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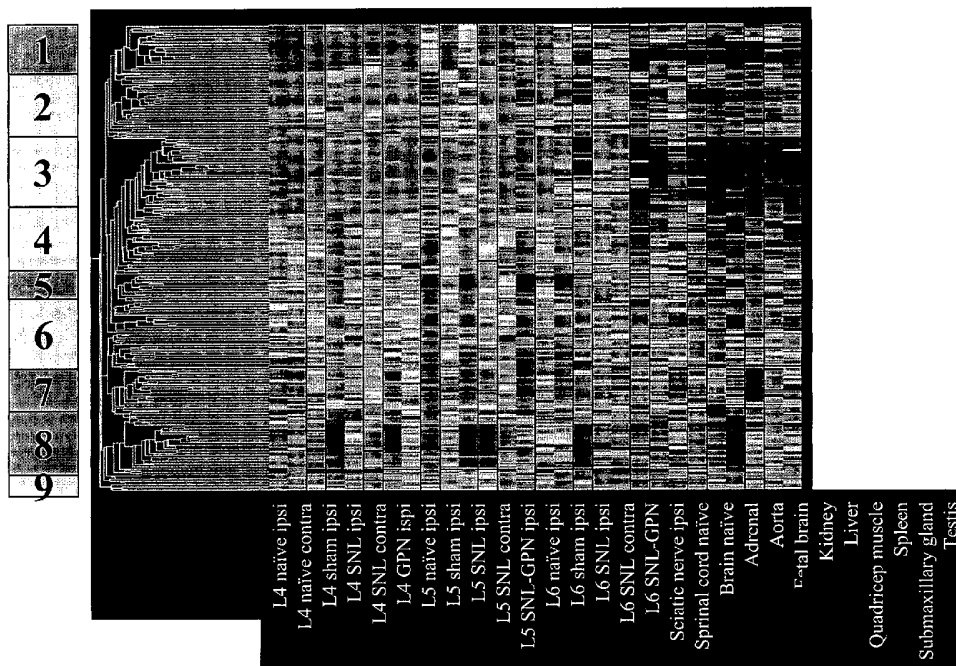
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(54) Title: GENES ASSOCIATED WITH RESPONSES TO NEUROPATHIC PAIN



(57) Abstract: The present relates to methods that are useful for the identification of therapeuticsw for the treatment of neuropathic pain. The invention specifically provides for identification and characterization of genes that are differentially expressed in a model oif neuropathic pain. The genes and their protein products can be used in screening methods to identify agonists and antagonists for the gene or gene product as potential therapeutic candidates.



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GENES ASSOCIATED WITH RESPONSES TO NEUROPATHIC PAIN

This application claims priority to provisional application Serial No. 60/485,101 filed on July 3, 2003, herein incorporated by reference in its entirety.

1. TECHNICAL FIELD

The present invention relates to methods that are useful for the identification of therapeutics for the treatment of neuropathic, inflammatory, cancer and other types of chronic pain. The invention specifically provides for identification and characterization of genes that are differentially expressed in a model of neuropathic pain. The genes and their protein products can be used in screening methods to identify agonists and antagonists for the gene or gene product as potential therapeutic candidates. The invention also relates to using the genes to generate animal models of pain using transgenic and recombinant technology to further elucidate a role for the genes in neuropathic pain. The present invention also relates to antibodies generated against the protein products of the genes to be used as diagnostic tools or therapeutics.

2. BACKGROUND ART

Pain is the most common symptom for which patients seek medical help, and can be classified as either acute or chronic. Acute pain is precipitated by immediate tissue injury (e.g. a burn or a cut), and is usually self-limited. Acute pain is a natural defense mechanism in response to immediate tissue injury, preventing further use of the injured body part, and withdrawal from the painful stimulus. Acute pain is amenable to traditional pain therapeutics, including non-steroidal antiinflammatory drugs (NSAIDs) and opioids. Conversely, chronic pain persists for 3 or more months, persisting after the injury has healed, and can lead to significant changes in a patient's life (e.g. functional ability and quality of life) (Foley, *Pain*, in *Cecil Textbook of Medicine* 100-107 (JC Bennett and F. Plum eds., 20th ed., Goldman Bennet 1996)).

Chronic pain can be classified as either "nociceptive" or "neuropathic", as defined below. Nociceptive pain includes tissue injury-induced pain (e.g. a cut or burn), and inflammatory pain (such as arthritis). Neuropathic pain refers to pain induced by damage to either the peripheral or central nervous system, and is characterized by aberrant somatosensory processing.

Chronic, debilitating pain represents a significant medical dilemma. In the United States, approximately 40 million suffer from chronic recurrent headaches, 35 million from persistent back pain, 20 million from osteoarthritis, 2.1 million from rheumatoid arthritis, and 5 million from cancer-related pain (Brower, *Nature Biotechnology* 2000; 18: 387-391).

5 Cancer-related pain involves both inflammation and nerve damage. In addition, analgesics are often associated with debilitating side effects such as nausea, dizziness, constipation, respiratory depression and cognitive dysfunction (Brower, *Nature Biotechnology* 2000; 18: 387-391, 2000).

10 2.1 Nociceptive Pain

“Nociceptive pain” is due to activation of pain-sensitive nerve fibers, either somatic or visceral. Nociceptive pain is generally a response to direct tissue damage. The initial trauma typically causes the release of several chemicals including bradykinin, serotonin, substance P, histamine, and prostaglandin. When somatic nerves are involved, the pain is
15 typically experienced as an aching or pressure-like sensation.

Nociceptive pain has traditionally been managed by administering non-opioid analgesics. These analgesics include acetylsalicylic acid, choline magnesium trisalicylate, acetaminophen, ibuprofen, fenoprofen, diflusal, and naproxen, among others. Opioid analgesics, such as morphine, hydromorphone, methadone, levorphanol, fentanyl,
20 oxycodone and oxymorphone, may also be used (Foley, *Pain, In: Cecil Textbook of Medicine*, pp.100-107, Bennett and Plum eds., 20th ed., 1996).

2.2 Neuropathic Pain

The term “neuropathic pain” refers to pain that is due to injury or disease of the central or peripheral nervous system (McQuay, *Acta Anaesthesiol. Scand.* 1997; 41(1 Pt 2): 175-83; Portenoy, *J. Clin. Oncol.* 1992; 10: 1830-2). In contrast to the immediate pain caused by
25 tissue injury, neuropathic pain can develop days or months after a traumatic injury. Furthermore, while pain caused by tissue injury is usually limited in duration to the period of tissue repair, neuropathic pain frequently is long-lasting or chronic. Moreover, neuropathic pain can occur spontaneously or as a result of stimulation that normally is not
30 painful.

Neuropathic pain is associated with chronic sensory disturbances, including spontaneous pain, hyperalgesia (*i.e.*, sensation of more pain than the stimulus would warrant), and allodynia (*i.e.*, a condition in which ordinarily painless stimuli induce the experience of

pain). In humans, prevalent symptoms include cold hyperalgesia and mechanical allodynia. Sensitivity to heat is rarely reported. Descriptors that are often used to describe such pain include "lancinating," "burning," or "electric". It is estimated that approximately 4 million people in North America suffer from chronic neuropathic pain, and of these no more than half achieve adequate pain control (Hansson, *Pain Clinical Updates* 1994; 2(3)).

Examples of neuropathic pain syndromes include those due to disease progression, such as diabetic neuropathy, multiple sclerosis, or post-herpetic neuralgia (shingles); those initiated by injury, such as amputation (phantom-limb pain), or injuries sustained in an accident (e.g., avulsions); and those caused by nerve damage, such as from chronic alcoholism, viral infection, hypothyroidism, uremia, or vitamin deficiencies. Traumatic nerve injuries can also cause the formation of neuromas, in which pain occurs as a result of aberrant nerve regeneration. Stroke (spinal or brain) and spinal cord injury can also induce neuropathic pain. Cancer-related neuropathic pain results from tumor growth compression of adjacent nerves, brain, or spinal cord. In addition, cancer treatments, including chemotherapy and radiation therapy, can also cause nerve injury.

Unfortunately, neuropathic pain is often resistant to available drug therapies. Treatments for neuropathic pain include opioids, anti-epileptics (e.g., gabapentin, carbamazepine, valproic acid, topiramate, phenytoin), NMDA antagonists (e.g., ketamine, dextromethorphan), topicals such as Lidocaine (for post-herpetic neuralgia), and tricyclic anti-depressants (e.g., fluoxetine (Prozac®), sertraline (Zoloft®), amitriptyline, among others). However, none of these treatments is particularly effective, achieving clinical significance of less than 50% (Bridges *et al.*, *Br. J. Anaes.* 2001; 87: 12-26). Neuropathic pain is frequently only partially relieved by high doses of opioids, which are the most commonly used analgesics (Cherny *et al.*, *Neurology* 1994; 44: 857-61.; MacDonald, *Recent Results Cancer Res.* 1991; 121: 24-35.; McQuay, 1997, *supra*). Current therapies may also have serious side effects such as cognitive changes, sedation, or nausea. Many patients suffering from neuropathic pain are elderly or have other medical conditions that limit their tolerance of such side-effects.

2.3 Inflammatory Pain

Chronic somatic pain generally results from inflammatory responses to tissue injury such as nerve entrapment, surgical procedures, cancer or arthritis (Brower, *Nature Biotechnology* 2000; 18: 387-391). Although many types of inflammatory pain are currently treated with NSAIDs, there is much room for improved therapies.

The inflammatory process is a complex series of biochemical and cellular events activated in response to tissue injury or the presence of foreign substances (Levine, *Inflammatory Pain, In: Textbook of Pain*, Wall and Melzack eds., 3rd ed., 1994). Inflammation often

occurs at the site of injured tissue, or foreign material, and contributes to the process of tissue repair and healing. The cardinal signs of inflammation include erythema (redness), heat, edema (swelling), pain and loss of function (ibid.). The majority of patients with inflammatory pain do not experience pain continually, but rather experience enhanced pain when the inflamed site is moved or touched.

Tissue injury induces the release of inflammatory mediators from damaged cells. These inflammatory mediators include ions (e.g., H^+ , K^+), bradykinin, histamine, serotonin (5-HT), ATP and nitric oxide (NO) (Kidd and Urban, *Br. J. Anaesthesia* 2001, 87: 3-11), among others. The production of prostaglandins and leukotrienes is initiated by activation of the arachidonic acid (AA) pathway. Via activation of phospholipase A_2 , AA is converted to prostaglandins by cyclooxygenases (COX-1 and COX-2), and to leukotrienes by 5-lipoxygenase. The NSAIDs exert their therapeutic action by inhibiting cyclooxygenases. Recruited immune cells release further inflammatory mediators, including cytokines and growth factors, and also activate the complement cascade. Some of these inflammatory mediators (e.g., bradykinin) activate nociceptors directly, leading to spontaneous pain. Others act indirectly via inflammatory cells, stimulating the release of additional pain-inducing (algogenic) agents. Application of inflammatory mediators (e.g., bradykinin, growth factors, prostaglandins) has been shown to produce pain, inflammation and hyperalgesia (increased responsiveness to normally noxious stimuli).

2.4 Players in Pain

Chronic pain, including neuropathic pain, has been shown to be mediated via several neurotransmitter systems, as well as ion channels and intracellular second messengers. Neuropathic pain is thought to induce neuronal plasticity, creating a hyper-excitable state. Here, some of the mechanisms proposed to be involved in chronic pain, and neuropathic pain in particular, are briefly discussed.

Glutamate is the most commonly studied neurotransmitter involved in pain states. A large body of evidence has accumulated showing the involvement of several types of glutamate receptors in neuropathic pain (Fundytus, *CNS Drugs* 2001; 15: 29-58; Neugebauer, *Expert Rev. Neurotherapeutics* 2001; 1: 207-224). Release of glutamate is enhanced in the spinal cord of neuropathic rats (Al-Ghoul *et al.*, *Brain Res. Bull.* 1993; 30: 453-459). The most commonly studied glutamate receptor is the N-methyl-D-aspartate (NMDA) receptor. It has been shown in animal models of neuropathic pain that NMDA antagonists reduce hyperalgesia and allodynia associated with nerve injury (Chaplan *et al.*, *J. Pharmacol. Ex. Ther.* 1997; 280: 829-838; Mayer *et al.*, *NIDA Res. Monogr.* 1995; 147: 269-298; Yamamoto & Yaksh, *Pain* 1992; 49: 121-128). Glutamate also acts at a class of G-protein

coupled receptors (GPCRs) termed metabotropic glutamate receptors (mGluRs). It has been shown that inhibition of group I mGluRs with either antagonist (Fisher et al., *Pain* 1998; 77: 59-66), selective antibodies (Fundytus et al., *NeuroReport* 1998; 9: 731-735), or antisense oligonucleotides (Fundytus et al., *Br. J. Pharmacol.* 2001; 132: 354-367)

5 significantly alleviates hyperalgesia and allodynia associated with a chronic constriction injury of the sciatic nerve.

There is also a considerable body of evidence showing that glutamate is involved in inflammatory pain (reviewed in Fundytus, *CNS Drugs* 2001; 15: 29-58; Neugebauer *Expert Rev. Neurotherapeutics* 2001; 1: 207-224). For example, administration of NMDA

10 antagonists reduces thermal hyperalgesia and mechanical allodynia (pain-like response to normally non-noxious stimuli) in animal models of persistent and chronic inflammation (Ren and Dubner, *Neurosci. Lett.* 1993; 163: 22-26; Eisenberg, LaCross, and Strassman, *Eur. J. Pharmacol.* 1994; 255: 123-129; Ren et al., *Pain* 1992; 50: 331-334; Ma, Allchrone, and Woolf, *Pain* 1998; 77: 49-57; Price et al., *Pain* 1996; 68: 119-127).

15 NMDA receptor expression is also modulated by chronic inflammation (Carlton and Coggeshall, *Brain Research* 1999; 820: 63-70; Renno, *Neurobiology (Bp)* 1998; 6: 333-357; Wang et al., *Neurosci. Lett.* 1999; 265: 195-198). Antagonists at two other types of ionotropic glutamate receptors, the AMPA and kainate receptors, attenuate hyperalgesia related to chronic inflammation (Szekely et al., *Eur. J. Pharmacol.* 1997; 336: 143-154; Coutinho, Meller and Gebhart, *Brain Res.* 1996; 736: 7-15). Recent evidence suggests a
20 role for group I mGluRs in inflammatory pain. Administration of an antisense oligonucleotide targeting mGluR1 alleviates hyperalgesia and allodynia induced by chronic inflammation (Fundytus et al., *Pharmacol. Biochem. & Behav.* 2002; 73: 401-410). In addition, antagonists to mGluR5 also attenuate inflammatory pain (Walker et al.,
25 *Neuropharmacology* 2000; 40: 1-9).

NMDA receptors are coupled to Ca^{2+} ion channels, and thus, activation of these receptors allows influx and subsequent increase in intracellular concentration of Ca^{2+} . Group I mGluRs are positively coupled to phosphatidylinositol (PI) hydrolysis via activation of phospholipase C (PLC), and activation of these receptors also leads to an increase of
30 intracellular Ca^{2+} as well as production of diacylglycerol (DAG). Both DAG and Ca^{2+} promote the translocation and activation of protein kinase C (PKC). PKC has been demonstrated to be involved in neuropathic pain. PKC is upregulated in the spinal cord dorsal horn following nerve injury (Miletic et al., *Neuroscience Letters* 288: 199-202, 2000; Fundytus et al., *Br. J. Pharmacol.* 132: 354-367, 2001). In addition, PKC knockout
35 mice do not develop neuropathic pain (Malmberg et al., *Science* 1997; 278: 279-283; Ohsawa et al., *Eur. J. Pharmacol.* 2001; 429: 157-160). Thus, PKC appears to be intimately involved in neuropathic pain. PKC has also been shown to play a role in

inflammatory pain. Antisense oligonucleotide knockdown of PKC γ has been shown to reduce pain responses in persistent inflammatory pain induced by intra-paw injection of a dilute solution of formalin (Wu et al., *Anesthesiology* 2000; 95: 216-240). A major inhibitory neurotransmitter, gamma-amino-butyric acid (GABA), is also involved in neuropathic pain. GABA levels are decreased in the spinal cord following sciatic nerve axotomy (Bridges et al., *supra*). Moreover, the anti-nociceptive effect of the GABA-B receptor agonist, baclofen, is enhanced in nerve-injured animals (Smith et al., *Neuropharmacology* 1994; 33: 1103-1108; Hwang & Yaksh, *Pain* 1997; 70: 15-22).

Recent evidence indicates a role for the cannabinoid (CB) system in neuropathic pain. Cannabinoid receptors are distributed in areas of the spinal cord associated with nociception (Bridges et al., *supra*). Moreover, cannabinoid agonists have been shown to attenuate the sensory changes associated with nerve injury (Bridges et al., *supra*).

Cannabinoid receptors also are involved in inflammatory pain. Topical and systemic application of cannabinoid agonists attenuates inflammatory pain (Kidd and Urban, *British Journal of Anaesthesia* 2001; 87: 3-11). Two subtypes of cannabinoid receptors are known, CB1 and CB2. CB1 receptors are found on central and peripheral neurons, are negatively coupled to adenylate cyclase, and activation of these receptors reduces the excitability of neurons. CB2 receptors are present on immune cells, and activation of these receptors may exert analgesia by inhibition of immune cell function.

The vanilloid receptor (VR1) is another receptor involved in both neuropathic and inflammatory pain. VR1 is the receptor for capsaicin, which is the "hot" substance in peppers. VR1 is activated by extracellular acidification and heat, as well as by agonists such as capsaicin. VR1 is a ligand-gated, non-selective cation channel, having six transmembrane spanning segments with a pore loop between domains five and six (Marzo, Blumber and Szallasi, *Current Opinion in Neurobiology* 2002; 12: 372-379). Under resting conditions, the VR1 channel is closed, but upon activation it enters the open state, allowing the influx of divalent cations (particularly Ca²⁺) through the cell membrane (Ibid). Inflammatory mediators such as bradykinin and nerve growth factors release VR1 from the inhibitory control of phosphatidylinositol-4, 5-bisphosphate (Ibid). There is evidence suggesting that both the sensitivity and density of VR1 are enhanced during the inflammation process (Ibid). Moreover, VR1 antagonists attenuate hyperalgesia and allodynia associated with inflammatory pain models. There is recent evidence suggesting that VR1 is also up-regulated in neuropathic pain (Ibid). Capsaicin is used for the treatment of post-herpetic neuralgia. Although initially application of the agonist induces excitation of the receptors, continued administration leads to de-sensitization, and reduced pain sensitivity.

Sodium channels have also been shown to be involved in neuropathic pain. Following nerve injury, there is an upregulation of type III tetrodotoxin (TTX) sensitive channel gene expression, which is not normally expressed by DRG cells (Bridges et al., *supra*). There is a concurrent down-regulation of gene expression of the TTX-resistant sodium channels, SNS (also called PN3) and NaN (also called SNS2) (Bridges et al., *supra*).

Although sodium channels are also involved in inflammatory pain, regulation of these channels is different from that in neuropathic pain states. During chronic inflammation, the amount of SNS/PN3 protein is increased (Kidd and Urban, *British Journal of Anaesthesia* 2001; 87: 3-11). Inflammatory mediators, including prostaglandin E2 (PGE2), adenosine and 5-HT enhance sodium channel sensitivity. Spinal antisense oligonucleotide knockdown of SNS/PN3 ameliorates inflammation-induced hyperalgesia (Porreca et al., *Proc. Natl. Acad. Sci.* 1999; 96: 7640-7644). Interestingly, local anesthetics such as lidocaine, and anticonvulsants such as carbamazepine, exert their therapeutic effects by blocking sodium channels.

Calcium channels have also been shown to mediate hyperalgesia and allodynia associated with nerve injury. Selective inhibitors of N-type Ca^{2+} channels have been shown to attenuate hyperalgesia and allodynia associated with animal models of nerve injury (Bridges et al., *supra*). Furthermore, an N-type Ca^{2+} channel inhibitor, ziconotide, is used in the clinic to relieve neuropathic pain in humans. The commonly used drug, gabapentin, is thought to exert its therapeutic effect via action on calcium channels.

2.5 Genetics

Recent efforts to treat neuropathic pain have focused on identification of genes that are differentially regulated in response to pain stimuli. Using rat models of neuropathic pain, changes in gene and protein expression in DRG neurons from the injured side of the animal (ipsilateral) compared with the uninjured side of the animal (contralateral) or uninjured neurons have been reported (Wang et al., *Neuroscience* 2002; 114:520-46; Kim et al., *NeuroReport* 2001; 12:3401-05; Xiao et al., *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99: 8361-65; Costigan et al., *BMC Neuroscience* 2002; 3:16; and Sun et al., *BMC Neuroscience*; 2002; 3: 11). Genes that were found to be upregulated in injured neurons include genes that encode cell-cycle and apoptosis-related proteins; genes encoding pro-inflammatory cytokines or lymphokines, including complement proteins; genes encoding ion channels and their receptors; genes encoding transcription factors; and genes encoding structural or glycoproteins involved in tissue remodeling (Wang et al., *supra*). Genes that were down-regulated compared with uninjured neurons include genes encoding the following: neuropeptides such as somatostatin and substance P; the serotonin 5HT-3

receptor; the glutamate receptor 5 (GluR5); sodium and potassium channels; calcium signaling molecules; and synaptic proteins (Wang et al., *supra*).

Neuronal transcription factors are also differentially regulated in injured neurons.

Transcription factors found to be differentially expressed include JunD, NGF1-A and

MRG1 (Xiao et al., *supra*; Sun et al., *supra*). The present invention is the first to show injury-induced transcription of DREAM, a transcription factor that represses expression of an endogenous opioid gene. However, it has been shown that targeted inactivation of DREAM resulted in mice with attenuated responses to noxious stimuli (Cheng et al., *Cell* 2002; 108:31-43). Despite the identification of certain genes that are up or down-regulated in models of neuropathic pain, there remains a need to identify other response genes in this condition.

3. DISCLOSURE OF THE INVENTION

The present invention provides a method of detecting a pain response in a first cell by determining the expression level of a nucleic acid molecule corresponding to one or more of 249 genes (**SEQ ID NOS: 1-249**, including species orthologs listed in Tables 5 and 6), and comparing the expression level to the expression level of the same nucleic acid molecule in a second cell from an animal model of neuropathic pain. An expression level of the one or more nucleic acid molecules in the first cell that is similar or identical to the expression level of the one or more nucleic acid molecules in the second cell indicates that the first cell is experiencing a pain response.

The present invention further provides a method of detecting a pain response in a first cell by determining the expression level or the activity level of one or more gene products of one or more nucleic acid molecules corresponding to **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6), and comparing the expression level or activity level of said gene product(s) to the expression level or activity level of the same gene product(s) in a second cell from an animal model of neuropathic pain. An expression level or activity level of said gene product(s) in said first cell that is similar or identical to the expression level or activity level of said gene product(s) in the second cell indicates that the first cell is experiencing a pain response.

The invention also provides a method for identifying a candidate compound for the treatment of pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) comprising (i) contacting a neuronal test cell with a test compound; (ii) determining the expression level in the test cell of one or more nucleic acid molecules which correspond to those depicted in **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6); and (iii) comparing the

expression level of the one or more nucleic acid molecules in the test cell to the expression level of the one or more nucleic acid molecules in a control cell not contacted with the test compound, wherein a detectable change in the expression level of one or more of the nucleic acid molecules in the test cell in response to contact with the test compound compared to the expression level of one or more of the nucleic acid molecules in the control cell indicates that the test compound modulates the expression level of one or more nucleic acid molecules and is a candidate compound. In one embodiment, the compound is a candidate for treating neuropathic pain.

The present invention also provides a method for identifying a candidate compound for the treatment of pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) comprising (i) contacting a neuronal test cell with a test compound; (ii) determining the expression level or the activity level in the test cell of the gene product(s) of one or more nucleic acid molecules corresponding to **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6); and (iii) comparing the expression level or the activity level of said gene product(s) in the test cell to the expression level or activity level of said gene product(s) in a control cell not contacted with the test compound, wherein a detectable change in the expression level or activity level of one or more of said gene product(s) in the test cell in response to contact with the test compound compared to the expression level or activity level of the one or more gene product(s) in the control cell indicates that the test compound modulates the expression level or activity level of one or more of said gene product(s) and is a candidate compound. In one embodiment, the compound is a candidate for treating neuropathic pain.

The present invention further provides a method for identifying a candidate compound for the treatment of pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) comprising (i) contacting a first set of neuronal cells with an analgesic compound; (ii) contacting a second set of neuronal cells with a test compound; (iii) determining the expression level in the first and second sets of neuronal cells of one or more nucleic acid molecules corresponding to the sequences depicted in **SEQ ID NOS: 1-249** (and species orthologs listed in Tables 5 and 6); and (iv) comparing the expression level of the one or more nucleic acid molecules in said second set of cells with the expression level in the first set of cells, wherein an expression level of one or more of said nucleic acid molecules in said first set of cells in response to contact with the analgesic compound that is similar to the expression level of one or more of said nucleic acid molecules in said second set of cells in response to contact with the test compound indicates that the test compound is producing an effect that is similar to the effect of the analgesic compound and that the test compound is a candidate compound. This method may further comprise comparing the expression levels of the one

or more nucleic acid molecules in the first and second sets of neuronal cells to the expression level of the one or more nucleic acid molecules in a set of control cells not contacted with the analgesic compound or test compound, wherein an expression level that is similar between the first and second set of cells, but different from the expression level in the set of control cells not contacted with either compound, further indicates that the test compound has an effect that is similar to the analgesic compound and is a candidate compound. In one embodiment, the test compound is a candidate for treating neuropathic pain.

The present invention further provides a method for identifying a candidate compound for the treatment of pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) comprising (i) contacting a first set of neuronal cells with an analgesic compound; (ii) contacting a second set of neuronal cells with a test compound; (iii) determining the expression level or activity level in the first and second set of neuronal cells of one or more of the gene product(s) of one or more of the nucleic acid molecules depicted in **SEQ ID NOS: 1-249** (and species orthologs listed in Tables 5 and 6); and (iv) comparing the expression level or activity level of one or more of the gene product(s) of one or more of the nucleic acid molecules in said second set of cells with the expression level or activity level of said gene product(s) of one or more of the nucleic acid molecules in the first set of cells, wherein an expression level or activity level of one or more of said gene products in said first set of cells that is similar to the expression level or activity level of the one or more gene products in the second set of cells indicates that the test compound is producing an effect that is similar to the effect of the analgesic compound, and that the test compound is a candidate compound. This method may further comprise comparing the expression level or activity level of one or more of the said gene product(s) in the first and second sets of neuronal cells to the expression level or activity of one or more said gene product(s) in a set of control cells not contacted with either the analgesic compound or the test compound, wherein an expression level or activity level that is similar between the first and second set of cells, but that is different from the expression level in the set of control cells not contacted with either compound, further indicates that the test compound has an effect that is similar to the analgesic compound and is a candidate compound. In one embodiment, the test compound is a candidate for treating neuropathic pain.

Also provided by the present invention is a method of diagnosing pain by determining the expression level of one or more nucleic acid molecules corresponding to one or more of 249 genes (**SEQ ID NOS.: 1-249**, including species orthologs listed in Tables 5 and 6) in

a first cell, and comparing the expression level to the expression level of the same one or more nucleic acid molecules in a second cell from an animal model of pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) and/or from a control cell from an individual not having, or not suspected of having, a condition involving pain or a related disease, or having a pain condition. In one embodiment, the first cell is from a patient having a pain condition. In another embodiment, the animal model is a model of neuropathic pain.

The present invention further provides a method of diagnosing pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) in a subject by determining in a first cell the expression level or activity level of the gene product(s) of one or more of the nucleic acid molecules corresponding to **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6), and comparing the expression level or activity level of said gene product(s) in the first cell to the expression level or activity of the same gene product(s) in a second cell from an animal model of pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) and/or from a control cell from a subject not having, or not suspected of having, a condition involving pain or a related disease, or a pain condition. In one embodiment, the first cell is from a patient having, or suspected of having, a pain condition. In another embodiment, the animal model is a model of neuropathic pain.

The present invention also provides a method for identifying new candidate targets for treating pain or a related disease (*i.e.*, a disease having similar physiological mechanisms as pain, as described below in “*Other Indications*”) by determining the interaction of one or more of the gene products, or fragments thereof, encoded by any one of **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6), herein the first protein, with one or more second proteins, or fragments thereof, that are either known or novel, wherein a measurable interaction between the first and second proteins indicates that the second protein is a ligand or binding partner for the first protein, and thus a candidate target for treating pain. Appropriate methods for identifying protein-protein interactions are known to one of skill in the art, for example including, but not limited to, co-immunoprecipitation and yeast two-hybrid assays.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a heat map of gene expression data with a dendrogram indicating the relatedness between two specific gene expression patterns. Each gene was represented by a row of shaded tiles, reflective of gene expression for the indicated conditions. The degree

of relatedness, defined by a Euclidean metric (see experimental procedures), was proportional to the distance of the connecting branches between two specific gene expression patterns. The major branches of the dendrogram segregated nine transcript regulation classes (left panel of Figure 1) 1-9 (as described in Table 3). The transcript class descriptions were based on annotations of the known genes represented within in each group.

Figure 2 summarizes experimental timelines for surgery, treatment and behavioral testing.

5. MODES OF THE INVENTION

The present invention provides nucleic acid molecules and proteins for screening new compounds useful for treating pain or a related disease and for diagnosis of pain or related disease conditions in an animal, including humans.

The present inventors have subdivided the nucleic acid molecules representing genes into transcript classes representing functionally related proteins using gene expression hierarchical clustering algorithms. By using these algorithms, the functional relevance of regulated genes was determined based on their gene expression data not only from the apparent up- or down- regulation between two conditions (such as treated and control) or a few conditions, but also from their entire expression pattern across 16 conditions in an animal pain model and expression distribution across 12 normal tissues, or 28 total conditions.

According to the present invention, the nucleic acid molecules can be used individually, *e.g.*, to monitor the level of expression of an individual gene, or they can be provided in a microarray format, which provides a powerful tool for evaluating the pain response state of a cell, such as dorsal root ganglion cells. The nucleic acid molecules can be used to design antisense oligonucleotides and short-interfering RNA (siRNA), ribozymes, and other molecules useful for modifying gene expression, for diagnostic, screening, and therapeutic purposes. Furthermore, the nucleic acid molecules can be used to express the encoded proteins. One skilled in the art can also design peptide antigens based on the nucleic acid sequences.

The proteins are useful as targets for drug discovery, *e.g.*, to identify lead compounds that agonize or antagonize their activity, as described below. In addition, the proteins can be used to generate antibodies or other specific binding agents for development of reagents for evaluating or treating pain responses. These specific binding agents may be used for

diagnostic, screening or therapeutic purposes. These specific binding agents can be used in microarray format, *e.g.*, for proteomic based diagnostics. In addition to diagnostics, the nucleic acid arrays or protein binding arrays are also useful for drug development. Gene expression analysis at either the mRNA or protein level provides an indication of a cellular response to pain stimuli, or related disease state, *e.g.*, whether the subject shows the expression characteristics of neuropathic pain, as well as cellular responses to pain treatment.

Proteins can be probed directly using, for example, yeast two-hybrid assays for protein-protein interactions to identify additional therapeutic targets. These characteristics can be evaluated in cells obtained from animals or from tissue culture. Briefly, the two-hybrid assay is a genetic assay for detecting interactions between two proteins or polypeptides. It can be used to identify proteins that bind to a known protein of interest, or to delineate domains or residues critical for an interaction. Variations on this methodology have been developed to clone genes that encode DNA binding proteins, to identify peptides that bind to a protein and to screen for drugs. The two-hybrid system exploits the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA binding domain that binds to an upstream activation sequence (UAS) of a reporter gene, and is generally performed in yeast. The assay requires the construction of two hybrid genes encoding (1) a DNA-binding domain that is fused to a first protein and (2) an activation domain fused to a second protein. The DNA-binding domain targets the first hybrid protein to the UAS of the reporter gene; however, because most proteins lack an activation domain, this DNA-binding hybrid protein does not activate transcription of the reporter gene. The second hybrid protein, which contains the activation domain, cannot by itself activate expression of the reporter gene because it does not bind the UAS. However, when both hybrid proteins are present, the non-covalent interaction of the first and second proteins tethers the activation domain to the UAS, activating transcription of the reporter gene.

