or more internal nucleotides of the short interfering nucleic acids; modifications that make the short interfering nucleic acids resistant to nuclease digestion; or substitution of one or more nucleotides in the short interfering nucleic acids with deoxyribonucleotides. The term "cognate", as used herein, means a nucleic acid from another mammalian species. It is understood that human $Na_v1.8$ mRNA may contain target sequences in common with their respective cognates or mutants. A single short interfering nucleic acid comprising such a common targeting sequence can therefore induce RNAi-mediated degradation of different RNA types that contain the common targeting sequence.

The short interfering nucleic acid of the invention can be targeted to any stretch of approximately 19-25 contiguous nucleotides in any target mRNA sequence. Techniques for selecting target sequences for short interfering nucleic acids are known in the art. In addition, a target sequence

on the target mRNA can be selected from a given cDNA sequence corresponding to the target mRNA, preferably beginning 50 to 100 nucleotides downstream, *i.e.*, in the 3' direction, from the start codon. The target sequence can, however, be located in the 5' or 3' untranslated regions, or in the region nearby the start codon. Suitable target sequences in the $Na_v1.8$ cDNA sequence are given in Example 1.

The short interfering nucleic acid of the invention can comprise partially purified nucleic acid, substantially pure nucleic acid, synthetic nucleic acid or recombinantly produced nucleic acid. The term "substantially pure" is defined herein to mean a short interfering nucleic acid that is free from other contaminating proteins, nucleic acids, and other biologicals derived from an original source organism or recombinant DNA expression system. Purity may be assayed by standard methods and will typically exceed at least about 50%, preferably at least about 75%, more preferably at least about 90%, and most preferably at least about 95% purity. The purity evaluation may be made on a mass or molar basis.

The short interfering nucleic acids of the invention can be obtained using any one of a number of techniques known to those of skill in the art. In addition, the short interfering nucleic acids may also be synthesized as two separate, complementary nucleic acid molecules, or as a single nucleic acid molecule with two complementary regions. For example, the short interfering nucleic acids of the invention are chemically synthesized using appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA synthesizer or other well known methods. In addition, the short interfering nucleic acids may be produced by a commercial supplier, such as Proligo (Hamburg, Germany), Dharmacon Research (Lafayette, CO, USA), Pierce Chemical (part of Perbio Science, Rockford, IL, USA), Glen Research (Sterling, VA, USA), ChemGenes (Ashland, MA, USA) and Cruachem (Glasgow, UK).

Alternatively, short interfering nucleic acids may also be expressed from a recombinant expression vector, such as a circular or linear DNA plasmid, using any suitable promoter. Recombinant expression vectors are

typically self-replicating DNA or RNA constructs comprising nucleic acids encoding one of the short interfering nucleic acids, usually operably linked to suitable genetic control elements that are capable of regulating expression of the nucleic acids in compatible host cells. Genetic control elements may include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, a translation initiation site, a polyadenylation site, sequences that terminate transcription and translation. Expression vectors may also contain an origin of replication that allows the vector to replicate independently of the host cell, or a selection gene, such as a gene conferring resistance to an antibiotic.

Vectors that could be used in this invention include microbial plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles that may facilitate integration of the nucleic acids into the genome of the host. Plasmids are a commonly used form of vector, but all other forms of vectors that serve an equivalent function and which are, or become, known in the art are suitable for use herein. See, *e.g.*, Pouwels *et al.*, *Cloning Vectors: A Laboratory Manual,* 1985 and Supplements, Elsevier, N.Y., and Rodriguez *et al.* (eds.), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses,* 1988, Buttersworth, Boston, MA.

Suitable promoters for expressing short interfering nucleic acids of the invention from a plasmid include, for example, the U6 promoter, the H1 RNA pol III promoter, and the cytomegalovirus promoter. Selection of other suitable promoters is within the skill in the art. The recombinant plasmids of the invention may also comprise an inducible or regulatable promoter for expression of the short interfering nucleic acid in a particular tissue or in a particular intracellular environment.

The short interfering nucleic acid expressed from recombinant plasmids may either be isolated from cultured cell expression systems by standard techniques, or can be expressed intracellularly. The short interfering

nucleic acid of the invention can be expressed from a recombinant plasmid either as two separate, complementary RNA molecules, or as a single RNA molecule with two complementary regions. Selection of plasmids suitable for expressing short interfering nucleic acids of the invention, methods for inserting nucleic acid sequences for expressing the short interfering nucleic acids into the plasmid, and methods of delivering the recombinant plasmid to the cells of interest are within the skill in the art. See, for example, Tuschl, *Nat. Biotechnol*, vol. 20, pp. 446-448 (2002); Brummelkamp *et al.*, *Science*, vol. 296, pp. 550-553 (2002); Miyagishi *et al.*, *Nat. Biotechnol.*, vol. 20, ppl. 497-500 (2002); Paddison *et al.*, *Genes Dev.*, vol. 16, pp. 948-958 (2002); Lee *et al.*, *Nat. Biotechnol.*, vol. 20, pp. 500-505 (2002); Paul *et al.*, *Nat. Biotechnol.*, vol. 20, pp. 505-508 (2002); and Sui et al., *Proc. Natl. Acad. Sci.* vol 99, 2002, pp5515-5520).

By way of example, a plasmid may comprise a sense RNA strand coding sequence in operable connection with a polyT termination sequence under the control of a human U6 RNA promoter, and an antisense RNA strand coding sequence in operable connection with a polyT termination sequence under the control of a human U6 RNA promoter.

As used herein, "in operable connection with" means that the nucleic acid sequences encoding the sense or antisense strands are adjacent to another sequence. Preferably, the sequence encoding the sense or antisense strands are immediately adjacent to the poly T termination signal in the 5' direction. Therefore, during transcription of the sense or antisense sequences from the plasmid, the polyT termination signals act to terminate transcription.

As used herein, "under the control of a promoter" means that the nucleic acid sequences encoding the sense or antisense strands are located 3' of the promoter, so that the promoter can initiate transcription of the sense or antisense coding sequences.

Expression of the short interfering nucleic acids can be carried out by conventional methods in either prokaryotic or eukaryotic cells. Prokaryotes include both gram negative and positive organisms, *e.g.*, *E. coli* and *B.*

*subtilis*. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, *e.g.*, insect cells, and birds, and of mammalian origin, *e.g.*, human, primates, and rodents. Many suitable host cells are known in the art. Preferred host cells are HEK293 cells and the neuroblastoma/DRG cell line ND7/23.

The short interfering nucleic acids of the invention may also be expressed from recombinant viral vectors. The recombinant viral vectors of the invention comprise sequences encoding the short interfering nucleic acids of the invention and any suitable promoter for expressing the short interfering nucleic acid sequences. Suitable promoters for expressing short interfering nucleic acids from a viral vector include, for example, the U6 promoter, the H1 RNA pol III promoter, and the cytomegalovirus promoter. Selection of other suitable promoters is within the skill in the art. The recombinant viral vectors of the invention can also comprise inducible or regulatable promoters for expression of the short interfering nucleic acids in a particular tissue or in a particular intracellular environment. The use of recombinant viral vectors to deliver short interfering nucleic acids of the invention to cells *in vivo* is discussed in more detail in Example 5.

The short interfering nucleic acids of the invention can be expressed from a recombinant viral vector either as two separate, complementary nucleic acid molecules, or as a single nucleic acid molecule with two complementary regions.

Any viral vector capable of accepting the coding sequences for the short interfering nucleic acid molecule(s) to be expressed can be used, such as, vectors derived from adenovirus (AV), adeno-associated virus (AAV), retroviruses, herpes virus, and the like. In addition, the tropism of the viral vectors may also be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the short interfering nucleic acids into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example,

Domburg, *Gene Therap.*, vol. 2, pp. 301-310 (1995); Eglitis, *Biotechniques*, vol. 6, pp. 608-614 (1988); Miller, *Hum Gene Therap.*, vol. 1, pp. 5-14 (1990); and Anderson, *Nature*, vol. 392, pp. 25-30 (1998).

Preferred viral vectors are those derived from adenovirus and adeno-associated virus. In a particularly preferred embodiment, a short interfering nucleic acid of the invention is expressed as a single-stranded nucleic acid molecule from a recombinant adenoviral vector comprising the U6 promoter. Preferred viral vectors are also herpes viral vectors. See for e.g., Burton, E.A. et al., (2005) *Curr. Opin. Mol. Ther.*. Aug: 7(4):326-36 and Yeomans D.D. et al .(2005)- *Hum Gene Therap* Feb:16(2):271-7. By way of example, and not limitation, the expressed single stranded nucleic acid molecule can comprise two complementary regions connected by a single stranded hairpin area. The single stranded nucleic acid molecule can further comprise a 3' overhang.

***In Vitro* and *In Vivo* Methods**

The ability of a short interfering nucleic acid to cause RNAi-mediated degradation of the target mRNA can be evaluated using standard techniques for measuring the levels of RNA or protein in cells. For example, short interfering nucleic acids may be delivered to cultured cells, and the levels of target mRNA can be measured by Northern blot, dot-blotting techniques, or by quantitative RT-PCR. Alternatively, the levels of $Na_v1.8$ protein in the cultured cells can be measured by ELISA or Western blot. A suitable cell culture system for measuring the effect of the present short interfering nucleic acids on target mRNA or protein levels is described in Example 2 below.

As discussed above, the short interfering nucleic acids of the invention targets and causes the RNAi-mediated degradation of $Na_v1.8$ mRNA, or alternative splice forms, mutants or cognates thereof. Degradation of the target mRNA by the present short interfering nucleic acids reduces the production of a functional gene product from the $Na_v1.8$ gene. Thus, the invention provides a method of inhibiting expression of $Na_v1.8$ in a subject, a method for inhibiting translation of an mRNA, a method for inhibiting expression of a polypeptide, a method for blocking the $Na_v1.8$ derived

membrane potential in a cell, and a method for blocking the $Na_v1.8$ derived sodium current in a cell. In the methods of the invention, blocking includes, but is not limited to an abolition or reduction in the $Na_v1.8$ derived membrane potential or the $Na_v1.8$ derived sodium current Although these methods are more thoroughly detailed in the Examples, they all share a few common characterisitics.

A step of each of the above methods involves contacting a cell with a short interfering nucleic acid. *In vivo*, the contacting step involves administering a short interfering nucleic acid in a pharmaceutical composition to a subject. *In vitro*, the contacting step involves bringing the cell and short interfering nucleic acid into close physical proximity such that the cell and the short interfering nucleic acid may contact each other. This contacting step will allow the short interfering nucleic acid to enter the cell and cause RNAi-induced degradation of mRNA from the $Na_v1.8$ sodium channel gene.

Preferably, the contacting step utilizes the short interfering nucleic acids of SEQ ID NOs: 1-11. The short interfering nucleic acids of SEQ ID NOs: 1, 2, 3, 5, 10 and 11 are preferable. The short interfering nucleic acids of SEQ ID NOs: 2 and 5 are more preferable. The short interfering nucleic acids of SEQ ID NOs: 1 and 3 are the most preferable. One or more of the short interfering nucleic acids of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 can also be utilized in the methods of the invention. By way of example, and not limitation, one or more of the short interfering nucleic acids of SEQ ID NOs; 1, 2, 3, 5, 10 and 11 can be used in the methods of the invention. Furthermore, in the practice of the present methods, it is understood that more than one short interfering nucleic acids of the invention can be administered simultaneously or sequentially to a cell or to a subject. This invention further provides the short interfering nucleic acids of the invention complexed with one or more proteins and/or target nucleic acid and a cell comprising one or more short interfering nucleic acids of the invention.

Pharmaceutical Compositions

The short interfering nucleic acids and siRNAs of the present invention can be used therapeutically to treat chronic pain.. Various compounds of the present invention may be incorporated into pharmaceutical compositions. For example, a pharmaceutical composition may comprise a single-stranded short interfering nucleic acid, a single-stranded short interfering nucleic acid that has a 3' overhang, a double-stranded short interfering nucleic acid, or a double-stranded short interfering nucleic acid wherein each strand has a 3'overhang. Preferably, the pharmaceutical composition comprises the short interfering nucleic acids of SEQ ID NOs: 1-11. The short interfering nucleic acids of SEQ ID NOs: 2 and 5 are more preferable, while the short interfering nucleic acids of SEQ ID NOs: 1 and 3 are the most preferable. One or more of the short interfering nucleic acids of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 can also be utilized in the pharmaceutical compositions of the invention. By way of example, and not limitation, one or more of the short interfering nucleic acids of SEQ ID NOs; 1, 2, 3, 5, 10 and 11 can be used in the pharmaceutical compositions of the invention.

Typical protocols for the therapeutic administration of such substances are well known in the art. Although the compositions of the present invention may be administered in simple solution, they are more typically administered in combination with other materials, such as carriers, preferably pharmaceutical acceptable carriers. The term "pharmaceutically acceptable carrier" means any compatible, non-toxic substance that is suitable for delivering the compositions of the invention to a subject. For example, sterile water, alcohol, fats, waxes, and inert solids may be included in a carrier. Pharmaceutically acceptable adjuvants, such as buffering agents and dispersing agents, may also be incorporated into the pharmaceutical composition. Generally, compositions useful for parenteral administration of such drugs are well known; *e.g. Remington's Pharmaceutical Science*, 17th Ed. (Mack Publishing Company, Easton, PA, 1990).