For example, in an assay to identify proteins that interact with one of the proteins of the present invention, a fusion polynucleotide encoding both one of the proteins, or a fragment thereof, of the present invention, and a UAS binding domain may be used. A large number of hybrid genes each encoding a different second protein fused to an activation domain are produced and screened in the assay. Typically, the second protein is encoded by one or more members of a total cDNA or genomic DNA fusion library, with each second protein coding region being fused to the activation domain. This system is applicable to a wide variety of proteins, and it is not even necessary to know the identity or function of the second binding protein. The system is highly sensitive and can detect interactions not revealed by other methods; even transient interactions may trigger

transcription to produce a stable mRNA that can be repeatedly translated to yield the reporter protein. This assay is described in detail in U.S. Patent No. 5,468,614 and U.S. Patent No. 5,667,973, both of which are incorporated by reference herein in their entirety.

In addition, when one of the proteins, or a fragment thereof, is known to interact with another protein or nucleic acid, the two-hybrid system assay can be used to detect agents that interfere with the binding interaction. Expression of the reporter gene is monitored as different test agents are added to the system. The presence of an inhibitory agent, for example, results in lack of or a reduction in a reporter signal.

Proteins encoded by the nucleic acid molecules of the present invention include G-protein (guanine nucleotide regulatory) coupled receptors (GPCRs), ion channels, and enzymes. The activity level of the encoded proteins can be assessed by any method known to one of skill in the art.

When a specific ligand binds to its corresponding GPCR, the ligand typically stimulates the receptor to activate a specific G-protein that is coupled to the intracellular portion or region of the receptor. The G-protein, in turn, transmits a signal to an effector molecule. These effector molecules include second messenger signal transduction pathways, such as adenylate cyclase and phospholipases, as well as ion channels. Non-limiting examples of assays to assess GPCR activity are described below.

Adenylate cyclase is an enzyme that promotes the production of cyclic adenosine monophosphate (cAMP) by catalyzing the conversion of ATP to cAMP, which in turn increases cellular excitability. Adenylate cyclase activity can be assessed by detecting and measuring the accumulation of cAMP, in the presence or absence of a test compound. In one non-limiting example, cells expressing a protein encoded by any one of the nucleic acid molecules of the present invention (corresponding to **SEQ ID NOs: 1-249**, including species orthologs listed in Tables 5 and 6) can be incubated in culture medium with or without ligand for 30 minutes, then the medium can be removed and cells lysed by treatment with 1 M perchloric acid. The cAMP levels in the lysate are measured by radioimmunoassay using methods well-known in the art. Changes in the levels of cAMP in the lysate from cells exposed to test compound compared to those with test compound indicate that the test compound modulates the encoded protein.

In another non-limiting example the activity of a GPCR coupled to phospholipase C (which hydrolyzes phosphoinositol-4,5-bisphosphate (PIP₂)) can be assessed by measuring the accumulation of products of phosphoinositol hydrolysis, such as diacylglycerol (DAG), inositol-1,4,5-trisphosphate (IP₃) or activated protein kinase C (PKC). To measure changes in inositol levels, cells expressing the receptor of interest are grown in 24-well plates

containing 1×10^5 cells per well and incubated with inositol-free media and [^3H]myoinositol, 2 mCi/well, for 48 hours. The culture medium is removed, and the cells washed with buffer containing 10 mM LiCl, followed by addition of ligand. The reaction is stopped by addition of perchloric acid. Inositol phosphates are extracted and separated on Dowex
5 AG1-X8 (Bio-Rad) anion exchange resin, and the total labeled inositol phosphates counted by liquid scintillation. Changes in the levels of labeled inositol phosphate from cells exposed to ligand compared to those without ligand indicate relative activity of the GPCR, and modulation by addition of test compounds can be assessed.

As described in *Enzyme Assays: A practical approach*, 1993, ed. R. Eisinger and M.J.

10 Danson, the activity of an enzyme may be measured by determining the rate of product formation or substrate utilization during the enzyme-catalysed reaction. For many enzymes, there are several alternative assay procedures that are known to one of skill in the art. Direct continuous assays measure any difference between the properties of the substrates and products that can be directly measured. Changes in absorbance,
15 fluorescence, pH, optical rotation, conductivity, enthalpy, viscosity or volume of the reaction mixture are examples of direct continuous assays known to one of skill in the art. Alternatively, indirect assays involve some further treatment of the reaction mixture either to produce a measurable product or to increase the sensitivity of the assay procedure. Discontinuous indirect assays, also called sampling assays, involve stopping the reaction
20 after a fixed time and treating the reaction mixture to separate product for analysis (e.g. by liquid chromatography), or to produce a change in the properties of either substrate or product which can then be measured. A continuous indirect assay can be employed by adding to the reaction mixture a reagent that reacts with one of the products of the reaction to form a detectable compound. An example of this type of reaction is the detection of
25 carnitine acyltransferases.

The hemolysis assay is used to measure complement activity in serum (isolated from blood). Sheep Blood Erythrocytes are sensitized by incubating them with a polyclonal antibody that recognizes sheep red blood cells. When serum is added to the sensitized erythrocytes (EA), the complement components present in serum bind to the antibody bound to the EA and
30 activate the complement pathway. Activation of the complement pathway leads to the formation of the complement membrane attack complex (MAC) on the cells, causing them to lyse and release hemoglobin. The amount of hemoglobin released in the serum sample, measured at A540, compared to EA incubated with water (100% lysis) is a measurement of the complement activity. The amount of serum giving 50% lysis is designated as the CH50.

35 The level of activity for each of the components in the complement pathway can be measured

by adding to your sample, serum that is depleted in a particular complement component. The amount of lysis achieved with the component-depleted serum is a measurement of the level or activity of that component in your serum sample.

- 5 Activation of ligand-gated ion channels elicits opening of the ion channel, which forms a central pore through the receptor structure (for review see *Textbook of Receptor Pharmacology*, 1996, ed J.C. Foreman and T. Johansen). Positively charged ions such as Na^+ , K^+ and Ca^{2+} flow through cationic channels (formed by for e.g. glutamate receptors, nicotinic receptors, 5HT_3 receptors, or ATP receptors). Ionic currents via cationic
10 channels are generally excitatory, and thus lead to depolarization of the cell. The main charge carrier via activation of inhibitory receptors such as GABA and glycine is Cl^- , with some contribution from HCO_3^- ions. Electrophysiological techniques enable the study of the effect of agonists and antagonists on ligand-gated ion channels. The response current through open ion channels when measured with for e.g. voltage or patch-clamp techniques,
15 is directly proportional to receptor activation.

The present invention is based on gene expression profiles obtained from a spinal nerve ligation (SNL) model of neuropathic pain (Kim & Chung, *Pain* 1992; 50:355-63). This model consists of tightly ligating the L5 and L6 spinal nerves in the rat. The rat L5/6 intervertebral disc is innervated by L1 to L6 dorsal root ganglia (DRG). Symptoms
20 induced by this injury include sensitivity to cold and mechanical stimuli, as well as sensitivity to heat. Behaviors indicative of spontaneous pain, such as sudden licking, gentle biting, or pulling of the nails on the operated side, are also observed. These symptoms are attenuated by drugs commonly used in the clinic to treat neuropathic pain (e.g., gabapentin).

- 25 Microarrays containing oligonucleotide probe sets representing 26,000 unique rat genes (GeneChip®, Rat Genome U34 A, B, and C arrays, Affymetric, Santa Clara, CA) were used initially to identify mRNAs regulated in rats subjected to the SNL model with or without gabapentin treatment. 249 known genes (Table 1) were selected on the basis of their regulation in the injured and non-injured dorsal root ganglia (DRGs), and assigned to
30 a transcript class based on their gene expression pattern (see Tables 2 and 3, respectively) and function activity described in the literature. Further refinement of the genes most significant as targets for pain therapeutics were those in transcript class 1 and 5, and included 22 genes co-regulated with genes known to be important as molecular mediators of pain perception and/or restricted in their expression across a panel of 12 normal tissues
35 to the target organ for pain, DRGs. For 19 of these 22 genes, apparent regulation in the

SNL model of identified genes was validated using quantitative real time PCR (TaqMan®, Applied Biosciences, Inc., Foster City, CA) on mRNA samples isolated from an independent pool of animals (see Table 4).

5.1 Regulated Genes Based on Evidence Collected from Current Literature

5 Calsenilin/(KChIP3)

Calsenilin/KChIP3 binds and modulates rapidly inactivating (A-type) voltage-gated potassium channels, notably the Kv4.2 alpha-subunit of potassium channels (An et al., *Nature* 2000; 403:553-556, and Spreafico et al., *Mol. Cell. Neurosci.* 2001; 17(1):1-16). Calsenilin, which also binds presenilin (Buxbaum et al., *Nat. Med.* 1998; 4:1177-81), is a substrate for caspase-3 (Choi et al., *J. Biol. Chem.* 2001; 276(22):19197-204) and its expression was shown to be induced coincident with the morphological and biochemical features of apoptosis, including cell shrinkage, DNA laddering, and caspase activation (Jo et al., *FASEB J.* 2001; 15(3):589-91).

The co-expression of Kv4.2 and KChIP3 was shown to inversely correlate with the frequency of neuronal "firing" (*i.e.*, excitability) in dopaminergic neurons and neurons found in the substantia nigra (An et al., *Nature* 2000; 403:553-556). The mechanism of KChIP3 in enhancing potassium currents is postulated to involve increasing surface expression of Kv4.2, and/or modulating PKA phosphorylation of the channel.

Calsenilin reverses presenilin-mediated enhancement of calcium signaling (Leissring et al., *PNAS.* 2000; 97: 8590-3), and enhances apoptosis by altering endoplasmic reticulum calcium signaling (Chan et al., *Mol. Cell. Neurosci.* 2002; 19(4):552-9). Therefore, Calsenilin is not only a calcium sensor, but also a calcium modulator.

Targeting Rationale. Finding new interacting proteins for calsenilin/KChIP3 that block or enhance its interactions with potassium channels, presenilin, caspases, calcium or other proteins will be used to further characterize its precise role in pain and to identify additional drug targets for therapeutics. The identified drug targets would be screened to optimally enhance or interfere with specific interactions to reduce pain.

Calsenilin/KChIP3 interactions with potassium channels can also be directly targeted for drug discovery. Since decreased amounts of calsenilin/KChIP3 are associated with more pain, putatively due to increased neuronal excitability, then compounds/peptides that enhance calsenilin/KChIP3 expression and/or interactions with potassium channels or compounds/peptides that directly activate potassium channel activity would be candidates for therapeutics.

DREAM

Downstream Regulatory Element Antagonist Modulator, or DREAM is a Ca^{2+} -regulated transcriptional repressor that binds to downstream regulatory element (DRE) sequences of pain-associated genes in the absence of calcium (Carrion et al., *Nature* 1999;

5 398(6722):80-4). It is a splice variant of calsenilin/KChIP3 that is translated from a different ATG start codon and contains a deletion in the region spanning exons 2 and 3 of the DREAM mRNA. Therefore, it is identical to calsenilin, except for the presence of 30 additional residues at the N-terminus.

DREAM has been specifically implicated in the repression of the prodynorphin gene in
10 spinal cord neurons (Cheng et al., *Cell* 2002; 108:31-48). Prodynorphin is the precursor for dynorphin, a pain-associated secreted peptide that binds opiate receptors and decreases inflammatory pain hypersensitivity in spinal cord neurons (Ji et al., *J. Neurosci.* 2002; 22(2):478-85). Upon stimulation by Ca^{2+} , DREAM's ability to bind to the DRE and its repressor function are inhibited. DREAM knock-out mice exhibited marked attenuation in
15 pain response to acute, chemical and inflammatory noxious stimuli as well as attenuation of neuropathic pain responses (Cheng et al., *supra*), in the absence of other observable phenotypes or side-effects. The authors observed that activation of the κ -opioid receptor was found to be causal to the reduced pain responses in the DREAM knock-out mice.

Targeting Rationale. The DREAM isoform of the DREAM/calsenilin/KChIP3 gene can
20 also be used to identify interacting proteins, *i.e.*, antagonists or antibodies, which inhibit DREAM expression or activity thereby decreasing pain responses. More ideally, binding partners (*e.g.* small molecules) that keep DREAM out of the nucleus would result in disinhibition of dynorphin-mediated analgesia, and via DREAM/KChIP3 –shared domains, increase KChIP3-mediated potassium channel activation reducing neuronal excitability.
25 Such a therapeutic would simultaneously target two molecular mechanisms and result in more powerful pain relief.

Kainate-sensitive glutamate receptor (GluR5)

The excitatory amino acid glutamate is the primary excitatory neurotransmitter in the central nervous system. The role of glutamate in both acute and chronic pain is well
30 established. Glutamate acts at several receptors, both those that are directly coupled to ion channels (ionotropic), as well as those directly coupled to second messengers via G-proteins (metabotropic). The ionotropic receptors include those selective for N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate (KA).

The subunits GluR5-7 are KA-selective (GluR1-4 are selective for AMPA). KA receptors are coupled to ion channels permeable to Na⁺, K⁺, and, in some instances, Ca²⁺ (when the GluR2 subunit is not present), and have 4 transmembrane domains similar to other ligand-gated ion channels. This family of receptors mediates fast excitatory postsynaptic potentials. There is also a family of KA binding proteins (KA1, KA2, KBP-f, KBP-c) that do not form functional channels, and have little sequence similarity to the GluR genes. The GluR5-7 subunits are capable of forming either homomeric or heteromeric units; the KA binding proteins may also form heteromeric channels with the GluR5-7 subunits.

There is a considerable body of evidence showing the involvement of KA receptors in pain (for review see Fundytus, 2001, *supra*). For example, exposure of the spinal cord to KA enhances dorsal horn neuronal responses to mechanical stimulation, while application of KA to the rat hind paw induces activation of primary afferent neurons. KA receptors desensitize at concentrations of agonist that are much lower than those required to elicit currents, raising the possibility of using agonists as functional antagonists (Chittajallu et al., *Trends Pharmacol. Sci.* 1999; 20(1):26-35).

Furthermore, both pre-synaptic and post-synaptic localization of KA receptors has been demonstrated. KA receptors are found on the post-synaptic membrane of neurons that respond to high-threshold dorsal root fiber stimulation (Li et al., *Nature* 1999; 397(6715):161-4; Kerchner et al., *J. Neurosci.* 2002; 22(18):8010-7). Pre-synaptic KA receptors regulate both excitatory and inhibitory neurotransmitter release. On a subset of DRG neurons expressing pre-synaptic KA receptors, activation of KA receptors regulates glutamate release (Hwang et al., *Neurosci. Lett.* 2001; 312(3):137-40; Kerchner et al., *supra*). Activation of pre-synaptic KA receptors on DRG cells attenuates glutamatergic transmission onto the spinal cord dorsal horn in rats (Kerchner et al., *J. Neurosci.* 2001; 21(1):59-66.). In GluR5 knockout mice, KA receptor function was abolished in DRG neurons (Kerchner et al., 2002, *supra*). Northern blot analysis and pharmacological analysis indicate that GluR5 is the predominant subunit expressed in DRGs (Kerchner et al., 2002, *supra*; Partin et al., *Neuron* 1993; 11(6):1069-82; Swanson et al., *J. Physiol.* 1998; 513 (Pt 3):639-46; Wilding & Huettner, *J. Physiol.* 2001; 532(Pt 2):411-21). In the spinal cord dorsal horn, pre-synaptic KA receptors responding to glutamate released from dorsal root sensory fibers regulate GABA and glycine release by direct depolarization of interneuron terminals (Kerchner et al., 2001, *supra*; Kerchner et al., 2002, *supra*). In spinal cord dorsal horn, application of the selective GluR5 agonist ATPA reliably triggered the quantal release of GABA and glycine in GluR6 knockout mice, further supporting the functional role of the GluR5 subunit in the spinal cord (Kerchner, et al., 2002, *supra*).

Behaviorally, the role of GluR5 in pain transmission is less clear. The selective GluR5 agonist ATPA reduced nociceptive reflexes *in vitro*, but failed to affect latency in the mouse hotplate test (Procter et al., 1998). However, in another study, ATPA was shown to not only inhibit nociceptive motoneurone responses in anesthetized rats, as well as wind-up elicited by repetitive dorsal root stimulation, and nociceptive responses in hemisectioned spinal cords, but also to be anti-hyperalgesic in rats with an inflammatory injury (Mascias et al., 2002). Antagonists selective for GluR5 (LY294486 and LY382884) reduced nociceptive responses both *in vitro* and *in vivo*, but only at doses that also affected responses to exogenous AMPA (Procter et al., *Neuropharmacology* 1998; 37(10-11):1287-97). Systemic administration of a selective GluR5 antagonist (LY382884) attenuated nociceptive responses in the rat formalin test. The compound SYM-2081 has been purported to be a GluR5/6 agonist (Mascias et al., *Neuropharmacology* 2002; 43(3):327-39.; Tocris Cookson catalog) and a selective GluR5 antagonist. SYM-2081 was anti-hyperalgesic in inflamed (Mascias et al., 2002, *supra*), and neuropathic animals.

Targeting Rationale. In the brain, iGluR5 mRNA is limited mainly to the subiculum, CA1 region of the hippocampus (weakly expressed in CA1 and CA3 regions) and the Purkinje cell layer of the cerebellum (Chitajallu et al., 1999, *supra*). In the peripheral nervous system, GluR5 is expressed on small- and medium-diameter neurons of the dorsal root ganglion (DRG). Although DRG neurons also express GluR5, GluR6, and KA2, it appears that the majority of native KA receptors in this region are composed of homomeric assemblies of edited GluR5 (Chitajallu et al., 1999, *supra*). Agonists, *e.g.*, ATPA and antagonists, *e.g.*, LY compounds such as LY382884, to the GluR5 can be evaluated for *in vivo* efficacy in acute, neuropathic, and other chronic pain models.

Serotonin Receptor (5HT3)

Serotonin is a major neurotransmitter involved in information processing of sensory neurons. Serotonin induces analgesia by inhibiting dorsal horn neuronal responses to noxious stimuli through activation of specific receptor sub-types. 5-HT3 receptors are ligand-gated multi-subunit channels and are expressed at low levels in sensory neurons of the DRG (Nicholson et al., *Neuroscience Letters* 2003; 337: 119-22). Expression of the 5-HT3 receptor mRNA was shown to be significantly increased in the rat lumbar DRG following injection of Freund's complete adjuvant (FCA), a pro-inflammatory cocktail (Wu et al., *Neuroscience Letters* 2001; 307:183-6). 5-HT3 receptors have also been shown to facilitate nociceptive transmission of acute pain upon peripheral application of serotonin on skin. The 5-HT3B subunit specifically associates with 5-HT3A receptor subunit to form a functional channel (Davies et al., *Nature* 1999; 397:359-363). Mutant mice lacking the 5HT3A gene displayed reduced pain responses in the formalin test (Zeitz et al., *J.*

Neurosci. 2002; 22(3):1010-9). On the other hand, agents that increase synaptic serotonin levels, such as selective serotonin reuptake inhibitors (SSRIs), are also effective against some types of neuropathic pain.

Targeting Rationale. Agonists or antagonists specific for the 5HT_{3A} receptor can be tested for their ability to block or promote pain behavior in animal models of acute and chronic pain. This will resolve the discrepancies between animal knockout and clinical human studies on the role of 5HT_{3A} in pain. One possibility is that acute pain perception is a peripheral, serotonin-agonized process, while the role of serotonin in neuropathic pain may be a more centrally acting, analgesic process. Thus, compounds affecting the 5HT_{3A} as agonists or antagonists will have different therapeutic indications for central or peripherally mediated pain mechanisms.

Nicotinic acetylcholine receptor subunit $\alpha 3$

Nicotinic receptors are expressed in muscle, and in the central and peripheral nervous systems. The neuronal nicotinic acetylcholine receptors (nAChR) are predominantly heteromeric (pentameric) cation-gated ligand channel receptors formed from combinations of subunits $\alpha 3$, $\beta 2$, $\beta 4$, $\alpha 4$, $\alpha 5$ and $\alpha 7$ (Oxale et al., *J. Pharmacology and Exp. Ther.* 1997; 283:675-83). The $\alpha 3$ subunit is expressed in combination with the $\beta 2$, $\beta 4$ and $\alpha 5$ subunits. Nicotinic acetylcholine receptor agonists such as epibatidine have been shown to produce anti-nociceptive effects in numerous animal models of neuropathic pain (Decker et al., *Biochem. Pharmacol.* 1999; 1 58:917-23). Antisense knock-down of the $\alpha 4$ subunit in $\alpha 4$ -containing nAChR in rats resulted in attenuation of the anti-nociceptive effects of a nAChR agonist, indicating a role for this subunit in mediating analgesia (Bitner et al., *Brain Res.* 2001; 871: 66-74). Knock-out mice for the $\alpha 3$ subunit exhibit autonomic nervous system defects, megacystis, mydriasis and altered acetylcholine responses in neurons of the superior cervical ganglion (Marubio et al., *J. Pharmacol.* 2000; 393: 113-21). It has been suggested that the release of specific neurotransmitters plays a critical role in eliciting the anti-nociceptive effects of nAChR.

Targeting Rationale. Since many of the nAChR agonists are toxic, carry risks of chemical dependency, or have other adverse effects, there remains a need in the art to identify agonists or antagonists that exert anti-nociceptive effects with reduced adverse effects. Screening for $\alpha 3$ -agonists or antagonists in cells that co-express the $\beta 2$, $\beta 4$ and $\alpha 5$ subunits is contemplated.

Vanilloid receptor (VR1)

Vanilloid receptor type 1 (VR1) is a ligand-gated ion channel expressed on sensory nerves. VR1 expression in A-fibers of neurons in mouse models of neuropathic pain increase following nerve injury (Rashid et al., *J. Pharmacol. Exp. Ther.* 2003; 304(3):940-8). VR1
5 in the DRG neurons and spinal dorsal horn is a channel that appears to confer responsiveness to heat, acidification and chemical stimuli (Ohtori et al., *J. Orthop. Sci.* 2003; 8(1):84-7), and local inflammation induces VR1 expression in small and medium-sized neurons (Amaya et al., *Brain Res.* 2003; 963(1-2):190-6). A VR1 antagonist, capzazepine, prevented the development of mechanical hyperalgesia induced by intraplantar
10 injection of VR1 agonist capsaicin in mice, rats and guinea pigs, suggesting a role for this receptor in inflammatory hyperalgesia (Walker et al., *J. Pharmacol. Exp. Ther.* 2003; 304(1): 56-62). As discussed earlier, continued application of the agonist capsaicin to post-herpetic neuralgia patients induces desensitization of the VR1 and thus reduced pain. Combined data also support a functional role for VR1 in the regulation of cytosolic
15 calcium. Specifically, activation of VR1 allows the influx of Ca^{2+} , increasing the intracellular concentration, leading to excitability of the neuron. VRI knockout mice exhibit reduced inflammatory hyperalgesia. Moreover, the density of VR1 in the colon tissue from patients with inflammatory bowel disease is increased (Y. Yiangou et al., *Lancet* 2001; 357(9265): 1338-1339).

20 **Targeting rationale.** Downregulation of VR1 expression and/or activity is a promising therapeutic strategy for novel analgesic drugs, as well as therapeutics for other indications such as inflammatory bowel disease. Reduction of VR1 activity may be achieved via the use of selective antagonists, antibodies, antisense oligonucleotides, siRNA or ribozymes. Decreased activation of VR1 can also be induced by continued, chronic application of
25 selective agonists, leading to desensitization of the receptor. Manipulating activity at VR1 in screening assays may also allow the examination of what other proteins interact with the receptor, elucidating additional targets for the treatment of acute and chronic pain, as well as other indications.

Sodium channel (NaN or SNS/PN3)

30 Numerous sodium channels, with distinct physiological properties, are expressed within small dorsal root ganglion (DRG) neurons, which include nociceptive cells. There is a dramatic change in sodium channel expression in DRG neurons, with down-regulation of the SNS/PN3 and NaN sodium channel genes and up-regulation of the Type III sodium channel gene, following injury to the axons of DRG neurons. This is accompanied by
35 electrophysiological changes in DRG neurons which cause spontaneous firing, or firing at

inappropriate high frequencies. Changes in sodium channel gene expression are also seen in experimental models of inflammatory pain (Waxman, *Pain* 1999; Suppl. 6:S133-40).

In the rat SNL model of neuropathic pain, described above, and in several other animal models of neuropathic pain, the expression of SNS/PN3 protein was abolished in all injured DRG neurons (Decostered et al., *Pain* 2002; 96(3): 269-77).

Targeting Rationale. Since particular subtypes of sodium channels including SNS/PN3 are down-regulated in the Chung model, pharmacological approaches to enhancing the expression and/or activity of these channels may be useful for the treatment of diseases such as neuropathic pain.

Regulator of G-Protein Signaling 4 (RGS4)

Activation of G-protein coupled receptors (GPCRs) promotes guanine nucleotide exchange (GTP replaces GDP) on $G\alpha$ subunits, and consequent dissociation of the tightly bound $G\beta\gamma$ complex from $G\alpha$. Subsequently, free $G\alpha$ -GTP and $G\beta\gamma$ regulate the activity of effector proteins.

These effector proteins then mobilize intracellular second messengers and ions to direct cell and tissue physiology. Activity of GPCRs is modulated by a family of proteins called Regulator of G-Protein Signaling (RGS).

Members of the RGS family of proteins contain an RGS homology domain. The RGS domains bind directly to activated $G\alpha$ subunits and act as GTPase-activating proteins (GAPs), and serve to attenuate or modulate hormone and neurotransmitter induced signaling by both $G\alpha$ -GTP and $G\beta\gamma$ (Heppler, *TIPS* 20: 376-382, 1999). Nearly all RGS family members tested act selectively as GAPs for one or more members of the $G_{i\alpha}$ and/or $G_{q\alpha}$ family, but not $G_{s\alpha}$ or $G_{12\alpha}$ (Ibid), and in most cases RGS proteins appear to be important for desensitization of GPCR signaling.

RGS4 is expressed in central nervous system tissues known to be involved in pain (Zhong et al., *J. Pharmacol. Exp. Ther.* 297: 837-845; Gold et al, *J. Neurosci.* 1997; 17: 8024-8037). Moreover, RGS4 interacts with neurotransmitter systems known to be involved in pain. For example, group I metabotropic glutamate receptors (mGluRs; mGluR1 and mGluR5) have been shown to be involved in neuropathic and inflammatory pain (Fundytus, *CNS Drugs* 2001; 15: 29-58; Fundytus et al., *Br. J. Pharmacol.* 2001; 132: 354-367; Fundytus et al., *Pharmacol. Biochem. Behav.* 2002; 73: 401-410), and, in *Xenopus* oocytes, purified RGS4 virtually abolishes group I mGluR mediated activation of

a calcium chloride current (Saugstad et al., *J. Neurosci.* 1998; 18: 905-913). RGS4 also markedly attenuates the mGluR-mediated inhibition of potassium currents in hippocampal CA1 neurons (Ibid). In addition, RGS4 modulates opioid receptor activity. Opioids are the most commonly used analgesics, but with continued use, tolerance may develop, and one mechanism by which this may occur is desensitization. In HEK293 cells transfected with human opioid receptors, RGS4 overexpression reduced the inhibitory activity of DAMGO (μ -opioid receptor agonist), DPDPE (δ -opioid receptor agonist) and BRL 52656 (κ -opioid receptor agonist) (Gamier et al., *J. Pharmacol. Exp. Ther.* 2003; 304: 1299-1306). The inhibitory effect of RGS4 can be partially overcome by increasing concentrations of opioid agonists. In addition, in PC 12 cells stably expressing either cloned μ or κ -opioid receptors, treatment with either morphine, DAMGO or U69,593 for 0.5-24 hr significantly and transiently increased the expression of RGS4 mRNA by 140-170% of the control in a dose-dependent manner.

There is also some evidence that RGS4 is involved in chronic pain. In the partial sciatic nerve ligation model of neuropathic pain, RGS4 mRNA expression showed a significant 2-fold increase in spinal cord of neuropathic rats at 7 days after injury (Gamier et al., *J. Pharmacol. Exp. Ther.* 2003; 304: 1299-1306). Taken together, these data suggest a role for RGS4 in the modulation of neurotransmitter systems involved in pain transmission.

Targeting Rationale. Identifying inhibitors of the RGS4 interaction with the G-proteins that couple to opioid receptors should increase the sensitivity, and thus signaling, of opioid receptors to agonists and inhibit desensitization. As an alternative, enhancing RGS4 interactions with group I mGluRs (or possibly an as yet unidentified receptor) would reduce cellular excitability and pain transmission in chronic pain states.

Galanin

Neuropeptides present in primary afferent neurons and the dorsal horn of the spinal cord have an important role in the mediation of nociceptive input under normal conditions. Galanin is a 29 amino acid neuropeptide that is normally expressed in less than 5% of sensory neurons in the adult dorsal root ganglia. There is a substantial body of work to indicate that galanin plays an important biological role as a developmental and trophic factor to subsets of neurons in the nervous and neuroendocrine systems, and as a regulator of neurotransmitter and hormone release in the adult (Wynick and Bacon, *Neuropeptides* 2002;36(2-3):132-44). After nerve transection (axotomy), chronic constriction of the sciatic nerve, or partial ligation of the sciatic nerve, the galanin content of the dorsal root ganglia increases dramatically and the peptide is then expressed in more than 50% of neurons.

The effect of galanin is mediated by three subtypes of receptors (GALRs). GALRs are seven transmembrane GPCR receptors. GALR1 is negatively coupled to cAMP production, and activation of this receptor leads to a reduction in the concentration of cAMP.

5 Activation of GALR1 also opens G-protein coupled inwardly rectifying K⁺ currents, and activates MAPK in a pertussis toxin (PTX) sensitive manner. These actions suggest that GALR1 couples to Gi, and that activation of GALR1 is inhibitory. Rat GALR1 mRNA is expressed in the brain, including in regions known to be involved in pain transmission (e.g., thalamus), as well as in the dorsal horn of the spinal cord and dorsal root ganglia (DRG) (Wynick et al, *Neuropeptides* 36: 132-144, 2002). In DRG, GALR1 expression is
10 almost exclusively in large sensory neurons, and is limited to the superficial laminae I and II (with much lower levels in the medial dorsal aspect of the dorsal horn (DH)). The level of expression of GALR1 decreases in the DRG after inflammation or peripheral nerve injury (Ibid).

GALR2 has a more widespread expression than GALR1, including several brain regions
15 and DRG, as well as in the periphery (Wynick et al., 2002, *supra*). Activation of GALR2 stimulates phospholipase C (PLC) activity and leads to hydrolysis of inositol phosphate in a PTX-resistant manner, and has also been shown to be important for mobilization of intracellular Ca²⁺ and Ca²⁺-dependent activation of chloride channels. GALR2 has also been shown to stimulate MAPK in a manner consistent with G_o mediated signaling.

20 GALR2 inhibition of cAMP accumulation is variable. In DRG, GALR2 mRNA is mostly in small CGRP-positive neurons (Wiesenfeld-Hallin & Xu, *Eur. J. Pharmacol.* 429: 49-59, 2001). GALR2 mRNA is downregulated after axotomy, and transiently upregulated after inflammation in DRG neurons (Ibid).

GALR3 is less well understood. The distribution of this receptor is controversial. Some
25 groups have found expression in the brain, spinal cord and DRG, while others have not (Wynick et al., 2002, *supra*). Within the cell, GALR3 has been shown to signal via activation of ATP-sensitive K⁺ channels and inwardly rectifying K⁺ (GIRK) channels.

The effects of galanin appear to be biphasic. The antinociceptive effect of intrathecal (i.t.)
galanin is usually observed only at high doses and is moderate. Intrathecal galanin can
30 potentiate the antinociceptive effects of morphine, while GALR antagonists reduce the spinal effect of morphine (Wiesenfeld-Hallin & Xu, 2001, *supra*). Galanin reduces the spinal excitability and pronociceptive effects induced by substance P (SP). However, low dose i.t. galanin facilitates the spinal reflex, and nociceptive behaviors (Ibid).