Therapeutic formulations may be administered. Formulations typically comprise at least one active ingredient, together with one or more pharmaceutically acceptable carriers. Formulations may include those

suitable for oral, rectal, nasal, transdermal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, *e.g.*, Gilman *et al.* (eds.) (1990), *The Pharmacological Bases of Therapeutics*, 8th Ed., Pergamon Press; and *Remington's Pharmaceutical Sciences*, *supra*, Easton, Penn.; Avis *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications* Dekker, New York; Lieberman *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets* Dekker, New York; and Lieberman *et al.* (eds.) (1990), *Pharmaceutical Dosage Forms: Disperse Systems* Dekker, New York.

By way of example, any of the short interfering nucleic acids or vectors of the invention may be deliverable transdermally. The transdermal compositions may take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose. See, *e.g. Remington's Pharmaceutical Science*, 17th Ed. (Mack Publishing Company, Easton, PA, 1990).

The dosage regimen involved in a therapeutic application will be determined by the attending physician, considering various factors that may modify the action of the therapeutic substance, *e.g.*, the condition, body weight, sex and diet of the patient, the severity of any infection, time of administration, and other clinical factors. Often, treatment dosages are titrated upward from a low level to optimize safety and efficacy. Generally, daily dosages will fall within a range of 100-500 μg per kilogram of body weight. Typically, the dosage range will be 150-250 μg per kilogram of body weight. Preferably, the dosage will be 200 μg per kilogram of body weight. Dosages may be adjusted to account for the smaller molecular sizes and possibly decreased half-lives (clearance times) following administration. An "effective amount" of a composition of the invention is an amount that will ameliorate chronic pain in a subject.

Chronic pain may include one or more of the following characteristics: pain present for about three or more months, pain that is not fully relieved by routine medical management or pain that continues beyond a normal recovery period. Examples of chronic pain include, but are not limited to, chronic neuropathic pain and chronic inflammatory pain. Examples of chronic neuropathic and/or chronic inflammatory conditions, include, but are not limited to, post-herpetic neuralgia, painful diabetic neuropathy, radiculopathy, nerve compression injuries (e.g., carpal tunnel syndrome, trigeminal neuralgia, tumor-related nerve compressions), upper and low back pain (e.g., arising from disc herniation injuries, ankylosing spondylitis or cases of unknown pathology), complex regional pain syndromes types I and II, nerve trauma pain (e.g., phantom-limb pain, other painful conditions resulting from limb amputation), nerve-root avulsion injuries, HIV-associated pain, neuropathies arising from chemotherapeutic strategies, retinopathies, sciatica, hyperalgesia, hyperpathia and ongoing burning pain (e.g., wound-associated pain, including, but not limited to post-operative pain), joint pain, rheumatoid and osteoarthritis pain, fibromyalgia, burn pain, neuritis, sciatica, tendonitis, bone pain, migraine pain, urinogenital pain and neuropathic conditions attributed to bladder hyperreflexia.

## Examples

The present invention may be better understood by reference to the following non-limiting examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the invention, and should in no way be construed as limiting the broad scope of the invention. Unless otherwise indicated, percentages given below for solids in solid mixtures, liquids in liquids, and solids in liquids are on a wt/wt, vol/vol and wt/vol basis, respectively. Sterile conditions were generally maintained during cell culture.

### Materials and General Methods

Standard molecular biological methods were used, as described, *e.g.*, in Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, 1982, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, (2d ed.), Vols 1-3, 1989, Cold Spring Harbor Press, NY; Ausubel *et al.*, *Biology*, Greene Publishing Associates, Brooklyn, NY; or Ausubel, *et al.* (1987 and Supplements), *Current Protocols in Molecular Biology*, Greene/Wiley, New York; and Innis *et al.* (eds.) *PCR Protocols: A Guide to Methods and Applications*, 1990, Academic Press, N.Y.

Example 1

This example illustrates the design of siRNAs against $Na_v1.8$. Putative siRNA sequences against both rat and human $Na_v1.8$ coding sequences were identified using Tuschl's prediction rules. See Tuschl *et al.*, "Targeted mRNA degradation by double-stranded RNA in vitro", *Genes Dev.*, vol. 13, no. 24, pp. 3191-3197 (Dec. 15, 1999); and Elbashir *et al.*, "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells", *Nature*, vol. 411, pp. 494-498 (2001). Table 2 identifies putative siRNA sequences against the human $Na_v1.8$ coding sequence. Also shown are the target sequences, the position of each target sequence in the gene, and the percentage of guanine/cytosine in the target sequence. Table 3 identifies putative siRNA sequences against the rat $Na_v1.8$ coding sequence. Also shown are the target sequences and the position of each target sequence in the gene.

Table 2

Human PN3 siRNAs

| Target | Target sequence | position in gene | % GC |
|---|---|---|---|
| 1 | AATTCCCCATTGGATCCCTCG (SEQ ID NO: 12) | 5 | 52.4 |
| 2 | AAACTAACAACTTCCGTCGCT (SEQ ID NO: 13) | 26 | 42.9 |
| 3 | AACAACTTCCGTCGCTTTACT (SEQ ID NO: 14) | 31 | 42.9 |
| 4 | AACTTCCGTCGCTTTACTCCG (SEQ ID NO: 15) | 34 | 52.4 |
| 5 | AAGCAAATTGCTGCCAAGCAG (SEQ ID NO: 16) | 76 | 47.6 |

| | | | |
|---|---|---|---|
| 6 | AAATTGCTGCCAAGCAGGGAA (SEQ ID NO: 17) | 80 | 47.6 |
| 7 | AAGCAGGGAACAAAGAAAGCC (SEQ ID NO: 18) | 91 | 47.6 |
| 8 | AACAAAGAAAGCCAGAGAGAA (SEQ ID NO: 19) | 99 | 38.1 |
| 9 | AAAGAAAGCCAGAGAGAAGCA (SEQ ID NO: 20) | 102 | 42.9 |
| 10 | AAAGCCAGAGAGAAGCATAGG (SEQ ID NO: 21) | 106 | 47.6 |
| 11 | AAGCATAGGGAGCAGAAGGAC (SEQ ID NO: 22) | 118 | 52.4 |
| 12 | AAGGACCAAGAAGAGAAGCCT (SEQ ID NO: 23) | 133 | 47.6 |
| 13 | AAGAAGAGAAGCCTCGGCCCC (SEQ ID NO: 24) | 140 | 61.9 |
| 14 | AAGAGAAGCCTCGGCCCCAGC (SEQ ID NO: 25) | 143 | 66.7 |
| 15 | AAGCCTCGGCCCCAGCTGGAC (SEQ ID NO: 26) | 148 | 71.4 |
| 16 | AAAGCCTGCAACCAGCTGCCC (SEQ ID NO: 27) | 172 | 61.9 |
| 17 | AACCAGCTGCCCAAGTTCTAT (SEQ ID NO: 28) | 181 | 47.6 |
| 18 | AAGTTCTATGGTGAGCTCCCA (SEQ ID NO: 29) | 193 | 47.6 |
| 19 | AACTGATCGGGGAGCCCCTGG (SEQ ID NO: 30) | 218 | 66.7 |
| 20 | AACAAAGGGAGGACCATTTCC (SEQ ID NO: 31) | 286 | 47.6 |
| 21 | AAAGGGAGGACCATTTCCCGG (SEQ ID NO: 32) | 289 | 57.1 |
| 22 | AACCTGATCAGAAGAACGGCC (SEQ ID NO: 33) | 349 | 52.4 |
| 23 | AAGAACGGCCATCAAAGTGTC (SEQ ID NO: 34) | 360 | 47.6 |
| 24 | AACGGCCATCAAAGTGTCTGT (SEQ ID NO: 35) | 363 | 47.6 |
| 25 | AAAGTGTCTGTCCACTCGTGG (SEQ ID NO: 36) | 373 | 52.4 |
| 26 | AATTGTGTGTGCATGACCCGA (SEQ ID NO: 37) | 427 | 47.6 |
| 27 | AACTGACCTTCCAGAGAAAAT (SEQ ID NO: 38) | 447 | 38.1 |
| 28 | AAAATTGAATATGTCTTCACT (SEQ ID NO: 39) | 463 | 23.8 |
| 29 | AATTGAATATGTCTTCACTGT (SEQ ID NO: 40) | 465 | 28.6 |
| 30 | AATATGTCTTCACTGTCATTT (SEQ ID NO: 41) | 470 | 28.6 |
| 31 | AAGCCTTGATAAAGATACTGG (SEQ ID NO: 42) | 500 | 38.1 |
| 32 | AAAGATACTGGCAAGAGGATT (SEQ ID NO: 43) | 510 | 38.1 |
| 33 | AAGAGGATTTTGTCTAAATGA (SEQ ID NO: 44) | 522 | 28.6 |
| 34 | AAATGAGTTCACGTACCTGAG (SEQ ID NO: 45) | 537 | 42.9 |
| 35 | AACTGGCTGGATTTTAGCGTC (SEQ ID NO: 46) | 568 | 47.6 |
| 36 | AATAGATCTCCGTGGGATCTC (SEQ ID NO: 47) | 615 | 47.6 |
| 37 | AAAAACAGTTTCTGTGATCCC (SEQ ID NO: 48) | 669 | 38.1 |
| 38 | AAACAGTTTCTGTGATCCCAG (SEQ ID NO: 49) | 671 | 42.9 |
| 39 | AAGGTCATTGTGGGGGCCCTG (SEQ ID NO: 50) | 697 | 61.9 |
| 40 | AAGAAACTGGCTGATGTGACC (SEQ ID NO: 51) | 730 | 47.6 |
| 41 | AAACTGGCTGATGTGACCATC (SEQ ID NO: 52) | 733 | 47.6 |
| 42 | AAGTGTTTTTGCCTTGGTGGG (SEQ ID NO: 53) | 771 | 47.6 |
| 43 | AACTCTTCAAGGGCAACCTCA (SEQ ID NO: 54) | 797 | 47.6 |
| 44 | AAGGGCAACCTCAAAAATAAA (SEQ ID NO: 55) | 805 | 33.3 |
| 45 | AACCTCAAAAATAAATGTGTC (SEQ ID NO: 56) | 811 | 28.6 |
| 46 | AAAAATAAATGTGTCAAGAAT (SEQ ID NO: 57) | 817 | 19 |
| 47 | AAATAAATGTGTCAAGAATGA (SEQ ID NO: 58) | 819 | 23.8 |
| 48 | AAATGTGTCAAGAATGACATG (SEQ ID NO: 59) | 823 | 33.3 |
| 49 | AAGAATGACATGGCTGTCAAT (SEQ ID NO: 60) | 832 | 38.1 |
| 50 | AATGACATGGCTGTCAATGAG (SEQ ID NO: 61) | 835 | 42.9 |
| 51 | AATGAGACAACCAACTACTCA (SEQ ID NO: 62) | 850 | 38.1 |
| 52 | AACCAACTACTCATCTCACAG (SEQ ID NO: 63) | 858 | 42.9 |
| 53 | AACTACTCATCTCACAGAAAA (SEQ ID NO: 64) | 862 | 33.3 |
| 54 | AAAACCAGATATCTACATAAA (SEQ ID NO: 65) | 879 | 23.8 |
| 55 | AACCAGATATCTACATAAATA (SEQ ID NO: 66) | 881 | 23.8 |
| 56 | AAATAAGCGAGGCACTTCTGA (SEQ ID NO: 67) | 897 | 42.9 |

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|---|---|---|---|
| 57 | AAGCGAGGCACTTCTGACCCC (SEQ ID NO: 68) | 901 | 61.9 |
| 58 | AATGGATCTGACTCAGGCCAC (SEQ ID NO: 69) | 934 | 52.4 |
| 59 | AAAACTTCTGACAACCCGGAT (SEQ ID NO: 70) | 979 | 42.9 |
| 60 | AACTTCTGACAACCCGGATTT (SEQ ID NO: 71) | 981 | 42.9 |
| 61 | AACCCGGATTTTAACTACACC (SEQ ID NO: 72) | 991 | 42.9 |
| 62 | AACTACACCAGCTTTGATTCC (SEQ ID NO: 73) | 1003 | 42.9 |
| 63 | AACGCCTCTACCAGCAGACCC (SEQ ID NO: 74) | 1076 | 61.9 |
| 64 | AAAATCTATATGATCTTTTTT (SEQ ID NO: 75) | 1111 | 14.3 |
| 65 | AATCTATATGATCTTTTTTGT (SEQ ID NO: 76) | 1113 | 19 |
| 66 | AATCTTCCTGGGATCTTTCTA (SEQ ID NO: 77) | 1140 | 38.1 |
| 67 | AACTTGATCTTGGCTGTAGTC (SEQ ID NO: 78) | 1168 | 42.9 |
| 68 | AACCAGGCAACCACTGATGAA (SEQ ID NO: 79) | 1210 | 47.6 |
| 69 | AACCACTGATGAAATTGAAGC (SEQ ID NO: 80) | 1218 | 38.1 |
| 70 | AAATTGAAGCAAAGGAGAAGA (SEQ ID NO: 81) | 1229 | 33.3 |
| 71 | AAGCAAAGGAGAAGAAGTTCC (SEQ ID NO: 82) | 1235 | 42.9 |
| 72 | AAAGGAGAAGAAGTTCCAGGA (SEQ ID NO: 83) | 1239 | 42.9 |
| 73 | AAGAAGTTCCAGGAGGCCCTC (SEQ ID NO: 84) | 1246 | 57.1 |
| 74 | AAGTTCCAGGAGGCCCTCGAG (SEQ ID NO: 85) | 1249 | 61.9 |
| 75 | AAGGAGCAGGAGGTGCTAGCA (SEQ ID NO: 86) | 1279 | 57.1 |
| 76 | AACCTCTCTCCACTCCCACAA (SEQ ID NO: 87) | 1317 | 52.4 |
| 77 | AATGGATCACCTTTAACCTCC (SEQ ID NO: 88) | 1336 | 42.9 |
| 78 | AACCTCCAAAAATGCCAGTGA (SEQ ID NO: 89) | 1350 | 42.9 |
| 79 | AAAAATGCCAGTGAGAGAAGG (SEQ ID NO: 90) | 1357 | 42.9 |
| 80 | AAATGCCAGTGAGAGAAGGCA (SEQ ID NO: 91) | 1359 | 47.6 |
| 81 | AAGGCATAGAATAAAGCCAAG (SEQ ID NO: 92) | 1374 | 38.1 |
| 82 | AATAAAGCCAAGAGTGTCAGA (SEQ ID NO: 93) | 1383 | 38.1 |
| 83 | AAAGCCAAGAGTGTCAGAGGG (SEQ ID NO: 94) | 1386 | 52.4 |
| 84 | AAGAGTGTCAGAGGGCTCCAC (SEQ ID NO: 95) | 1392 | 57.1 |
| 85 | AAGACAACAAATCACCCCGCT (SEQ ID NO: 96) | 1415 | 47.6 |
| 86 | AACAAATCACCCCGCTCTGAT (SEQ ID NO: 97) | 1420 | 47.6 |
| 87 | AAATCACCCCGCTCTGATCCT (SEQ ID NO: 98) | 1423 | 52.4 |
| 88 | AACCAGCGCAGGATGTCTTTT (SEQ ID NO: 99) | 1447 | 47.6 |
| 89 | AAAACGCCGGGCTAGTCATGG (SEQ ID NO: 100) | 1485 | 57.1 |
| 90 | AACGCCGGGCTAGTCATGGCA (SEQ ID NO: 101) | 1487 | 61.9 |
| 91 | AAAGCCATCGGGGCTCTCTGC (SEQ ID NO: 102) | 1592 | 61.9 |
| 92 | AAGGCCCCCTCCCTAGAAGCC (SEQ ID NO: 103) | 1637 | 66.7 |
| 93 | AAGCCCTCTTCCTCAACCCAG (SEQ ID NO: 104) | 1653 | 57.1 |
| 94 | AACCCAGCAACCCTGACTCCA (SEQ ID NO: 105) | 1667 | 57.1 |
| 95 | AACCCTGACTCCAGGCATGGA (SEQ ID NO: 106) | 1675 | 57.1 |
| 96 | AAGATGAACACCAACCGCCGC (SEQ ID NO: 107) | 1697 | 57.1 |
| 97 | AACACCAACCGCCGCCCACTA (SEQ ID NO: 108) | 1703 | 61.9 |
| 98 | AACCGCCGCCCACTAGTGAGC (SEQ ID NO: 109) | 1709 | 66.7 |
| 99 | AAAAGAAGACTTTCTTGTCAG (SEQ ID NO: 110) | 1772 | 33.3 |
| 100 | AAGAAGACTTTCTTGTCAGCA (SEQ ID NO: 111) | 1774 | 38.1 |
| 101 | AAGACTTTCTTGTCAGCAGAA (SEQ ID NO: 112) | 1777 | 38.1 |
| 102 | AATACTTAGATGAACCTTTCC (SEQ ID NO: 113) | 1796 | 33.3 |
| 103 | AACCTTTCCGGGCCCAAAGGG (SEQ ID NO: 114) | 1808 | 61.9 |
| 104 | AAAGGGCAATGAGTGTTGTCA (SEQ ID NO: 115) | 1823 | 42.9 |
| 105 | AATGAGTGTTGTCAGTATCAT (SEQ ID NO: 116) | 1830 | 33.3 |
| 106 | AACCTCCGTCCTTGAGGAACT (SEQ ID NO: 117) | 1851 | 52.4 |
| 107 | AACTCGAGGAGTCTGAACAGA (SEQ ID NO: 118) | 1868 | 47.6 |

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|---|---|---|---|
| 108 | AACAGAAGTGCCCACCCTGCT (SEQ ID NO: 119) | 1883 | 57.1 |
| 109 | AAGTGCCCACCCTGCTTGACC (SEQ ID NO: 120) | 1888 | 61.9 |
| 110 | AAGTATCTGATCTGGGATTGC (SEQ ID NO: 121) | 1921 | 42.9 |
| 111 | AAGCTCAAGACAATTCTCTTT (SEQ ID NO: 122) | 1957 | 33.3 |
| 112 | AAGACAATTCTCTTTGGGCTT (SEQ ID NO: 123) | 1963 | 38.1 |
| 113 | AATTCTCTTTGGGCTTGTGAC (SEQ ID NO: 124) | 1968 | 42.9 |
| 114 | AACACCATCTTCATGGCCATG (SEQ ID NO: 125) | 2032 | 47.6 |
| 115 | AAGCCATGCTCCAGATAGGCA (SEQ ID NO: 126) | 2081 | 52.4 |
| 116 | AACATCGTCTTTACCATATTT (SEQ ID NO: 127) | 2101 | 28.6 |
| 117 | AAATGGTCTTCAAAATCATTG (SEQ ID NO: 128) | 2132 | 28.6 |
| 118 | AAAATCATTGCCTTCGACCCA (SEQ ID NO: 129) | 2143 | 42.9 |
| 119 | AATCATTGCCTTCGACCCATA (SEQ ID NO: 130) | 2145 | 42.9 |
| 120 | AAGAAGTGGAATATCTTTGAC (SEQ ID NO: 131) | 2179 | 33.3 |
| 121 | AAGTGGAATATCTTTGACTGC (SEQ ID NO: 132) | 2182 | 38.1 |
| 122 | AATATCTTTGACTGCATCATC (SEQ ID NO: 133) | 2188 | 33.3 |
| 123 | AAGAAGGGAAGCCTGTCTGTG (SEQ ID NO: 134) | 2242 | 52.4 |
| 124 | AAGGGAAGCCTGTCTGTGCTG (SEQ ID NO: 135) | 2245 | 57.1 |
| 125 | AAGCCTGTCTGTGCTGCGGAG (SEQ ID NO: 136) | 2250 | 61.9 |
| 126 | AAGCTGGCCAAATCCTGGCCC (SEQ ID NO: 137) | 2293 | 61.9 |
| 127 | AAATCCTGGCCCACCTTAAAC (SEQ ID NO: 138) | 2302 | 47.6 |
| 128 | AAACACACTCATCAAGATCAT (SEQ ID NO: 139) | 2319 | 33.3 |
| 129 | AAGATCATCGGAAACTCAGTG (SEQ ID NO: 140) | 2332 | 42.9 |
| 130 | AAACTCAGTGGGGGCACTGGG (SEQ ID NO: 141) | 2343 | 61.9 |
| 131 | AACCTCACCATCATCCTGGCC (SEQ ID NO: 142) | 2365 | 57.1 |
| 132 | AAGCAGCTCCTAGGGGAAAAC (SEQ ID NO: 143) | 2416 | 52.4 |
| 133 | AAAACTACCGTAACAACCGAA (SEQ ID NO: 144) | 2432 | 38.1 |
| 134 | AACTACCGTAACAACCGAAAA (SEQ ID NO: 145) | 2434 | 38.1 |
| 135 | AACAACCGAAAAAATATCTCC (SEQ ID NO: 146) | 2443 | 33.3 |
| 136 | AACCGAAAAAATATCTCCGCG (SEQ ID NO: 147) | 2446 | 42.9 |
| 137 | AAAAAATATCTCCGCGCCCCA (SEQ ID NO: 148) | 2451 | 47.6 |
| 138 | AAAATATCTCCGCGCCCCATG (SEQ ID NO: 149) | 2453 | 52.4 |
| 139 | AATATCTCCGCGCCCCATGAA (SEQ ID NO: 150) | 2455 | 52.4 |
| 140 | AAGACTGGCCCCGCTGGCACA (SEQ ID NO: 151) | 2474 | 66.7 |
| 141 | AACATGTGGGCCTGCATGGAA (SEQ ID NO: 152) | 2557 | 52.4 |
| 142 | AAGTTGGCCAAAAATCCATAT (SEQ ID NO: 153) | 2576 | 33.3 |
| 143 | AAAAATCCATATGCCTCATCC (SEQ ID NO: 154) | 2585 | 38.1 |
| 144 | AAATCCATATGCCTCATCCTT (SEQ ID NO: 155) | 2587 | 38.1 |
| 145 | AACCTGGTGGTGCTTAACCTG (SEQ ID NO: 156) | 2632 | 52.4 |
| 146 | AACCTGTTCATCGCCCTGCTA (SEQ ID NO: 157) | 2647 | 52.4 |
| 147 | AACTCTTTCAGTGCTGACAAC (SEQ ID NO: 158) | 2671 | 42.9 |
| 148 | AACCTCACAGCCCCGGAGGAC (SEQ ID NO: 159) | 2689 | 66.7 |
| 149 | AACAACCTGCAGGTGGCCCTG (SEQ ID NO: 160) | 2722 | 61.9 |
| 150 | AACCTGCAGGTGGCCCTGGCA (SEQ ID NO: 161) | 2725 | 66.7 |
| 151 | AAACAGGCTCTTTGCAGCTTC (SEQ ID NO: 162) | 2773 | 47.6 |
| 152 | AAGGCAGAGCCTGAGCTGGTG (SEQ ID NO: 163) | 2824 | 61.9 |
| 153 | AAACTCCCACTCTCCAGCTCC (SEQ ID NO: 164) | 2848 | 57.1 |
| 154 | AAGGCTGAGAACCACATTGCT (SEQ ID NO: 165) | 2869 | 47.6 |
| 155 | AACCACATTGCTGCCAACACT (SEQ ID NO: 166) | 2878 | 47.6 |
| 156 | AACACTGCCAGGGGGAGCTCT (SEQ ID NO: 167) | 2893 | 61.9 |
| 157 | AAGCTCCCAGAGGCCCCAGGG (SEQ ID NO: 168) | 2924 | 71.4 |
| 158 | AATCCGACTGTGTGGGTCTCT (SEQ ID NO: 169) | 2968 | 52.4 |