Sciatic nerve transection (axotomy) induces a significant increase of galanin-like
35 immunoreactivity in rat DRG neurons, with detection rising from about 5% in the intact

animal, to about 50% of DRG neurons of all sizes in axotomized animals (Wiesenfeld Hallin & Xu, 2001, *supra*). Increase of both galanin-like immunoreactivity and galanin mRNA is seen within 24 hr after nerve injury, reaches a maximum within 7 days, and remains high in the absence of nerve regeneration (*ibid*). After partial nerve injury, galanin levels are also increased, and the extent of galanin upregulation is inversely correlated to the severity of pain-like behavior (*Ibid*).

The pattern of galanin expression is somewhat different in models of inflammation. Carrageenan-induced inflammation is associated with an upregulation of galanin in the superficial DH, but the expression of galanin in sensory neurons, which is already low, is further reduced (Wiesenfeld-Hallin & Xu, 2001, *supra*).

Targeting rationale: It has been suggested that upregulation of galanin in DRG neurons following nerve injury results in antinociceptive effects via stimulation of GALR1 on dorsal horn neurons, and that the pro-nociceptive effects of galanin are related to presynaptic GALR2 neurons on primary afferent neurons (Liu & Hokfelt, *TiPS* 2002; 23: 468474). Thus, it is desirable to produce therapeutics agents which are either GALR1 agonists, or GALR2 antagonists.

5.2 Differentially Regulated Genes with a Novel Association to Pain Based on Regulation

Decay Accelerating Factor GPI-Form (DAF/CD55)

Decay Accelerating Factor (or CD55) is a major regulator of the alternative and classical pathways of complement activation and is expressed on all serum-exposed cells. It is used by pathogens, including many enteroviruses and uropathogenic *E. coli*, as a receptor prior to infection. DAF is a GPI-anchored member of the RCA protein family that protects autologous cells from complement damage. CD55 regulates complement by binding and accelerating the decay of the C3 convertases, the enzymes that link the three activation pathways and the downstream effectors. This central role in regulating complement has led to the development of transgenic animals that express human CD55 as a potential source of organs for xenotransplantation. DAF has a non-complement-related interaction with CD97, which affects cell activation, migration and adhesion under inflammatory conditions.

Peripheral nerve damage exposes proteins, P0 and P2 that are not normally exposed to immune cells and complement components (Watkins and Maier, *Physiol. Rev.* 2002; 82: 981-1011). These proteins are seen as “non-self” by the immune system, thus initiating an immune response and inflammatory cascade. In addition to attack by macrophages and

mast cells, the complement system is also activated. As a result of activation of complement components, macrophages and neutrophils are recruited from the general circulation into nerve, "coat" bound antibody to enhance its destruction by phagocytes, and disrupt Schwann cell function. Complement activation also causes formation of membrane attack complexes (MACs), which insert into the lipid membranes, forming cation pores. This action kills pathogens, but may also damage the host tissues. Although most of the host's cells are protected from MAC-induced cell death by cytoplasmic factors that quickly expel MACs, the myelin sheath is not. MAC insertion into the myelin sheath causes morphological changes, where the sheath's lamellae are split, and show signs of decompaction. This in turn leads to more exposure of P0 and P2, thus activating the complement cascade, and creating a positive feedback loop that preserves peripheral nerve damage.

Targeting Rationale. Neurogenic inflammation associated with nerve injury may promote unregulated complement attack of neuronal cells. DAF analogues that target either convertase or CD97, as well as modulators specific to other components of the complement pathway, could attenuate neuronal damage by protecting against complement attack and possible immune cell mediated inflammation.

Potassium channel (Kv9.1)

Modulatory potassium channel α -subunit Kv9.1 was cloned by screening a rat brain cDNA library using conserved sequences from cloned voltage-gated potassium channels (Stocker et al., *Biochem. Biophys. Res. Comm.* 1998; 248: 927-34). These authors found that rat Kv9.1 is expressed exclusively in the central nervous system. Differential expression of potassium channels on sensory neurons contributes to sensory neuron signaling and nociceptive responses. In the Chung SNL model, described *infra*, there is a dramatic reduction in potassium channel expression in injured DRG neurons (Rasband et al., *Proc. Nat. Acad. Sci. U.S.A.* 2001; 98: 13373-78). Analgesic agents such as diclofenac have been shown to increase activity of potassium channels in neuropathic pain, and thus, induce anti-nociception. The anti-nociceptive effect could be blocked by pre-administration of potassium channel antagonists (Ortiz et al., *Eur. J. Pharmacol.* 2002; 438: 85-91).

Targeting Rationale. Changes in ion channel expression, localization, or activity may contribute to the onset or persistence of neuropathic pain following nerve injury, as well as chronic, inflammatory pain. Numerous modulators of potassium channels are known in the art. For a review, see Coghlan et al., *J. Med. Chem.* 2001; 44: 1628-53. *In situ* hybridization will be performed to determine the specificity of potassium channel expression in DRG.

Gene knockdown or inhibition of protein expression by gene targeting or RNA interferences will be performed to assess the impact of Kv9.1, and/or associated potassium channel(s) subunit activity such as Kv2.1, in chronic pain models. The present invention further contemplates screening for compounds that selectively modify the DRG-specific Kv9.1 and/or associated potassium channel(s) subunit activity for evaluation of their effects on neuropathic and inflammatory pain. Since the Kv9.1 potassium channel is specific to DRG nociceptive neurons, activation would be sufficient to relieve pain at the level of the DRG, without causing side effects associated with potassium channel activation in other tissues (such as the heart).

10 **Regenerating Gene Protein-2 (Reg-2)/Pancreatic Associated Protein-1 (PAP-1)**

Reg-2 was first isolated from regenerating pancreatic islet β cells and has been implicated in the replication, growth and maturation of islet β cells (Kobayashi et al., *J. Biological Chem.* 2000; 275: 10723-21). Reg-2 is a 16 kD secreted protein that is also expressed in motor neurons during development. Expression of Reg-2 is regulated by cytokines such as ciliary neurotrophic factor (CNTF) and related cytokines (Nishimune et al., *Nature Cell Biology* 2000; 2:906-14), leukemia inhibitory factor (LIF) and cardiotrophin (CT-1). Both cytokines signal through the IL-6 receptor. Reg-2 is also upregulated in a specific population of sensory DRG neurons following peripheral nerve injury (e.g., sciatic nerve crush), the IB4/P2X2, GDNF-sensitive population, and has been shown to have regenerative capabilities in the neurons (Averill et al., *J. Neuroscience* 2002; 22: 7493-7501). Antibodies to Reg-2 inhibit regeneration of sensory/motor neurons. Reg-2 also has been found to have a mitogenic effect on Schwann cells *in vitro* (Livesey et al., *Nature* 1997; 390: 614-18). Reg-2 is not normally expressed in the adult rat CNS or PNS, and therefore expression of Reg-2 following injury may be an example of a homeostatic mechanism, whereby the body is trying to regenerate (heal) itself.

Targeting Rationale. Reg-2, a secreted protein, was upregulated following peripheral nerve injury, and is therefore an attractive candidate for having a specific role in pain. Effects of purified Reg-2 protein and antibodies that specifically bind to Reg-2 can be evaluated in pain models, as well as other models of chronic disease. Regulation of Reg-2 with antisense oligonucleotides, siRNA and ribozymes will also be evaluated in pain and other disease models. Screening for and evaluating small molecules that block or enhance Reg-2 interaction with its receptor, such as EXTL1, are also contemplated. Additional cellular binding partners for Reg-2, *i.e.*, receptors, will be identified, as well as any other proteins (e.g., signal transduction molecules) that are affected by Reg-2 activity.

Heparanase

Heparanase is an enzyme that degrades heparan sulphate proteoglycans (HSPGs), which are the main components of the extracellular matrix. Tumors rely on heparanase to invade tissue. Cell surface localization and secretion of heparanase markedly stimulated tumor angiogenesis (Goldschmidt et al., *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99(15): 10031-6). On the other hand, since HSPGs play an important role in signal transduction, degradation of HSPGs by secreted heparanase may suppress tumor cell growth (Ikeguchi et al., *Eur. J. Cancer* 2003; 39: 86-90). Therefore, it is very important to know where and when heparanase is produced. Besides the cell surface, heparanase is also localized within lysosomes and the Golgi apparatus (Goldschmidt et al., *Exp. Cell. Res.* 2002; 281(1):50-62). When secreted, heparanases are thought to degrade basement membrane HSPGs at sites of injury or inflammation, allowing extravasion of immune cells into nonvascular spaces and releasing factors that regulate cell proliferation (Bame, *Glycobiology* 2001; 11(6): 91R-98R). For example, basic fibroblast growth factor is bound to heparan sulfate in the extracellular matrix and is released in an active form when the HSPG is degraded by heparanase.

Targeting Rationale. Corresponding heparanase enzyme activity during animal models of pain will be measured to determine whether heparanase activation or inhibition leads to an analgesic effect. Since secreted heparanases enhance the immune response, it is likely that blocking heparanase activity at the site of injury would reduce the inflammatory component of pain. Alternatively, increases or decreases in heparanase activity at the site of injury could modulate the release of molecules or growth factors that affect pain signal transduction. Enzyme activity assays can be used to find inhibitors or enhancers of heparanase as analgesics.

Channel-Interacting PDZ Domain Protein (CIPP)

CIPP is a potassium channel-interacting protein that was identified using a yeast two-hybrid system. CIPP was also found to interact with the NMDA-type glutamate receptors and cell surface molecules present in synaptic membranes through multiple protein binding domains (Kurschner et al., *Mol. And Cell. Neurosci.* 1998; 11: 161-72). Channel activity of potassium channels was found to be increased in CIPP transfected COS-7 cells (Kurschner, *supra*). CIPP has also been found to interact with proton-gated acid-sensing ion channel (ASIC) subunit 3 in sensory neurons (Anzai et al., *J. Biol. Chem.* 2002: 277: 16655-16661). ASIC3 subunits are expressed primarily in small nociceptive neurons. Channel activity of ASIC3-containing ion channels was increased in cells co-transfected with CIPP as evidenced by increases in current density.

Targeting Rationale. CIPP has been shown to interact physically with several receptors implicated in pain. Thus, CIPP may serve to integrate pain signals mediated by these receptors. It therefore would be of interest to identify compounds that disrupt the association of CIPP with ASIC3 and/or other receptors, ion channels, or signal transduction mechanisms, and examine the effect on channel function and nociceptive signaling *in vivo* and *in vitro*. In addition, CIPP could be used to screen for compounds which disrupt other PDZ-mediated CIPP-protein interactions and evaluate their effects *in vitro* and *in vivo*. CIPP may act as a scaffolding protein that brings different proteins into adjacent and functional positions at the cell membrane (Kurschner, *supra*). By virtue of its interaction with receptors and ion channels known to be involved in pain, CIPP inhibitors may be of therapeutic value in chronic pain states.

Thymic stroma-derived lymphopoietin (TSLP)

TSLP is a 140 amino acid protein isolated from a thymic stromal cell line that has mitogenic effects on the proliferation of B and T cells, and also positively affects the differentiation of B cells from B cell precursors. TSLP is also expressed in the brain (Sims et al., *J. Exp. Med.* 2000; 192: 671-80). TSLP is within the IL-2 family of cytokines, and human TSLP regulates immune responses by activating dendritic cells and attracting inflammatory cytokines known to be involved in pain (Leonard, *Nature Immunology* 2002; 3: 605-607). TSLP has also been associated with inflammation *in vivo* in allergic reactions. The TSLP receptor (TSLPR) comprises the IL-7R α subunit along with a 359 amino acid polypeptide (Park et al., *J. Exp. Med.* 2000; 192: 659-69).

Targeting Rationale. TSLP may be involved in neurogenic inflammation associated with nerve injury and could be used as a marker to detect injury. In addition, inhibition of TSLP or TSLP receptor may reduce inflammatory damage of neurons. Effects of purified TSLP protein and antibodies that specifically bind to TSLP can be evaluated in pain models, as well as other models of chronic disease. Regulation of TSLP with antisense oligonucleotides, siRNA and ribozymes will also be evaluated in pain and other disease models. Screening for and evaluating small molecules that block or enhance TSLP interaction with its receptor, such as TSLP-R and IL7-R, are also contemplated. Additional cellular binding partners for TSLP, *i.e.* receptors, will be identified, as well as any other proteins that are affected by TSLP activity (*e.g.*, downstream cytokine signal transduction molecules).

Kinesin-Like 4/KIF22B

The kinesin superfamily comprises about 45 highly-conserved members (reviewed by Miki et al. *PNAS* 2001; 98:7004) that have diverse functions, including the transport of

organelles, protein complexes, and mRNAs in a microtubule and ATP dependent manner. Kinesins also participate in chromosomal and spindle movement during mitosis and meiosis. Kinesin function is important in neuronal cells due to their elongated shape, polarity and dendritic protrusions. Kinesins are selective with respect to the molecules and organelles they transport, and targeted disruption of individual kinesin genes in mice, *e.g.*, KIF1A, resulted in immediate post-natal death within one day of birth caused by impaired transport of synaptic vesicle precursors and consequential decreases in synaptic vesicle density (Yonekawa et al., *J. Cell Biol.* 1998; 141: 431-41). It has been suggested that kinesins bind to known proteins with non-motor ability that are present in the cell, and that these proteins have dual activity which includes a role as a docking receptor protein for kinesins (Goldstein, *Proc. Nat. Acad. Sci. U.S.A.* 2001; 98: 6999-7003).

Kinesin KIF17 is involved in the transport of vesicles containing NMDA receptor 2-b along microtubules (Setou et al., *Science* 2000; 288: 1796-1801). KIF17 binds to receptor mLin-10 on the membranes, and in Lin-10 mutated *C. elegans*, the glutamate receptor GluR1 is mislocalized. Diseases linked to kinesin mutations include retinitis pigmentosa, Charcot-Marie-Tooth disease type 2A (peripheral neuropathy), and FTDP taudementia.

Targeting Rationale. Since kinesins are involved in axonal transport, they may be involved in synaptic remodeling that leads to chronic pain. *In situ* hybridization will be performed to determine the specificity of kinesin expression in DRG. Agonists, such as sabeluzole, increase fast axonal transport in a kinesin-microtubule dependent manner, and antagonists, such as AS2, inhibit related kinesin EG5. These demonstrate that there are compounds that can target kinesins directly, and will be evaluated for their effect in chronic pain models. Other compounds that affect the Kinesin-like 4 activity or binding to its specific cargo will be screened for their effect on pain.

Knockdown of gene or protein expression by gene targeting or RNA interference, as well as screening for Kinesin-like 4 binding partners will also be performed.

Pervin-Advillin

Pervin (peripheral nervous system villin-like protein) is an 820 amino acid protein that has identity to gelsolin/villin family members and is a homolog of a mouse protein, advillin.

Pervin is expressed in a subset of DRG sensory neurons in the rat peripheral nervous system. These proteins are actin-binding proteins and function in regulating neurite initiation and elongation. Overexpression of pervin in one-day-old cultures of neonatal rat DRG resulted in neurons with increased neurite length (Ravenall et al., *Eur. J. Neurosci.* 2002; 15: 281-90). Embryonic hippocampal neurons from gelsolin knockout mice exhibit enhanced NMDA receptor activation, calcium intake, and cell death in response to

glutamate. Human advillin has been implicated in inflammatory bowel disease (Tumer et al., *Gene* 2002; 18: 179-85).

Targeting Rationale. Gene targeting or knockdown technology will be used to evaluate the role of pervin in pain. Since gelsolins are involved in neurite outgrowth, family members may also function in synaptic remodeling that leads to chronic pain. Compounds that alter pervin binding to actin and other interacting proteins will be screened for their effects on pain.

Prostatic acid phosphatase (PAP)

Prostatic acid phosphatase (PAP) has been reported as a marker for human prostatic gland cancer. Currently, a role for prostatic acid phosphatase signaling in neurons has not been identified. Kinases and phosphatases have been implicated in changing the phosphorylation status of signal transduction proteins and key transcription factors and thereby modulating their function. Changes of PAP levels may alter the cellular response to pain stimulus.

Targeting Rationale. Since prostatic acid phosphatase is an enzyme, an activity assay could be used to screen for inhibitors or activators. Alternatively, prostatic acid phosphatase substrates could be identified to find additional drug targets for pain therapeutics. The identified drug targets would be screened to optimally enhance or interfere with specific interactions to reduce pain.

G_{olf}-alpha (G_{olfα})

G_{olfα} is a GTP binding protein that was first identified in olfactory receptor neurons (Jones and Reed, *Science* 1989; 244: 790-795) where it was found to mediate olfactory receptor signaling. Expression of G_{olfα} has recently been identified in other regions of the brain, including the striatum (Drinnan et al., *Mol. Cell Neurosci.* 1991; 2: 66-70). G_{olfα} has been shown to mediate dopamine receptor signaling through adenylate cyclase. G_{olfα} shares 88% homology amino acid composition with G_{sα} (Zhuang, Belluscio and Hen. *J. Neurosci.* 2000; 20: RC91 (1-5)). Similarly to G_{sα}, activation of G_{olfα}, via receptor coupling, stimulates adenylate cyclase and thus enhances production of cAMP (a signal transduction molecule that has been shown to be important for opioid effects and pain). Recently, G_{olfα} expression and regulation were identified in the spinal cord (Sun et al., *BMC Neuroscience* 2002; 3: 11), and were found to be up-regulated following nerve injury. This is the first demonstration that there is expression of G_{olfα} in DRG. Recently, a G_{olfα} knockout mouse has been developed (Belluscio et al., *Neuron* 1998; 20: 69-81). These G_{olfα} knockout mice

are anosmic, and exhibit no hypolocomotor response to a dopamine receptor agonist or cocaine (Zhuang et al., *J. Neurosci.* 2000; 20: RC91; Belluscio et al., *Neuron* 1998; 20:69-81). The majority of G_{olfr} knockout mice are also unable to nurse and die shortly after birth, and surviving mutant mice exhibit poor maternal behavior and are hyperactive.

- 5 **Targeting Rationale.** Coupling of G_{olfr} to dopamine receptors suggests that it functions similarly to G_{sa} , stimulating cAMP production. Antagonists would therefore reduce cAMP production and cellular excitability, thus reducing pain transmission. In addition, G_{olfr} could be used to discover DRG-specific GPCRs or regulators of G protein signaling, using yeast 2-hybrid assays, cDNA expression libraries, or biochemical interaction screening.
- 10 Gene knockdown or inhibition of protein expression by gene targeting or RNA interference will be performed to assess the impact of G_{olfr} and/or associated signal transduction activity such as adenylate cyclase production, in chronic pain models.

5.3 Other indications

- It is a common observation that mechanisms and mediators of pain, including chronic pain,
- 15 nociceptive pain, neuropathic pain (including all types of hyperalgesia (i.e. sensation of more pain than the stimulus would warrant) and allodynia (i.e. a condition in which ordinarily painless stimuli induce the experience of pain)), inflammatory and cancer pain, are also involved in several other indications. These include addiction, seizures (including epilepsy), stroke or ischemia, neurodegenerative disorders (Parkinson's disease, multiple
 - 20 sclerosis (MS), amyotrophic lateral sclerosis (ALS), Huntington's chorea), headache (e.g. general, migraine, cluster or tension), anxiety, depression, asthma, rheumatic disease, osteoarthritis, retinopathy, inflammatory eye disorders, pruritis, ulcers (gastric and duodenal), gastric lesions (e.g. induced by a necrotizing agent), uncontrollable urination (e.g. incontinence), an inflammatory or unstable bladder disorder, and inflammatory bowel
 - 25 disease (e.g. Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS) including irritable bowel disease (IBD), gastroesophageal reflux disease (GERD), functional dyspepsia (e.g. ulcer-like dyspepsia, dysmotility-like dyspepsia, functional heartburn and non-ulcer dyspepsia), functional chest pain of presumed oesophageal origin, functional dysphagia, non-cardiac chest pain, symptomatic gastroesophageal disease,
 - 30 gastritis, aerophagia, functional constipation, functional diarrhea, burbulence, chronic functional abdominal pain, recurrent abdominal pain (RAP), functional abdominal bloating, functional biliary pain, functional incontinence, functional ano-rectal pain, chronic pelvic pain, pelvic floor dyssnergia, unspecified functional ano-rectal disorder, cholecystalgia, interstitial cystitis, dysmenorrhea, or dyspareunia.

For example, in addition to playing a role in pain, the excitatory amino acid glutamate has also been shown to play a role in opioid tolerance and dependence. Antagonists of NMDA and mGluR receptors reduce the development of opioid tolerance that occurs with chronic administration, and attenuate symptoms of opioid withdrawal (reviewed in Fundytus, *CNS Drugs* 15: 29-58, 2001).

Furthermore, glutamate excitotoxicity has been implicated in seizure activity and epilepsy, involving NMDA, AMPA/kainate and metabotropic glutamate receptors (Loscher, *Prog. Neurobiol.* 1998; 54: 721-741). Many anti-convulsants inhibit glutamate release via actions at sodium channels. As discussed above, sodium channels are also involved in the mediation of chronic pain.

Glutamate has been shown to play a key role in central nervous system trauma induced by stroke or ischemia. In *in vivo* animal models of both focal cerebral ischemia (model of stroke) and global ischemia (*e.g.* resembling that seen in humans after a heart attack or during thoracoabdominal surgery) it has been shown that glutamate release is enhanced and that glutamate receptor antagonists attenuate neuronal damage (Caragine et al., *Brain Res.* 1998; 793: 255-264; O'Neill et al., *Neuropharmacology* 1998; 37: 1211-1222; Minger et al., *Brain Res.* 1998; 810: 1881-1899; Dexter et al., *Anesthesia & Analgesia* 1998; 87: 72-78; Lang-Lazdunski et al., *J. Thoracic & Cardiovascular Surgery* 1999; 117: 881-889). Moreover, it has been suggested that active modulation of mGluR activity in cerebral ischemia may prevent neuronal injury (Maiese, *Clinical Neuropharmacology* 1998; 21: 1-7). In the clinic, there are increased cerebrospinal fluid (CSF) levels of glutamate in encephalitis (Launes et al., *NeuroReport* 1998;9: 577-581), and in blood and CSF of acute stroke patients (Castillo et al., *Lancet* 1997; 349: 70-83).

Many neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, mitochondrial encephalomyopathies, spinocerebellar degeneration syndromes, motor neuron diseases, and even schizophrenia have been associated with glutamate excitotoxicity (Massieu and Garcia, *Neurobiology (Budapest)* 1998; 6: 99-108; Arias et al., *Neurobiology (Budapest)* 1998; 6: 33-43; Bittagau and Ikonomidou, *Journal of Child Neurology* 1997; 12: 471-485; Myhrer, *Neuroscience & Biobehavioral Reviews* 1998; 2391: 131-139). AIDS dementia may also be associated with glutamate induced neuronal injury (Lipton, *Ann. Rev. Pharmacol. Toxicol.* 1997; 38: 159-177). Selective antagonists of the mGluR5 receptor have also been shown to exert anti-Parkinson activity *in vivo* (K.J. Ossowska et al., *Neuropharmacology* 2001; 41: 413-420; P.J.M. Will et al., *Trends in Pharmacological Sciences* 2001; 22: 331-337).

In addition to glutamate, other mediators of pain have also been shown to be involved in other indications. A good example of this is VR1. In support of a role for VR1 in inflammatory bowel diseases, it has been shown that the density of VR1 in the colon tissue from patients with inflammatory bowel disease is increased (Y. Yiangou et al., *Lancet* 2001; 357(9265): 1338-1339).

5.4 Definitions

As used herein, the term "pain" is art recognized and includes a bodily sensation elicited by noxious chemical, mechanical, or thermal stimuli, in a subject, *e.g.*, a mammal such as a human. The term "pain" includes chronic pain such as lower back pain; pain due to arthritis, *e.g.*, osteoarthritis; joint pain, *e.g.*, knee pain or carpal tunnel syndrome; myofascial pain, and neuropathic pain. The term "pain" further includes acute pain, such as pain associated with muscle strains and sprains; tooth pain; headaches; pain associated with surgery; or pain associated with various forms of tissue injury, *e.g.*, inflammation, infection, and ischemia.

"Neuropathic pain" refers to pain caused by injury or disease of the central or peripheral nervous system. In contrast to the immediate (acute) pain caused by tissue injury, neuropathic pain can develop days or months after a traumatic injury. Neuropathic pain frequently is long-lasting or chronic and is not limited in duration to the period of tissue repair. Neuropathic pain can occur spontaneously or as a result of stimulation that normally is not painful. Neuropathic pain is sustained by aberrant somatosensory processing, and is associated with chronic sensory disturbances, including spontaneous pain, hyperalgesia (*i.e.*, sensation of more pain than the stimulus would warrant) and allodynia (*i.e.*, a condition in which ordinarily painless stimuli induce the experience of pain). Neuropathic pain includes but is not limited to pain caused by peripheral nerve trauma, viral infection, diabetes mellitus, causalgia, plexus avulsion, neuroma, limb amputation, vasculitis, nerve damage from chronic alcoholism, hypothyroidism, uremia, and vitamin deficiencies, among other causes. Neuropathic pain is one type of pain associated with cancer. Cancer pain can also be "nociceptive" or "mixed."

"Chronic pain" can be defined as pain lasting longer than three months (Bonica, *Semin. Anesth.* 1986, 5:82-99), and may be characterized by unrelenting persistent pain that is not fully amenable to routine pain control methods. Chronic pain includes, but is not limited to, inflammatory pain, postoperative pain, cancer pain, osteoarthritis pain associated with metastatic cancer, trigeminal neuralgia, acute herpetic and post-herpetic neuralgia, diabetic neuropathy, pain due to arthritis, joint pain, myofascial pain, causalgia, brachial plexus avulsion, occipital neuralgia, reflex sympathetic dystrophy, fibromyalgia, gout, phantom

limb pain, burn pain, pain associated with spinal cord injury, multiple sclerosis, reflex sympathetic dystrophy and lower back pain and other forms of neuralgia, neuropathic, and idiopathic pain syndromes.

5 “Nociceptive pain” is due to activation of pain-sensitive nerve fibers, either somatic or visceral. Nociceptive pain is generally a response to direct tissue damage. The initial trauma causes the release of several chemicals including bradykinin, serotonin, substance P, histamine, and prostaglandin. When somatic nerves are involved, the pain is typically experienced as an aching or pressure-like sensation.

10 The “dorsal root ganglion” or “DRG” is the cluster of neurons just outside the spinal cord, made of cell bodies of afferent spinal neurons that comprise the peripheral nervous system (PNS). The cell bodies of sensory nerves that convey somatosensory (sense of touch) information to the brain are found in the DRG. These neurons are unipolar, where the axon splits in two, sending one branch to the sensory receptor and the other to the brain for processing.

15 The term “ipsilateral” refers to the side of the animal on which the L5 and L6 nerves are ligated (wounded) in the rat SNL model of neuropathic pain. The corresponding “ipsilateral” side in a sham-operated animal is the side on which the nerve is exposed but not injured, and the “ipsilateral” side in a naïve animal is the side that would have been injured (*e.g.*, the left side as described in the Example section, *infra*). The term
20 “contralateral” refers to the unligated (unwounded) side of the animal.

In the context of the present invention insofar as it relates to pain, the terms “treat”, “treatment”, and the like refer to a means to relieve or alleviate the perception of a pain. The terms “treat”, “treatment”, and the like may mean to relieve or alleviate the intensity and/or duration of a pain (*e.g.*, burning sensation, tingling, electric-shock-like feelings,
25 etc.) experienced by a subject in response to a given stimulus (*e.g.*, pressure, tissue injury, cold temperature, etc.). Treatment can occur in a subject (*e.g.*, a human or companion animal) suffering from a pain condition or having one or more symptoms of another condition that can be treated according to the present invention, or in an animal model of pain, such as the SNL rat model of neuropathic pain described herein, or any other animal
30 model of pain. In the context of the present invention insofar as it relates to any of the other conditions recited herein (other than pain), the terms “treat”, “treatment”, and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition.

35 An “analgesic” refers to any compound (*e.g.*, small inorganic or organic molecule, polypeptide, nucleic acid molecule, etc.) that is either known or novel, and useful to treat

pain. Specific categories of analgesics include but are not limited to opioids (*e.g.*, morphine, hydromorphone, methadone, levorphanol, fentanyl, oxycodone, oxymorphone), antidepressants (*e.g.*, fluoxetine (Prozac®), sertraline (Zoloft®), amitriptyline), anti-convulsants (*e.g.*, gabapentin, carbamazepine, valproic acid, topiramate, phenytoin), non-steroidal anti-inflammatory drugs (NSAIDs) and anti-pyretics (such as, *e.g.*, acetaminophen, ibuprofen, fenoprofen, diflusal, and naproxen, aspirin and other salicylates (*e.g.*, choline magnesium trisalicylate)), NMDA antagonists (*e.g.*, ketamine, dextromethorphan), and topical Lidocaine (*see also* Sindrup *et al.*, *Pain* 1999; 83: 389-400).

10 The term "subject" as used herein refers to a mammal (*e.g.*, a rodent such as a mouse or rat, a pig, a primate, or a companion animal (*e.g.*, dog or cat, etc.). In particular, the term refers to humans.

"Expression profile" refers to any description or measurement of one or more of the genes that are expressed by a cell, tissue, or organism under or in response to a particular condition. Expression profiles can identify genes that are up-regulated, down-regulated, or unaffected under particular conditions. Gene expression can be detected at the nucleic acid level or at the protein level. The expression profiling at the nucleic acid level can be accomplished using any available technology to measure gene transcript levels. For example, the method could employ *in situ* hybridization, Northern hybridization or hybridization to a nucleic acid microarray, such as an oligonucleotide microarray, or a cDNA microarray. Alternatively, the method could employ reverse transcriptase-polymerase chain reaction (RT-PCR) such as fluorescent dye-based quantitative real time PCR (TaqMan® PCR). In the Examples section provided below, nucleic acid expression profiles were obtained by (i) hybridization of labeled cRNA derived from total cellular mRNA to Affymetrix GeneChip® oligonucleotide microarrays, and (ii) TaqMan® PCR using gene-specific PCR primers. The expression profiling at the protein level can be accomplished using any available technology to measure protein levels, *e.g.*, using peptide-specific capture agent arrays (*see, e.g.*, International PCT Publication No. WO 00/04389).

The term "expressed sequence tag" or "EST" refers to short (usually approximately 200-600 nt) single-pass sequence reads from one or both ends of a cDNA clone. Typically, ESTs are produced in large batches by performing a single, automated, sequencing read of cDNA inserts in a cDNA library using a primer based on the vector sequence. As a result, ESTs often correspond to relatively inaccurate (around 2% error) partial cDNA sequences. Since most ESTs are short, they probably will not contain the entire coding region of a large gene (exceeding 200-600 nt in ORF length). Alternatively, or in addition, ESTs may contain non-coding sequences corresponding to untranslated regions of mRNA. ESTs may

provide information about the location, expression, and function of the entire gene they represent. They are useful (*e.g.*, as hybridization probes and PCR primers) in identifying full-length genomic and coding sequences as well as in mapping exon-intron boundaries, identifying alternatively spliced transcripts, non-translated transcripts, truly unique genes, and extremely short genes. For a review, *see* Yuan *et al.*, *Pharmacology and Therapeutics* 2001, 91:115-132. In the present application, the term "EST clone" is used to indicate the entire cloned cDNA segment of which only a portion has been initially end-sequenced to produce the "EST" or "EST sequence" that may be stored in public domain sequence databases (*e.g.*, dbEST at NCBI, available on the WorldWideWeb at ncbi.nlm.nih.gov/dbEST/). As with other public domain DNA sequences, these ESTs or EST sequences have accession numbers, and can be analyzed by sequence comparison algorithms such as BLAST, FASTA, DNA Strider, GCG, etc. The Affymetrix GeneChip arrays used in the Examples section below include probesets (consisting of 25 nt oligonucleotides) designed to measure mRNA levels of the gene encompassing the EST and are annotated by Affymetrix with the accession number for the relevant EST sequence. Herein, such probesets are referred to by their EST accession number.

The terms "array" and "microarray" are used interchangeably and refer generally to any ordered arrangement (*e.g.*, on a surface or substrate) of different molecules, referred to herein as "probes." Each different probe of an array is capable of specifically recognizing and/or binding to a particular molecule, which is referred to herein as its "target," in the context of arrays. Examples of typical target molecules that can be detected using microarrays include mRNA transcripts, cDNA molecules, cRNA molecules, and proteins.