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|---|---|---|---|
| 159 | AATCTGATCTTGATGACTTGG (SEQ ID NO: 170) | 3008 | 38.1 |
| 160 | AAGATGCTCAGAGCTTCCAGC (SEQ ID NO: 171) | 3044 | 52.4 |
| 161 | AAGTGATCCCCAAAGGACAGC (SEQ ID NO: 172) | 3068 | 52.4 |
| 162 | AAAGGACAGCAGGAGCAGCTG (SEQ ID NO: 173) | 3079 | 57.1 |
| 163 | AAGTCGAGAGGTGTGGGGACC (SEQ ID NO: 174) | 3104 | 61.9 |
| 164 | AACATCTTCTGAGGACCTGGC (SEQ ID NO: 175) | 3153 | 52.4 |
| 165 | AAAGATGAGTCTGTTCCTCAG (SEQ ID NO: 176) | 3196 | 42.9 |
| 166 | AAGCTCCTCTGAGGGCAGCAC (SEQ ID NO: 177) | 3243 | 61.9 |
| 167 | AAATCCTGAGGAAGATCCCTG (SEQ ID NO: 178) | 3287 | 47.6 |
| 168 | AAGATCCCTGAGCTGGCAGAT (SEQ ID NO: 179) | 3298 | 52.4 |
| 169 | AAGAACCAGATGACTGCTTCA (SEQ ID NO: 180) | 3326 | 42.9 |
| 170 | AACCAGATGACTGCTTCACAG (SEQ ID NO: 181) | 3329 | 47.6 |
| 171 | AAGGATGCATTCGCCACTGTC (SEQ ID NO: 182) | 3350 | 52.4 |
| 172 | AAACTGGATACCACCAAGAGT (SEQ ID NO: 183) | 3379 | 42.9 |
| 173 | AAGAGTCCATGGGATGTGGGC (SEQ ID NO: 184) | 3394 | 57.1 |
| 174 | AAGACTTGCTACCGTATCGTG (SEQ ID NO: 185) | 3427 | 47.6 |
| 175 | AAGACTATTACCTGGACCAGA (SEQ ID NO: 186) | 3515 | 42.9 |
| 176 | AAGCCCACGGTGAAAGCTTTG (SEQ ID NO: 187) | 3535 | 52.4 |
| 177 | AAAGCTTTGCTGGAGTACACT (SEQ ID NO: 188) | 3547 | 42.9 |
| 178 | AAGTGGGTGGCCTATGGCTTC (SEQ ID NO: 189) | 3610 | 57.1 |
| 179 | AAAAAGTACTTCACCAATGCC (SEQ ID NO: 190) | 3631 | 38.1 |
| 180 | AAAGTACTTCACCAATGCCTG (SEQ ID NO: 191) | 3633 | 42.9 |
| 181 | AATGCCTGGTGCTGGCTGGAC (SEQ ID NO: 192) | 3646 | 61.9 |
| 182 | AATATCTCACTGATAAGTCTC (SEQ ID NO: 193) | 3679 | 33.3 |
| 183 | AAGTCTCACAGCGAAGATTCT (SEQ ID NO: 194) | 3693 | 42.9 |
| 184 | AAGATTCTGGAATATTCTGAA (SEQ ID NO: 195) | 3706 | 28.6 |
| 185 | AATATTCTGAAGTGGCTCCCA (SEQ ID NO: 196) | 3716 | 42.9 |
| 186 | AAGTGGCTCCCATCAAAGCCC (SEQ ID NO: 197) | 3725 | 57.1 |
| 187 | AAAGCCCTTCGAACCCTTCGC (SEQ ID NO: 198) | 3739 | 57.1 |
| 188 | AACCCTTCGCGCTCTGCGGCC (SEQ ID NO: 199) | 3750 | 71.4 |
| 189 | AAGGCATGCGGGTGGTGGTGG (SEQ ID NO: 200) | 3794 | 66.7 |
| 190 | AATGTCCTCCTCGTCTGCCTC (SEQ ID NO: 201) | 3847 | 57.1 |
| 191 | AACCTCTTCGCAGGGAAGTTT (SEQ ID NO: 202) | 3901 | 47.6 |
| 192 | AAGTTTTGGAGGTGCATCAAC (SEQ ID NO: 203) | 3916 | 42.9 |
| 193 | AACTATACCGATGGAGAGTTT (SEQ ID NO: 204) | 3934 | 38.1 |
| 194 | AATAACAAGTCTGACTGCAAG (SEQ ID NO: 205) | 3979 | 38.1 |
| 195 | AACAAGTCTGACTGCAAGATT (SEQ ID NO: 206) | 3982 | 38.1 |
| 196 | AAGTCTGACTGCAAGATTCAA (SEQ ID NO: 207) | 3985 | 38.1 |
| 197 | AAGATTCAAAACTCCACTGGC (SEQ ID NO: 208) | 3997 | 42.9 |
| 198 | AAAACTCCACTGGCAGCTTCT (SEQ ID NO: 209) | 4004 | 47.6 |
| 199 | AACTCCACTGGCAGCTTCTTC (SEQ ID NO: 210) | 4006 | 52.4 |
| 200 | AATGTGAAAGTCAACTTTGAT (SEQ ID NO: 211) | 4033 | 28.6 |
| 201 | AAAGTCAACTTTGATAATGTT (SEQ ID NO: 212) | 4039 | 23.8 |
| 202 | AACTTTGATAATGTTGCAATG (SEQ ID NO: 213) | 4045 | 28.6 |
| 203 | AATGTTGCAATGGGTTACCTT (SEQ ID NO: 214) | 4054 | 38.1 |
| 204 | AATGGGTTACCTTGCACTTCT (SEQ ID NO: 215) | 4062 | 42.9 |
| 205 | AACCTTTAAAGGCTGGATGGA (SEQ ID NO: 216) | 4092 | 42.9 |
| 206 | AAAGGCTGGATGGACATTATG (SEQ ID NO: 217) | 4099 | 42.9 |
| 207 | AACATGCAACCCAAGTGGGAG (SEQ ID NO: 218) | 4147 | 52.4 |
| 208 | AACCCAAGTGGGAGGACAACG (SEQ ID NO: 219) | 4154 | 57.1 |
| 209 | AAGTGGGAGGACAACGTGTAC (SEQ ID NO: 220) | 4159 | 52.4 |

| | | | |
|---|---|---|---|
| 210 | AACGTGTACATGTATTTGTAC (SEQ ID NO: 221) | 4171 | 33.3 |
| 211 | AATCTCTTTGTTGGGGTCATA (SEQ ID NO: 222) | 4231 | 38.1 |
| 212 | AATTGACAACTTCAATCAACA (SEQ ID NO: 223) | 4251 | 28.6 |
| 213 | AACTTCAATCAACAGAAAAAA (SEQ ID NO: 224) | 4258 | 23.8 |
| 214 | AATCAACAGAAAAAAAAGTTA (SEQ ID NO: 225) | 4264 | 19 |
| 215 | AACAGAAAAAAAAGTTAGGGG (SEQ ID NO: 226) | 4268 | 33.3 |
| 216 | AAAAAAAAGTTAGGGGGCCAG (SEQ ID NO: 227) | 4273 | 42.9 |
| 217 | AAAAAAGTTAGGGGGCCAGGA (SEQ ID NO: 228) | 4275 | 47.6 |
| 218 | AAAAGTTAGGGGGCCAGGACA (SEQ ID NO: 229) | 4277 | 52.4 |
| 219 | AAGTTAGGGGGCCAGGACATC (SEQ ID NO: 230) | 4279 | 57.1 |
| 220 | AAGAAATACTACAATGCCATG (SEQ ID NO: 231) | 4318 | 33.3 |
| 221 | AAATACTACAATGCCATGAAG (SEQ ID NO: 232) | 4321 | 33.3 |
| 222 | AATGCCATGAAGAAGTTGGGC (SEQ ID NO: 233) | 4330 | 47.6 |
| 223 | AAGAAGTTGGGCTCCAAGAAG (SEQ ID NO: 234) | 4339 | 47.6 |
| 224 | AAGTTGGGCTCCAAGAAGCCC (SEQ ID NO: 235) | 4342 | 57.1 |
| 225 | AAGAAGCCCCAGAAGCCCATC (SEQ ID NO: 236) | 4354 | 57.1 |
| 226 | AAGCCCCAGAAGCCCATCCCA (SEQ ID NO: 237) | 4357 | 61.9 |
| 227 | AAGCCCATCCCACGGCCCCTG (SEQ ID NO: 238) | 4366 | 71.4 |
| 228 | AACAAGTTCCAGGGTTTTGTC (SEQ ID NO: 239) | 4387 | 42.9 |
| 229 | AAGTTCCAGGGTTTTGTCTTT (SEQ ID NO: 240) | 4390 | 38.1 |
| 230 | AAGCTTTTGACATCACCATCA (SEQ ID NO: 241) | 4427 | 38.1 |
| 231 | AACATGATCACCATGATGGTG (SEQ ID NO: 242) | 4465 | 42.9 |
| 232 | AAAGTGAAGAAAAGACGAAAA (SEQ ID NO: 243) | 4499 | 28.6 |
| 233 | AAGAAAAGACGAAAATTCTGG (SEQ ID NO: 244) | 4505 | 33.3 |
| 234 | AAAAGACGAAAATTCTGGGCA (SEQ ID NO: 245) | 4508 | 38.1 |
| 235 | AAGACGAAAATTCTGGGCAAA (SEQ ID NO: 246) | 4510 | 38.1 |
| 236 | AAAATTCTGGGCAAAATCAAC (SEQ ID NO: 247) | 4516 | 33.3 |
| 237 | AATTCTGGGCAAAATCAACCA (SEQ ID NO: 248) | 4518 | 38.1 |
| 238 | AAAATCAACCAGTTCTTTGTG (SEQ ID NO: 249) | 4528 | 33.3 |
| 239 | AATCAACCAGTTCTTTGTGGC (SEQ ID NO: 250) | 4530 | 42.9 |
| 240 | AACCAGTTCTTTGTGGCCGTC (SEQ ID NO: 251) | 4534 | 52.4 |
| 241 | AATGTGTCATGAAGATGTTCG (SEQ ID NO: 252) | 4565 | 38.1 |
| 242 | AAGATGTTCGCTTTGAGGCAG (SEQ ID NO: 253) | 4576 | 47.6 |
| 243 | AAATGGCTGGAATGTGTTTGA (SEQ ID NO: 254) | 4608 | 38.1 |
| 244 | AATGTGTTTGACTTCATTGTG (SEQ ID NO: 255) | 4618 | 33.3 |
| 245 | AATTCTTAAGTCACTTCAAAG (SEQ ID NO: 256) | 4674 | 28.6 |
| 246 | AAGTCACTTCAAAGTTACTTC (SEQ ID NO: 257) | 4681 | 33.3 |
| 247 | AAAGTTACTTCTCCCCAACGC (SEQ ID NO: 258) | 4691 | 47.6 |
| 248 | AACGCTCTTCAGAGTCATCCG (SEQ ID NO: 259) | 4707 | 52.4 |
| 249 | AATTGGCCGCATCCTCAGACT (SEQ ID NO: 260) | 4737 | 52.4 |
| 250 | AAGGGGATCCGCACACTGCTC (SEQ ID NO: 261) | 4771 | 61.9 |
| 251 | AACATCGGGCTGTTGCTATTC (SEQ ID NO: 262) | 4825 | 47.6 |
| 252 | AACTTCCAGACCTTCGCCAAC (SEQ ID NO: 263) | 4927 | 52.4 |
| 253 | AACAGCATGCTGTGCCTCTTC (SEQ ID NO: 264) | 4945 | 52.4 |
| 254 | AACACAGGGCCCCCCTACTGT (SEQ ID NO: 265) | 5014 | 61.9 |
| 255 | AATCTGCCCAACAGCAATGGC (SEQ ID NO: 266) | 5041 | 52.4 |
| 256 | AACAGCAATGGCACCAGAGGG (SEQ ID NO: 267) | 5050 | 57.1 |
| 257 | AATGGCACCAGAGGGGACTGT (SEQ ID NO: 268) | 5056 | 57.1 |
| 258 | AACATGTACATTGCAGTGATT (SEQ ID NO: 269) | 5143 | 33.3 |
| 259 | AACTTCAATGTGGCCACGGAG (SEQ ID NO: 270) | 5170 | 52.4 |
| 260 | AATGTGGCCACGGAGGAGAGC (SEQ ID NO: 271) | 5176 | 61.9 |

| | | | |
|---|---|---|---|
| 261 | AAGTTTGACCCAGAGGCCACT (SEQ ID NO: 272) | 5248 | 52.4 |
| 262 | AATCCCAAAACCCAATCGAAA (SEQ ID NO: 273) | 5328 | 38.1 |
| 263 | AAAACCCAATCGAAATATACT (SEQ ID NO: 274) | 5334 | 28.6 |
| 264 | AACCCAATCGAAATATACTGA (SEQ ID NO: 275) | 5336 | 33.3 |
| 265 | AATCGAAATATACTGATCCAG (SEQ ID NO: 276) | 5341 | 33.3 |
| 266 | AAATATACTGATCCAGATGGA (SEQ ID NO: 277) | 5346 | 33.3 |
| 267 | AAGATCCACTGCTTGGACATC (SEQ ID NO: 278) | 5389 | 47.6 |
| 268 | AAGAATGTCCTAGGAGAATCC (SEQ ID NO: 279) | 5425 | 42.9 |
| 269 | AATGTCCTAGGAGAATCCGGG (SEQ ID NO: 280) | 5428 | 52.4 |
| 270 | AATCCGGGGAGTTGGATTCTC (SEQ ID NO: 281) | 5441 | 52.4 |
| 271 | AAGGCAAATATGGAGGAGAAG (SEQ ID NO: 282) | 5464 | 42.9 |
| 272 | AAATATGGAGGAGAAGTTTAT (SEQ ID NO: 283) | 5469 | 28.6 |
| 273 | AAGTTTATGGCAACTAATCTT (SEQ ID NO: 284) | 5482 | 28.6 |
| 274 | AACTAATCTTTCAAAATCATC (SEQ ID NO: 285) | 5493 | 23.8 |
| 275 | AATCTTTCAAAATCATCCTAT (SEQ ID NO: 286) | 5497 | 23.8 |
| 276 | AAAATCATCCTATGAACCAAT (SEQ ID NO: 287) | 5505 | 28.6 |
| 277 | AATCATCCTATGAACCAATAG (SEQ ID NO: 288) | 5507 | 33.3 |
| 278 | AACCAATAGCAACCACTCTCC (SEQ ID NO: 289) | 5519 | 47.6 |
| 279 | AATAGCAACCACTCTCCGATG (SEQ ID NO: 290) | 5523 | 47.6 |
| 280 | AACCACTCTCCGATGGAAGCA (SEQ ID NO: 291) | 5529 | 52.4 |
| 281 | AAGCAAGAAGACATTTCAGCC (SEQ ID NO: 292) | 5545 | 42.9 |
| 282 | AAGAAGACATTTCAGCCACTG (SEQ ID NO: 293) | 5549 | 42.9 |
| 283 | AAGACATTTCAGCCACTGTCA (SEQ ID NO: 294) | 5552 | 42.9 |
| 284 | AAAAGGCCTATCGGAGCTATG (SEQ ID NO: 295) | 5576 | 47.6 |
| 285 | AAGGCCTATCGGAGCTATGTG (SEQ ID NO: 296) | 5578 | 52.4 |
| 286 | AACACCCCATGTGTGCCCAGA (SEQ ID NO: 297) | 5623 | 57.1 |
| 287 | AAGGTTTTGTTGCATTCACAG (SEQ ID NO: 298) | 5675 | 38.1 |
| 288 | AAATGAAAATTGTGTACTCCC (SEQ ID NO: 299) | 5697 | 33.3 |
| 289 | AAAATTGTGTACTCCCAGACA (SEQ ID NO: 300) | 5702 | 38.1 |
| 290 | AATTGTGTACTCCCAGACAAA (SEQ ID NO: 301) | 5704 | 38.1 |
| 291 | AAATCTGAAACTGCTTCTGCC (SEQ ID NO: 302) | 5722 | 42.9 |
| 292 | AAACTGCTTCTGCCACATCAT (SEQ ID NO: 303) | 5729 | 42.9 |
| 293 | AACATGAGGACATCTAGCTCA (SEQ ID NO: 304) | 5797 | 42.9 |
| 294 | AATACAAAATGAAGATGAAGC (SEQ ID NO: 305) | 5817 | 28.6 |
| 295 | AAAATGAAGATGAAGCCACCA (SEQ ID NO: 306) | 5822 | 38.1 |
| 296 | AATGAAGATGAAGCCACCAGT (SEQ ID NO: 307) | 5824 | 42.9 |
| 297 | AAGATGAAGCCACCAGTATGG (SEQ ID NO: 308) | 5828 | 47.6 |
| 298 | AAGCCACCAGTATGGAGCTGA (SEQ ID NO: 309) | 5834 | 52.4 |