Microarrays are useful for simultaneously detecting the presence, absence and quantity of a plurality of different target molecules in a sample (such as an mRNA preparation isolated from a relevant cell, tissue, or organism, or a corresponding cDNA or cRNA preparation). The presence and quantity, or absence, of a probe's target molecule in a sample may be readily determined by analyzing whether (and how much of) a target has bound to a probe at a particular location on the surface or substrate.

In a preferred embodiment, arrays used in the present invention are "addressable arrays" where each different probe is associated with a particular "address". For example, in a preferred embodiment where the probes are immobilized on a surface or a substrate, each different probe of the addressable array is immobilized at a particular, known location on the surface or substrate. The presence or absence of that probe's target molecule in a sample may therefore readily be determined by simply detecting whether a target has bound to that particular location on the surface or substrate.

The arrays according to the present invention are preferably nucleic acid arrays (also referred to herein as “transcript arrays” or “hybridization arrays”) that comprise a plurality of nucleic acid probes immobilized on a surface or substrate. The different nucleic acid probes are complementary to, and therefore can hybridize to, different target nucleic acid molecules in a sample. Thus, such probes can be used to simultaneously detect the presence and quantity of a plurality of different nucleic acid molecules in a sample, to determine the expression level of a plurality of different genes, *e.g.*, the presence and abundance of different mRNA molecules, or of nucleic acid molecules derived therefrom (for example, cDNA or cRNA).

There are two major types of microarray technology; spotted cDNA arrays and manufactured oligonucleotide arrays. The Examples section below describes the use of high density oligonucleotide Affymetrix GeneChip® arrays.

The arrays are preferably reproducible, allowing multiple copies of a given array to be produced and the results from each easily compared to each other. Preferably the microarrays are small, usually smaller than 5 cm², and are made from materials that are stable under binding (*e.g.*, nucleic acid hybridization) conditions. A given binding site or unique set of binding sites in the microarray will specifically bind the target (*e.g.*, the mRNA of a single gene in the cell). Although there may be more than one physical binding site (hereinafter “site”) per specific target, for the sake of clarity the discussion below will assume that there is a single site. It will be appreciated that when cDNA complementary to the RNA of a cell is made and hybridized to a microarray under suitable hybridization conditions, the level or degree of hybridization to the site in the array corresponding to any particular gene will reflect the prevalence in the cell of mRNA transcribed from that gene. For example, when detectably labeled (*e.g.*, with a fluorophore) cDNA complementary to the total cellular mRNA is hybridized to a microarray, any site on the array corresponding to a gene (*i.e.*, capable of specifically binding a nucleic acid product of the gene) that is not transcribed in the cell will have little or no signal, while a gene for which the encoded mRNA is highly prevalent will have a relatively strong signal.

By way of example, GeneChip expression analysis (Affymetrix, Santa Clara, CA) generates data for the assessment of gene expression profiles and other biological assays. Oligonucleotide expression arrays simultaneously and quantitatively “interrogate” thousands of mRNA transcripts (genes or ESTs), simplifying large genomic studies. Each transcript can be represented on a probe array by multiple probe pairs to differentiate among closely related members of gene families. Each probe set contains millions of copies of a specific oligonucleotide probe, permitting the accurate and sensitive detection of

even low-intensity mRNA hybridization patterns. After hybridization intensity data is captured, *e.g.*, using optical detection systems (*e.g.*, a scanner), software can be used to automatically calculate intensity values for each probe cell. Probe cell intensities can be used to calculate an average intensity for each gene, which correlates with mRNA abundance levels. Expression data can be quickly sorted based on any analysis parameter and displayed in a variety of graphical formats for any selected subset of genes. Gene expression detection technologies include, among others, the research products manufactured and sold by Hewlett-Packard, Perkin-Elmer and Gene Logic.

An “antisense” nucleic acid molecule or oligonucleotide is a single stranded nucleic acid molecule, which may be DNA, RNA, a DNA-RNA chimera, or a derivative thereof, which, upon hybridizing under physiological conditions with complementary bases in an RNA or DNA molecule of interest, inhibits the expression of the corresponding gene by inhibiting, *e.g.*, mRNA transcription, mRNA splicing, mRNA transport, or mRNA translation or by decreasing mRNA stability. As presently used, “antisense” broadly includes RNA-RNA interactions, RNA-DNA interactions, and RNase-H mediated arrest. Antisense nucleic acid molecules can be encoded by a recombinant gene for expression in a cell (see, *e.g.*, U.S. Patents No. 5,814,500 and 5,811,234), or alternatively they can be prepared synthetically (see, *e.g.*, U.S. Patent No. 5,780,607). According to the present invention, the involvement of a protein or peptide encoded by a nucleic acid molecule comprising any one of **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6), in the regulation of any particular pain condition may be identified, modulated and studied using antisense nucleic acids derived on the basis of nucleic acid molecules comprising any one of **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6) of the present invention.

The term “ribozyme” is used to refer to a catalytic RNA molecule capable of cleaving RNA substrates. Ribozyme specificity is dependent on complementary RNA-RNA interactions (for a review, see Cech and Bass, *Annu. Rev. Biochem.* 1986; 55: 599-629). Two types of ribozymes, hammerhead and hairpin, have been described. Each has a structurally distinct catalytic center. The present invention contemplates the use of ribozymes designed on the basis of any one of the nucleic acid molecules of the invention comprising **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6) to induce catalytic cleavage of the corresponding mRNA, thereby inhibiting expression of the corresponding gene. Ribozyme technology is described further in *Intracellular Ribozyme Applications: Principals and Protocols*, Rossi and Couture ed., Horizon Scientific Press, 1999.

The term "RNA interference" or "RNAi" refers to the ability of double stranded RNA (dsRNA) to suppress the expression of a specific gene of interest in a homology-dependent manner. It is currently believed that RNA interference acts post-transcriptionally by targeting mRNA molecules for degradation. RNA interference commonly involves the use of dsRNAs that are greater than 500 bp; however, it can also be mediated through small interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs), which can be 10 or more nucleotides in length and are typically 18 or more nucleotides in length. For reviews, see Bosner and Labouesse, *Nature Cell Biol.* 2000; 2: E31-E36 and Sharp and Zamore, *Science* 2000; 287: 2431-2433.

10 The term "nucleic acid hybridization" refers to anti-parallel hydrogen bonding between two single-stranded nucleic acids, in which A pairs with T (or U if an RNA nucleic acid) and C pairs with G. Nucleic acid molecules are "hybridizable" to each other when at least one strand of one nucleic acid molecule can form hydrogen bonds with the complementary bases of another nucleic acid molecule under defined stringency conditions. Stringency of hybridization is determined, *e.g.*, by (i) the temperature at which hybridization and/or washing is performed, and (ii) the ionic strength and (iii) concentration of denaturants such as formamide of the hybridization and washing solutions, as well as other parameters. Hybridization requires that the two strands contain substantially complementary sequences. Depending on the stringency of hybridization, however, some degree of mismatches may be tolerated. Under "low stringency" conditions, a greater percentage of mismatches are tolerable (*i.e.*, will not prevent formation of an anti-parallel hybrid). As the stringency increases, only duplexes with fewer mismatches per length of duplex nucleic acid remain hybridized (see *Molecular Biology of the Cell*, Alberts *et al.*, 3rd ed., New York and London: Garland Publ., 1994, Ch. 7).

25 Typically, hybridization of two strands at high stringency requires that the sequences exhibit a high degree of complementarity over an extended portion of their length. Examples of high stringency conditions include: hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65°C, followed by washing in 0.1x SSC/0.1% SDS (where 1x SSC is 0.15 M NaCl, 0.15 M Na citrate) at 68°C or for oligonucleotide molecules washing in 6xSSC/0.5% sodium pyrophosphate at about 37°C (for 14 nucleotide-long oligos), at about 48°C (for about 17 nucleotide-long oligos), at about 55°C (for 20 nucleotide-long oligos), and at about 60°C (for 23 nucleotide-long oligos)).

Conditions of intermediate or moderate stringency (such as, for example, an aqueous solution of 2xSSC at 65°C; alternatively, for example, hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65°C, and washing in 0.2 x SSC/0.1% SDS at 42°C) and low stringency (such as, for example, an aqueous solution of 2xSSC at

55°C), require correspondingly less overall complementarity for hybridization to occur between two sequences. Specific temperature and salt conditions for any given stringency hybridization reaction depend on the concentration of the target DNA and length and base composition of the probe, and are normally determined empirically in preliminary experiments, which are routine (see Southern, *J. Mol. Biol.* 1975; 98: 503; Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 2, ch. 9.50, CSH Laboratory Press, 1989; Ausubel *et al.* (eds.), 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3).

As used herein, the term "standard hybridization conditions" refers to hybridization conditions that allow hybridization of two nucleotide molecules having at least 75% sequence identity. According to a specific embodiment, hybridization conditions of higher stringency may be used to allow hybridization of only sequences having at least 80% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or at least 99% sequence identity.

Nucleic acid molecules that "hybridize" to any one of the nucleic acid molecules comprising **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6) of the present invention may be of any length. In one embodiment, such nucleic acid molecules are at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, and at least 70 nucleotides in length. In another embodiment, nucleic acid molecules that hybridize are of about the same length as the particular nucleic acid molecule.

The term "homologous" as used in the art commonly refers to the relationship between nucleic acid molecules or proteins that possess a "common evolutionary origin," including nucleic acid molecules or proteins within superfamilies (*e.g.*, the immunoglobulin superfamily) and nucleic acid molecules or proteins from different species (Reeck *et al.*, *Cell* 1987; 50: 667). Such nucleic acid molecules or proteins have sequence homology, as reflected by their sequence similarity, whether in terms of substantial percent similarity or the presence of specific residues or motifs at conserved positions.

The terms "percent (%) sequence similarity", "percent (%) sequence identity", and the like, generally refer to the degree of identity or correspondence between different nucleotide sequences of nucleic acid molecules or amino acid sequences of proteins that may or may not share a common evolutionary origin (see Reeck *et al.*, *supra*). In addition to the sequences of known genes having novel pain association according to the present invention (**SEQ ID NOS: 1-249**) the present invention includes sequences having about 95-99.9% identity to any one of the listed sequences. Sequence identity can be determined using any of a number of publicly available sequence comparison algorithms, such as

BLAST, FASTA, DNA Strider, GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin), etc.

To determine the percent identity between two amino acid sequences or two nucleic acid molecules, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, percent identity = number of identical positions/total number of positions (*e.g.*, overlapping positions) x 100). In one embodiment, the two sequences are, or are approximately, of the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent sequence identity, typically exact matches are counted.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 1990, 87:2264, modified as in Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 1993, 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, *J. Mol. Biol.* 1990; 215: 403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to sequences of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to protein sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res.* 1997, 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationship between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See ncbi.nlm.nih.gov/BLAST/ on the WorldWideWeb. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, *CABIOS* 1988; 4: 11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

In a preferred embodiment, the percent identity between two amino acid sequences is determined using the algorithm of Needleman and Wunsch (*J. Mol. Biol.* 1970, 48:444-453), which has been incorporated into the GAP program in the GCG software package (Accelrys, Burlington, MA; available at accelrys.com on the WorldWideWeb) using either

a Blossum 62 matrix or a PAM250 matrix, a gap weight of 16, 14, 12, 10, 8, 6, or 4, and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package using a NWSgapdna.CMP matrix, a gap weight of 40, 50, 60, 70, or 80, and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and one that can be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

In addition to the cDNA sequences encoding rat, mouse and human proteins, comprising any one of **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6), the present invention further provides polynucleotide molecules comprising nucleotide sequences having certain percentage sequence identities to any of the aforementioned sequences. Such sequences preferably hybridize under conditions of moderate or high stringency as described above, and may include species orthologs.

As used herein, the term "orthologs" refers to genes in different species that apparently evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function through the course of evolution. Identification of orthologs can provide reliable prediction of gene function in newly sequenced genomes. Sequence comparison algorithms that can be used to identify orthologs include without limitation BLAST, FASTA, DNA Strider, and the GCG pileup program. Orthologs often have high sequence similarity.

The present invention encompasses all orthologs of any one of **SEQ ID NOS: 1-249**. In addition to rat, mouse and human orthologs, particularly useful orthologs of the present invention are monkey, porcine, canine (dog), and guinea pig orthologs.

As used herein, the term "isolated" means that the material being referred to has been removed from the environment in which it is naturally found, and is characterized to a sufficient degree to establish that it is present in a particular sample. Such characterization can be achieved by any standard technique, such as, *e.g.*, sequencing, hybridization, immunoassay, functional assay, expression, size determination, or the like. Thus, a biological material can be "isolated" if it is free of cellular components, *i.e.*, components of the cells in which the material is found or produced in nature. For nucleic acid molecules, an isolated nucleic acid molecule or isolated polynucleotide molecule, or an isolated oligonucleotide, can be a PCR product, an mRNA transcript, a cDNA molecule, or a restriction fragment. A nucleic acid molecule excised from the chromosome that it is naturally a part of is considered to be isolated. Such a nucleic acid molecule may or may

not remain joined to regulatory, or non-regulatory, or non-coding regions, or to other regions located upstream or downstream of the gene when found in the chromosome. Nucleic acid molecules that have been spliced into vectors such as plasmids, cosmids, artificial chromosomes, phages and the like are considered isolated. In a particular
5 embodiment, a nucleic acid molecule comprising any one of **SEQ ID NOS: 1-249** (and species orthologs listed in Tables 5 and 6) spliced into a recombinant vector, and/or transformed into a host cell, is considered to be "isolated".

Isolated nucleic acid molecules and isolated polynucleotide molecules of the present invention do not encompass uncharacterized clones in man-made genomic or cDNA
10 libraries.

A protein that is associated with other proteins and/or nucleic acids with which it is associated in an intact cell, or with cellular membranes if it is a membrane-associated protein, is considered isolated if it has otherwise been removed from the environment in which it is naturally found and is characterized to a sufficient degree to establish that it is
15 present in a particular sample. A protein expressed from a recombinant vector in a host cell, particularly in a cell in which the protein is not naturally expressed, is also regarded as isolated.

An isolated organelle, cell, or tissue is one that has been removed from the anatomical site (cell, tissue or organism) in which it is found in the source organism.

20 An isolated material may or not be "purified". The term "purified" as used herein refers to a material (*e.g.*, a nucleic acid molecule or a protein) that has been isolated under conditions that detectably reduce or eliminate the presence of other contaminating materials. Contaminants may or may not include native materials from which the purified material has been obtained. A purified material preferably contains less than about 90%,
25 less than about 75%, less than about 50%, less than about 25%, less than about 10%, less than about 5%, or less than about 2% by weight of other components with which it was originally associated.

Methods for purification are well-known in the art. For example, nucleic acids or polynucleotide molecules can be purified by precipitation, chromatography (including
30 preparative solid phase chromatography, oligonucleotide hybridization, and triple helix chromatography), ultracentrifugation, and other means. Polypeptides can be purified by various methods including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reverse-phase HPLC, gel filtration, affinity chromatography, ion exchange and partition chromatography, precipitation and salting-out chromatography,
35 extraction, and counter-current distribution. Cells can be purified by various techniques,

including centrifugation, matrix separation (*e.g.*, nylon wool separation), panning and other immunoselection techniques, depletion (*e.g.*, complement depletion of contaminating cells), and cell sorting (*e.g.*, fluorescence activated cell sorting (FACS)). Other purification methods are possible. The term “substantially pure” indicates the highest degree of purity that can be achieved using conventional purification techniques currently known in the art. In the context of analytical testing of the material, “substantially free” means that contaminants, if present, are below the limits of detection, or are detected at levels that are low enough to be acceptable for use in the relevant art, for example, no more than about 2-5% (w/w), using techniques currently known in the art. Accordingly, with respect to the purified material, the term “substantially pure” or “substantially free” means that the purified material being referred to is present in a composition where it represents 95% (w/w) or more of the weight of that composition. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, or any other appropriate method known in the art.

The terms “about” and “approximately” mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within an acceptable standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to $\pm 20\%$, preferably up to $\pm 10\%$, more preferably up to $\pm 5\%$, and more preferably still up to $\pm 1\%$ of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term “about” is implicit and in this context means within an acceptable error range for the particular value.

The term “degenerate variants” of a polynucleotide sequence are those in which a change of one or more nucleotides in a given codon position results in no alteration in the amino acid encoded at that position.

The term “modulator” refers to a compound that differentially affects the expression or activity of a gene or gene product (*e.g.*, nucleic acid molecule or protein), for example, in response to a stimulus that normally activates or represses the expression or activity of that gene or gene product when compared to the expression or activity of the gene or gene product not contacted with the stimulus. In one embodiment, the gene or gene product the expression or activity of which is being modulated includes a gene, cDNA molecule or mRNA transcript that encodes a mammalian protein encoded by any one of the nucleic acid molecules comprising any one of **SEQ ID NOS: 1-249** (including species orthologs listed

in Tables 5 and 6) such as, *e.g.*, a rat, mouse, companion animal, or human protein. Examples of modulators of the nucleic acid molecules of the present invention include without limitation antisense nucleic acids, ribozymes, and RNAi oligonucleotides.

5 A “test compound” is a molecule that can be tested for its ability to act as a modulator of a gene or gene product. Test compounds can be selected without limitation from small inorganic and organic molecules (*i.e.*, those molecules of less than about 2 kD, and more preferably less than about 1 kD in molecular weight), polypeptides (including native ligands, antibodies, antibody fragments, and other immunospecific molecules), oligonucleotides, polynucleotide molecules, and derivatives thereof. In various
10 embodiments of the present invention, a test compound is tested for its ability to modulate the expression of a mammalian nucleic acid molecule comprising any one of **SEQ ID NOS: 1-249** (including species orthologs listed in Tables 5 and 6), or a protein encoded by said nucleic acid molecules, or to bind to a said mammalian protein. A compound that modulates a nucleic acid or protein of interest is designated herein as a “candidate
15 compound” or “lead compound” suitable for further testing and development. Candidate compounds include, but are not necessarily limited to, the functional categories of agonist and antagonist.

An “agonist” is defined herein as a compound that interacts with (*e.g.*, binds to) a nucleic acid molecule or protein, and promotes, enhances, stimulates or potentiates the biological
20 expression or function of the nucleic acid molecule or protein. The term “partial agonist” is used to refer to an agonist which interacts with a nucleic acid molecule or protein, but promotes only partial function of the nucleic acid molecule or protein. A partial agonist may also inhibit certain functions of the nucleic acid molecule or protein with which it interacts. An “antagonist” interacts with (*e.g.*, binds to) and inhibits or reduces the
25 biological expression or function of the nucleic acid molecule or protein.

The phrase “similar or identical expression” (and the like) as used herein refers to an expression level of a gene or gene product (*i.e.*, an mRNA transcript or protein) in a first cell that is $\pm 30\%$, preferably $\pm 20\%$, and more preferably $\pm 10\%$ of a given numerical value of the expression level of the same gene or gene product in a second comparator (or
30 control) cell as determined by any quantitative assay known in the art. Preferably, the second cell is either the same type of cell, and preferably from the same type of tissue, as the first cell, or the second cell is from the same cell line as the first cell. This second cell is also referred to herein as a “control cell” or “corresponding cell”. The first and second cells are preferably, but need not be, otherwise incubated and treated under the same
35 conditions.

The term “detectable change” as used herein in relation to an expression level of a gene or gene product means any statistically significant change and preferably at least a 1.5-fold change as measured by any available technique such as hybridization or quantitative PCR.

5 As used herein, the term “specific binding” refers to the ability of one molecule, typically an antibody, polynucleotide, polypeptide, or a small molecule ligand to contact and associate with another specific molecule, even in the presence of many other diverse molecules. “Immunospecific binding” refers to the ability of an antibody to specifically bind to (or to be “specifically immunoreactive with”) its corresponding antigen.

10 “Amplification” of DNA as used herein denotes the use of exponential amplification techniques known in the art such as the polymerase chain reaction (PCR), and non-exponential amplification techniques such as linked linear amplification, that can be used to increase the concentration of a particular DNA sequence present in a mixture of DNA sequences. For a description of PCR, see Saiki *et al.*, *Science* 1988, 239:487 and U.S. Patent No. 4,683,202. For a description of linked linear amplification, see U.S. Patent
15 Nos. 6,335,184 and 6,027,923; Reyes *et al.*, *Clinical Chemistry* 2001; 47: 131-40; and Wu *et al.*, *Genomics* 1989; 4: 560-569.

As used herein, the phrase “sequence-specific oligonucleotides” refers to oligonucleotides that can be used to detect the presence of a specific nucleic acid molecule, or that can be used to amplify a particular segment of a specific nucleic acid molecule for which a
20 template is present. Such oligonucleotides are also referred to as “primers” or “probes.” In a specific embodiment, “probe” is also used to refer to an oligonucleotide, for example about 25 nucleotides in length, attached to a solid support for use on “arrays” and “microarrays” described below.

The term “host cell” refers to any cell of any organism that is selected, modified,
25 transformed, grown, used or manipulated in any way so as, *e.g.*, to clone a recombinant vector that has been transformed into that cell, or to express a recombinant protein. Host cells are useful in screening and other assays, as described below.

As used herein, the terms “transfected cell” and “transformed cell” both refer to a host cell that has been genetically modified to express or over-express a nucleic acid encoding a
30 specific gene product of interest or a fragment thereof. Any eukaryotic or prokaryotic cell can be used, although eukaryotic cells are preferred, vertebrate cells are more preferred, and mammalian cells are the most preferred. In the case of multi-subunit ion channels, nucleic acids encoding the several subunits are preferably co-expressed by the transfected or transformed cell to form a functional channel. Transfected or transformed cells are
35 suitable to conduct an assay to screen for compounds that modulate the function of the gene

product. A typical "assay method" of the present invention makes use of one or more such cells, *e.g.*, in a microwell plate or some other culture system, to screen for such compounds. The effects of a test compound can be determined on a single cell, or on a membrane fraction prepared from one or more cells, or on a collection of intact cells
5 sufficient to allow measurement of activity.

The term "recombinantly engineered cell" refers to any prokaryotic or eukaryotic cell that has been genetically manipulated to express or over-express a nucleic acid of interest by any appropriate method, including transfection, transformation or transduction. The term "recombinantly engineered cell" also refers to a cell that has been engineered to activate an
10 endogenous nucleic acid, in a rat, mouse or human cell, which cell would not normally express that gene product or would express the gene product at only a sub-optimal level.

The terms "vector", "cloning vector" and "expression vector" refer to recombinant constructs including, *e.g.*, plasmids, cosmids, phages, viruses, and the like, with which a nucleic acid molecule (*e.g.*, a nucleic acid molecule comprising any one of **SEQ ID NOS:**
15 **1-249** or an siRNA-expressing nucleic acid) can be introduced into a host cell so as to, *e.g.*, clone the vector or express the introduced nucleic acid molecule. Vectors may further comprise selectable markers.

The terms "mutant", "mutated", "mutation", and the like, refer to any detectable change in genetic material, (*e.g.*, DNA), or any process, mechanism, or result of such a change.
20 Mutations include gene mutations in which the structure (*e.g.*, DNA sequence) of the gene is altered; any DNA or other nucleic acid molecule derived from such a mutation process; and any expression product (*e.g.*, the encoded protein) exhibiting a non-silent modification as a result of the mutation.

As used herein, the term "genetically modified animal" encompasses all animals into which
25 an exogenous genetic material has been introduced and/or whose endogenous genetic material has been manipulated. Examples of genetically modified animals include without limitation transgenic animals, *e.g.*, "knock-in" animals with the endogenous gene substituted with a heterologous gene or an ortholog from another species or a mutated gene, "knockout" animals with the endogenous gene partially or completely inactivated, or
30 transgenic animals expressing a mutated gene or overexpressing a wild-type or mutated gene (*e.g.*, upon targeted or random integration into the genome) and animals containing cells harboring a non-integrated nucleic acid construct (*e.g.*, viral-based vector, antisense oligonucleotide, shRNA, siRNA, ribozyme, etc.), including animals wherein the expression of an endogenous gene has been modulated (*e.g.*, increased or decreased) due to
35 the presence of such construct.

As used herein, a "transgenic animal" is a nonhuman animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal include a transgene. Other examples of transgenic animals include nonhuman primates, sheep, dogs, pigs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal.

As used herein, a "homologous recombinant animal" is a nonhuman animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A "knock-in animal" is an animal (*e.g.*, a mammal such as a mouse or a rat) in which an endogenous gene has been substituted in part or in total with a heterologous gene (*i.e.*, a gene that is not endogenous to the locus in question; see Roamer *et al.*, *New Biol.* 1991, 3: 331). This can be achieved by homologous recombination (see "knockout animal" below), transposition (Westphal and Leder, *Curr. Biol.* 1997; 7: 530), use of mutated recombination sites (Araki *et al.*, *Nucleic Acids Res.* 1997; 25: 868), PCR (Zhang and Henderson, *Biotechniques* 1998; 25: 784), or any other technique known in the art. The heterologous gene may be, *e.g.*, a reporter gene linked to the appropriate (*e.g.*, endogenous) promoter, which may be used to evaluate the expression or function of the endogenous gene (see, *e.g.*, Elegant *et al.*, *Proc. Natl. Acad. Sci. USA* 1998; 95: 11897).

A "knockout animal" is an animal (*e.g.*, a mammal such as a mouse or a rat) that has had a specific gene in its genome partially or completely inactivated by gene targeting (see, *e.g.*, U.S. Patents Nos. 5,777,195 and 5,616,491). A knockout animal can be a heterozygous knockout (*i.e.*, with one defective allele and one wild type allele) or a homozygous knockout (*i.e.*, with both alleles rendered defective). Preparation of a knockout animal typically requires first introducing a nucleic acid construct (a "knockout construct"), that will be used to decrease or eliminate expression of a particular gene, into an undifferentiated cell type termed an embryonic stem (ES) cell. The knockout construct is typically comprised of: (i) DNA from a portion (*e.g.*, an exon sequence, intron sequence, promoter sequence, or some combination thereof) of a gene to be knocked out; and (ii) a selectable marker sequence used to identify the presence of the knockout construct in the ES cell. The knockout construct is typically introduced (*e.g.*, electroporated) into ES cells so that it can homologously recombine with the genomic DNA of the cell in a double crossover event. This recombined ES cell can be identified (*e.g.*, by Southern

hybridization or PCR reactions that show the genomic alteration) and is then injected into a mammalian embryo at the blastocyst stage. In a preferred embodiment where the knockout animal is a mammal, a mammalian embryo with integrated ES cells is then implanted into a foster mother for the duration of gestation (see, *e.g.*, Zhou *et al.*, *Genes and Dev.* 1995; 9: 2623-34).

The phrases “disruption of the gene”, “gene disruption”, and the like, refer to: (i) insertion of a different or defective nucleic acid sequence into an endogenous (naturally occurring) DNA sequence, *e.g.*, into an exon or promoter region of a gene; or (ii) deletion of a portion of an endogenous DNA sequence of a gene; or (iii) a combination of insertion and deletion, so as to decrease or prevent the expression of that gene or its gene product in the cell as compared to the expression of the endogenous gene sequence.

In accordance with the present invention, there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. See, *e.g.*, Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989 (herein “Sambrook *et al.*, 1989”); *DNA Cloning: A Practical Approach, Volumes I and II* (Glover ed. 1985); *Oligonucleotide Synthesis* (Gait ed. 1984); *Nucleic Acid Hybridization* (Hames and Higgins eds. 1985); *Transcription And Translation* (Hames and Higgins eds. 1984); *Animal Cell Culture* (Freshney ed. 1986); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); Ausubel *et al.* eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. 1994; among others.

5.5 Screening for Agonists and Antagonists

The present invention provides nucleic acid molecules and gene products (proteins) that can be used in screening assays to identify compounds for use as therapeutics for the treatment of pain, including inflammatory and cancer-related pain, and especially neuropathic pain, as well as therapeutics for other indications such as addiction, seizures and epilepsy, stroke and ischemia, neurodegenerative disorders (Parkinson’s disease, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Huntington’s chorea), ulcers, and inflammatory bowel disease (Crohn’s disease and ulcerative colitis), as well as others. For instance, the invention provides methods and compositions that use a nucleic acid identified in Tables 1, 2, and 4 *infra*, and any of their respective gene products, to screen and/or test compounds to identify candidate compounds that may be useful for treating symptoms of neuropathic, inflammatory or cancer pain, addiction, seizures, epilepsy, stroke, ischemia, neurodegenerative disorders (Parkinson’s disease, multiple sclerosis (MS), amyotrophic

lateral sclerosis (ALS), Huntington's chorea), headache (e.g. general, migraine, cluster or tension), anxiety, depression, asthma, rheumatic disease, osteoarthritis, retinopathy, inflammatory eye disorders, pruritis, ulcers (gastric or duodenal), gastric lesions (e.g. induced by a necrotizing agent), uncontrollable urination (e.g. incontinence), an
5 inflammatory or unstable bladder disorder, inflammatory bowel disease (Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS) such as irritable bowel disease (IBD), gastroesophageal reflux disease (GERD), functional dyspepsia (e.g. ulcer-like dyspepsia, dysmotility-like dyspepsia, functional heartburn, or non-ulcer dyspepsia), functional chest pain of presumed oesophageal origin, functional dysphagia, non-cardiac
10 chest pain, symptomatic gastroesophageal disease, gastritis aerophagia, functional constipation, functional diarrhea, burbulence, chronic functional abdominal pain, recurrent abdominal pain (RAP), functional abdominal bloating, functional biliary pain, functional incontinence, functional ano-rectal pain, chronic pelvic pain, pelvic floor dyssnergia, unspecified functional ano-rectal disorder, cholecystalgia, interstitial cystitis,
15 dysmenorrhea, or dyspareunia.

The proteins encoded by the nucleic acid molecules described herein can be used in binding and functional assays to screen for lead compounds for treating pain. As discussed for each gene product, the ability to identify either an antagonist or agonist would provide for development of new treatments for neuropathic, and other types of pain, and the indications
20 listed above.

Thus, the nucleic acid molecules are useful for expressing the proteins to be isolated and used in direct binding assays. Protein expression can be carried out in any host cell system, e.g., plants, prokaryotes (e.g., *E. coli*), yeast, insect cells (e.g., Sf9 cells, using baculovirus vectors), or mammalian cells (e.g., CHO, COS etc.) Techniques for the
25 isolation and purification of the protein products are well known to one skilled in the art.

Protein products, or fragments thereof (e.g., proteolytic fragments or synthetic fragments), can be used to generate specific antibodies for directly detecting protein expression, e.g., through immunoassay.

Gene expression profiles may be used in screening for compounds that modulate the mRNA or protein expression of the differentially expressed genes shown in Tables 1, 2 and
30 4. Such a differentially expressed gene is referred to as the "gene of interest" and such modulating compounds are referred to as modulators that may up- or down-regulate mRNA transcription, or agonize or antagonize the activity of the protein. Such compounds are useful, e.g., for inhibiting or stimulating the expression of genes found to be regulated in
35 DRG neurons in the SNL model of neuropathic pain. Compounds that modulate the

expression profile of one or more of the genes may be readily identified using numerous screening methods known in the art. As used herein, the expression of a gene can be determined by measuring mRNA levels, protein levels, or protein activity using standard techniques.

- 5 In a preferred embodiment of the invention, the individual nucleic acid and/or gene product is initially used in a high-throughput screen (see below) to identify "candidate compounds" that bind specifically to the particular gene or gene product. Once identified, the candidate compound can be further used in cell-based or whole animal-based assays to determine its effect on expression of the particular nucleic acid, or expression or activity (*i.e.*, function)
- 10 of the gene product, relative to an untreated control cell or animal expressing the same nucleic acid and/or gene product. For the present invention, expression can also be detected in cells further treated or untreated with drugs commonly used to treat pain, *e.g.*, opioids or gabapentin. Cell culture assays using neuronal cells, *e.g.*, DRG neurons, may be used to determine whether a test compound functions as a modulator of expression. In a
- 15 specific embodiment, cells are contacted with a test compound and the effect of the compound on the expression is evaluated relative to a corresponding cell not contacted with a test compound. As used herein, the term "corresponding cell" refers to a cell in a separate sample from that of the test sample that is preferably of the same cell-type from the same tissue-type as the cell being tested.
- 20 A compound that is found to modulate expression may be designated a lead compound with potential therapeutic ability to treat pain and related disease states. Examples of lead compounds may include: (i) small inorganic molecules; (ii) small organic molecules, including natural products compounds; (iii) peptides or peptide analogs or mimetics; (iv) synthetic compounds, analogs or mimetics; and (v) antibodies, including recombinant
- 25 humanized antibodies. Alternatively, the gene products identified herein can themselves serve as therapeutic agents, by administering a gene product to a subject to treat a pain state or other indication.

Classes of compounds that may be identified by such screening assays include, but are not limited to, small molecules (*e.g.*, organic or inorganic molecules which are less than about

30 2 kd in molecular weight, more preferably less than about 1 kd in molecular weight, and/or are able to cross the blood-brain barrier or gain entry into an appropriate cell and affect the expression of the relevant gene or the activity of the relevant gene product. Compounds identified by these screening assays may also include polypeptides, such as soluble peptides, fusion peptides, members of combinatorial libraries (such as those described by

35 Lam et al., *Nature* 1991, 354:82-84; and by Houghten et al., *Nature* 1991, 354:84-86); members of libraries derived by combinatorial chemistry, such as molecular libraries of D-

and/or L-configuration amino acids; phosphopeptides, such as members of random or partially degenerate, directed phosphopeptide libraries (see, *e.g.*, Songyang et al., *Cell* 1993, 72:767-778); peptide libraries derived from the "phage method" (Scott and Smith, *Science* 1990, 249:386-390; Cwirla, et al., *Proc. Natl. Acad. Sci. USA* 1990, 87:6378-6382; Devlin et al., *Science* 1990, 49:404-406); chemicals from other chemical libraries (Geysen et al., *Molecular Immunology* 1986, 23:709-715; Geysen et al., *J. Immunologic Methods* 1987, 102:259-274; Fodor et al., *Science* 1991, 251:767-773; Furka et al., *14th International Congress of Biochemistry* 1988, Volume #5, Abstract FR:013; Furka, *Int. J. Peptide Protein Res.* 1991, 37:487-493; U.S. Patent No. 4,631,211; U.S. Patent No. 5,010,175; Needels et al., *Proc. Natl. Acad. Sci. USA* 1993, 90:10700-4; Ohlmeyer et al., *Proc. Natl. Acad. Sci. USA* 1993, 90:10922-10926; PCT Publication No. WO 92/00252; and PCT Publication No. WO 94/28028); and large libraries of synthetic or natural compounds available from a variety of sources, including Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), Microsource (New Milford, CT), Aldrich (Milwaukee, WI), Pan Laboratories (Bothell, WA), and MycoSearch (NC) (see, *e.g.*, Blondelle et al., *TIBTech* 1996, 14:60).

Neuronal cell cultures. DRG neuronal cultures can be produced using ordinary techniques known in the art. The screening methods of the present invention can use cultured cells or cell lines to screen for candidate therapeutic compounds. The cells are preferably neurons or neuronal cells. In another embodiment, transformed neuronal cell lines, such as those created with tetracarcinoma cell lines, can also be used.

Cultured post-mitotic or neuronal precursors can be obtained using various methods. As one example, primary neurons or neural progenitor cells are extracted and cultured according to methods known in the art (see, *e.g.*, U.S. Patent 5,654,189). Examples of the neurons useful for the methods of the present invention include those in brain tissue collected from mammals, and neuronal cell lines in which the nerve projections are extended by the induction of growth factors such as NGF (nerve growth factor; neurotrophic factor), IGF (insulin-like growth factor). For example, DRG neurons from rats can be dissociated (Caldero et al., *J. Neurosci.* 1998; 18: 356-370). Following dissociation, neurons are placed on tissue-culture dishes or microwells coated, *e.g.*, with ornithine-laminin, medium supplemented with glutamine, fetal bovine serum (FBS), putrescine, sodium selenite, progesterone and antibiotics (see, for example, Baudet et al., *Development* 2000; 127: 4335-4344). Growth factors such as NGF, FGF (fibroblast growth factor), EGF (epidermal growth factor), interleukin 6, etc. (*Ann. Rev. Pharmacol. Toxicol.* 1991; 31:205-228); IGF (*The Journal of Cell Biology* 1986; 102:1949-1954) and those which are disclosed in *Cell Culture in the Neurosciences*, New York: Plenum Press, pages 95-123 (1955), can also be included. In another embodiment, clonal cell lines may

be isolated from a conditionally-immortalized neural precursor cell line (See *e.g.*, U.S. Patent No. 6,255,122 to Sah et al.). In a preferred embodiment, the neural cells are primary cultures of neurons. A skilled artisan will readily appreciate that cells or cell cultures used in the methods of this invention should be carefully controlled for parameters
5 such as cell passage number, cell density, the methods by which the cells are dispensed, and growth time after dispensing.

5.6 Screening Assays for Binding and Function

5.6.1 High-Throughput Screen

Lead compounds that specifically bind to the genes or gene products according to the
10 invention may be identified by screening in high-throughput assays, including without limitation cell-based and cell-free assays, against individual protein targets. It will be appreciated by those skilled in the art that different types of assays can be used to detect different types of agents. Several methods of automated assays have been developed in recent years to permit screening of tens of thousands of compounds in a short period of
15 time (see, *e.g.*, U.S. Patent Nos. 5,585,277, 5,679,582, and 6,020,141). Such high-throughput screening methods are particularly preferred.

In a preferred embodiment, the differentially expressed genes of the present invention will be used in screening methods comprising microarrays.

Cell-based arrays combine the technique of cell culture in conjunction with the use of
20 fluidic devices for measurement of cell response to analytes (*i.e.*, test compounds) in a sample of interest, screening of samples for identifying molecules or organisms that induce a desired effect in cultured cells, and selection and identification of cell populations with novel and desired characteristics. High-content screens can be performed on either fixed cells, using fluorescently labeled antibodies, biological ligands, and/or nucleic acid
25 hybridization probes, or live cells using multicolor fluorescent indicators and biosensors. The choice of fixed or live cell screens depends on the specific cell-based assay required.

There are numerous single- and multi-cell-based array techniques known in the art. Recently developed techniques such as micro-patterned arrays (described in WO 97/45730, WO 98/38490) and microfluidic arrays provide valuable tools for comparative cell-based
30 analysis. Transfected cell microarrays are a complementary technique in which array features comprise clusters of cells overexpressing defined cDNAs. Complementary DNAs cloned in expression vectors are printed on microscope slides, which become "living arrays" after the addition of a lipid transfection reagent and adherent mammalian cells (Bailey et al., *Drug Discov. Today* 2002; 7 (18 Suppl.): S113-8). Cell-based arrays are

described in detail in, *e.g.*, Beske, *Drug Discov. Today* 2002;7 (18 Suppl.) :S131-5; Sundberg et al., *Curr. Opin. Biotechnol.* 2000; 11(1):47-53; Johnston et al., *Drug Discov. Today* 2002; 7 (6): 353-63; U.S. Patent Nos. 6,406,840 and 6,103,479, and U.S. published patent application 2002/0197656. For cell-based assays specifically used to screen for modulators of ligand-gated ion channels, see Mattheakis et al., *Curr. Opin. Drug Discov. Devel.* 2001; (1): 124-34 and Baxter et al., *J. Biomol. Screen.* 2002; 7(1):79-85.

5.6.1.1 Protein Arrays

- 10 Protein arrays are solid-phase, ligand binding assay systems using immobilized proteins on surfaces which include glass, membranes, microtiter wells, mass spectrometer plates, and beads or other particles. The assays are highly parallel and often miniaturized. Their advantages include being rapid and automatable, capable of high sensitivity, economical on reagents, and producing an abundance of data from a single experiment.
- 15 Automated multi-well formats are the best developed high-throughput screening systems. Automated 96-well plate-based screening systems are the most widely used. The current trend in plate based screening systems is to reduce the volume of the reaction wells further, thereby increasing the density of the wells per plate (96-well to 384-, and up to 1536-wells per plate). The reduction in reaction volumes results in increased throughput, dramatically decreased bioreagent costs, and a decrease in the number of plates that need to be managed by automation. For a description of protein arrays that can be used for high-throughput screening, see U.S. Patent Nos. 6,475,809; 6,406,921; and 6,197,599; and PCT Publication Nos. WO 00/04389 and WO 00/07024 herein incorporated by reference.

For construction of arrays, sources of proteins include cell-based expression systems for recombinant proteins, purification from natural sources, production *in vitro* by cell-free translation systems, and synthetic methods for peptides. For capture arrays and protein function analysis, it is important that proteins should be correctly folded and functional; this is not always the case, *e.g.*, where recombinant proteins are extracted from bacteria under denaturing conditions, whereas other methods (isolation of natural proteins, cell free synthesis) generally retain functionality. However, arrays of denatured proteins are useful in screening antibodies for cross-reactivity, identifying auto-antibodies and selecting ligand binding proteins.

The immobilization method used should be reproducible, applicable to proteins of different properties (size, hydrophilic, hydrophobic), amenable to high throughput and automation,

and compatible with retention of fully functional protein activity. Both covalent and noncovalent methods of protein immobilization are used. Substrates for covalent attachment include glass slides coated with amino- or aldehyde-containing silane reagents (Telechem). In the Versalinx™ system (Prolinx), reversible covalent coupling is achieved by interaction between the protein derivatized with phenyldiboronic acid, and salicylhydroxamic acid immobilized on the support surface. Covalent coupling methods providing a stable linkage can be applied to a range of proteins. Noncovalent binding of unmodified protein occurs within porous structures such as HydroGel™ (PerkinElmer), based on a 3-dimensional polyacrylamide gel.

For screening for agents that bind to ligand-gated ion channels, see, *e.g.*, Sinclair et al., *Anal. Chem.* 2002; 15; 74(24): 6133-8. This technique utilizes a microfluidics-patch clamp platform for performing high-throughput screening using a microfluidic chip consisting of multiple channels entering an open volume with standard patch clamp equipment for electrophysiological measurements.

5.6.1.2 Detection

For detection of molecules using screening assays, a molecule (*e.g.*, antibody or polynucleotide probe) can be detectably labeled by virtue of containing an atom (*e.g.*, radionuclide), molecule (*e.g.*, fluorescein), or complex that, due to a physical or chemical property, indicates the presence of the molecule. A molecule may also be detectably labeled when it is covalently bound to or otherwise associated with a “reporter” molecule (*e.g.*, a biomolecule such as an enzyme) that acts on a substrate to produce a detectable atom, molecule or other complex. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Labels useful in the present invention include biotin for staining with labeled avidin or streptavidin conjugate, magnetic beads (*e.g.*, Dynabeads™), fluorescent dyes (*e.g.*, fluorescein, fluorescein-isothiocyanate (FITC), Texas red, rhodamine, green fluorescent protein, enhanced green fluorescent protein, lissamine, phycoerythrin, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, FluorX [Amersham], SyBR Green I & II [Molecular Probes], and the like), radiolabels (*e.g.*, ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (*e.g.*, hydrolases, particularly phosphatases such as alkaline phosphatase, esterases and glycosidases, or oxidoreductases, particularly peroxidases such as horse radish peroxidase, and the like), substrates, cofactors, inhibitors, chemiluminescent groups, chromogenic agents, and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, etc.) beads. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

Means of detecting such labels are well known to those of skill in the art. Thus, for example, chemilluminant and radioactive labels may be detected using photographic film or scintillation counters, and fluorescent markers may be detected using a photodetector to detect emitted light (*e.g.*, as in fluorescence-activated cell sorting). Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting a colored reaction product produced by the action of the enzyme on the substrate. Colorimetric labels are detected by simply visualizing the colored label. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter, photographic film as in autoradiography, or storage phosphor imaging. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Also, simple colorimetric labels may be detected by observing the color associated with the label. Fluorescence resonance energy transfer has been adapted to detect binding of unlabeled ligands, which may be useful on arrays.

5.7 Functional Characterization: Inhibition of Expression or Activity

As stated above, the effects of the above-described differentially regulated genes can be evaluated by inhibiting the expression of the target gene and/or activity of the gene product in models of persistent and chronic pain. Both *in vitro* methods using cultured cells and *in vivo* methods using genetically engineered animals can be employed to achieve this objective. Technologies that block expression at the protein or mRNA level are referred to as gene “knockdown.” “Knockout” involves disrupting the target at the gene level. Combining microarray and “knockout” technology allows the profiling of tissue-specific gene expression changes and compensatory changes for individual genes.

5.7.1 Antibodies

Recent advances in antibody engineering have allowed the genes encoding antibodies to be manipulated, so that engineered antigen binding molecules can be expressed within mammalian cells. Suitable antibodies may be polyclonal, monoclonal, or recombinant. Application of gene technologies to antibody engineering has enabled the synthesis of single-chain fragment variable (scFv) antibodies that combine within a single polypeptide chain the light and heavy chain variable domains of an antibody molecule covalently joined by a predesigned peptide linker. Examples of useful fragments include separate heavy chains, light chains, Fab, F(ab')₂, Fabc, and Fv fragments. Fragments can be produced by

enzymatic or chemical separation of intact immunoglobulins or by recombinant DNA techniques. Fragments may be expressed in the form of phage-coat fusion proteins (see, e.g. International PCT Publication Nos. WO 91/17271, WO 92/01047 and WO 92/06204). Typically, the antibodies, fragments, or similar binding agents bind a specific antigen with an affinity of at least 10^7 , 10^8 , 10^9 , or 10^{10} M⁻¹.

The present invention provides an isolated antibody directed against a polypeptide of the present invention. In a specific embodiment, antibodies can be raised against a protein of the invention using known methods in view of this disclosure. Various host animals selected, e.g. from pigs, cows, horses, rabbits, goats, sheep, rats, or mice, can be immunized with a partially or substantially purified protein of the invention, or with a peptide homolog, fusion protein, peptide fragment, analog or derivative thereof, as described above. An adjuvant can be used to enhance antibody production.

Polyclonal antibodies can be obtained and isolated from the serum of an immunized animal and tested for specificity against the antigen using standard techniques. Alternatively, monoclonal antibodies can be prepared and isolated using any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, *Nature* 1975; 256: 495-497; the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 1983; 4: 72; Cote et al., *Proc. Natl. Acad. Sci. USA* 1983; 80: 2026-2030); and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., 1985, pp 77-96). Alternatively, techniques described for the production of single chain antibodies (see e.g. U.S. Patent No. 4,946,778) can be adapted to produce specific single chain antibodies.

Antibody fragments that contain specific binding sites for a polypeptide of the present invention are also encompassed within the present invention, and can be generated by known techniques. Such fragments include but are not limited to F(ab')₂ fragments, which can be generated by pepsin digestion of an intact antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., *Science* 1989; 246: 1275-1281) to allow rapid identification of Fab fragments having the desired specificity to the particular protein.

Techniques for the production and isolation of monoclonal antibodies and antibody fragments are known in the art, and are generally described, among other places, in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory,

1988, and in Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, London, 1986.

Antibodies or antibody fragments can be used in methods known in the art relating to the localization and activity of a protein of the present invention, e.g. Western blotting, *in situ* imaging, measuring levels thereof in appropriate physiological samples, etc. Immunoassay techniques using antibodies include radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), “sandwich” immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (using, e.g. colloidal gold, enzyme or radioisotope labels), precipitation reactions, agglutination assays (e.g. gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. Antibodies can also be used in microarrays (see, e.g. International PCT Publication No. WO 00/04389).

Recent advances in antibody engineering have allowed the genes encoding antibodies to be manipulated, so that antigen-binding molecules can be expressed within mammalian cells. Application of gene technologies to antibody engineering has enabled the synthesis of single-chain fragment variable (scFv) antibodies that combine within a molecule covalently joined by a pre-designed peptide linker. Intracellular antibodies (or intrabodies) can be used to target molecules involved in essential cellular pathways for modification or ablation of protein function. Antibody genes for intracellular expression can be derived either from murine or human monoclonal antibodies or from phage display libraries. For intracellular expression, small recombinant antibody fragments containing the antigen recognizing and binding regions can be used. Intrabodies can be directed to different intracellular compartments by targeting sequences attached to the antibody fragments.

Various methods have been developed to produce intrabodies. Techniques described for the production of single chain antibodies (U.S. Patent Nos. 5,476,786 and 5,132,405 U.S. Patent 4,946,778) can be adapted to produce polypeptide-specific single chain antibodies. Another method called intracellular antibody capture (IAC) is based on a genetic screening approach (Tanaka et al., *Nucleic Acids Res.* 2003 Mar 1; 31(5): e23). Using this technique, consensus immunoglobulin variable frameworks are identified, which can form the basis of intrabody libraries for direct screening. The procedure comprises *in vitro* production of a single antibody gene fragment from oligonucleotides and diversification of CDRs of the immunoglobulin variable domain by mutagenic PCR to generate intrabody libraries. This method obviates the need for *in vitro* production of antigen for pre-selection of antibody fragments and also yields intrabodies with enhanced intracellular stability.

These intrabodies can be used to modulate cellular physiology and metabolism through a variety of mechanisms, including the blocking, stabilizing, or mimicking of protein-protein interactions, by altering enzyme function, or by diverting proteins from their usual intracellular compartments. Intrabodies can be directed to the relevant cellular compartments by modifying the genes that encode them to specify N- or C-terminal polypeptide extensions for providing intracellular-trafficking signals.

Antibodies and intrabodies can be used as therapeutics for the treatment of pain and the other indications listed *infra* by modulating the function or activity of the gene product to which they specifically bind.

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5.7.2 Antisense

In a specific embodiment, gene function is inhibited by use of antisense nucleic acids. The present invention provides the analytic, therapeutic or prophylactic use of nucleic acids that are antisense to a gene or cDNA encoding a polypeptide.

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An antisense oligonucleotide is typically 18 to 25 bases in length and is designed to bind to a sequence in a selected mRNA population. This binding prevents translation of that specific mRNA, inhibiting production of the protein product encoded by that mRNA. This technology provides rapid identification of the protein function for a newly sequenced gene. Antisense technology can also be used to predict if developing a drug targeted against that gene product would likely produce the desired therapeutic effect. The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA, or a chimera or other modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous introduced sequences.

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In one embodiment, the invention is directed to methods for inhibiting the expression of a target nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense nucleic acid.

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The antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., *Proc.*

Natl. Acad. Sci. USA 1989; 86:6553-6556; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 1987; 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., *BioTechniques* 1988; 6:958-976) or intercalating agents (see, *e.g.*, Zon, *Pharm. Res.* 1988; 5:539-549).

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety, *e.g.* a sugar moiety selected from the group consisting of arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., *Nucl. Acids Res.* 1987; 15:6625-6641).

Morpholino antisense oligonucleotides are RNA oligos in which the bases are linked to 6-membered morpholine rings, which are connected to other morpholine-linked bases via non-ionic phosphorodiamidate intersubunit linkages. Morpholino oligonucleotides are resistant to nucleases and act by sterically blocking transcription of the target mRNA.

The oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer.

- 5 In a specific embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA). Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed
- 10 to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive.
- 15 Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature* 1981; 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 1980; 22:787-797), the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA* 1981; 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature* 1982;
- 20 296:39-42), etc.

- In another embodiment, "naked" antisense RNA can be delivered. Scrape delivery to adherent cells involves adding the antisense oligonucleotide to the cells, scraping the cells from the culture plate; and transferring the scraped cells to another plate where they are allowed to re-adhere. Scraping the cells from the plate pulls adhesion plaques from the cell
- 25 membrane, generating small holes which allow oligonucleotides to directly enter the cytosol for up to one minute, by which time the holes have resealed. Transferring the cells to another plate assures that any cells that were not scraped are not included in subsequent stages of the experiment.

- The antisense nucleic acids of the invention comprise a sequence complementary to at least
- 30 a portion of an RNA transcript of a target gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex
- 35 formation may be assayed. The ability to hybridize will depend on both the degree of

complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA encoding it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

As stated above for antibodies and intrabodies, antisense oligonucleotides can be used as therapeutics for the treatment of pain and the other indications listed *infra* by inhibiting the expression of the gene to which the antisense oligonucleotide binds.

5.7.3 Ribozyme Inhibition

In another embodiment, the expression of one or more genes of the present invention can be inhibited by ribozymes based on the nucleotide sequence thereof.

According to the present invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA has the following sequence of two bases: 5'-UG-3'. The construction of hammerhead ribozymes is known in the art, and described more fully in Myers, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, 1995 (see especially Figure 4, page 833) and in Haseloff and Gerlach, *Nature* 1988; 334: 585-591.

Preferably, the ribozymes of the present invention are engineered so that the cleavage recognition site is located near the 5' end of the corresponding mRNA, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts. In another embodiment the level of gene expression and/or gene product activity may be decreased by using target gene sequences in conjunction with well-known gene "knock-out," ribozyme and/or triple helix methods to decrease the level of gene expression. Such molecules may be designed to reduce or inhibit either unimpaired, or if appropriate, mutant target gene activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

Ribozyme molecules designed to catalytically cleave gene mRNA transcripts can be used to prevent translation of target gene mRNA and, therefore, expression of the gene product. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA (for a review, see Rossi, *Current Biology* 1994; 4: 469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The

composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

- 5 While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy mRNAs encoding a target gene, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and
- 10 production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff and Gerlach, *Nature* 1988; 334, 585-591, which is incorporated herein by reference in its entirety.
- 15 Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA encoding the target protein, *i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

- As in the antisense approach, ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, etc.), and can be delivered to mammalian cells,
- 20 preferably mouse, rat, or human cells, that express the target protein *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous mRNA encoding the protein and inhibit translation. Because ribozymes, unlike antisense molecules, are
- 25 catalytic, a lower intracellular concentration is required for efficacy.

Ribozymes can be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. Ribozyme technology is described further in *Intracellular Ribozyme Applications: Principals and Protocols*, Rossi and Couture eds., Horizon Scientific Press, 1999.

30

5.7.4 Triple Helix Formation

Nucleic acid molecules to be used in triplex helix formation for the inhibition of transcription should be single stranded and composed of deoxynucleotides. The base composition of these oligonucleotides should be designed to promote triple helix formation

via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC + triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as, for example, solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines. Ribozyme technology is described further in "Intracellular Ribozyme Applications: Principals and Protocols," Ed. Rossi and Couture, 1999, Horizon Scientific Press. Therapeutic use of ribozymes to inhibit expression of a gene product is also contemplated.

5.7.5 RNA Interference (RNAi or siRNA)

RNA interference (RNAi) is a process of sequence-specific post-transcriptional gene silencing by which double stranded RNA (dsRNA) homologous to a target locus can specifically inactivate gene function in plants, fungi, invertebrates, and vertebrates, including mammals (Hammond et al, *Nature Genet.* 2001; 2: 110-119; Sharp, *Genes Dev.*

1999; 13: 139-141). This dsRNA-induced gene silencing is mediated by short double-stranded small interfering RNAs (siRNAs) generated from longer dsRNAs by ribonuclease III cleavage (Bernstein et al, *Nature* 2001; 409: 363-366 and Elbashir et al, *Genes Dev.* 2001; 15: 188-200). RNAi-mediated gene silencing is thought to occur via sequence-specific mRNA degradation, where sequence specificity is determined by the interaction of an siRNA with its complementary sequence within a target mRNA (see e.g. Tuschl, *Chem. Biochem.* 2001; 2: 239-245).

For mammalian systems, RNAi commonly involves the use of dsRNAs that are greater than 500 bp; however, it can also be activated by introduction of either siRNAs (Elbashir, et al., *Nature* 2001; 411: 494-498) or short hairpin RNAs (shRNAs) bearing a fold back stem-loop structure (Paddison et al., *Genes Dev.* 2002; 16: 948-958; Sui et al., *Proc. Natl. Acad. Sci. USA* 2002; 99: 5515-5520; Brummelkamp et al., *Science* 2002; 296: 550-553; Paul et al., *Nature Biotechnol.* 2002; 20: 505-508).

The siRNAs to be used in the methods of the present invention are preferably short double stranded nucleic acid duplexes comprising annealed complementary single stranded nucleic acid molecules. In preferred embodiments, the siRNAs are short dsRNAs comprising annealed complementary single strand RNAs. However, the invention also encompasses embodiments in which the siRNAs comprise an annealed RNA:DNA duplex, wherein the sense strand of the duplex is a DNA molecule and the antisense strand of the duplex is a RNA molecule.

Preferably, each single stranded nucleic acid molecule of the siRNA duplex is of from about 19 nucleotides to about 27 nucleotides in length. In preferred embodiments, duplexed siRNAs have a 2 or 3 nucleotide 3' overhang on each strand of the duplex. In preferred embodiments, siRNAs have 5'-phosphate and 3'-hydroxyl groups.

The RNAi molecules to be used in the methods of the present invention comprise nucleic acid sequences that are complementary to the nucleic acid sequence of a portion of the target locus. In certain embodiments, the portion of the target locus to which the RNAi probe is complementary is at least about 15 nucleotides in length. In preferred embodiments, the portion of the target locus to which the RNAi probe is complementary is at least about 19 nucleotides in length. The target locus to which an RNAi probe is complementary may represent a transcribed portion of the gene of interest or an untranscribed portion thereof (e.g., intergenic regions, repeat elements, etc.).

The RNAi molecules may include one or more modifications, either to the phosphate-sugar backbone or to the nucleoside. For example, the phosphodiester linkages of natural RNA may be modified to include at least one heteroatom other than oxygen, such as nitrogen or

sulfur. In this case, for example, the phosphodiester linkage may be replaced by a phosphothioester linkage. Similarly, bases may be modified to block the activity of adenosine deaminase. Where the RNAi molecule is produced synthetically, or by *in vitro* transcription, a modified ribonucleoside may be introduced during synthesis or
5 transcription.

According to the present invention, siRNAs may be introduced to a target cell as an annealed duplex siRNA, or as single stranded sense and anti-sense nucleic acid sequences that, once within the target cell, anneal to form the siRNA duplex. Alternatively, the sense and anti-sense strands of the siRNA may be encoded on an expression construct that is
10 introduced to the target cell. Upon expression within the target cell, the transcribed sense and antisense strands may anneal to reconstitute the siRNA.

The shRNAs to be used in the methods of the present invention comprise a single stranded "loop" region connecting complementary inverted repeat sequences that anneal to form a double stranded "stem" region. Structural considerations for shRNA design are discussed,
15 for example, in McManus *et al.*, *RNA* 2002; 8: 842-850. In certain embodiments the shRNA may be a portion of a larger RNA molecule, *e.g.*, as part of a larger RNA that also contains U6 RNA sequences (Paul *et al.*, *supra*).

In preferred embodiments, the loop of the shRNA is from about 1 to about 9 nucleotides in length. In preferred embodiments the double stranded stem of the shRNA is from about 19
20 to about 33 base pairs in length. In preferred embodiments, the 3' end of the shRNA stem has a 3' overhang. In particularly preferred embodiments, the 3' overhang of the shRNA stem is from 1 to about 4 nucleotides in length. In preferred embodiments, shRNAs have 5'-phosphate and 3'-hydroxyl groups.

Although the RNAi molecules useful according to the invention preferably contain
25 nucleotide sequences that are fully complementary to a portion of the target locus, 100% sequence complementarity between the RNAi probe and the target locus is not required to practice the invention.

RNA molecules useful for RNAi may be chemically synthesized, for example using appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA
30 synthesizer. RNAs produced by such methodologies tend to be highly pure and to anneal efficiently to form siRNA duplexes or shRNA hairpin stem-loop structures. Following chemical synthesis, single stranded RNA molecules are deprotected, annealed to form siRNAs or shRNAs, and purified (*e.g.*, by gel electrophoresis or HPLC).

Alternatively, standard procedures may be used for *in vitro* transcription of RNA from DNA templates carrying RNA polymerase promoter sequences (*e.g.*, T7 or SP6 RNA polymerase promoter sequences). Efficient *in vitro* protocols for preparation of siRNAs using T7 RNA polymerase have been described (Donzé and Picard, *Nucleic Acids Res.* 2002; 30: e46; and Yu *et al.*, *Proc. Natl. Acad. Sci. USA* 2002; 99: 6047-6052).
5 Similarly, an efficient *in vitro* protocol for preparation of shRNAs using T7 RNA polymerase has been described (Yu *et al.*, *supra*). The sense and antisense transcripts may be synthesized in two independent reactions and annealed later, or may be synthesized simultaneously in a single reaction.

10 RNAi molecules may be formed within a cell by transcription of RNA from an expression construct introduced into the cell. For example, both a protocol and an expression construct for *in vivo* expression of siRNAs are described in Yu *et al.*, *supra*. Similarly, protocols and expression constructs for *in vivo* expression of shRNAs have been described (Brummelkamp *et al.*, *supra*; Sui *et al.*, *supra*; Yu *et al.*, *supra*; McManus *et al.*, *supra*;
15 Paul *et al.*, *supra*).

The expression constructs for *in vivo* production of RNAi molecules comprise RNAi encoding sequences operably linked to elements necessary for the proper transcription of the RNAi encoding sequence(s), including promoter elements and transcription termination signals. Preferred promoters for use in such expression constructs include the polymerase-
20 III HI-RNA promoter (see, *e.g.*, Brummelkamp *et al.*, *supra*) and the U6 polymerase-III promoter (see, *e.g.*, Sui *et al.*, *supra*; Paul, *et al.* *supra*; and Yu *et al.*, *supra*). The RNAi expression constructs can further comprise vector sequences that facilitate the cloning of the expression constructs. Standard vectors that may be used in practicing the current invention are known in the art (*e.g.*, pSilencer 2.0-U6 vector, Ambion Inc., Austin, TX).

25 Antisense and siRNA technologies have broad applications for preclinical drug development. In particular, antisense and siRNAs are beginning to be used in combination with expression profiling to evaluate drug specificity and mechanism-of-action, aiding in the identification of better candidates earlier in the drug development process.

30 5.7.6 In vivo Testing Using Genetically Modified Animals

Transgenic animals, including transgenic mammals, may be prepared for evaluating the molecular mechanism(s) of the genes associated with responses to persistent and chronic pain, including neuropathic, inflammatory and cancer pain. Responses in animal models can also be assessed for addiction, seizures and epilepsy, stroke and ischemia,
35 neurodegenerative disorders (Parkinson's disease, multiple sclerosis (MS), amyotrophic

lateral sclerosis (ALS), Huntington's chorea), headache (e.g. general, migraine, cluster or tension), anxiety, depression, asthma, rheumatic disease, osteoarthritis, retinopathy, inflammatory eye disorders pruritis, ulcers (gastric or duodenal), gastric lesions (e.g. induced by a necrotizing agent), uncontrollable urination (e.g. incontinence),

5 aninflammatory or unstable bladder disorder, inflammatory bowel disease (Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS) including irritable bowel disease (IBD), gastroesophageal reflux disease (GERD), functional dyspepsia (e.g. ulcer-like dyspepsia, dysmotility-like dyspepsia, fnctional heartburn, and non-ulcer dyspepsia), functional chest pain of presumed oesophageal origin, functional dysphagia, non-cardiac

10 chest pain, symptomatic gastroesophageal disease, gastritis, aerophagia, functional constipation, functional diarrhea, burbulence, chronic functional abdominal pain, recurrent abdominal pain (RAP), functional abdominal bloating, functional biliary pain, functional incontinence, functional ano-rectal pain, chronic pelvic pain, pelvic floor dyssnergia, unspecified functional ano-rectal disorder, cholecystalgia, interstitial cystitis, dysmenorrhea

15 or dyspareunia. Such animals provide excellent models for screening and/or testing drug candidates as therapeutics for pain. Mammalian "knock-in" animals can be prepared for evaluating the molecular biology to this system in greater detail than is possible with human subjects. It is also possible to evaluate compounds or diseases in "knockout" animals, e.g., to identify specific roles for the genes determined to be differentially regulated in

20 neuropathic pain models and to identify compounds that can compensate for the defect in gene activity. Both technologies permit manipulation of single units of genetic information in their natural position in a cell genome and to examine the results of that manipulation in the background of a terminally differentiated organism. Transgenic mammals can be prepared by any method, including but not limited to modification of embryonic stem (ES)

25 cells and heteronuclear injection into blast cells.