Table 3

## Rat PN3 siRNA's

| Target | Target sequence | position in gene |
|---|---|---|
| 1 | AACTACCAATTTCAGACGGTT (SEQ ID NO: 310) | 27 |
| 2 | AATTTCAGACGGTTCACTCCA (SEQ ID NO: 311) | 34 |
| 3 | AAGCAGATTGCTGCTCACCGC (SEQ ID NO: 312) | 76 |

| | | |
|---|---|---|
| 4 | AAGAAGGCCAGAACCAAGCAC (SEQ ID NO: 313) | 103 |
| 5 | AAGGCCAGAACCAAGCACAGA (SEQ ID NO: 314) | 106 |
| 6 | AACCAAGCACAGAGGACAGGA (SEQ ID NO: 315) | 114 |
| 7 | AAGCACAGAGGACAGGAGGAC (SEQ ID NO: 316) | 118 |
| 8 | AAGGGCGAGAAGCCCAGGCCT (SEQ ID NO: 317) | 139 |
| 9 | AAGCCCAGGCCTCAGCTGGAC (SEQ ID NO: 318) | 148 |
| 10 | AAAGCCTGTAACCAGCTGCCC (SEQ ID NO: 319) | 172 |
| 11 | AACCAGCTGCCCAAGTTCTAT (SEQ ID NO: 320) | 181 |
| 12 | AAGTTCTATGGTGAGCTCCCA (SEQ ID NO: 321) | 193 |
| 13 | AACTGGTCGGGGAGCCCCTGG (SEQ ID NO: 322) | 218 |
| 14 | AATAAAAGCAGGACCATTTCC (SEQ ID NO: 323) | 286 |
| 15 | AAAAGCAGGACCATTTCCAGA (SEQ ID NO: 324) | 289 |
| 16 | AAGCAGGACCATTTCCAGATT (SEQ ID NO: 325) | 291 |
| 17 | AACCTGATCAGAAGAACAGCC (SEQ ID NO: 326) | 349 |
| 18 | AAGAACAGCCATCAAAGTGTC (SEQ ID NO: 327) | 360 |
| 19 | AACAGCCATCAAAGTGTCTGT (SEQ ID NO: 328) | 363 |
| 20 | AAAGTGTCTGTCCATTCCTGG (SEQ ID NO: 329) | 373 |
| 21 | AACTGCGTGTGCATGACCCGA (SEQ ID NO: 330) | 427 |
| 22 | AACTGATCTTCCAGAGAAAGT (SEQ ID NO: 331) | 447 |
| 23 | AAAGTCGAGTACGTCTTCACT (SEQ ID NO: 332) | 463 |
| 24 | AAGATACTGGCAAGAGGGTTT (SEQ ID NO: 333) | 511 |
| 25 | AAGAGGGTTTTGTCTAAATGA (SEQ ID NO: 334) | 522 |
| 26 | AAATGAGTTCACTTATCTTCG (SEQ ID NO: 335) | 537 |
| 27 | AACTGGCTGGACTTCAGTGTC (SEQ ID NO: 336) | 568 |
| 28 | AATCTCAGGCCTGCGGACATT (SEQ ID NO: 337) | 630 |
| 29 | AAAACTGTTTCTGTGATCCCA (SEQ ID NO: 338) | 670 |
| 30 | AACTGTTTCTGTGATCCCAGG (SEQ ID NO: 339) | 672 |
| 31 | AAGGTCATCGTGGGAGCCCTG (SEQ ID NO: 340) | 697 |
| 32 | AAGCTGGCCGACGTGACTATC (SEQ ID NO: 341) | 733 |
| 33 | AAGGGGAACCTTAAGAACAAA (SEQ ID NO: 342) | 805 |
| 34 | AACCTTAAGAACAAATGCATC (SEQ ID NO: 343) | 811 |
| 35 | AAGAACAAATGCATCAGGAAC (SEQ ID NO: 344) | 817 |
| 36 | AACAAATGCATCAGGAACGGA (SEQ ID NO: 345) | 820 |
| 37 | AAATGCATCAGGAACGGAACA (SEQ ID NO: 346) | 823 |
| 38 | AACGGAACAGATCCCCACAAG (SEQ ID NO: 347) | 835 |
| 39 | AACAGATCCCCACAAGGCTGA (SEQ ID NO: 348) | 840 |
| 40 | AAGGCTGACAACCTCTCATCT (SEQ ID NO: 349) | 853 |
| 41 | AACCTCTCATCTGAAATGGCA (SEQ ID NO: 350) | 862 |
| 42 | AAATGGCAGAATACATCTTCA (SEQ ID NO: 351) | 875 |
| 43 | AATACATCTTCATCAAGCCTG (SEQ ID NO: 352) | 884 |
| 44 | AAGCCTGGTACTACGGATCCC (SEQ ID NO: 353) | 898 |
| 45 | AATGGGTCTGATGCTGGTCAC (SEQ ID NO: 354) | 931 |
| 46 | AAAACTCCTGACAACCCGGAT (SEQ ID NO: 355) | 976 |
| 47 | AACTCCTGACAACCCGGATTT (SEQ ID NO: 356) | 978 |
| 48 | AACCCGGATTTTAACTACACC (SEQ ID NO: 357) | 988 |
| 49 | AACTACACCAGCTTTGATTCC (SEQ ID NO: 358) | 1000 |
| 50 | AAAATGTACATGGTCTTTTTC (SEQ ID NO: 359) | 1108 |
| 51 | AATGTACATGGTCTTTTTCGT (SEQ ID NO: 360) | 1110 |
| 52 | AATTTGATCTTGGCCGTGGTC (SEQ ID NO: 361) | 1165 |
| 53 | AAGAGCAGAGCCAGGCAACAA (SEQ ID NO: 362) | 1199 |
| 54 | AACAATTGCAGAAATCGAAGC (SEQ ID NO: 363) | 1215 |

| | | |
|---|---|---|
| 55 | AATTGCAGAAATCGAAGCCAA (SEQ ID NO: 364) | 1218 |
| 56 | AAATCGAAGCCAAGGAAAAAA (SEQ ID NO: 365) | 1226 |
| 57 | AAGCCAAGGAAAAAAAGTTCC (SEQ ID NO: 366) | 1232 |
| 58 | AAGGAAAAAAAGTTCCAGGAA (SEQ ID NO: 367) | 1237 |
| 59 | AAAAAAAGTTCCAGGAAGCCC (SEQ ID NO: 368) | 1241 |
| 60 | AAAAAGTTCCAGGAAGCCCTT (SEQ ID NO: 369) | 1243 |
| 61 | AAAGTTCCAGGAAGCCCTTGA (SEQ ID NO: 370) | 1245 |
| 62 | AAGCCCTTGAGGTGCTGCAGA (SEQ ID NO: 371) | 1256 |
| 63 | AAGGAACAGGAGGTGCTGGCA (SEQ ID NO: 372) | 1276 |
| 64 | AACAGGAGGTGCTGGCAGCCC (SEQ ID NO: 373) | 1280 |
| 65 | AAAAACGCCAATGAGAGAAGA (SEQ ID NO: 374) | 1354 |
| 66 | AAACGCCAATGAGAGAAGACC (SEQ ID NO: 375) | 1356 |
| 67 | AATGAGAGAAGACCCAGGGTG (SEQ ID NO: 376) | 1363 |
| 68 | AAGACCCAGGGTGAAATCAAG (SEQ ID NO: 377) | 1371 |
| 69 | AAATCAAGGGTGTCAGAGGGC (SEQ ID NO: 378) | 1384 |
| 70 | AAGGGTGTCAGAGGGCTCCAC (SEQ ID NO: 379) | 1389 |
| 71 | AACAGGTCACCCCAATCTGAC (SEQ ID NO: 380) | 1417 |
| 72 | AATCTGACCCTTACAACCAGC (SEQ ID NO: 381) | 1430 |
| 73 | AACCAGCGCAGGATGTCTTTC (SEQ ID NO: 382) | 1444 |
| 74 | AAGACGCAGGGCTAGCCACGG (SEQ ID NO: 383) | 1482 |
| 75 | AAGACATCTCATTTCCTGACG (SEQ ID NO: 384) | 1532 |
| 76 | AAAGCCGTCGAGGTTCCATAT (SEQ ID NO: 385) | 1589 |
| 77 | AACCCTGGCCGTAGACATGGA (SEQ ID NO: 386) | 1672 |
| 78 | AAGAGGGACAGCTCGGAGTGC (SEQ ID NO: 387) | 1694 |
| 79 | AAGGCCCGGCACTCGACACTA (SEQ ID NO: 388) | 1745 |
| 80 | AAGAGCTTCCTGTCTGCGGGC (SEQ ID NO: 389) | 1774 |
| 81 | AACGAACCTTTCCGAGCACAG (SEQ ID NO: 390) | 1801 |
| 82 | AACCTTTCCGAGCACAGAGGG (SEQ ID NO: 391) | 1805 |
| 83 | AAGAGTCTAAGCTGAAGTGCC (SEQ ID NO: 392) | 1871 |
| 84 | AAGCTGAAGTGCCCACCCTGC (SEQ ID NO: 393) | 1879 |
| 85 | AAGTGCCCACCCTGCTTGATC (SEQ ID NO: 394) | 1885 |
| 86 | AAGTATCTGATCTGGGAGTGC (SEQ ID NO: 395) | 1918 |
| 87 | AAGTGGAGGAAGTTCAAGATG (SEQ ID NO: 396) | 1945 |
| 88 | AAGTTCAAGATGGCGCTGTTC (SEQ ID NO: 397) | 1954 |
| 89 | AAGATGGCGCTGTTCGAGCTG (SEQ ID NO: 398) | 1960 |
| 90 | AACACCGTCTTCATGGCCATG (SEQ ID NO: 399) | 2029 |
| 91 | AAGCCGGCAACATTGTCTTCA (SEQ ID NO: 400) | 2090 |
| 92 | AACATTGTCTTCACCGTGTTT (SEQ ID NO: 401) | 2098 |
| 93 | AATGGAGATGGCCTTCAAGAT (SEQ ID NO: 402) | 2124 |
| 94 | AAGATCATTGCCTTCGACCCC (SEQ ID NO: 403) | 2140 |
| 95 | AAGAAGTGGAATATCTTCGAC (SEQ ID NO: 404) | 2176 |
| 96 | AAGTGGAATATCTTCGACTGT (SEQ ID NO: 405) | 2179 |
| 97 | AATATCTTCGACTGTGTCATC (SEQ ID NO: 406) | 2185 |
| 98 | AAGAAGGGCAGCCTGTCTGTG (SEQ ID NO: 407) | 2239 |
| 99 | AAGGGCAGCCTGTCTGTGCTC (SEQ ID NO: 408) | 2242 |
| 100 | AAGCTGGCCAAGTCCTGGCCC (SEQ ID NO: 409) | 2290 |
| 101 | AAGTCCTGGCCCACCCTGAAC (SEQ ID NO: 410) | 2299 |
| 102 | AACACCCTCATCAAGATCATC (SEQ ID NO: 411) | 2317 |
| 103 | AAGATCATCGGGAACTCCGTG (SEQ ID NO: 412) | 2329 |
| 104 | AACTCCGTGGGGGCCCTGGGC (SEQ ID NO: 413) | 2341 |
| 105 | AACCTGACCTTTATCCTGGCC (SEQ ID NO: 414) | 2362 |