A "knock-in" animal is an animal (e.g., a mammal such as a mouse or rat) in which an endogenous gene is substituted with a heterologous gene (Roamer et al., *New Biol.* 1991, 3:331). Preferably, the heterologous gene is "knocked into" a locus of interest, either the subject of evaluation (in which case the gene may be a reporter gene; see Elegant et al.,

30 *Proc. Natl. Acad. Sci. USA* 1998, 95:11897) of expression or function of a homologous gene, thereby linking the heterologous gene expression to transcription from the appropriate promoter. This can be achieved by homologous recombination (Westphal and Leder, *Curr. Biol.* 1997, 7:530), using mutant recombination sites (Araki et al., *Nucleic Acids Res.* 1997, 25:868) or PCR (Zhang and Henderson, *Biotechniques* 1998, 25:784).

35 The term "knockout" refers to partial or complete suppression of the expression of at least a portion of a protein encoded by an endogenous DNA sequence in a cell. The term "knockout construct" refers to a nucleic acid sequence that is designed to decrease or

suppress expression of a protein encoded by endogenous DNA sequences in a cell. The nucleic acid sequence used as the knockout construct is typically comprised of: (1) DNA from some portion of the gene (exon sequence, intron sequence, and/or promoter sequence) to be suppressed; and (2) a marker sequence used to detect the presence of the knockout construct in the cell. The knockout construct is inserted into a cell, and integrates with the genomic DNA of the cell in such a position so as to prevent or interrupt transcription of the native DNA sequence. Such insertion usually occurs by homologous recombination. Generally, for homologous recombination, the DNA will be at least about 1 kilobase (kb) in length and preferably 3-4 kb in length, thereby providing sufficient complementary sequence for recombination when the knockout construct is introduced into the genomic DNA of the ES cell (discussed below). The knockout construct nucleic acid sequence may comprise: (1) a full or partial sequence of one or more exons and/or introns of the gene to be suppressed; (2) a full or partial promoter sequence of the gene to be suppressed; or (3) combinations thereof. Typically, the knockout construct is inserted into an embryonic stem cell (ES cell) and is integrated into the ES cell genomic DNA, usually by the process of homologous recombination. This ES cell is then injected into, and integrates with, the developing embryo.

A "knockout animal" is an animal (*e.g.*, a mammal such as a mouse or rat) that contains within its genome a specific gene that has been inactivated by the method of gene targeting (see, *e.g.*, US Patents Nos. 5,777,195 and 5,616,491). A knockout animal includes both a heterozygous knockout (*i.e.*, one defective allele and one wild type allele) and a homozygous knockout. Preparation of a knockout animal requires first introducing a nucleic acid construct that will be used to suppress expression of a particular gene into an undifferentiated cell type termed an embryonic stem cell. This cell is then injected into a mammalian embryo. In preferred embodiments where the knockout animal is a mammal, a mammalian embryo with an integrated cell is then implanted into a foster mother for the duration of gestation. Zhou, et al. (*Genes and Development*, 1995, 9:2623-34) describes protective protein/cathepsin A (PPCA) knockout mice.

The phrases "disruption of the gene" and "gene disruption" refer to insertion of a nucleic acid sequence into one region of a native DNA sequence (usually one or more exons) and/or the promoter region of a gene so as to decrease or prevent expression of that gene in the cell as compared to the wild type or naturally occurring sequence of the gene. By way of example, a nucleic acid construct can be prepared containing a DNA sequence encoding an antibiotic resistance gene which is inserted into the DNA sequence that is complementary to the DNA sequence (promoter and/or coding region) to be disrupted. When this nucleic acid construct is then transfected into a cell, the construct will integrate into the genomic DNA. Thus, many progeny of the cell will no longer express the gene at

least in some cells, or will express it at a decreased level, as the DNA is now disrupted by the antibiotic resistance gene.

Included within the scope of this invention is an animal, preferably a mammal (*e.g.*, a mouse) in which two or more neuropathic pain-associated genes identified according to the present invention have been knocked out or knocked in, or both. Such animals can be generated by repeating the procedures set forth herein for generating each knockout construct, or by breeding two animals, each with a single gene knocked out, to each other, and screening for those with the double knockout genotype.

Regulated knockout animals can be prepared using various systems, such as the tet-repressor system (see US Patent No. 5,654,168) or the Cre-Lox system (see US Patents No. 4,959,317 and 5,801,030).

In another series of embodiments, transgenic animals are created in which: (i) a human counterpart of the rat gene(s) is (are) stably inserted into the genome of the target animal; and/or (ii) the endogenous rat genes are inactivated and replaced with their human counterparts (see, *e.g.*, Coffman, *Semin. Nephrol.* 1997, 17:404; Esther et al., *Lab. Invest.* 1996, 74: 953; Murakami et al., *Blood Press. Suppl.* 1996, 2: 36). In one aspect of these embodiments, a human ortholog of a gene inserted into and/or expressed by the transgenic animal is a wild-type gene. In another aspect, the human gene or genes inserted into and/or expressed by the transgenic animal comprise a mutant or variant gene. In a particularly preferred aspect of these embodiments, the mutation is one that is found to be associated with neuropathic pain and for which there is a need in the art to identify therapeutics. Such transgenic animals can be treated with candidate compounds and monitored for attenuation of pain.

5.8 Diagnostic Applications

A variety of methods can be employed for the diagnostic evaluation of pain or a related disease, and for the identification of subjects having pain (*e.g.*, neuropathic pain) or a related disease. These methods may utilize reagents such as nucleic acid sequences that correspond to the genes specified in Tables 1 and 2, *infra* (including orthologs and homologs thereof, and their complementary sequences). The methods may also utilize gene products, or fragments thereof, that are encoded by those nucleic acids. In preferred embodiments, such reagents may be used specifically for: (1) the detection of either an over- or an under-expression of any of the nucleic acids relative to expression in an unaffected state (*e.g.*, in a subject or individual not having neuropathic pain); (2) the detection of either an over- or an under-abundance of a gene product encoded by any of the nucleic acids relative to abundance in an unaffected state; and (3) the detection of an

aberrant gene product activity relative to the unaffected state (*e.g.*, the detection of an under- or over-abundance of activity, which may be indicative of an over- or under-expression of either the gene or gene product).

5 The methods described herein are performed using at least one nucleic acid corresponding to one or more genes recited in Tables 1, 2, and 4, or, alternatively, may comprise one or more antibody reagents that specifically recognize one or more of the gene products encoded by those nucleic acids. In a preferred embodiment, the method utilizes a microarray that comprises a nucleic acid that can specifically hybridize to at least one of the genes recited in Tables 1, 2 and 4, and more preferably comprises probes for a
10 plurality of those genes. Such microarrays, as well as methods and systems for their use and manufacture, are described in *supra*.

A sample comprising a neuronal cell or any neural cell type or tissue of any tissue type in which the nucleic acids are capable of being expressed may be used in the diagnostic methods of this invention, *e.g.*, for detection of gene expression or for the detection of
15 gene products. For example, in preferred embodiments, cultured neuronal cells, such as neurons, may be used.

Detection of nucleic acids. The diagnostic methods of the invention include methods for assaying the levels of gene expression. For example, RNA from a cell type or tissue, such as DRG neurons, that are known or suspected to express a gene of interest such as those
20 listed in Tables 1, 2 and 4 may be isolated and tested utilizing hybridization or PCR techniques such as those described here. The isolated cells may be, for example, cells derived from a cell culture or from a patient. To assay levels of nucleic acid sequences in a sample, a variety of standard methods may be employed.

For example, nucleic acid sequences may be used in hybridization or amplification assays
25 of such biological samples to detect abnormalities in gene structure. As an example, and not by way of limitation, diagnostic methods of the invention can involve contacting and incubating nucleic acids (including recombinant DNA molecules, cloned genes or degenerate variants thereof) obtained from a sample with one or more labeled nucleic acid reagents, such as recombinant DNA molecules, cloned genes or degenerate variants
30 thereof, under conditions favorable for the specific annealing or hybridization of these reagents to their complementary sequences in the sample nucleic acids. Preferably, the lengths of these labeled nucleic acid reagents are at least 15 to 30 nucleotides. After incubation, all non-annealed or non-hybridized nucleic acids are removed. The presence of any nucleic acids that have hybridized is then detected.

In a preferred embodiment of such a detection scheme, the nucleic acids from the cell type or tissue of interest may be hybridized to nucleic acids attached to a solid support such as a membrane or a plastic surface (for example, on a microtiter plate or on polystyrene beads). In particularly preferred embodiments, the nucleic acid is immobilized on a solid surface as part of a microarray. After incubation, non-annealed, labeled nucleic acid reagents may be easily removed and detection of the remaining, annealed, labeled nucleic acid reagents may be accomplished using standard techniques that are well-known in the art. Alternative diagnostic methods for the detection of specific nucleic acids in patient samples or in other cell sources may involve their amplification, *e.g.*, by PCR (see, for example, the experimental embodiment taught in U.S. Patent No. 4,683,202) followed by detection of the amplified molecules using techniques that are well known to those of skill in the art.

In one preferred embodiment of such a detection scheme, a cDNA molecule is synthesized from an RNA molecule of interest (*e.g.*, by reverse transcription). A sequence within the cDNA may then be used as a template for a nucleic acid amplification reaction such as PCR. Nucleic acid reagents used as synthesis initiation reagents (*e.g.*, primers) in the reverse transcription and amplification steps of such an assay are preferably chosen from the nucleic acid sequences described herein or are fragments thereof. Preferably, the nucleic acid reagents are at least about 9 to 30 nucleotides in length. The amplification may be performed using, *e.g.*, radioactively labeled or fluorescently labeled nucleotides, for detection. Alternatively, enough amplified product may be made such that the product can be visualized by standard ethidium bromide or other staining methods.

Gene expression assays of the invention may also be performed *in situ* (*i.e.*, directly upon tissue sections of patient tissue, which may be fixed and/or frozen), thereby eliminating the need of nucleic acid purification. Nucleic acid reagents may be used as probes or as primers for such *in situ* procedures (see, for example, *Nuovo, PCR in situ Hybridization: Protocols and Application*, 1992, Raven Press, New York). Alternatively, if a sufficient quantity of the appropriate cells can be obtained, standard Northern analysis can be performed to determine the level of gene expression by detecting levels of mRNA molecules corresponding to the particular gene or genes of interest.

Detection of gene products. The diagnostic and prognostic methods of the invention also include methods that comprise detecting levels of proteins or other polypeptides encoded by a gene or genes associated with pain (*e.g.*, the genes recited in Tables 1 and 2). For example, antibodies directed against gene products or peptide fragments thereof can be used as diagnostic reagents for neuropathic pain. Such reagents may be used, for example, to detect abnormalities in the level of gene product synthesis or expression. Antibodies and immunoassay methods such as those described herein below also have important *in vitro*

applications for assessing the efficacy of treatments for pain. For example, antibodies, or fragments of antibodies, can be used in screens of potentially therapeutic compounds *in vitro* to ascertain a compound's effects on gene expression and polypeptide production.

Compounds that may have beneficial effects on a gene associated with pain can be

5 identified and a therapeutically effective dose for such compounds may be determined using such assays.

Generally the tissue or cell types analyzed using such methods will include cells, such as DRG neurons, that are known to express one or more of the gene products of interest.

Protein isolation methods such as those described by Harlow & Lane (*Antibodies: A*

10 *Laboratory Manual*, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) may be employed. The isolated cells may be cells derived from cell culture or from tissues of an individual subject.

As one example, antibodies or fragments of antibodies may be used to detect the presence of a gene product, for example, by immunofluorescence techniques employing a

15 fluorescently labeled antibody coupled with light microscopic, flow cytometric or fluorimetric detection methods. Such techniques are particularly preferred for detecting gene products on the surface of cells.

Antibodies or fragments thereof may also be employed histologically, for example, in

immunofluorescence or immunoelectron microscopy techniques, for *in situ* detection of a

20 gene product. *In situ* detection may be accomplished by removing a histological specimen (*e.g.*, a tissue sample) from a patient and applying thereto a labeled antibody of the present invention or a fragment of such an antibody (or primary and labeled secondary antibodies or fragments). The antibody or antibody fragment is preferably applied by overlaying the labeled antibody or antibody fragment onto a biological sample. Through the use of such a

25 procedure, it is possible to detect, not only the presence of particular gene products, but also the gene products' distribution in the examined tissue. A wide variety of histological methods that are well known in the art can be readily modified by those skilled in the art to achieve such *in situ* detection.

Immunoassays for gene products will typically comprise incubating a biological sample (for

30 example, a biological fluid, a tissue extract, freshly harvested cells or cell lysates) in the presence of detectably labeled antibodies that are capable of specifically binding to the gene product or products of interest. The bound antibody may then be detected by any of a number of techniques well known in the art.

6. EXAMPLE

The present invention is now described, in detail, by way of the following particular examples. However, the use of such examples is illustrative only and in no way limits the scope or meaning of this invention or of any exemplified term. Nor is the invention limited to any particular preferred embodiment(s) described herein. Indeed, many modifications and variations of the invention will be apparent to those skilled in the art upon reading this specification, and such "equivalents" can be made without departing from the invention in spirit or scope. The invention is therefore limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

EXAMPLE 1: Identification of Genes Differentially Expressed in Neuropathic Pain

Materials and Methods and Results

Animals and surgery. Rats having the L5-L6 spinal nerves ligated (SNL) according to the method of Chung et al., *Pain* 1992; 50:355-63 were used in this experiment. Briefly, nerve injury was induced by tight ligation of the left L5 and L6 spinal nerves, producing symptoms of neuropathic pain as described *infra*. The advantage of this model is that it allows the investigation of dorsal root ganglia that are injured (L5 and L6), versus dorsal root ganglia that are not injured (L4). Thus, it is possible to see changes in gene expression specifically in response to nerve injury. Surgery was performed under isoflurane/O₂ inhalation anesthesia. Following induction of anesthesia, a 3 cm incision was made, just lateral to the spinal vertebrae. The left paraspinal muscles were separated from the spinous process at the L4-S2 levels. The L6 transverse process was carefully removed with a pair of small rongeurs to visually identify the L4-L6 spinal nerves. The left L5 and L6 spinal nerves were isolated and tightly ligated with 7-0 silk suture. A complete hemostasis was confirmed, and the wound was sutured using non-absorbable sutures, such as 4-0 Vicryl.

The rats receiving the surgery were allowed to recover for 14 days when behavioral measurements of pain are no longer responsive to non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin, indicating that the remaining pain is neuropathic in nature. Gabapentin dosing was started after the 14 days recovery, and, as described above, administered intraperitoneally with a chronic dosing regimen. Naïve and sham surgery controls were carried out in parallel. Behavioral tests for neuropathic mechanical hyperalgesia by the paw pressure test were carried out to ensure that the surgery and drug treatment exhibited the expected effects.

Both naïve and sham-operated animals were used as controls. Sham-operation consisted of exposing the spinal nerves without ligation or manipulation. After surgery, animals were weighed and administered a subcutaneous injection of Ringers lactate solution. Following injection, the wound area was dusted with antibiotic powder and the animals were kept on a warm pad until recovery from anesthesia. Animals were then returned to their home cages until behavioral testing. The naïve control group consisted of rats that were not operated on. Eight to twelve rats in each group were evaluated.

Drug Treatment. Some rats from the SNL and naïve groups were also treated with gabapentin (GPN) as described herein. GPN, which is an anti-convulsant, has been shown in the clinic to be quite effective for the treatment of neuropathic pain (Melleger et al., *The Clinical Journal of Pain* 2001; 17: 284-295; Rose & Kam, *Anaesthesia* 2002; 57: 451-462). The present inventors set out to use the L4, L5 and L6 DRGs from the SNL model of neuropathic pain to identify genes involved in mediating and responding to pain (including genes affected by GPN treatment) by using expression profiling, which is based on identifying probes on a "genome-scale" microarray that are differentially expressed in SNL DRGs as compared to DRGs of naïve and sham-operated animals. Rats were tested 12-14 days after surgery, divided into the following treatment groups, and treated for 7 days: 1) naïve + vehicle; 2) naïve + GPN; 3) sham + vehicle; 4) SNL + vehicle; 5) SNL + GPN. Vehicle and GPN were administered intraperitoneally (i.p.) in a volume of 2 ml/kg, and GPN was dosed at 100 mg/kg.

Table 8 summarizes five experimental groups consisting of sham surgery, naïve or SNL surgery with or without GPN treatment:

Group Number	Experimental Group Name	Surgery	Drug Treatment
1	naïve + vehicle	none performed	vehicle
2	naïve + GPN	none performed	gabapentin
3	sham + vehicle	sham	vehicle
4	SNL + vehicle	SNL	vehicle
5	SNL + GPN	SNL	gabapentin

Behavioral testing. Mechanical sensitivity was assessed using the paw pressure test. This test measures mechanical hyperalgesia. Hind paw withdrawal thresholds ("PWT")

(measured in grams) in response to a noxious mechanical stimulus were determined using an analgesymeter (Model 7200, commercially available from Ugo Basile of Italy), as described in Stein, *Biochemistry & Behavior* 1988; 31: 451-455. The rat's paw was placed on a small platform, and weight was applied in a graded manner up to a maximum of 250 grams. The endpoint was taken as the weight at which the paw was completely withdrawn. PWT was determined once for each rat at each time point, and only the injured ipsilateral paw (*i.e.*, the hind paw on the same side of the animal as the ligation in SNL animals, or the side of the animal where the nerve was exposed but not injured in sham-operated animals) was used in the test. For naïve animals, the left paw or the side that "would have been" subjected to surgery (herein also referred to as "ipsilateral") was used for the test.

Rats were tested prior to injury (SNL or sham surgery; naïve rats were tested at the same time) to determine a baseline, or normal, PWT. To verify that the surgical procedure was successful, rats were again tested at 12-14 days after surgery. At this time, rats with an SNL injury should exhibit a significantly reduced PWT compared to their baseline PWT, while sham-operated and naïve rats should have PWT that is not significantly different from their baseline PWT. Only rats that met these criteria were included in further behavioral testing and the gene expression study.

Rats that met the behavior criteria were divided into the treatment groups (described above): 1) naïve + vehicle; 2) naïve + GPN; 3) sham + vehicle; 4) SNL + vehicle; 5) SNL + GPN (Table 8). Vehicle (0.9% saline) and GPN (dissolved in 0.9% saline) were administered intraperitoneally (i.p.) in a volume of 2 ml/kg. The dose of GPN was 100 mg/kg. The rats in the above treatment groups were treated each day for 7 days (with either vehicle or GPN as per their group), and on the last (7th) treatment day (corresponding to 19-21 days post surgery), rats were again assessed for mechanical sensitivity using the paw pressure test described above, in particular to confirm the reversal of neuropathic pain with GPN treatment. Following testing, tissues were collected as described below. See Figure 2 for a summary of the experimental timelines for surgery, treatment, and testing.

Tissue collection and RNA preparation. Rats were sacrificed 19-21 days after nerve injury, following 7 days of drug treatment as described above, and the following tissues were collected: brain, hemisected spinal cord cut into ipsilateral (same side) to injury and contralateral (opposite side) to injury, mid-thigh sciatic nerve, and L4, L5 and L6 dorsal root ganglia (DRG), both ipsilateral and contralateral to injury. Samples were rapidly frozen on dry ice. Next, for each experimental group and tissue (5 groups x 6 tissues = 30 total), the samples were separated into two pools (Pool 1 and Pool 2), consisting of half or 4-6 animals each.

Total RNA from each sample was prepared using Tri-Reagent (Sigma, St. Louis, MO), and quantified by measuring absorption at 260 nm. RNA quality was assessed by measuring absorption at 260 nm/280 nm and by capillary electrophoresis on an RNA Lab-on-chip Bioanalyzer 2100 (Agilent, Palo Alto, CA) to ensure that the ratio of 260 nm/280 nm was > 2.0 , and that the ratio 28S/18S was > 1.0 for each sample. Two pools of RNA were prepared. Pool 1 was used for an Affymetrix microarray hybridization, and Pool 2 was used for TaqMan[®] analysis.

Affymetrix microarray hybridization and staining GeneChip[®] analysis. GeneChip[®]

(Affymetrix) technology allows comparative analysis of the relative expression of thousands of genes under numerous experimental conditions. Each gene is represented by a “probeset” consisting of multiple pairs of oligonucleotides (25 nt in length) with sequence complementary to the gene sequence of EST sequence of interest, and the same oligonucleotide sequence with a one base-pair mismatch. These probeset pairs allow for the detection of gene-specific nucleic acid hybridization signals as described below.

Affymetrix Rat U34 A, B and C arrays used for the described analysis contain probesets representing approximately 26, 000 genes including more than 1200 cDNAs (corresponding to mRNA) that are of known relevance to the field of neurobiology, were hybridized with the RNA isolated from test animals as described above. These arrays include cDNA specific for kinases, cell surface receptors, cytokines, growth factors and oncogenes, among others.

Hybridization probes were prepared according to the Affymetrix Technical Manual (available on the WorldWideWeb at affymetrix.com/support/technical/manual/expression_manual.affx). To prime first strand cDNA synthesis from each total RNA sample (10 µg), 5 mM of oligonucleotide primer encoding the T7 RNA polymerase promoter linked to oligo-dT₂₄ primer was used. cDNA synthesis reactions were carried out at 42°C using Superscript II - reverse transcriptase (Invitrogen, Carlsbad, California). Second strand cDNA synthesis was finished using DNA polymerase I and T4 DNA ligase. Each double-stranded cDNA sample was purified by sequential Phase Lock Gels (Brinkman Instrument, Westbury, NY) and Phenol/Chloroform extraction (Ambion, Austin, TX). Half of each cDNA sample was transcribed *in vitro* into the copy RNA (cRNA) labeled with biotin-UTP and biotin-CTP using the BioArray High Yield RNA Transcript Labeling Kit (Enzo Biochemicals, New York, NY). These cRNA transcripts were purified using RNeasy[™] columns (Qiagen, Hilden Germany) and quantitated by measuring absorption at 260nm/280nm. 15 µg aliquots of each cRNA sample were fragmented at 95°C for 35 min in 40 mM Tris-acetate, pH 8.0, 100 mM KOAc, and 30 mM MgOAc to a mean size of ~50-150 nucleotides. Hybridization buffer

(0.1 M MES, pH6.7, 1M NaCl, 0.01% Triton, 0.5 mg/ml BSA, 0.1 mg/ml H. sperm DNA, 50 pM control oligo B2, and 1X eukaryotic hybridization control) was added to each sample. Samples were then hybridized to RG-U34A, B, and C microarrays (Affymetrix) at 45°C for 16 h. Microarrays were washed and sequentially incubated with streptavidin phycoerythrin (Molecular Probes), biotinylated anti-streptavidin antibody (Vector Laboratories, Inc., Burlingame, CA), and streptavidin phycoerythrin on the Fluidic Station (Affymetrix). Finally, the microarrays were scanned with a dedicated Gene Array Scanner (Hewlett Packard Instruments, TX) to capture a fluorescence image. Microarray Suite 5.0 software (Affymetrix) was used to extract gene expression signal from the scanned array image.

Genes that were found to be more than 2x under- or over-expressed relative to controls were selected for further evaluation using TaqMan® as described below.

TaqMan® quantitative real-time PCR. 10 ng total RNA was used to synthesize cDNA with random hexamers using TaqMan® Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time PCR analysis was performed on an Applied Biosystems ABI Prism 7700 Sequence Detection System. Matching primers and fluorescence probes were designed for each of the genes using the Primer Express® program provided by Applied Biosystems. Both forward and reverse primers were used at 200 nM. In all cases, the final probe concentration was 200 nM. The PCR reaction was performed in a final volume of 25 µl using TaqMan® Universal PCR Master Mix containing AmpliTaq® Gold DNA Polymerase, AmpErase® UNG, dNTPs (with dUTP), Passive Reference 1, optimized buffer components (proprietary formulation) and 5 µl of cDNA template. Three replicates of RT and PCR for each RNA sample were performed on the same reaction plate. A control lacking a DNA template, and controls using reference genes with stable expressions in all samples in this SNL study, were included on the same plate to reduce the reaction variations.

Normal Tissue Panel. Tissue specific expression for the genes was performed for 27 rat organs using 81 rat GeneChips® (Affymetrix). Validation of organ expression was performed using Taqman® Real-Time PCR. Results for 12 tissues are presented in Table 1B below.

Annotation. Affymetrix sequences were blasted against a human transcript database generated from a human genome annotation downloaded from NCBI. Blast output was processed, and the top hits were reported. The top hits were categorized as follows: score =100, hit length =100, percent identity =85. EST sequences that did not having a

convincing hit, were further analyzed in one of two ways: EST walking, or a “25-mer method.”

For EST walking, sequences were first blasted against human/rat/mouse EST database. Overlapping hits of high sequence identity were assembled along with the query sequence using Sequencher. An assembled consensus sequence was then blasted against an NR (non-redundant) database. Top hits of high sequence identity were reported.

A 25-mer is an oligonucleotide used by Affymetrix on a chip to detect the expression of a particular transcript. For 25-mer analysis, 25-mers of probe sets of interest were selected based on their behavior in various experimental conditions (for example changed 3 fold in SNL animals in either L4 or L5 and L6, and consistent with the overall pattern of the probeset). Selected probe sets were blasted against the NR database. Identical subject sequence(s) or those with at most one mismatch were considered as potential hits. Since multiple “good” 25-mers could be picked for a particular probeset, consistency of potential hit(s) among 25-mers for a single probeset was very important in deciding the final hit(s).

If by one of the two methods described above, a known gene was identified for an EST designated by Affymetrix, the new annotation based on the identified known gene was used for analysis and reported in Table 2 (italicized).

Statistical Criteria. Based on historical statistical analysis of replicate sample data (not shown), it was determined that the reproducibility of GeneChip data is dependent on the intensity of the signal. For intensities above 130, the reproducibility exhibits a coefficient of variation (CV: standard deviation divided by the average intensity) of 0.2 or better. Below 130, the reproducibility quickly falls off to CVs approaching infinity. For genes having a gene expression intensity greater than 130, there is a high confidence (> 2 standard deviations) in apparent fold-changes of three-fold. Therefore, in order to identify genes most likely to be regulated by the SNL, for L4 the following criteria were applied:

Maximum value for L4 sham ipsi, SNL ipsi, and SNL contra > 130

AND, $L4\ SNL\ ipsi \div L4\ sham\ ipsi > \text{three-fold regulation}$

AND, $L4\ SNL\ ipsi \div L4\ SNL\ contra > \text{three-fold regulation}$

In the injured neurons, L5 and L6, the differential expression observed was more robust and the following criteria were applied:

Maximum value for L5 sham ipsi, L5 SNL ipsi, L6 sham ipsi, and L6 SNL ipsi > 130

AND, $L5\ SNL\ ipsi \div L5\ sham\ ipsi > \text{three-fold regulation}$

AND, L6 SNL ipsi $\div$ L6 sham ipsi > three-fold regulation

Results

Mechanical hyperalgesia is a common symptom of neuropathic pain syndromes. The pilot chronic dosing experiment showed that neuropathic (SNL) rats displayed mechanical hyperalgesia, indicated by a large decrease in paw withdrawal threshold 21 days after surgery prior to drug injection, while there was no change in paw withdrawal threshold in sham-operated and naïve rats. SNL rats treated with gabapentin, either chronically or acutely only on test day, had increased paw withdrawal thresholds compared to vehicle-treated SNL rats 1 hour after treatment, indicating the gabapentin attenuated neuropathic mechanical hyperalgesia in neuropathic rats. This experiment indicated that rats could be chronically dosed with gabapentin, and tolerance would not develop to the effects of gabapentin. Rats were then prepared for tissue collection, divided into the treatment groups listed above. In this experiment, neuropathic (SNL) rats displayed mechanical hyperalgesia, as indicated by a decrease in paw withdrawal threshold 19-21 days after surgery, while there was no change in paw withdrawal threshold in sham-operated or naïve rats.

These results indicate that the SNL model is a valid model of neuropathic pain for this experiment. Following the last behavioral testing one-hour post-gabapentin treatment on the seventh day, the rats were sacrificed and whole dorsal root ganglia (DRG) was obtained from the ipsilateral (ipsi) and contralateral (contra) sides at lumbar L4, L5, and L6.

Analysis using both sets of criteria demonstrated that 337 sequences in total (from 26,000 rat genes represented on the array by probe sets) were up or down-regulated three-fold in the sciatic nerve ligation model (Table 1). Of the 337, a subset of 249 represented known genes (Table 2). These 249 genes were annotated as “known” based on annotations provided by Affymetrix, as well as those further annotated by in-house bioinformatics criteria, as described in the methods (summary of annotations and filtering criteria in Table 2). Given the strict selection criteria used, and based on significant regulation at the mRNA level, these 249 genes are likely to be involved in mediating molecular mechanisms of nociception and/or neuronal repair following injury ligation, and could potentially be utilized as targets for drug discovery of analgesics and/or therapeutics to treat pain or to promote or prevent neuronal repair.

In order to further subdivide the discovered genes into transcript classes representing functionally related proteins, hierarchical clustering algorithms were applied to the 337 regulated probe sets. High-density gene expression arrays including Affymetrix GeneChips

have been successfully used to characterize global RNA regulation during cellular processes such as cell-cycle regulation (Cho et al., *Molecular Cell* 1998; 2(1): 65-73; Spellman et al., *Genome Biol.* 2002; 3(9): RESEARCH0046), fibroblast growth control (Iyer et al., *Science* 1999;283(5398):83-7), metabolic responses to growth medium (DeRisi et al., *Clin Exp Immunol.* 1997; 107 Suppl. 1:33-40), and germ cell development (Cho et al., 1998, *supra*). The program of gene expression delineated in these studies demonstrated a correlation between shared functional pathways and temporally coupled expression. Clustering analysis of temporal expression profiles also provided a dynamic overall picture of the cell processes engaged (reviewed by (Brown and Botstein, *Nat. Genet.* 1999; 21 (1 Suppl.): 33-7). Clustering algorithms then were applied, as described in methods, to the 337 probe sets from genome profiling of the SNL model.

Using hierarchical clustering algorithms, the regulated genes were ordered based on their gene expression pattern not only across 16 SNL conditions (gene expression values summarized in Table 1A, but also across the 12 organ recital samples (Table 1B). In Figure 1, each gene was represented by one row of shaded tiles, reflective of gene expression values for the indicated conditions. In the dendrogram of Figure 1, the relatedness between two gene expression patterns defined by a Euclidean metric (see experimental procedures) was proportional to the distance of the connecting branch. The major branches of the dendrogram segregated nine transcript regulation classes (left panel of **Figure 1**) that were designated as (1) known and novel DRG-specific pain targets, (2) neuronal cellular signal transduction proteins, (3) neuronal markers, (4) cellular signal transduction proteins, (5) known and novel neuropeptides or secreted molecules, (6) inflammatory response genes A, (7) inflammatory response genes B, (8) markers of muscle tissue, and (9) unknown (see Table 3). The transcript class descriptions were based on annotations of the known genes represented in each group.

Almost half of the genes fall into transcript classes 1, 2 and 3, exhibiting a gene expression profile primarily of down-regulation in the L5 and L6 affected neurons (ipsilateral SNL + vehicle, and ipsilateral SNL + GPN). Transcript class 1 distinguishes itself from 2 and 3, mostly due to higher expression in DRG in the naïve conditions, and reduced expression in other neuronal tissues such as brain and spinal cord. In addition, eight out of the twenty-three “known” genes (as opposed to unannotated ESTs) in transcript class 1 code for proteins described in the literature to be associated with pain (the vanilloid receptor, two voltage-gated sodium channels NaV and SNS/PN3, the ionotropic glutamate receptor, the serotonin receptor, DREAM, the nicotinic acetylcholine receptor, and regulator of G protein signaling 4). Since these known pain genes, such as the vanilloid receptor (**SEQ ID NO: 2**) and the voltage-gated sodium channel (**SEQ ID NO: 5**) are known drug targets for pain therapeutics, it is postulated that the other sixteen known genes, such as DAF, newly

associated with SNL-induced gene expression by our study, are also useful as drug targets, due to the functional implications of tight co-regulation by hierarchical clustering methods applied to gene expression data. Their restricted expression to the DRG also indicates their usefulness as drug targets with limited side effects since they are very specifically
5 expressed in the pain target organ. Transcript class 1 is designated as “known and novel DRG-specific pain targets”.