| | | |
|---|---|---|
| 106 | AAAGCAGCTTCTCTCAGAGGA (SEQ ID NO: 415) | 2412 |
| 107 | AAGGACGGCGTCTCCGTGTGG (SEQ ID NO: 416) | 2446 |
| 108 | AACGGCGAGAAGCTCCGCTGG (SEQ ID NO: 417) | 2467 |
| 109 | AAGCTCCGCTGGCACATGTGT (SEQ ID NO: 418) | 2476 |
| 110 | AATCCTCTGCGGGGAGTGGAT (SEQ ID NO: 419) | 2529 |
| 111 | AACATGTGGGTCTGCATGGAG (SEQ ID NO: 420) | 2554 |
| 112 | AAATCCATCTGCCTCATCCTC (SEQ ID NO: 421) | 2584 |
| 113 | AACCTAGTGGTGCTCAACCTT (SEQ ID NO: 422) | 2629 |
| 114 | AACCTTTTCATCGCTTTACTG (SEQ ID NO: 423) | 2644 |
| 115 | AACTCCTTCAGCGCGGACAAC (SEQ ID NO: 424) | 2668 |
| 116 | AACCTCACGGCTCCAGAGGAT (SEQ ID NO: 425) | 2686 |
| 117 | AACAACTTGCAGTTAGCACTG (SEQ ID NO: 426) | 2719 |
| 118 | AACTTGCAGTTAGCACTGGCC (SEQ ID NO: 427) | 2722 |
| 119 | AAGGTGGAGACCCAGCTGGGC (SEQ ID NO: 428) | 2821 |
| 120 | AAGCCCCCACTCACCAGCTCA (SEQ ID NO: 429) | 2845 |
| 121 | AAGAACCACATTGCCACTGAT (SEQ ID NO: 430) | 2872 |
| 122 | AACCACATTGCCACTGATGCT (SEQ ID NO: 431) | 2875 |
| 123 | AACCTGACAAAGCCAGCTCTC (SEQ ID NO: 432) | 2914 |
| 124 | AAAGCCAGCTCTCAGTAGCCC (SEQ ID NO: 433) | 2922 |
| 125 | AAGGAGAACCACGGGGACTTC (SEQ ID NO: 434) | 2944 |
| 126 | AACCACGGGGACTTCATCACT (SEQ ID NO: 435) | 2950 |
| 127 | AACGTGTGGGTCTCTGTGCCC (SEQ ID NO: 436) | 2977 |
| 128 | AATCTGACCTCGACGAGCTCG (SEQ ID NO: 437) | 3011 |
| 129 | AAGATATGGAGCAGGCTTCGC (SEQ ID NO: 438) | 3035 |
| 130 | AAGAGGACCCCAAGGGACAGC (SEQ ID NO: 439) | 3071 |
| 131 | AAGGGACAGCAGGAGCAGTTG (SEQ ID NO: 440) | 3082 |
| 132 | AAGTCCAAAAGTGTGAAAACC (SEQ ID NO: 441) | 3107 |
| 133 | AAAAGTGTGAAAACCACCAGG (SEQ ID NO: 442) | 3113 |
| 134 | AAGTGTGAAAACCACCAGGCA (SEQ ID NO: 443) | 3115 |
| 135 | AAAACCACCAGGCAGCCAGAA (SEQ ID NO: 444) | 3122 |
| 136 | AACCACCAGGCAGCCAGAAGC (SEQ ID NO: 445) | 3124 |
| 137 | AAGCCCAGCCTCCATGATGTC (SEQ ID NO: 446) | 3141 |
| 138 | AAGAGGAAGGATAGCCCTCAG (SEQ ID NO: 447) | 3199 |
| 139 | AAGGATAGCCCTCAGGTCCCT (SEQ ID NO: 448) | 3205 |
| 140 | AAATCCTGAGGAAGATCCCCG (SEQ ID NO: 449) | 3290 |
| 141 | AAGATCCCCGAGCTGGCAGAT (SEQ ID NO: 450) | 3301 |
| 142 | AAGGCTGCACTCGCCGCTGTC (SEQ ID NO: 451) | 3353 |
| 143 | AACGTGAATACTAGCAAGTCT (SEQ ID NO: 452) | 3382 |
| 144 | AATACTAGCAAGTCTCCTTGG (SEQ ID NO: 453) | 3388 |
| 145 | AAGTCTCCTTGGGCCACAGGC (SEQ ID NO: 454) | 3397 |
| 146 | AAGACCTGCTACCGCATCGTG (SEQ ID NO: 455) | 3430 |
| 147 | AACTACCTGGAAGAGAAACCC (SEQ ID NO: 456) | 3523 |
| 148 | AAGAGAAACCCCGAGTGAAGT (SEQ ID NO: 457) | 3533 |
| 149 | AAACCCCGAGTGAAGTCCGTG (SEQ ID NO: 458) | 3538 |
| 150 | AAGTCCGTGCTGGAGTACACT (SEQ ID NO: 459) | 3550 |
| 151 | AAGTGGGTAGCCTATGGCTTC (SEQ ID NO: 460) | 3613 |
| 152 | AAAAAGTATTTCACCAATGCC (SEQ ID NO: 461) | 3634 |
| 153 | AAAGTATTTCACCAATGCCTG (SEQ ID NO: 462) | 3636 |
| 154 | AATGCCTGGTGCTGGCTGGAC (SEQ ID NO: 463) | 3649 |
| 155 | AACATCTCCCTGACAAGCCTC (SEQ ID NO: 464) | 3682 |
| 156 | AAGCCTCATAGCGAAGATCCT (SEQ ID NO: 465) | 3696 |

| | | |
|---|---|---|
| 157 | AAGATCCTTGAGTATTCCGAC (SEQ ID NO: 466) | 3709 |
| 158 | AAAGCCCTTCGGACTCTCCGT (SEQ ID NO: 467) | 3742 |
| 159 | AAGGCATGAGGGTAGTGGTGG (SEQ ID NO: 468) | 3797 |
| 160 | AACGTCCTCCTCGTCTGCCTC (SEQ ID NO: 469) | 3850 |
| 161 | AACCTCTTCGCCGGGAAATTT (SEQ ID NO: 470) | 3904 |
| 162 | AAATTTTCGAAGTGCGTCGAC (SEQ ID NO: 471) | 3919 |
| 163 | AAGTGCGTCGACACCAGAAAT (SEQ ID NO: 472) | 3928 |
| 164 | AAATAACCCATTTTCCAACGT (SEQ ID NO: 473) | 3945 |
| 165 | AACCCATTTTCCAACGTGAAT (SEQ ID NO: 474) | 3949 |
| 166 | AACGTGAATTCGACGATGGTG (SEQ ID NO: 475) | 3961 |
| 167 | AATTCGACGATGGTGAATAAC (SEQ ID NO: 476) | 3967 |
| 168 | AATAACAAGTCCGAGTGTCAC (SEQ ID NO: 477) | 3982 |
| 169 | AACAAGTCCGAGTGTCACAAT (SEQ ID NO: 478) | 3985 |
| 170 | AAGTCCGAGTGTCACAATCAA (SEQ ID NO: 479) | 3988 |
| 171 | AATCAAAACAGCACCGGCCAC (SEQ ID NO: 480) | 4003 |
| 172 | AAAACAGCACCGGCCACTTCT (SEQ ID NO: 481) | 4007 |
| 173 | AACAGCACCGGCCACTTCTTC (SEQ ID NO: 482) | 4009 |
| 174 | AACGTCAAAGTCAACTTCGAC (SEQ ID NO: 483) | 4036 |
| 175 | AAAGTCAACTTCGACAACGTC (SEQ ID NO: 484) | 4042 |
| 176 | AACTTCGACAACGTCGCTATG (SEQ ID NO: 485) | 4048 |
| 177 | AACGTCGCTATGGGCTACCTC (SEQ ID NO: 486) | 4057 |
| 178 | AACCTTCAAAGGCTGGATGGA (SEQ ID NO: 487) | 4095 |
| 179 | AAAGGCTGGATGGACATAATG (SEQ ID NO: 488) | 4102 |
| 180 | AATGTATGCAGCTGTTGATTC (SEQ ID NO: 489) | 4119 |
| 181 | AACAGTCAGCCTAACTGGGAG (SEQ ID NO: 490) | 4150 |
| 182 | AACTGGGAGAACAACTTGTAC (SEQ ID NO: 491) | 4162 |
| 183 | AACAACTTGTACATGTACCTG (SEQ ID NO: 492) | 4171 |
| 184 | AACTTGTACATGTACCTGTAC (SEQ ID NO: 493) | 4174 |
| 185 | AATCTCTTTGTTGGGGTCATA (SEQ ID NO: 494) | 4234 |
| 186 | AATCGACAACTTCAACCAACA (SEQ ID NO: 495) | 4254 |
| 187 | AACTTCAACCAACAGAAAAAA (SEQ ID NO: 496) | 4261 |
| 188 | AACCAACAGAAAAAAAAGCTA (SEQ ID NO: 497) | 4267 |
| 189 | AACAGAAAAAAAAGCTAGGAG (SEQ ID NO: 498) | 4271 |
| 190 | AAAAAAAAGCTAGGAGGCCAG (SEQ ID NO: 499) | 4276 |
| 191 | AAAAAAGCTAGGAGGCCAGGA (SEQ ID NO: 500) | 4278 |
| 192 | AAAAGCTAGGAGGCCAGGACA (SEQ ID NO: 501) | 4280 |
| 193 | AAGCTAGGAGGCCAGGACATC (SEQ ID NO: 502) | 4282 |
| 194 | AAGAGCAGAAGAAGTACTACA (SEQ ID NO: 503) | 4313 |
| 195 | AAGAAGTACTACAATGCCATG (SEQ ID NO: 504) | 4321 |
| 196 | AAGTACTACAATGCCATGAAG (SEQ ID NO: 505) | 4324 |
| 197 | AATGCCATGAAGAAGCTGGGC (SEQ ID NO: 506) | 4333 |
| 198 | AAGAAGCTGGGCTCCAAGAAA (SEQ ID NO: 507) | 4342 |
| 199 | AAGCTGGGCTCCAAGAAACCC (SEQ ID NO: 508) | 4345 |
| 200 | AAGAAACCCCAGAAGCCCATC (SEQ ID NO: 509) | 4357 |
| 201 | AAACCCCAGAAGCCCATCCCA (SEQ ID NO: 510) | 4360 |
| 202 | AAGCCCATCCCACGGCCCCTG (SEQ ID NO: 511) | 4369 |
| 203 | AATAAGTACCAAGGCTTCGTG (SEQ ID NO: 512) | 4390 |
| 204 | AAGTACCAAGGCTTCGTGTTT (SEQ ID NO: 513) | 4393 |
| 205 | AAGGCTTCGTGTTTGACATCG (SEQ ID NO: 514) | 4400 |
| 206 | AAGCCTTTGACATCATCATCA (SEQ ID NO: 515) | 4430 |
| 207 | AACATGATCACCATGATGGTG (SEQ ID NO: 516) | 4468 |

| | | |
|---|---|---|
| 208 | AAGACGAAGGTTCTGGGCAGA (SEQ ID NO: 517) | 4513 |
| 209 | AAGGTTCTGGGCAGAATCAAC (SEQ ID NO: 518) | 4519 |
| 210 | AATCAACCAGTTCTTTGTGGC (SEQ ID NO: 519) | 4533 |
| 211 | AACCAGTTCTTTGTGGCCGTC (SEQ ID NO: 520) | 4537 |
| 212 | AAGATGTTCGCCCTGCGACAG (SEQ ID NO: 521) | 4579 |
| 213 | AACGGCTGGAACGTGTTCGAC (SEQ ID NO: 522) | 4612 |
| 214 | AACGTGTTCGACTTCATAGTG (SEQ ID NO: 523) | 4621 |
| 215 | AATCCTTAAGTCACTGGAAAA (SEQ ID NO: 524) | 4677 |
| 216 | AAGTCACTGGAAAACTACTTC (SEQ ID NO: 525) | 4684 |
| 217 | AAAACTACTTCTCCCCGACGC (SEQ ID NO: 526) | 4694 |
| 218 | AACTACTTCTCCCCGACGCTC (SEQ ID NO: 527) | 4696 |
| 219 | AAGGGGATTCGCACGCTGCTC (SEQ ID NO: 528) | 4774 |
| 220 | AACATCGGCCTCCTCCTCTTC (SEQ ID NO: 529) | 4828 |
| 221 | AACGTCGTGGACGAGGCCGGC (SEQ ID NO: 530) | 4894 |
| 222 | AACTTCAAGACCTTTGGCAAC (SEQ ID NO: 531) | 4930 |
| 223 | AAGACCTTTGGCAACAGCATG (SEQ ID NO: 532) | 4936 |
| 224 | AACAGCATGCTGTGCCTGTTC (SEQ ID NO: 533) | 4948 |
| 225 | AACACGGGGCCTCCCTACTGC (SEQ ID NO: 534) | 5017 |
| 226 | AACCTGCCCAACAGCAACGGC (SEQ ID NO: 535) | 5044 |
| 227 | AACAGCAACGGCTCCCGGGGG (SEQ ID NO: 536) | 5053 |
| 228 | AACGGCTCCCGGGGGAACTGC (SEQ ID NO: 537) | 5059 |
| 229 | AACTGCGGGAGCCCGGCGGTG (SEQ ID NO: 538) | 5074 |
| 230 | AACATGTACATCGCAGTGATT (SEQ ID NO: 539) | 5146 |
| 231 | AACTTCAACGTGGCCACCGAG (SEQ ID NO: 540) | 5173 |
| 232 | AACGTGGCCACCGAGGAGAGC (SEQ ID NO: 541) | 5179 |
| 233 | AAGTTCGACCCGGAGGCCACC (SEQ ID NO: 542) | 5251 |
| 234 | AATCCCCAAACCCAACCAGAA (SEQ ID NO: 543) | 5331 |
| 235 | AAACCCAACCAGAATATATTA (SEQ ID NO: 544) | 5338 |
| 236 | AACCAGAATATATTAATCCAG (SEQ ID NO: 545) | 5344 |
| 237 | AATATATTAATCCAGATGGAC (SEQ ID NO: 546) | 5350 |
| 238 | AATCCAGATGGACCTGCCGTT (SEQ ID NO: 547) | 5358 |
| 239 | AAGATCCACTGTCTGGACATC (SEQ ID NO: 548) | 5392 |
| 240 | AAAGAACGTCTTGGGAGAATC (SEQ ID NO: 549) | 5427 |
| 241 | AACGTCTTGGGAGAATCCGGG (SEQ ID NO: 550) | 5431 |
| 242 | AATCCGGGGAGTTGGACTCCC (SEQ ID NO: 551) | 5444 |
| 243 | AAGACCAATATGGAAGAGAAG (SEQ ID NO: 552) | 5467 |
| 244 | AATATGGAAGAGAAGTTTATG (SEQ ID NO: 553) | 5473 |
| 245 | AAGAGAAGTTTATGGCGACCA (SEQ ID NO: 554) | 5480 |
| 246 | AAGTTTATGGCGACCAATCTC (SEQ ID NO: 555) | 5485 |
| 247 | AATCTCTCCAAAGCATCCTAT (SEQ ID NO: 556) | 5500 |
| 248 | AAAGCATCCTATGAACCAATA (SEQ ID NO: 557) | 5509 |
| 249 | AACCAATAGCCACCACCCTCC (SEQ ID NO: 558) | 5522 |
| 250 | AATAGCCACCACCCTCCGGTG (SEQ ID NO: 559) | 5526 |
| 251 | AAGCAGGAAGACCTCTCAGCC (SEQ ID NO: 560) | 5548 |
| 252 | AAGACCTCTCAGCCACAGTCA (SEQ ID NO: 561) | 5555 |
| 253 | AAAAGGCCTACCGGAGCTACA (SEQ ID NO: 562) | 5579 |
| 254 | AAGGCCTACCGGAGCTACATG (SEQ ID NO: 563) | 5581 |
| 255 | AACACCCTGCATGTGCCCAGG (SEQ ID NO: 564) | 5626 |
| 256 | AAGGCTACGTTACATTCATGG (SEQ ID NO: 565) | 5678 |
| 257 | AAACAGTGGACTCCCGGACAA (SEQ ID NO: 566) | 5700 |
| 258 | AAATCAGAAACTGCCTCTGCT (SEQ ID NO: 567) | 5719 |

| | | |
|---|---|---|
| 259 | AAACTGCCTCTGCTACGTCTT (SEQ ID NO: 568) | 5726 |
| 260 | AACATTAACCCATCTAGCTCA (SEQ ID NO: 569) | 5794 |
| 261 | AACCCATCTAGCTCAATGCAA (SEQ ID NO: 570) | 5800 |
| 262 | AATGCAAAATGAAGATGAGGT (SEQ ID NO: 571) | 5814 |
| 263 | AAAATGAAGATGAGGTCGCTG (SEQ ID NO: 572) | 5819 |
| 264 | AATGAAGATGAGGTCGCTGCT (SEQ ID NO: 573) | 5821 |
| 265 | AAGATGAGGTCGCTGCTAAGG (SEQ ID NO: 574) | 5825 |
| 266 | AAGGAAGGAAACAGCCCTGGA (SEQ ID NO: 575) | 5842 |
| 267 | AAGGAAACAGCCCTGGACCTC (SEQ ID NO: 576) | 5846 |
| 268 | AAACAGCCCTGGACCTCAGTG (SEQ ID NO: 577) | 5850 |

Of the above siRNA sequences, five were selected for testing their ability to knock-down $Na_v1.8$ expression and function *in vitro*. The five siRNAs were selected so as to cover different regions of the entire 5874 nucleotide $Na_v1.8$ coding sequence. The five siRNA sequences, including the nucleotide position at which each sequence is located within the genome, are shown below in Table 4:

Table 4

| siRNA | Sequence | Nuc. Pos. |
|---|---|---|
| siRNA 1 | AAAAGCAGGACCAUUUCCAGA (SEQ ID NO: 1) | 289 |
| siRNA 2 | AAAGUGUCUGUCCAUUCCUGG (SEQ ID NO: 2) | 373 |
| siRNA 3 | AACUACACCAGCUUUGAUUCC (SEQ ID NO: 3) | 1000 |
| siRNA 4 | AAAUCCAUCUGCCUCAUCCUC (SEQ ID NO: 4) | 2584 |
| siRNA 5 | AAUAAGUACCAAGGCUUCGUG (SEQ ID NO: 5) | 4390 |

Example 2

The above five selected siRNA sequences, SEQ ID NOs: 1-5, were synthesized by Qiagen Inc., Valencia, CA. We then cloned the $Na_v1.8$ cDNA into a pcDNA3.1 mammalian expression plasmid (Invitrogen, Carlsbad, CA) to generate a pcDNA-$Na_v1.8$ control expression plasmid. Upon transfection of the control plasmid into HEK293 cells (Microbix Biosystems, Inc., Ontario, Canada M8Z3A8), the cells exhibited high levels of $Na_v1.8$ RNA and protein

expression. $Na_v1.8$ RNA expression was detected by Taqman® quantitative RT-PCR (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. $Na_v1.8$ protein expression in the HEK293 cells was detected by westernimmunoblot analysis using a $Na_v1.8$ specific peptide antibody, SOD1 (AnaSpec, San Jose, CA). The published peptide sequence was used for making the SOD1 antibody. See Novakovic *et al.*, "Distribution of the tetrodotoxin-resistant sodium channel PN3 in rat sensory neurons in normal and neuropathic conditions, *J. Neuroscience*, vol. 18, no. 6, pp. 2174-2187 (1998).

Knock-down of $Na_v1.8$ RNA

The five chemically synthesized siRNAs, SEQ ID NOs: 1-5, were individually co-transfected into HEK293 cells, along with the pcDNA-$Na_v1.8$ control expression plasmid. The final siRNA concentration in the transfected cells was maintained at 25 nM. 24 hours after transfection, the cells were lysed and the total RNA was purified from lysates using RNAeasy minicolumns (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total RNA purified from either cells cotransfected with the individual siRNAs or control cells were used for quantitative RT-PCR analysis using rat $Na_v1.8$ specific Taqman® primer and probe sets (Applied Biosystems, Foster City, CA). Any rat $Na_v1.8$ specific primer should work in this regard. The design of PCR primers is known in the art.

The expression level of $Na_v1.8$ RNA in siRNA transfected cells was compared with RNA expression in control cells. The control cells, which were transfected with pcDNA3.1-$Na_v1.8$ control expression plasmid, exhibited a relative $rNa_v1.8$ RNA expression level of 100%. The siRNA 1 cells, which were co-transfected with pcDNA3.1-$Na_v1.8$ control expression plasmid and the siRNA of SEQ ID NO: 1, exhibited a relative $rNa_v1.8$ RNA expression level of 20%. The siRNA 2 cells, which were co-transfected with pcDNA3.1-$Na_v1.8$ control expression plasmid and the siRNA of SEQ ID NO: 2, exhibited a relative $rNa_v1.8$ RNA expression level of 65%. The siRNA 3 cells, which were co-transfected with pcDNA3.1-$Na_v1.8$ control expression plasmid and the

siRNA of SEQ ID NO: 3, exhibited a relative $rNa_v1.8$ RNA expression level of 30%. The siRNA 4 cells, which were co-transfected with pcDNA3.1-$Na_v1.8$ control expression plasmid and the siRNA of SEQ ID NO: 4, exhibited a relative $rNa_v1.8$ RNA expression level of 115%. The siRNA 5 cells, which were co-transfected with pcDNA3.1-$Na_v1.8$ control expression plasmid and the siRNA of SEQ ID NO: 5, exhibited a relative $rNa_v1.8$ RNA expression level of 70%.

Cells co-transfected with either siRNA 1 or siRNA 3 showed high levels of $Na_v1.8$ RNA silencing while cells co-transfected with either siRNA 2 and siRNA 5 showed moderate levels of RNA silencing. No knock-down in $Na_v1.8$ RNA expression was seen in cells transfected with siRNA 4.

Knock-down of $Na_v1.8$ Protein

The five chemically synthesized siRNAs, SEQ ID NOs: 1-5, were individually co-transfected into HEK293 cells, along with the pcDNA-$Na_v1.8$ control expression plasmid. The final siRNA concentration in the transfected cells was maintained at 25 nM. 24 hours after transfection, the cells were lysed in a denaturing lysis buffer and the lysates were run on denaturation 12% TBE gels (Invitrogen, Carsbad, CA). The gels were blotted onto nitrocellulose sheets and probed with the $Na_v1.8$ specific antibody – SOD1 (AnaSpec, San Jose, CA).

Lysates from cells transfected with pcDNA-$Na_v1.8$ control expression plasmid, the control cells, showed high levels of $Na_v1.8$ protein expression. Lysates from cells co-transfected with pcDNA-$Na_v1.8$ control expression plasmid and either siRNA 1 or siRNA 3 showed almost complete abolition of $Na_v1.8$ protein expression. Lysates from cells co-transfected with pcDNA-$Na_v1.8$ control expression plasmid and either siRNA 2 or siRNA 5 showed moderate levels of $Na_v1.8$ protein expression. Lysates from cells co-transfected with pcDNA-$Na_v1.8$ control expression plasmid and siRNA 4 showed no reduction in $Na_v1.8$ protein expression.

Example 3

Next, we determined whether siRNA was capable of functionally knocking-down the $Na_v1.8$ sodium channel. This determination was made using a FlexStation® assay (Molecular Devices, Sunnyvale, CA) and voltage clamp measurements. We stably expressed, by retroviral integration, the $Na_v1.8$ coding sequence in the neuroblastoma/DRG fusion cell line ND7/23 (European Collection of Cell Cultures, Wiltshire, UK). The ND7/23 cell line is a mouse neuroblastoma and rat neurone hybrid, which is identified by European Collection of Cell Cultures No. 92090903. This ND7/23-$Na_v1.8$ cell line showed consistent and high levels of $Na_v1.8$ sodium current in both FlexStation® membrane potential assays and in whole cell voltage clamp measurements.

FlexStation® Assay

We individually transfected each of the above five selected siRNA sequences, SEQ ID NOs: 1-5, into the ND7/23-$Na_v1.8$ cell line. Functional knock-down of $Na_v1.8$ by the siRNAs was confirmed using the membrane potential assay on the FlexStation® according to the manufacturer's instructions. Readings on the FlexStation® were taken 1 day post transfection with individual siRNAs.

The luminescence level of control cells was compared to that of cells individually transfected with siRNAs 1-5. All cells were transfected with the $Na_v1.8$ coding sequence. The control cells exhibited a luminescence level of 175,000 units. The siRNA 1 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 1, exhibited a luminescence level of 48,000 units. The siRNA 2 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 2, exhibited a luminescence level of 70,000 units. The siRNA 3 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 3, exhibited a luminescence level of 45,000. The siRNA 4 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 4, exhibited a luminescence level of 151,000. The siRNA 5 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 5, exhibited a luminescence level of 80,000.

siRNAs 1 and 3 blocked $Na_v1.8$ derived membrane potential while siRNAs 2 and 5 showed moderate levels of blockage in membrane potential. siRNA 4 showed minimal or no blockage in membrane potential. The level of blockage in membrane potential by the individual siRNAs was similar to the level of both protein and RNA silencing by the siRNAs in the HEK293 system. In another example of this experiment, the control cells exhibited a luminescence level of 198,698 units. The siRNA 1 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 1, exhibited a luminescence level of 46,068 units (corresponding to 23% of control signal). The siRNA 2 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 2, exhibited a luminescence level of 71,523 units (corresponding to 36% of control). The siRNA 3 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 3, exhibited a luminescence level of 42,422 units (corresponding to 21% of control). The siRNA 4 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 4, exhibited a luminescence level of 151,067 units (corresponding to 76% of control). The siRNA 5 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 5, exhibited a luminescence level of 80,567 units (corresponding to 41% of control).

Voltage clamp measurements

To further confirm functional knock-down of $Na_v1.8$ sodium currents, we performed whole cell voltage clamp measurements in ND7/23-$Na_v1.8$ control cells that were subsequently transfected with siRNA 1. Briefly, ND7/23-$Na_v1.8$ cells were either mock transfected with non-silencing siRNAs, the control cells, or transfected with siRNA 1, the siRNA 1 cells. The siRNA 1 concentration was maintained at 25 nM. Successfully transfected cells, which were identified by cotransfection with Green Fluorescent Protein (GFP) (Clontech, Palo Alto, CA), were used for whole cell voltage clamp measurements. Measurements were made both 24 and 48 hours post transfection.