Transcript classes 2 and 3 also consist of genes down-regulated by SNL, but are highly expressed in brain and spinal cord, as well as DRG. These are designated as “neuronal cellular signal transduction proteins” and “neuronal markers”, respectively, because many
10 of the representative genes within these two branches of the dendrogram are primarily expressed in neurons, *e.g.* neuronal pentraxin (SEQ ID NO: 43), SNAP-25 isoforms (SEQ ID NOS: 65 and 66), small neurofilament protein (SEQ ID NO: 75), and synaptobrevin (SEQ ID NOS: 78 and 79). It is hypothesized that the apparent down-regulation partially reflects neuronal degeneration as a result of the nerve injury, and that genes within these
15 two transcript classes may be targets for neurodegenerative disorders (for *e.g.* Parkinson’s disease, Huntington’s chorea, amyotrophic lateral sclerosis (ALS)) as well as pain.

Most of the remaining genes are up regulated by SNL, including those from transcript classes 4, 5, 6, and 7. Transcript class 4 seems to represent general cellular signal transduction proteins that could be targeted for pain therapeutics. Transcript class 5
20 includes several genes that encode neuropeptides known to play a role in pain signaling, *e.g.* galanin, vasoactive intestinal peptide, and neuropeptide Y (SEQ ID NO: 123, SEQ ID NO: 125, and SEQ ID NO: 127, respectively). Neuropeptides are signaling peptides secreted by neurons that act as ligands, which can modulate neurotransmission. Transcript class 5 is therefore designated as “known and novel neuropeptides,” and it is postulated
25 that co-regulated genes in this class code for secreted molecules involved in neurotransmission during the pain state. Targets of these secreted molecules would be good candidates for therapeutic intervention.

Transcript classes 6 and 7 include many inflammatory response genes, *e.g.* complement component 1, (SEQ ID NOS.: 140 and 141) for class 6, and several MHC markers for
30 class 7 (SEQ ID NOS.: 170, 172, 173, and 178-83). Of particular interest is the observation that complement 3 (SEQ ID NOS.: 195 and 196) is up-regulated, while DAF (SEQ ID NO: 3), a gene previously described in transcript class 1, is down-regulated. Since complement 3 has been demonstrated in the literature to be inhibited by DAF, these gene expression results support a therapeutic approach for analgesia targeting the
35 complement :3 DAF receptor-ligand interaction, either by reducing complement 3 activation directly, or by providing DAF itself or a DAF mimetic to reduce complement 3

activation. Therapeutics down-regulating the activity of the inflammatory response genes in transcript classes 6 and 7 may serve to alleviate pain, particularly since their apparent regulation is observed at a time when pain is refractory to treatment by NSAIDs.

Transcript class 8 consists primarily of known marker genes of muscle tissues, and from the organ recital data were observed to be highly expressed in aorta and quadriceps.

Because these genes as a group were apparently highly up-regulated in certain biologically unrelated tissues, (*i.e.*, L4 SNL + vehicle ipsi, L5 naïve + vehicle ipsi, L5 SNL + vehicle ipsi, L5 SNL + GPN ipsi, and L6 naïve + vehicle ipsi), it was concluded that they represent muscle tissue that was accidentally carried over in the tissue extraction. Finally, “unknown” transcript class 9 consists of a small number of functionally unrelated genes that may have met the filtering criteria for clustering analysis (threefold regulation in L4, or threefold regulation in L5 and L6), but otherwise do not seem to form a functional cluster. Due to the large number of genes examined in this study (26,000 total), it is likely that these are false positives carried over into the analysis.

Further evaluation of the genes in transcript class 1 and class 5 was performed. Sequence specific probes for real-time PCR analysis (TaqMan) were designed to confirm the apparent regulation observed in Affymetrix GeneChip hybridizations (probe sequences listed in Table 4). The results summarized in Table 4 indicate that apparent regulation was confirmed for 19 out of 22 genes in RNA samples prepared from independent sets of animals, (pool 2). The TaqMan® probes designed for validated genes in transcript classes 1 and 5 may be used as diagnostics for both up-regulation and down-regulation of specific genes during the pain state. For *in vitro* or *in vivo* rat models, these diagnostics can be used in screening assays as surrogates for the pain state to find compounds that affect expression of the genes. The probes for the human orthologs of these genes (specific examples provided in Table 5) can be used both for diagnosing individuals having pain, particularly neuropathic pain, or a related disease as described above, and also for monitoring a therapy.

Identification of homologous sequences. Affymetrix probeset nucleotide sequences were “blasted” against the NCBI RefSeq DNA database with the program ‘BLASTN’ (see the definition of “identify”, *infra*, for an explanation of the algorithm). For each probe set, the top hits against human, mouse, and rat, with a score of 300 or higher, are presented in Table 5. See Table 7 for the Genbank index identifier (GI) and actual sequence (as identified by SEQ ID NOs) corresponding to the human, mouse and rat Refseq top hit sequences. Affymetrix probeset sequences also were blasted against the NCBI RefSeq protein database with the program ‘BLASTX’. For each probe set, the top hits against human, mouse, and rat, with a score of 150 or higher, are presented in Table 6. Also see

Table 7 for the Genbank index identifier (GI) and actual sequences corresponding to the human, mouse and rat Refseq top hit **SEQ ID NOs**.

All references cited herein are incorporated by reference in their entireties.

TABLE 1: Raw data (intensity) for each probe set that passed the filtering criteria in the samples specified from the Affymetrix hybridization experiment. *Definitions:* ipsi = ipsilateral (injured) side; contra = contralateral (non-injured) side; SNL = spinal nerve ligated (injured) group; sham = sham operated (non-injured) group; GPN = gabapentin treated.

A. Samples collected in the SNL / GPN experiment.

Sequence ID	Probe set ID	L4 naive ipsi	L4 naive contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naive ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naive ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
1	AA108277_at	232	307	195	132	332	262	301	291	52	229	81	297	332	55	178	94
2	AF029310_at	319	292	279	214	290	241	300	312	91	289	114	382	507	75	346	104
3	AF039583_s_at	786	915	445	619	707	1036	649	569	160	481	176	1002	964	81	1017	174
4	rc_A1639123_at	320	420	374	159	464	376	467	445	129	403	155	449	537	75	368	101
5	AF059030_at	1242	1516	1201	688	1068	995	1669	1384	220	994	250	1974	1831	69	1581	115
6	U01227_s_at	1301	1407	1032	721	1305	1147	1458	1137	323	1041	336	1753	1499	133	1714	157
7	Y17606_at	366	354	254	267	420	294	342	425	33	335	33	273	292	13	257	13
8	M24104_g_at	1077	1316	891	746	1326	571	881	963	270	757	335	784	965	122	732	132
9	M83561_s_at	214	216	146	103	121	146	222	204	37	190	38	384	279	6	152	23
10	L31621_s_at	324	307	281	250	364	286	298	380	98	406	106	423	420	29	414	64
11	rc_AA891631_at	260	257	245	165	253	205	266	246	71	197	96	265	267	43	242	69
12	rc_AA965272_at	85	83	36	73	154	169	80	132	15	162	8	164	231	18	24	27
13	U59672_at	327	333	214	116	219	299	375	216	68	495	63	486	342	50	293	56
14	U27767_at	1275	1715	1065	1085	1264	1265	1350	1165	356	1409	329	1563	1414	179	2127	167

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
15	rc_AI227592_at	1820	1907	1367	1001	1366	1210	1560	1249	400	1592	505	1255	1994	252	1391	361
16	rc_AI058904_at	177	225	178	161	204	60	113	252	71	270	113	107	499	17	131	62
17	X13804cds_at	4056	3700	3351	2093	3253	2704	3023	3365	717	2403	776	1894	3233	450	2477	672
18	rc_AI072454_at	5757	4832	4113	2493	3225	3704	5159	4334	1394	4380	1496	5759	6848	1079	4751	1396
19	X92184_at	945	1062	750	551	858	852	1043	848	231	663	235	1220	1045	151	1124	146
20	rc_AA848868_at	260	283	299	214	336	310	102	298	94	238	62	156	73	21	137	29
21	X74978exon_at	94	135	79	82	57	109	144	140	19	97	37	127	123	12	104	9
22	rc_AA900198_at	41	157	144	36	25	29	191	83	27	19	30	25	156	23	35	82
23	rc_AA957792_s_at	354	208	398	255	293	94	74	164	37	286	219	178	283	57	97	75
24	rc_AI011627_at	203	317	332	258	89	77	286	341	74	210	193	68	438	59	215	142
25	rc_AA955302_at	52	55	41	20	69	21	24	95	11	25	7	29	133	29	10	47
26	U10354_at	150	144	121	114	110	137	190	150	22	116	47	220	139	11	194	23
27	AB017544_g_at	335	358	438	252	305	272	379	246	53	224	147	343	349	82	322	216
28	rc_AI639118_at	124	174	132	119	148	195	140	136	23	161	62	171	167	49	208	53
29	M28647_g_at	4724	5368	5530	4128	4034	4494	5238	6379	1807	3929	1987	5082	6537	2052	5647	1707
30	M74494_g_at	3849	4924	4250	3660	4876	4461	4173	4889	1477	3778	1440	4407	8240	744	3994	1035

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naive ipsi	L4 naive contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naive ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naive ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
31	rc_AA955164_at	143	96	89	61	90	109	97	107	6	110	32	265	237	32	176	21
32	rc_AA893158_at	121	94	86	57	102	82	115	125	16	61	43	71	191	20	64	83
33	rc_AI010339_r_at	1951	1633	1944	1291	587	832	1229	895	254	1145	207	596	1015	242	1447	239
34	AA933181_at	282	218	210	149	197	281	196	268	73	312	95	289	386	55	257	35
35	rc_AI639443_at	115	82	90	103	73	102	59	114	26	80	69	63	161	36	102	29
36	AF060173_at	68	93	116	81	77	103	113	138	28	91	26	109	183	35	95	24
37	J05087_at	272	249	206	146	244	230	238	232	61	190	57	225	221	55	186	67
38	S56508_s_at	174	167	209	116	196	162	156	160	44	216	44	130	133	8	162	27
39	K02248cnds_s_at	416	534	394	170	404	392	414	387	33	328	20	657	718	19	634	28
40	M25890_at	842	1127	842	551	1144	813	992	948	165	1254	174	1589	1446	16	1302	26
41	rc_AI230211_s_at	144	182	161	102	135	130	157	135	13	145	6	223	237	6	149	4
42	rc_AI227989_at	167	157	164	37	124	84	159	178	64	111	8	179	304	7	132	12
43	rc_AI072943_at	197	154	143	137	175	178	196	160	48	418	62	236	216	3	171	34
44	M76426_at	337	501	318	237	595	323	485	430	128	309	126	428	564	74	421	105
45	U52103_at	494	598	467	313	362	382	626	481	47	314	34	730	722	74	604	32
46	S80376_at	175	182	87	99	138	130	123	134	41	133	38	134	221	16	118	48
47	X70662_at	180	276	135	157	281	266	195	213	31	249	40	241	381	42	212	36
48	rc_H33003_at	424	350	279	302	272	458	344	300	28	428	42	487	317	24	420	22
49	rc_AI171087_at	137	113	109	81	96	124	109	109	24	133	100	120	171	36	79	17

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNLGN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNLGN
50	rc_AA818595_at	154	168	131	30	134	102	169	145	74	130	128	118	190	58	132	95
51	rc_AA998089_at	185	134	90	24	184	190	132	78	95	159	106	168	221	122	118	158
52	rc_AI071289_at	160	144	145	32	148	180	151	128	157	143	128	143	105	140	104	133
53	rc_AA997117_at	91	133	60	118	128	53	88	170	48	159	84	51	174	44	144	28
54	rc_AI044249_at	154	153	132	127	185	80	105	149	46	133	123	151	123	37	103	36
55	rc_AI029180_at	140	70	63	35	84	33	19	162	41	98	156	35	120	37	31	56
56	rc_AI029290_at	208	239	154	188	232	158	247	197	52	291	297	141	353	67	228	121
57	rc_AA963649_at	21	136	39	62	95	53	41	95	31	116	90	101	172	34	20	63
58	rc_AI175539_at	1343	1644	1307	1085	1361	1300	1545	1142	229	878	1462	1156	208	43	665	97
59	AF054586_at	192	228	194	59	88	70	184	149	22	58	22	147	274	15	55	15
60	AF090133_at	113	150	146	12	93	55	75	82	6	58	7	62	33	6	109	5
61	U31554_at	88	67	245	153	26	132	11	40	11	31	12	80	161	17	207	10

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNLGPV ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNLGPV
62	rc_A1232425_at	99	37	17	131	36	82	29	13	51	34	12	4	40	10	37	24
63	U94322_s_at	89	122	118	132	36	78	88	26	203	30	30	102	141	30	169	119
64	AA686952_f_at	69	136	131	84	93	102	99	182	15	223	40	215	271	28	260	9
65	AB003991_g_at	1467	1931	1572	1552	2427	1905	1060	2081	545	1783	714	1201	1782	422	1656	483
66	AB003992_s_at	367	423	359	298	598	487	231	477	99	631	138	299	434	84	325	106
68	M22253_at	306	422	335	348	409	466	272	408	58	432	77	278	194	41	351	58
68	AF009603_at	171	204	200	152	276	221	174	247	76	229	76	184	325	51	254	37
69	rc_AA997651_at	3076	3132	2956	1867	1833	1798	2982	2449	599	2855	747	1963	2436	450	2462	636
70	rc_A1180306_at	3938	2993	2372	2273	2642	2892	2895	2610	677	3116	1080	3269	2816	323	3595	778
71	rc_A1030734_at	709	794	557	485	456	477	803	784	151	593	200	473	750	224	547	269
72	rc_A1059035_f_at	302	349	262	201	259	196	356	306	93	394	50	332	499	98	606	160
73	rc_AA955960_at	824	832	785	537	680	638	828	793	211	694	206	615	908	211	811	197

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naive ipsi	L4 naive contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naive ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNLGN ipsi	L6 naive ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNLGN
74	AF091834_at	183	291	158	196	349	231	166	281	81	227	101	211	337	59	259	96
75	M25638_s_at	8199	11267	9599	8395	9933	9434	7849	10149	2101	6896	2492	6762	10645	1613	9790	1723
76	rc_AA964581_at	1806	1947	1782	1304	1051	1371	1823	1931	636	1581	974	1536	2260	556	2008	943
77	rc_AI009372_at	955	907	667	640	800	763	932	816	173	667	206	1132	713	155	818	206
78	rc_AI229196_at	4530	4420	4321	2207	3352	3025	3769	3962	852	4306	1317	3540	4017	610	3653	722
79	rc_AI229196_g_at	4929	4425	4196	3176	4133	4314	4325	4628	1167	4779	1585	4563	4281	775	5308	1131
80	rc_AA943601_at	1335	1337	1379	924	948	915	1206	1185	315	1219	361	867	1573	68	1298	218
81	rc_AI235426_at	291	254	128	115	123	153	268	242	23	182	106	259	284	25	179	67
82	D10666_at	373	508	350	407	663	462	229	401	81	434	133	306	281	31	330	38
83	rc_AI227991_at	836	843	882	731	878	801	626	694	160	855	213	550	410	35	646	81
84	rc_AI228540_at	1154	985	870	709	818	1100	1022	1092	297	946	352	1051	1017	288	1075	398
85	rc_AI172335_at	149	128	271	112	180	142	173	163	34	133	44	161	213	59	191	47

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNLGPV ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNLGPV
86	rc_AA944398_at	2574	3390	3040	2336	2032	1659	3666	2801	628	2769	976	1518	2033	468	2227	667
87	rc_AI059141_at	2387	3198	2571	2015	2526	1936	2802	3088	911	2670	915	2058	3120	697	1878	1076
88	rc_AA943348_at	385	195	325	240	217	238	416	326	94	240	123	256	378	97	300	74
89	rc_AI232700_at	278	262	246	214	271	299	242	276	87	212	120	204	407	89	264	101
90	rc_AI009884_at	53	217	83	103	53	52	161	123	30	100	42	108	190	42	171	44
91	M27726_at	438	603	666	486	699	423	457	756	163	493	183	397	802	133	492	176
92	X59737mRNA_at	1651	2352	1950	1174	1734	1748	1858	1577	342	1231	575	1401	1446	365	1678	612
93	X59737mRNA_g_at	1119	1649	1433	785	1213	997	1314	1189	356	1145	457	935	957	308	1249	357
94	rc_AI012433_at	768	740	499	364	801	714	768	680	191	589	273	416	516	154	468	231
95	rc_AI072060_s_at	8791	8577	9849	4305	5578	6327	8979	8025	2620	7881	3073	8630	11676	1802	8118	2321
96	rc_AA999186_at	129	142	158	48	161	104	97	140	179	141	148	169	225	85	126	170

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
97	rc_AI013253_i_at	619	455	650	142	439	473	392	322	348	478	214	610	331	367	498	488
98	rc_AA818790_at	415	265	241	61	209	272	332	310	207	282	167	334	285	241	355	172
99	rc_AA926175_f_at	142	213	178	40	168	142	119	67	267	104	67	155	152	188	115	154
100	rc_AI235753_at	87	98	50	160	45	104	88	83	128	81	103	147	116	94	100	89
101	rc_AI230216_at	133	206	152	157	57	137	123	34	115	168	130	189	40	201	163	118
102	rc_AI058975_at	29	18	42	95	28	10	32	45	153	27	136	37	54	189	29	260
103	U23407_at	154	167	220	336	141	121	168	197	617	131	426	141	131	628	121	576
104	M22400_at	326	316	463	779	352	381	388	457	1858	359	774	467	409	1583	362	951
105	rc_AA925256_at	73	91	97	129	181	112	62	87	266	87	173	111	77	264	109	241
106	rc_AA817875_at	84	125	120	200	171	101	72	85	389	92	319	136	137	535	125	472
107	rc_AA964755_at	167	90	180	347	320	267	105	195	715	194	321	179	117	796	82	528
108	rc_AI177099_at	88	59	74	109	92	96	74	67	250	59	162	102	94	288	86	262
109	R47042_at	242	292	324	578	324	397	283	385	1323	282	478	392	305	916	301	542
110	rc_AA997590_at	55	88	93	120	81	84	44	65	311	61	87	83	75	275	46	104

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
111	rc_AI171281_at	694	579	665	1462	828	805	504	603	2838	860	1066	678	529	1983	623	975
112	rc_AI072183_at	97	102	49	199	92	68	100	171	521	137	446	112	195	588	133	545
113	rc_AA859389_at	28	62	46	98	79	28	47	74	273	40	143	38	9	235	49	162
114	rc_AA923946_at	103	203	178	582	183	267	201	231	1374	245	1313	203	160	1162	166	1447
115	rc_AA900699_at	53	80	59	110	84	120	73	27	170	83	184	94	43	192	84	114
116	rc_AA944384_at	108	71	36	109	127	134	90	52	183	80	97	18	41	163	36	82
117	rc_AA946142_at	80	75	82	85	130	165	14	19	159	86	77	84	28	99	113	94
118	rc_AI070342_at	55	62	30	64	98	166	9	13	214	18	194	109	70	264	10	344
119	rc_AI112057_at	62	85	79	165	99	123	45	12	198	91	74	50	37	185	51	90
120	rc_AI170314_at	54	37	49	167	71	96	28	57	312	65	187	50	61	370	51	197
121	rc_AI176592_at	55	44	49	63	30	42	32	10	83	45	98	29	26	131	42	94

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNLGPV ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNLGPV
122	rc_AI172290_i_at	78	59	29	197	49	207	54	102	69	76	117	113	34	207	25	134
123	J03624_at	153	119	271	858	91	531	126	309	1851	103	1982	73	220	2980	108	3175
124	M98049_s_at	6	5	20	219	8	70	12	21	211	7	280	18	28	71	5	291
125	rc_AI101739_at	7	34	78	356	51	188	9	95	1069	11	1403	13	32	2004	8	1817
126	X02341cds_at	5	17	27	77	9	58	6	28	308	22	313	14	21	490	4	471
127	M15880_at	241	190	326	2086	216	1301	161	478	4466	151	4935	145	298	8661	99	6760
128	rc_AA851392_g_at	234	218	352	670	257	317	278	349	2008	147	1795	312	239	2684	244	1965
129	rc_AA945867_at	177	170	224	393	133	287	167	199	617	129	676	170	171	829	185	774
130	rc_AI012030_at	1426	1046	2046	3397	2327	2357	1670	2033	8500	1549	3355	1591	2100	9448	1555	5218
131	M86621_at	155	141	190	431	176	357	175	218	757	138	745	286	121	985	158	1181
132	rc_AA963716_at	37	103	25	249	55	120	166	95	290	127	352	195	102	250	125	136
133	rc_AA900468_at	28	120	103	130	30	106	135	41	135	80	128	98	12	201	108	59

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
134	rc_AA801395_s_at	40	45	35	88	17	28	36	36	122	31	65	19	61	232	52	95
135	rc_AI146189_s_at	55	47	43	85	20	40	43	58	219	37	150	55	60	253	52	185
136	rc_AI012611_at	23	20	20	149	32	23	21	22	62	41	43	29	35	36	76	19
137	rc_AA819081_at	62	87	79	124	129	96	79	85	268	70	214	81	81	510	88	200
138	rc_AA901350_at	149	150	175	348	241	265	140	200	626	245	528	236	189	596	273	426
139	rc_AI010470_at	217	213	214	391	308	305	187	262	1037	242	536	256	208	982	157	725
140	rc_AA996499_at	370	335	439	672	355	397	338	466	1626	426	1362	363	555	2088	508	1785
141	rc_AI177119_at	163	151	218	306	176	190	144	213	759	203	648	158	247	800	185	605
142	rc_AI170394_at	102	75	93	195	24	147	80	118	544	67	407	57	104	547	81	394
143	rc_AA851961_at	93	21	81	110	39	43	22	56	178	59	214	36	24	272	69	292

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNLGN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNLGN
144	rc_AI179953_at	109	81	151	249	321	388	138	92	1128	111	132	101	109	1155	146	228
145	rc_AI029920_s_at	163	212	305	782	380	185	104	247	945	439	390	235	289	940	406	219
146	AF104362_at	41	47	51	97	67	99	60	54	206	56	66	87	39	231	46	75
147	S66184_s_at	79	69	78	194	81	115	63	76	319	75	186	101	68	427	89	251
148	D84418_s_at	89	104	126	91	71	70	90	100	303	67	251	71	73	631	170	274
149	rc_AA891302_g_at	4	16	12	19	4	25	21	25	77	8	72	25	5	138	14	95
150	rc_AA866404_at	59	83	71	109	30	73	67	40	153	19	121	42	32	204	23	204
152	rc_AI230572_at	58	72	94	174	51	130	71	40	202	138	172	104	44	227	162	144
152	C06892_at	22	19	18	35	38	41	16	23	133	19	22	15	108	26	19	64
153	L32591mrna_at	164	136	248	332	128	283	197	236	798	161	800	128	189	1224	121	935
154	rc_AI070295_g_at	60	77	91	203	78	118	126	106	332	90	426	64	97	558	73	477
155	rc_AI070295_at	54	49	32	85	51	116	57	71	275	106	379	77	34	163	33	418
156	rc_AI229785_at	3	1	25	29	9	7	3	24	108	25	77	2	16	139	2	132
157	M32062_at	51	51	76	90	68	67	32	84	266	89	132	46	58	362	71	201
158	M32062_g_at	33	43	65	102	71	90	28	72	242	74	164	42	93	325	77	201
159	M57263_at	10	6	2	69	9	11	9	7	103	13	108	4	15	144	5	206
160	M63282_at	138	139	205	593	103	320	140	240	1071	147	1173	125	142	1572	89	1799

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naive ipsi	L4 naive contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naive ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naive ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
161	rc_AI045030_s_at	14	17	17	45	24	18	17	19	90	19	115	30	14	172	18	163
162	U33935_at	20	13	37	22	26	14	15	20	140	25	133	35	6	260	11	217
163	rc_AA891911_at	55	50	63	119	76	94	38	74	246	98	314	54	48	300	61	397
164	X81193_at	58	47	63	125	67	88	48	91	329	112	458	37	39	542	42	547
165	rc_AA859447_at	17	8	41	71	24	24	11	12	106	56	91	23	28	186	19	116
166	rc_AI060313_at	17	11	17	13	20	16	8	20	86	7	100	13	33	136	6	136
167	D88250_at	151	112	189	330	173	164	119	278	1358	140	456	162	287	1033	114	572
168	rc_AI228153_s_at	9	16	58	79	6	19	6	46	286	14	354	11	55	416	8	237
169	X53054_at	34	34	85	213	51	99	39	75	376	42	359	30	69	487	49	296
170	K02815_s_at	276	231	392	637	341	449	99	394	1471	375	1292	244	323	2317	232	1278
171	X53054_g_at	148	146	332	627	194	331	103	291	1773	165	1432	128	292	1960	192	1562
172	M36151cds_s_at	121	128	164	396	172	265	100	216	836	203	709	123	164	1234	125	823
173	U65217_i_at	41	25	43	115	40	67	24	55	293	52	222	30	29	274	37	253
174	rc_AI071264_at	10	9	15	9	10	9	6	11	173	10	73	11	15	368	7	149
175	rc_AI136662_at	12	17	17	15	23	20	16	16	259	11	295	10	10	366	19	236

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
176	re_AI639103_s_at	5	5	11	23	14	14	4	4	131	5	56	5	3	136	12	48
177	U18729_at	122	191	173	228	97	212	201	166	528	34	355	151	198	831	221	536
178	X13044_at	384	275	514	1164	308	681	229	610	2879	322	2359	246	506	3586	372	2754
179	X13044_g_at	326	257	553	1514	411	720	234	722	4042	212	3210	310	529	5284	345	3888
180	X14254cds_g_at	127	171	253	806	249	294	135	304	1891	123	1789	142	301	2554	159	1746
181	X14254cds_at	21	66	128	329	49	183	24	204	979	32	810	19	129	1517	19	1028
182	rc_AI171966_at	86	103	121	177	116	148	81	133	480	35	416	105	107	776	84	567
183	U31599_g_at	12	31	12	51	11	28	12	14	154	15	117	9	28	293	13	233
184	rc_AA799323_at	43	22	6	48	13	27	8	30	111	28	116	11	13	191	32	99
185	Z49761_at	69	71	55	174	32	70	61	78	229	32	189	58	96	250	61	269
186	X71127_at	76	106	159	340	47	175	91	118	778	46	748	108	107	745	108	818
187	X71127_g_at	71	92	81	205	18	118	63	82	514	43	380	81	101	661	83	688
188	X73371_at	21	13	17	37	37	35	23	21	126	34	78	13	40	163	5	113
189	rc_AI113008_at	54	9	15	19	23	19	14	6	68	13	48	12	9	132	11	66
190	rc_AI011203_at	141	184	27	184	41	58	155	98	341	177	324	139	229	518	203	276
191	E12286cds_at	146	171	59	82	104	55	113	60	252	38	183	95	41	220	80	235
192	Y07534cds_s_at	58	255	51	50	71	38	47	54	168	67	54	52	44	179	43	50
193	M58364_at	48	53	31	76	17	66	46	44	151	69	170	45	41	175	52	185
194	J02720_at	15	8	18	50	18	25	10	9	113	54	138	25	45	170	8	139

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
195	M29866_s_at	5	4	8	8	5	8	5	10	143	6	154	4	4	210	12	221
196	X52477_at	15	28	51	82	18	65	14	44	194	44	135	16	10	282	17	217
197	rc_AI639117_s_at	10	37	65	29	20	17	19	30	157	34	39	17	43	133	24	51
198	S61865_s_at	59	40	35	112	7	64	36	46	186	41	187	48	52	188	42	275
199	D28561_s_at	53	23	50	155	33	60	86	18	50	61	96	73	58	72	16	36
200	rc_AA800206_at	16	58	9	140	18	43	119	11	81	50	181	126	10	73	23	11
201	X14812_at	22	61	38	270	12	12	59	41	110	26	55	51	20	35	7	10
202	X59736mRNA_g_at	52	80	44	271	79	61	160	49	77	87	168	153	32	55	46	21
203	rc_AA891522_f_at	10	46	10	210	16	5	24	7	71	7	14	54	9	7	7	6
204	X15939_f_at	77	97	41	407	77	61	132	77	172	83	148	116	61	76	43	49
205	J00692_at	721	480	157	3861	285	175	3475	238	2233	215	6115	4504	155	560	292	292
206	rc_AI010605_at	95	38	6	485	10	10	420	9	148	7	1111	724	9	65	26	7
207	rc_AA946094_at	21	32	13	664	26	23	547	12	48	18	1058	712	17	160	21	18
208	X02412_at	504	184	254	1287	230	208	1249	212	806	215	1732	2162	175	219	213	314
209	rc_AI010583_at	166	22	17	702	40	72	764	76	408	23	1889	1113	96	86	51	91
210	K02423cds_s_at	131	73	59	571	84	66	525	61	357	120	1484	934	46	86	51	83
211	X00975_at	230	84	99	1482	107	61	1212	89	1015	106	2896	2016	79	210	122	132
212	rc_AI136540_at	215	84	37	1277	94	58	1017	90	956	110	2185	2209	37	181	95	97
213	M10140_at	346	178	92	3210	164	101	2170	145	1678	108	4812	3482	63	189	208	136

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
214	M99223_at	167	40	13	1106	65	40	1465	42	535	36	3227	1990	30	54	58	63
215	S68736_at	173	28	10	477	35	19	972	33	198	42	1280	937	14	70	29	37
216	rc_AI639532_at	144	44	8	871	41	20	888	40	590	47	1846	1765	25	148	49	54
217	L24897_s_at	87	35	29	2170	56	24	916	50	1014	71	3545	3751	23	17	76	50
218	M73701_at	56	11	7	357	11	10	298	32	227	11	795	665	10	72	9	42
219	rc_AA851223_at	34	5	4	308	3	3	261	5	154	5	403	387	6	9	3	4
220	rc_AI639096_at	13	3	10	184	9	3	210	4	96	12	447	454	6	24	3	4
221	L00088expanded_cds#2_at	180	30	2	992	45	20	1283	22	658	18	3402	2244	2	154	33	66
222	X00975_g_at	129	18	14	1224	37	6	1063	35	598	9	2403	1917	4	165	38	63
223	rc_AI059389_at	59	47	12	353	47	17	294	65	182	17	454	434	79	163	68	16
224	rc_AA963366_at	319	160	86	1263	264	81	1344	51	512	77	2582	1032	54	303	94	113
225	rc_AI044544_at	20	71	12	395	17	22	93	30	211	61	590	313	59	67	11	45
226	L10669_at	96	60	73	415	101	68	380	67	289	100	683	529	85	74	103	83
227	X12554cds_s_at	262	177	145	641	159	133	488	180	329	216	684	609	178	161	146	152
228	L10669_g_at	94	42	69	396	40	47	433	81	245	33	645	462	71	83	83	65
229	rc_AA945951_at	15	14	10	152	22	20	79	11	123	9	221	109	15	12	21	20
230	rc_AI171640_at	90	14	19	163	40	43	106	21	31	14	180	172	18	50	18	15
231	Z17319_at	62	14	6	234	29	24	149	31	94	17	290	259	19	17	13	3
232	X59864mRNA_at	9	6	7	400	13	9	171	16	323	14	1740	625	8	90	8	133
233	X59864mRNA_g_at	242	84	92	784	161	172	525	206	652	166	2058	861	205	343	81	451

TABLE 1 A (continued)

Sequence ID	Probeset ID	L4 naïve ipsi	L4 naïve contra	L4 sham ipsi	L4 SNL ipsi	L4 SNL contra	L4 GPN ipsi	L5 naïve ipsi	L5 sham ipsi	L5 SNL ipsi	L5 SNL contra	L5 SNL GPN ipsi	L6 naïve ipsi	L6 sham ipsi	L6 SNL ipsi	L6 SNL contra	L6 SNL GPN
234	rc_AA819140_at	372	275	72	1261	239	99	845	66	422	58	1160	2258	84	285	97	86
235	J04993_at	12	35	41	131	41	25	52	30	92	74	44	49	54	40	44	65
236	X07314cds_at	2	12	1	148	5	2	2	2	9	2	3	2	2	7	2	2
237	X07314cds_g_at	12	50	10	303	13	8	75	26	113	63	90	34	15	42	12	47
238	X15939_i_at	3	44	2	166	4	4	34	2	61	3	9	22	12	31	1	4
239	D86556_i_at	33	91	57	78	92	50	7	144	33	40	33	43	33	8	40	12
240	E00903cds_s_at	98	103	119	114	73	157	76	142	35	220	78	111	123	24	34	25
241	rc_AA946199_at	111	136	23	29	48	51	22	41	169	40	47	48	161	51	198	154
242	S60953_s_at	193	87	125	40	144	50	107	97	121	186	67	93	75	50	78	51
243	rc_AI012505_at	142	176	76	46	47	44	267	129	32	80	113	44	178	29	22	121
244	D84336_at	143	102	100	30	149	130	100	132	112	211	112	84	67	135	157	47
245	L19180_at	246	97	246	72	187	74	200	388	44	104	123	214	313	63	151	72
246	M90514mRNA_at	178	171	156	163	242	108	144	46	164	158	181	171	125	29	115	119
247	rc_AA866439_at	411	232	266	85	344	190	145	271	234	355	174	143	225	157	207	165
248	X59608exon_at	59	36	110	39	128	34	87	103	22	124	61	122	150	23	65	73
249	rc_AA859983_at	67	115	81	25	153	27	23	78	30	70	23	39	65	15	24	21