At 24 hours post transfection, the control cells exhibited a peak amplitude of -900 while the siRNA 1 cells exhibited a peak amplitude of -175.

At 48 hours post transfection, the control cells exhibited a peak amplitude of -775 while the siRNA 1 cells exhibited a peak amplitude of -175. Therefore, we observed almost total blockage in sodium currents in siRNA 1 transfected cells. In fact, the blockage was greater than 85%. Furthermore, the specificity of $Na_v1.8$ block was confirmed by the observation that no block in tetrodotoxin-sensitive currents was seen in siRNA 1 treated ND7/23-$Na_v1.8$ cells.

In another example, at 24 hours post transfection, the control cells (sample size = 10) exhibited a mean peak whole-cell $Na_V1.8$ current amplitude of -912pA while the siRNA 1 cells (sample size = 11) exhibited a mean peak amplitude of -169pA. Thus, by 24h after transfection, siRNA1 had reduced the NaV1.8 current amplitude to 18.5% of control. Furthermore, the specificity of the siRNA effect to the intended tetrodotoxin-resistant $Na_v1.8$ sodium channel was confirmed by the observation that no reduction in the amplitude of the background tetrodotoxin-sensitive sodium currents was seen in siRNA 1 treated ND7/23-$Na_v1.8$ cells at either 24 or 48h post-transfection.

Example 4

In order to identify backup siRNAs that exhibit high levels of $Na_v1.8$ knock-down, we designed six additional siRNAs, SEQ ID NOs: 6-11, in the region of the $Na_v1.8$ coding sequences covering siRNA 1 and siRNA 3. These six additional siRNAs were designed using the same procedures outlined in Example 1, and have the following sequences, as shown in Table 5 below:

Table 5

| siRNA | Sequence |
|---|---|
| siRNA 6 | AAGAAGGCCAGAACCAAGCAC (SEQ ID NO: 6) |
| siRNA 7 | AAGUUCUAUGGUGAGCUCCCA (SEQ ID NO: 7) |
| siRNA 8 | AACUGGCUGGACUUCAGUGUC (SEQ ID NO: 8) |
| siRNA 9 | AACUGUUUCUGUGAUCCCAGG (SEQ ID NO: 9) |
| siRNA 10 | AAGGCUGACAACCUCUCAUCU (SEQ ID NO: 10) |

| | |
|---|---|
| siRNA 11 | AAGAGUCUAAGCUGAAGUGCC (SEQ ID NO: 11) |

All six additional siRNAs, SEQ ID NOs: 6-11, were screened for their ability to knock-down $Na_v1.8$ derived membrane potential in ND7/23 cells. Using procedures similar to those in Example 3, the luminescence level of control cells was compared to that of cells individually transfected with siRNAs 6-11. The control cells exhibited a luminescence level of 175,000 units. The siRNA 6 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 6, exhibited a luminescence level of 85,000 units. The siRNA 7 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 7, exhibited a luminescence level of 50,000 units. The siRNA 8 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 8, exhibited a luminescence level of 45,000. The siRNA 9 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 9, exhibited a luminescence level of 43,000. The siRNA 10 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 10, exhibited a luminescence level of 10,000. The siRNA 11 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 11, exhibited a luminescence level of 35,000. Using procedures similar to those in Examples 2 and 3, we determined that all six siRNAs were also capable of blocking $Na_v1.8$ expression and function, resulting in a collection of efficacious siRNAs.

In another example, the control cells exhibited a luminescence level of 198,698 units. The siRNA 6 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 6, exhibited a luminescence level of 86,105 units (43% of control cells). The siRNA 7 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 7, exhibited a luminescence level of 50,237 units (25% of control cells). The siRNA 8 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 8, exhibited a luminescence level of 44,038 units (22% of control cells). The siRNA 9 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 9, exhibited a luminescence level of 46,917 units (24% of control cells). The siRNA 10 cells, which were subsequently transfected with the siRNA of SEQ

ID NO: 10, exhibited a luminescence level of 21,847 (7% of control cells). The siRNA 11 cells, which were subsequently transfected with the siRNA of SEQ ID NO: 11, exhibited a luminescence level of 30,587 (15% of control cells).

Example 5

Adenoviral delivery of $Na_v1.8$ siRNA

For long term siRNA delivery to cells and knock-down of $Na_v1.8$ function, we designed an adenoviral vector for driving siRNA expression. Briefly, the construct was designed to express siRNA 3 (SEQ ID NO: 3) under the control of a U6 promoter cassette. The siRNA 3 expression cassette was then cloned into an E1-deleted pTG4213 adenoviral backbone (Transgene SA, France).

ND7/23-$Na_v1.8$ cells from Example 3 were infected with the above siRNA 3 adenoviral vector construct at a concentration of $1e^9$ particles/ml. Control cells were infected at the same concentration with a control adenovirus containing a U6 promoter cassette. Infected cells were lysed for total RNA purification in order to perform either Taqman® assays or FlexStation® assays.

For Taqman® assays, Infected cells were lysed at 6, 8 and 10 days post infection (dpi), and RNA purifed from lysates was used to measure $Na_v1.8$ RNA expression by quantitative RT-PCR analysis. At 6 days post infection, $Na_v1.8$ RNA expression, expressed as a percentage of non-silencing adenoviral siRNA, was 18%. At 8 days post infection, $Na_v1.8$ RNA expression, expressed as a percentage of non-silencing adenoviral siRNA, was 22%. At 10 days post infection, $Na_v1.8$ RNA expression, expressed as a percentage of non-silencing adenoviral siRNA, was 30%.

For FlexStation® assays, infected cells were used for measuring $Na_v1.8$ derived membrane potential on the FlexStation® at 2, 4, 6, 8 and 10 days post infection (dpi). At each time point, knock-down in membrane potential in siRNA 3 adenoviral vector construct infected cells was compared to membrane potential in control adenoviral infected cells. At 2 days post

infection, percent sodium current, as compared to non-silencing adenoviral-siRNA, was 45%. At 4 days post infection, percent sodium current, as compared to non-silencing adenoviral-siRNA, was 15%. At 6 days post infection, percent sodium current, as compared to non-silencing adenoviral-siRNA, was 8%. At 8 days post infection, percent sodium current, as compared to non-silencing adenoviral-siRNA, was 8%. At 10 days post infection, percent sodium current, as compared to non-silencing adenoviral-siRNA, was 4%.

To further confirm that the reduction in RNA expression seen with the viral-siRNA construct was representative of an attenuation in function NaV1.8 sodium channel activity, we performed a number of whole-cell voltage clamp experiments to directly measure NaV1.8-mediated current at various times post-infection. In each of these experiments, current amplitudes were measured in a sample of ND7/23-NaV1.8 cells that had been previously infected with either a control, non-silencing siRNA construct ot with the adenoviral-siRNA 3 construct. At 4 days post infection with the non-silencing viral construct total mean (10 cells sampled) whole cell NaV1.8 current was -821pA whilst that measured in siRNA3-virus infected cells was -101pA (corresponding to 12.3% of control). At 6 days post infection, with the non-silencing viral construct total mean (10 cells sampled) whole cell NaV1.8 current was -932pA whilst that measured in siRNA3-virus infected cells was -247.7pA (corresponding to 26.6% of control). At 10 days post infection, with the non-silencing viral construct total mean (10 cells sampled) whole cell NaV1.8 current was -976.7pA whilst that measured in siRNA3-virus infected cells was -542.7pA (corresponding to 55.6% of control).

Thus, Taqman® and FlexStation® and voltage-clamp assays showed knock-down of $Na_v1.8$ RNA expression and $Na_v1.8$ derived membrane potential that lasted for at least 8 dpi. Infection by siRNA expressing adenovirus resulted in at least 80% knock-down of $Na_v1.8$ expression and function. Thus, high levels of sustained $Na_v1.8$ block were demonstrated using viral vectors for siRNA delivery.

Example 6

Effect of $Na_V1.8$ siRNA in a Rat Model of Chronic Pain

The effect of $Na_V1.8$-siRNA was investigated in a rat model of chronic pain using siRNA 3. Hind paw tactile sensitivity was measured in a cohort of rats using graded von-Frey microfilaments (= baseline sensitivity). The same rats were then subjected to a surgical procedure that entailed exposure of the left sciatic nerve at mid thigh level followed by a loose ligation injury effected using standard suture material. The wound was closed and the animals allowed to recover from the procedure for a period of at least one week prior to any subsequent behavioral evaluation. The nerve trauma resulting from the procedure resulted in a tactile hypersensitivity in the left hind paw, a condition that is referred to as tactile allodynia. The degree of allodynia is readily quantified using the same von-Frey filament procedure as used for the baseline measurements, such measurements were taken 13 days after the surgical day. In order to evaluate the effects of siRNA 3 (SEQ ID No 3) on the allodynia, the siRNA was delivered as a duplex into the intrathecal space around the spinal cord via a permanent indwelling intrathecal catheter. Two separate injections of 2µg of siRNA 3 were made daily over a period of three days, control rats received an identical injection of vehicle only using the same timing protocol.

Baseline hindpaw sensitivities of rats used in these experiments ranged between 13 to 15 grams force. Thirteen days after the nerve trauma injury the hindpaw sensitivities were re-determined for each rat and were found to be in the range of 1.2 ± 0.4g in the cohort designated the siRNA group and 2.1 ±0.4g in the control cohort (cohort size = 6 rats in drug-treated group and 5 rats in the control group). Nerve injury resulted, therefore, in a profoundly painful tactile hypersensitivity (allodynia) that is typical of that seen in human subjects having suffered injuries that lead to a chronic neuropathic pain state. Regular assessments revealed the painful allodynic state to be maintained (typically < 2.1g) through days 13 to 21 in the control cohort of rats that received vehicle-only injections. By contrast, rats that were injected with

siRNA 3 for three days, commencing immediately after their day 13 assessment, showed a pronounced reversal of their painful allodynia (8.1 ± 2.1g, assessed on day 16). Rats treated with siRNA 3 showed a consistently improved pain score, (e.g., an amelioration of an experimentally-induced chronic pain state) compared to controls, over several subsequent days during which measurements were taken.

## Claims

We claim:

1. An isolated or recombinant short interfering nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, or an analogue thereof.

2. The isolated or recombinant short interfering nucleic acid of claim 1 comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 5, 9 and 10.

3. The isolated or recombinant short interfering nucleic acid of claim 1, further comprising a 3' overhang.

4. A pharmaceutical composition comprising a short interfering nucleic acid of either claim 1, or claim 3, and a pharmaceutically acceptable carrier.

5. The isolated or recombinant short interfering nucleic acid of either claim 1 or claim 3, further comprising a complementary nucleotide sequence thereto.

6. The complementary nucleotide sequence of claim 5, further comprising a 3' overhang.

7. A pharmaceutical composition comprising the short interfering nucleic acid and complementary nucleotide sequence of either claim 5 or claim 6, and a pharmaceutically acceptable carrier.

8. The isolated or recombinant short interfering nucleic acid of claim 5, wherein said nucleotide sequence and said complementary nucleotide sequence hybridize to form a duplex.

9. The duplex of claim 8, wherein said nucleotide sequence further comprises a 3' overhang and said complementary nucleotide sequence further comprises a 3' overhang.

10. A pharmaceutical composition comprising the duplex of either claim 8 or claim 9, and a pharmaceutically acceptable carrier.

11. An isolated or recombinant short interfering nucleic acid comprising a sense strand and an antisense strand, wherein said sense strand and said antisense strand hybridize to form a duplex, wherein said sense strand comprises a nucleotide sequence substantially identical to a target sequence, and wherein said target sequence is selected from the group consisting of SEQ ID NOs: 12-577.

12. The duplex of claim 11, wherein said sense strand further comprises a 3' overhang and said antisense strand further comprises a 3' overhang.

13. A pharmaceutical composition comprising the duplex of either claim 11 or claim 12, and a pharmaceutically acceptable carrier.

14. A recombinant vector comprising the nucleotide sequence of claim 1 or claim 3.

15. A method for inhibiting translation of an mRNA to a polypeptide comprising contacting a cell capable of expressing a $Na_v1.8$ mRNA with the short interfering nucleic acid of claim 1 or claim 3.

16. A method for inhibiting expression of a polypeptide comprising contacting a cell capable of expressing a $Na_v1.8$ polypeptide with the short interfering nucleic acid of claim 1 or claim 3.

17. A method for blocking the membrane potential in a cell comprising contacting a cell expressing a $Na_v1.8$ polypeptide with the short interfering nucleic acid of claim 1 or claim 3.

18. A method for blocking the sodium current in a cell comprising contacting a cell expressing a $Na_v1.8$ polypeptide with the short interfering nucleic acid of claim 1 or claim 3.

19. A method for inhibiting chronic pain comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of either claim 4, claim 7, claim 10 or claim 13.

20. The use of one or more of the short interfering nucleic acids of claim 1 or 3 in the manufacture of a medicament for inhibiting chronic pain.