TABLE 1B: Normal Tissue Panel

Sequence ID	Probeset ID	sciatic nerve	spinal cord	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
1	AA108277_at	51	120	89	73	58	103	82	43	71	9	10	33
2	AF029310_at	59	37	30	71	33	13	196	9	65	18	49	46
3	AF039583_s_at	71	19	5	5	16	15	138	3	207	12	23	35
4	rc_A1639123_at	179	184	143	50	89	93	292	128	54	182	126	154
5	AF059030_at	10	53	28	5	104	43	27	16	55	37	138	53
6	U01227_s_at	153	203	186	145	299	295	156	144	85	190	311	255
7	Y17606_at	6	9	41	4	12	16	5	11	9	15	30	17
8	M24104_g_at	93	571	249	67	171	111	82	89	227	94	230	192
9	M83561_s_at	10	22	35	2	46	26	11	7	17	12	5	31
10	L31621_s_at	11	63	61	45	37	72	67	11	19	113	47	22
11	rc_AA891631_at	68	96	190	56	93	39	20	8	42	83	101	14
12	rc_AA965272_at	20	18	19	2	20	14	4	4	2	3	13	3
13	U59672_at	42	39	21	26	149	9	5	4	12	8	33	29
14	U27767_at	49	73	225	151	67	41	9	4	10	9	22	14

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
15	rc_A1227592_at	40	247	802	15	21	18	18	18	17	8	32	44
16	rc_A1058904_at	3	59	63	2	6	7	3	12	2	2	13	4
17	X13804cds_at	8	873	224	55	29	12	14	63	18	90	150	20
18	rc_A1072454_at	80	15	11	9	11	5	5	3	3	3	8	19
19	X92184_at	44	36	6	5	34	26	4	27	18	8	14	52
20	rc_AA848868_at	21	19	33	3	36	57	5	12	3	132	33	14
21	X74978exon_at	8	7	3	3	9	8	5	36	7	5	28	10
22	rc_AA900198_at	29	31	16	4	12	8	14	87	3	5	26	4
23	rc_AA957792_s_at	56	307	341	9	42	40	19	69	17	14	33	11
24	rc_A1011627_at	57	284	54	13	36	180	76	31	14	12	31	8
25	rc_AA955302_at	8	35	4	5	6	4	30	5	9	16	9	7
26	U10354_at	17	61	13	13	32	96	823	19	135	35	51	29
27	AB017544_g_at	84	396	546	565	81	194	73	401	68	54	246	68
28	rc_A1639118_at	23	98	588	80	15	70	64	75	10	33	627	7
29	M28647_g_at	817	1370	2477	3278	618	423	5700	690	557	491	3573	483
30	M74494_g_at	97	571	858	2496	495	233	6311	454	286	277	5679	385

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
31	rc_AA955164_at	5	24	26	4	4	4	156	77	2	2	328	4
32	rc_AA893158_at	61	26	72	57	30	13	56	8	16	20	101	22
33	rc_AI010339_r_at	208	1318	1758	1114	1782	70	692	394	902	42	222	70
34	AA933181_at	17	75	70	10	47	128	23	36	17	11	107	185
35	rc_AI639443_at	11	58	24	8	17	158	23	9	23	14	124	100
36	AF060173_at	12	58	253	7	21	379	12	34	16	21	19	21
37	J05087_at	54	293	556	215	45	83	40	22	112	52	32	80
38	S56508_s_at	7	398	272	2	11	36	5	3	279	3	10	234
39	K02248cds_s_at	19	710	784	15	41	464	17	11	30	40	61	43
40	M25890_at	15	1572	1961	15	50	897	17	11	20	16	47	20
41	rc_AI230211_s_at	38	73	87	4	15	58	6	4	5	6	14	8
42	rc_AI227989_at	9	108	524	2	10	63	3	3	17	2	3	25
43	rc_AI072943_at	11	132	214	21	24	30	1	6	6	14	41	24
44	M76426_at	4	238	237	24	78	170	8	6	21	23	19	79
45	U52103_at	11	33	485	11	14	516	8	5	11	16	10	35
46	S80376_at	3	6	47	3	6	21	7	2	8	6	14	7
47	X70662_at	22	118	108	29	25	24	20	12	19	36	22	77
48	rc_H33003_at	46	377	236	38	72	34	53	45	104	42	135	70
49	rc_AI171087_at	93	103	88	18	9	53	55	31	53	38	37	104

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
50	re_AA818595_at	123	271	684	32	47	442	18	20	22	128	71	127
51	re_AA998089_at	85	151	128	33	92	69	35	31	6	40	89	308
52	re_AI071289_at	143	190	194	19	29	93	28	38	16	33	31	218
53	re_AA997117_at	27	57	14	28	14	60	7	6	18	13	12	118
54	re_AI044249_at	19	103	46	19	12	11	27	8	7	17	8	129
55	re_AI029180_at	30	96	50	23	14	8	14	5	5	9	17	71
56	re_AI029290_at	94	264	234	88	181	161	88	47	11	79	77	50
57	re_AA963649_at	126	74	38	10	55	66	12	14	39	6	22	120
58	RC_AI175539												
59	AF054586_at	14	247	802	15	28	63	19	14	19	23	49	53
60	AF090133_at	8	90	236	4	15	8	6	5	8	7	20	11
61	U31554_at	12	594	514	8	16	99	10	12	15	17	17	12

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
62	rc_AI232425_at	73	38	6	2	12	13	13	12	2	2	1	53
63	U94322_s_at	18	16	13	14	24	20	15	9	19	22	28	32
64	AA686952_f_at	3	105	247	2	1	105	3	2	3	1	8	5
65	AB003991_g_at	67	1878	1943	66	140	243	51	61	80	41	37	41
66	AB003992_s_at	3	436	360	2	54	41	2	8	6	4	11	6
67	M22253_at	1	510	404	2	2	62	5	1	15	2	5	5
68	AF009603_at	14	288	294	10	35	118	86	11	15	17	23	22
69	rc_AA997651_at	24	1869	1448	6	26	385	144	16	8	16	23	887
70	rc_AI180306_at	21	1792	1372	2	1	346	89	1	1	1	3	141
71	rc_AI030734_at	56	615	644	15	49	107	7	4	5	5	13	11
72	rc_AI059035_f_at	45	311	695	3	20	122	3	2	2	3	11	4
73	rc_AA955960_at	280	1224	1791	19	19	105	47	10	14	7	13	7

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
74	AF091834_at	6	181	166	75	9	33	12	6	13	9	15	11
75	M25638_s_at	16	2794	627	6	14	126	12	5	15	14	27	10
76	rc_AA964581_at	29	659	352	4	15	163	6	4	3	4	30	3
77	rc_AI009372_at	8	332	385	1	7	66	1	1	1	1	2	1
78	rc_AI229196_at	304	4349	1802	195	102	162	116	73	109	149	173	136
79	rc_AI229196_g_at	273	5661	2221	91	38	51	48	22	64	69	61	42
80	rc_AA943601_at	20	1496	2492	6	11	22	6	4	3	129	6	7
81	rc_AI235426_at	129	147	240	13	8	44	32	6	3	17	11	13
82	D10666_at	111	1144	1166	225	14	92	2	24	38	12	48	8
83	rc_AI227991_at	257	1966	2851	99	3	72	2	2	1	2	4	5
84	rc_AI228540_at	384	316	635	86	43	145	49	22	26	8	33	2
85	rc_AI172335_at	78	105	100	81	20	17	15	7	24	3	15	21

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
86	rc_AA944398_at	2072	269	255	17	71	77	14	9	91	9	23	10
87	rc_AI059141_at	838	236	173	26	65	273	69	8	32	29	46	26
88	rc_AA943348_at	301	781	892	11	63	48	24	5	17	16	29	26
89	rc_AI232700_at	199	260	160	13	23	23	11	3	9	9	5	18
90	rc_AI009884_at	67	70	173	5	17	15	10	5	5	5	18	10
91	M27726_at	10	314	147	12	33	181	38	6	13	14	396	17
92	X59737mRNA_at	124	1766	2192	77	185	242	765	62	76	134	2104	130
93	X59737mRNA_g_at	30	1318	1459	24	71	165	551	60	85	24	1307	32
94	rc_AI012433_at	123	334	116	18	38	38	223	6	5	15	143	2
95	rc_AI072060_s_at	1545	1637	2785	865	358	256	3745	288	68	187	1867	73
-	rc_AI028909_at	74	36	68	1	3	11	63	1	2	12	51	2
96	rc_AA999186_at	90	93	222	19	79	122	40	23	36	21	35	41

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
97	rc_AI013253_i_at	56	375	443	34	257	226	54	94	31	46	119	12
98	rc_AA818790_at	290	260	487	63	96	134	109	46	34	244	10	29
99	rc_AA926175_f_at	276	219	42	6	20	163	41	5	10	26	79	27
100	rc_AI235753_at	73	70	91	11	14	44	33	10	7	26	9	57
101	rc_AI230216_at	28	188	145	53	16	75	19	11	11	23	26	21
102	rc_AI058975_at	24	19	87	3	2	2109	10	1	1	12	3	2
103	U23407_at	358	96	111	11	33	771	11	8	11	11	54	8
104	M22400_at	2503	252	126	294	445	442	421	10	74	31	49	19
105	rc_AA925256_at	239	90	81	16	236	39	66	6	42	2	25	2
106	rc_AA817875_at	301	71	59	12	40	40	37	2	40	12	29	15
107	rc_AA964755_at	401	74	26	7	49	85	105	1	13	109	65	2
108	rc_AI177099_at	577	63	52	18	104	16	212	30	62	11	86	12
109	R47042_at	696	234	79	72	174	94	105	42	65	35	44	94
110	rc_AA997590_at	347	89	42	17	106	6	16	4	12	17	17	8

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
111	rc_AI171281_at	2699	844	324	236	601	167	203	62	127	170	133	121
112	rc_AI072183_at	1215	215	355	84	67	2	2	3	16	50	3	3
113	rc_AA859389_at	24	107	63	15	10	23	14	11	2	2	2	11
114	rc_AA923946_at	88	517	333	47	92	264	88	9	17	31	4	11
115	rc_AA900699_at	122	138	355	12	67	97	11	1	21	39	23	16
116	rc_AA944384_at	84	85	107	14	58	22	28	8	40	16	63	25
117	rc_AA946142_at	19	66	61	19	69	50	49	18	26	20	49	24
118	rc_AI070342_at	38	10	4	3	58	51	19	16	5	1	19	58
119	rc_AI112057_at	8	12	42	3	14	22	84	11	2	3	3	102
120	rc_AI170314_at	198	152	77	14	61	7	39	259	4	7	17	34
121	rc_AI176592_at	87	58	20	2	19	8	11	98	2	8	39	4

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
122	rc_AI172290_i_at	149	29	184	95	60	62	11	57	6	9	9	78
123	J03624_at	11	106	39	9	15	11	7	21	13	23	26	19
124	M98049_s_at	1	5	15	2	8	3	16	6	8	4	9	7
125	rc_AI101739_at	25	26	247	14	12	8	5	3	4	7	22	23
126	X02341cds_at	5	27	57	5	36	16	3	4	46	7	53	31
127	M15880_at	135	305	498	281	281	241	107	81	28	1033	19	26
128	rc_AA817953_at	125	180	188	25	29	251	43	13	14	16	22	34
-	rc_AA851392_g_at	460	184	148	159	104	293	61	37	26	78	72	80
129	rc_AA945867_at	288	107	142	992	99	79	87	17	191	60	181	9
130	rc_AI012030_at	3483	1016	350	2760	2485	237	1085	138	1306	1304	648	557
131	M86621_at	297	112	387	281	65	251	12	12	2080	61	28	68
132	rc_AA963716_at	601	183	185	29	42	145	30	36	502	20	13	16
133	rc_AA900468_at	76	185	60	44	33	95	37	21	48	40	5	25

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
134	rc_AA801395_s_at	75	97	39	8	55	16	72	5	2	130	28	1
135	rc_AI146189_s_at	119	224	131	3	39	11	15	29	11	188	19	3
136	rc_AI012611_at	34	343	215	6	16	11	6	6	5	85	5	8
137	rc_AA819081_at	141	88	65	27	74	24	23	24	4	311	27	68
138	rc_AA901350_at	512	152	90	90	119	45	41	126	37	332	44	23
139	rc_AI010470_at	725	364	134	110	174	39	56	58	21	172	152	33
140	rc_AA996499_at	1095	937	491	199	314	116	96	281	73	1081	180	52
141	rc_AI177119_at	413	226	219	95	104	56	54	146	19	474	81	61
142	rc_AI170394_at	126	254	148	211	25	16	19	234	1	284	40	27
143	rc_AA851961_at	65	25	57	77	23	15	231	146	5	61	73	5

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
144	rc_AI179953_at	160	484	297	24	27	94	2519	426	22	27	47	45
145	rc_AI029920_s_at	130	307	124	159	371	153	1118	6	621	100	403	33
146	AF104362_at	253	40	38	57	145	51	91	7	69	92	65	98
147	S66184_s_at	563	56	38	683	428	126	67	39	72	598	17	43
148	D84418_s_at	180	172	105	147	77	1560	91	38	34	1595	159	1020
149	rc_AA891302_g_at	57	16	19	8	25	44	29	8	8	1021	22	14
150	rc_AA866404_at	85	58	112	290	55	80	114	120	107	136	90	125
151	rc_AI230572_at	220	180	79	117	138	160	132	91	140	189	92	253
152	C06892_at	32	27	25	7	90	8	13	7	5	78	66	18
153	L32591mRNA_at	193	118	58	62	66	51	485	84	163	113	146	83
154	rc_AI070295_g_at	236	68	51	20	107	51	334	70	121	107	106	59
155	rc_AI070295_at	147	29	37	1	57	36	209	35	47	58	68	26
156	rc_AI229785_at	128	2	1	1	29	4	39	7	1	2	99	3
157	M32062_at	80	104	92	64	101	69	27	55	27	223	179	184
158	M32062_g_at	108	72	45	65	84	64	51	83	9	226	174	166
159	M57263_at	6	11	9	3	4	8	6	5	7	42	11	60
160	M63282_at	323	90	86	77	241	88	88	49	213	113	149	127

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
161	rc_AI045030_s_at	46	16	14	12	20	12	15	10	118	15	17	15
162	U33935_at	13	18	14	57	34	17	9	11	14	23	33	24
163	rc_AA891911_at	84	94	84	92	155	87	207	55	132	115	211	143
164	X81193_at	82	45	69	53	575	73	71	29	461	152	100	174
165	rc_AA859447_at	230	73	15	26	39	27	5	69	13	39	39	5
166	rc_AI060313_at	90	30	24	10	9	6	3	2	6	5	32	10
167	D88250_at	903	90	23	334	207	23	107	1277	100	766	165	137
168	rc_AI228153_s_at	96	16	2	11	19	1	1	31	4	219	1	34
169	X53054_at	108	44	10	61	73	8	58	90	7	1033	7	141
170	K02815_s_at	389	300	190	555	701	174	669	406	320	6098	628	352
171	X53054_g_at	436	151	98	398	455	84	722	359	196	6981	256	334
172	M36151cds_s_at	219	103	101	430	419	56	620	266	162	4456	260	642
173	U65217_i_at	175	8	3	119	112	3	258	31	7	1560	46	201
174	rc_AI071264_at	40	13	4	3	7	3	4	8	2	174	4	3
175	rc_AI136662_at	94	15	10	4	160	3	10	90	3	2871	5	10

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
176	rc_AI639103_s_at	39	23	4	15	11	4	17	29	5	545	7	13
177	U18729_at	429	176	117	142	157	42	693	157	56	2127	64	50
178	X13044_at	945	366	128	1335	1073	4	2163	611	257	11918	1018	113
179	X13044_g_at	1773	284	72	1016	793	10	2065	477	224	15366	697	67
180	X14254cds_g_at	420	143	23	969	802	3	1885	431	43	10019	426	20
181	X14254cds_at	212	31	12	334	97	15	270	221	28	2241	31	11
182	rc_AI171966_at	266	199	137	191	603	26	564	202	147	2591	174	59
183	U31599_g_at	20	22	14	75	276	12	192	42	55	1024	243	11
184	rc_AA799323_at	77	60	27	35	49	9	13	11	64	516	16	22
185	Z49761_at	167	183	167	77	64	26	47	114	27	573	19	19
186	X71127_at	550	513	326	367	319	66	75	562	268	2660	269	45
187	X71127_g_at	208	263	139	144	127	77	13	442	25	1934	133	19
188	X73371_at	81	47	21	24	151	27	9	295	42	661	65	45
189	rc_AI113008_at	121	17	21	3	16	6	6	203	48	19	6	12
190	rc_AI011203_at	441	133	82	433	543	13	107	405	158	209	179	196
191	E12286cds_at	13	164	20	1231	94	28	104	699	17	378	35	258
192	Y07534cds_s_at	44	74	50	1177	54	53	42	2072	70	59	43	59
193	M58364_at	31	61	50	213	40	40	4	887	8	382	32	16
194	J02720_at	11	48	42	11	47	34	67	2140	12	10	900	15

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
195	M29866_s_at	25	240	10	8	9	3	8	16412	6	28	13	6
196	X52477_at	43	267	77	9	60	16	13	15215	21	75	113	15
197	rc_A1639117_s_at	142	91	42	232	137	20	107	4467	20	177	317	40
198	S61865_s_at	53	21	109	45	62	227	88	424	28	101	276	17
199	D28561_s_at	259	60	87	145	910	89	182	19	1592	73	243	166
200	rc_AA800206_at	564	41	111	70	504	15	64	17	5478	20	30	19
201	X14812_at	134	28	30	11	143	10	23	47	2342	23	49	15
202	X59736mRNA_g_at	839	9	6	14	589	17	23	23	8447	47	68	26
203	rc_AA891522_f_at	164	9	24	3	1526	10	7	3	1452	6	16	11
204	X15939_f_at	180	65	94	50	2572	80	30	9	890	13	247	77
205	J00692_at	13292	91	214	63	1225	210	36	35	34266	70	103	45
206	rc_A1010605_at	6937	5	26	2	505	17	2	1	2889	5	15	3
207	rc_AA946094_at	3404	13	13	4	1405	9	6	4	3416	4	10	4
08	X02412_at	10092	387	127	68	2244	176	90	61	16956	124	149	101
209	rc_A1010583_at	9872	31	18	16	150	25	26	7	5494	6	50	19
210	K02423cds_s_at	4161	78	58	55	315	103	76	56	14800	103	151	125
211	X00975_at	9494	65	72	69	431	114	73	48	31153	107	129	134
212	rc_A1136540_at	10153	69	78	45	295	84	64	27	27962	141	77	27
213	M10140_at	16771	52	32	49	1235	113	92	74	41283	165	166	216

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
214	M99223_at	8682	19	30	15	480	41	41	10	26765	86	68	47
215	S68736_at	3734	24	17	26	325	19	20	19	7072	27	49	40
216	rc_AI639532_at	9384	146	113	25	351	44	30	27	26788	53	77	46
217	L24897_s_at	12048	10	28	40	96	40	28	43	19873	46	91	72
218	M73701_at	3512	5	8	24	155	5	47	11	15897	23	26	12
219	rc_AA851223_at	3278	2	5	2	19	2	5	2	8890	6	5	4
220	rc_AI639096_at	2484	3	11	11	482	12	10	3	14287	5	24	23
221	L00088expanded_cds#2_at	7785	4	2	5	404	30	19	1	10234	28	8	35
222	X00975_g_at	12120	32	6	9	276	13	8	5	25680	57	10	39
223	rc_AI059389_at	1407	20	65	21	569	7	21	32	2698	27	58	37
224	rc_AA963366_at	6328	122	173	15	216	38	18	59	5177	20	163	20
225	rc_AI044544_at	1234	43	34	2	22	10	17	11	2606	17	70	7
226	L10669_at	1252	578	345	56	521	84	102	71	19005	105	104	94
227	X12554cds_s_at	2142	300	312	192	1769	160	205	251	15744	221	349	272
228	L10669_g_at	1713	631	381	74	418	18	17	15	19231	165	44	20
229	rc_AA945951_at	728	124	70	13	75	7	7	5	641	12	15	4
230	rc_AI171640_at	344	36	47	11	20	8	12	8	881	19	11	25
231	Z17319_at	1273	12	15	19	350	14	14	14	3857	16	60	730
232	X59864mRNA_at	1773	8	7	9	55	639	11	8	24555	10	30	27
233	X59864mRNA_g_at	3561	50	57	90	280	1264	19	73	16769	36	175	62

TABLE 1B (continued)

Sequence ID	Probeset ID	sciatic nerve	spinal cord naïve	brain naïve	adrenal	aorta	brain	kidney	liver	quadriceps muscle	spleen	submaxillary gland	testis
234	rc_AA819140_at	7614	183	265	880	2483	27	195	560	958	36	49	82
235	J04993_at	60	54	20	52	67	44	29	46	268	72	42	104
236	X07314cds_at	2	2	2	2	52	2	2	3	997	4	4	4
237	X07314cds_g_at	72	14	9	13	258	49	38	22	1610	36	25	84
238	X15939_i_at	4	2	2	4	60	32	3	3	380	4	10	16
239	D86556_i_at	104	10	9	66	85	85	9	42	128	13	31	98
240	E00903cds_s_at	25	38	32	27	3006	70	38	25	41	56	169	53
241	rc_AA946199_at	259	113	129	39	104	20	46	9	12	13	17	22
242	S60953_s_at	927	182	133	132	56	105	51	52	50	44	60	145
243	rc_A1012505_at	584	167	253	115	2485	12	46	448	60	6	18	624
244	D84336_at	116	268	491	2298	76	1126	73	119	450	129	888	140
245	L19180_at	143	330	318	458	95	749	64	45	98	132	660	158
246	M90514mRNA_at	14	193	177	287	250	423	303	168	189	281	280	349
247	rc_AA866439_at	179	285	362	441	445	394	274	422	265	322	425	651
248	X59608exon_at	22	51	115	411	480	276	257	94	193	167	631	659
249	rc_AA859983_at	16	37	55	63	41	22	26	27	24	132	94	36

TABLE 2: Analysis (annotation and fold change) for each probeset corresponding to known genes that passed the filtering criteria from the Affymetrix hybridization experiment. Fold change values that met the criteria (≥ 3 fold for, L4 SNL ipsi/sham ipsi AND L4 SNL ipsi/SNL contra, OR, L5 SNL ipsi/sham ipsi AND L6 SNL ipsi/sham ipsi, either up-regulated (+ value) or down-regulated (- value)) are bolded. **Definitions:** ipsi = ipsilateral (injured) side; contra = contralateral (non-injured) side; SNL = spinal nerve ligated (injured) group; sham = sham operated (non-injured) group; N = no annotation. Italicized annotations refer to annotations determined by our own analysis (otherwise they were determined from Affymetrix long descriptions).

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
1	AA108277_at	<i>heat shock 105kD</i>	1	-1.5	-2.5	-5.6	-6.1
2	AF029310_at	VR1 (vanilloid receptor, capsaicin receptor)	1	-1.3	-1.4	-3.4	-6.8
3	AF039583_s_at	<i>decay accelerating factor GPI-form precursor (DAF)</i>	1	1.4	-1.1	-3.6	-11.9
4	rc_AI639123_at	CIPP	1	-2.4	-2.9	-3.5	-7.1
5	AF059030_at	Na channel alpha subunit NaN, voltage-gated	1	-1.7	-1.6	-6.3	-26.6
6	²¹ U01227_s_at	<i>5HT3 receptor</i>	1	-1.4	-1.8	-3.5	-11.3
7	Y17606_at	<i>potassium channel, alpha subunit (Kv9.1)</i>	1	1.1	-1.6	-13.0	-23.0
8	M24104_g_at	<i>vesicle associated membrane protein (VAMP-1)</i>	1	-1.2	-1.8	-3.6	-7.9
9	M83561_s_at	glutamate receptor subunit 5-2 (GluR5-2), kainate subtype	1	-1.4	-1.2	-5.6	-47.2
10	L31621_s_at	<i>nicotinic acetylcholine receptor alpha 3 subunit</i>	1	-1.1	-1.5	-3.9	-14.5
11	rc_AA891631_at	<i>KIAA1163 protein</i>	1	-1.5	-1.5	-3.5	-6.3
12	³¹ rc_AA965272_at	<i>calsenilin, presenilin binding protein, EF hand transcription factor; Kv channel interacting protein 1, DREAM</i>	1	2.0	-2.1	-8.8	-12.7
13	²¹ U59672_at	<i>5HT3 receptor</i>	1	-1.9	-1.9	-3.2	-6.8
14	U27767_at	<i>RGP4</i>	1	1.0	-1.2	-3.3	-7.9
15	³¹ rc_AI227592_at	<i>calsenilin, presenilin binding protein, EF hand transcription factor,</i>	1	-1.4	-1.4	-3.1	-7.9
16	rc_AI058904_at	hypothetical protein FLJ14735	1	-1.1	-1.3	-3.5	-28.7
17	X13804cds_at	<i>heavy neurofilament polypeptide NF-H C-terminus</i>	1	-1.6	-1.6	-4.7	-7.2
18	rc_AI072454_at	advillin	1	-1.6	-1.3	-3.1	-6.3

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
19	X92184_at	voltage-gated sodium channel (SNS/PN3/Nav1.8))	1	-1.4	-1.6	-3.7	-6.9
20	rc_AA848868_at	heparanase	1	-1.4	-1.6	-3.2	-3.6
21	X74978exon_at	<i>prostatic acid phosphatase</i> ,	1	1.0	1.4	-7.3	-10.3
22	rc_AA900198_at	poliovirus receptor-related 2 (herpesvirus entry mediator B)	1	-3.9	1.5	-3.0	-6.7
23	rc_AA957792_s_at	low density lipoprotein receptor-related protein 3	1	-1.6	-1.1	-4.4	-4.9
24	rc_AI011627_at	hypothetical protein MGC2628	1	-1.3	2.9	-4.6	-7.5
25	rc_AA955302_at	myosin IE	2	-2.0	-3.4	-9.0	-4.6
26	U10354_at	<i>kidney extracellular calcium-sensing receptor</i>	2	-1.1	1.0	-6.9	-12.9
27	AB017544_g_at	<i>peroxisomal membrane anchor protein</i>	2	-1.7	-1.2	-4.6	-4.3
28	rc_AI639118_at	<i>calcium-dependent actin-binding protein</i>	2	-1.1	-1.2	-5.8	-3.4
29	^{2,26} M28647_g_at	<i>Na,K-ATPase alpha-1 subunit</i>	2	-1.3	1.0	-3.5	-3.2
30	²⁶ M74494_g_at	<i>sodium/potassium ATPase alpha-1 subunit truncated isoform</i>	2	-1.2	-1.3	-3.3	-11.1
31	rc_AA955164_at	<i>p53 apoptosis-associated target</i>	2	-1.4	-1.5	-17.0	-7.3
32	rc_AA893158_at	<i>Anxa 7 (Annexin A7)</i>	2	-1.5	-1.8	-7.8	-9.8
33	rc_AI010339_r_at	<i>similar to a putative protein with homology to a sequence between V2 and V3P segments within T cell gamma receptor region</i>	2	-1.5	2.2	-3.5	-4.2
34	AA933181_at	<i>immunoglobulin superfamily, member 4</i>	2	-1.4	-1.3	-3.7	-7.0
35	rc_AI639443_at	<i>roundabout, axon guidance receptor, homolog 2 (Drosophila)</i>	2	1.2	1.4	-4.4	-4.5
36	AF060173_at	<i>SV2 related protein (SVOP)</i>	2	-1.4	1.1	-4.9	-5.2
37	J05087_at	<i>calmodulin-sensitive plasma membrane Ca²⁺-transporting ATPase (PMCA3)</i>	2	-1.4	-1.7	-3.8	-4.0
38	S56508_s_at	<i>phosphatidylinositol 4-kinase</i>	2	-1.8	-1.7	-3.6	-16.5
39	²² K02248cds_s_at	Somatostatin	2	-2.3	-2.4	-11.7	-38.8
40	²² M25890_at	somatostatin	2	-1.5	-2.1	-5.8	-93.3
41	rc_AI230211_s_at	<i>Kcnd3, potassium voltage gated channel</i>	2	-1.6	-1.3	-10.8	-39.5
42	rc_AI227989_at	similar to T19A6.3.p	2	-4.4	-3.3	-2.8	-45.4

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/ SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
43	rc_AI072943_at	<i>neuronal pentraxin I precursor</i>	2	-1.0	-1.3	-3.3	-63.4
44	M76426_at	<i>dipeptidyl aminopeptidase-related protein (dpp6)</i>	2	-1.3	-2.5	-3.4	-7.6
45	U52103_at	<i>rCRMP-3</i>	2	-1.5	-1.2	-10.2	-9.8
46	S80376_at	<i>G alpha olf=GTP-binding protein Golf alpha subunit</i>	2	1.1	-1.4	-3.3	-14.3
47	X70662_at	<i>K+ channel protein</i>	2	1.2	-1.8	-6.9	-9.1
48	rc_H33003_at	<i>beta-interferon-induced protein</i>	2	1.1	1.1	-10.6	-13.4
49	rc_AI171087_at	<i>hypothetical protein FLJ12565</i>	2	-1.3	-1.2	-4.5	-4.7
50	rc_AA818595_at	<i>B-cell CLL/lymphoma 11A</i>	2	-4.4	-4.5	-2.0	-3.3
51	rc_AA998089_at	<i>hypothetical protein MGC29761</i>	2	-3.7	-7.6	1.2	-1.8
52	rc_AI071289_at	<i>KIAA0779 protein</i>	2	-4.5	-4.6	1.2	1.3
53	rc_AA997117_at	<i>KIAA0144 gene product</i>	2	2.0	-1.1	-3.5	-4.0
54	rc_AI044249_at	<i>LOC148894</i>	2	-1.0	-1.5	-3.2	-3.4
55	rc_AI029180_at	<i>tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide</i>	2	-1.8	-2.4	-3.9	-3.2
56	rc_AI029290_at	<i>adenosine monophosphate deaminase 2 (isoform L)</i>	2	1.2	-1.2	-3.8	-5.3
57	rc_AA963649_at	<i>AD-012 protein</i>	2	1.6	-1.5	-3.0	-5.1
58	rc_AI175539_at	<i>parvalbumin</i>	2	-1.2	-1.3	-5.0	-4.8
59	AF054586_at	<i>brain finger protein (BFP)</i>	2	-3.3	-1.5	-6.7	-18.0
60	AF090133_at	<i>lin-7-A mRNA</i>	2	-12.1	-7.8	-13.7	-5.5
61	U31554_at	<i>limbic system-associated membrane protein</i>	2	-1.6	5.9	-3.6	-9.8
62	rc_AI232425_at	<i>ZC250.2.p</i>	2	7.5	3.6	4.1	-4.2
63	U94322_s_at	<i>galanin receptor type 2</i>	2	1.1	3.7	7.8	-4.7
64	AA686952_f_at	brain expressed, X-linked 1	2	-1.6	-1.1	-12.5	-9.8
65	¹ AB003991_g_at	<i>SNAP-25A</i>	3	-1.0	-1.6	-3.8	-4.2
66	¹ AB003992_s_at	<i>SNAP-25B</i>	3	-1.2	-2.0	-4.8	-5.1
67	M22253_at	<i>sodium channel I</i>	3	1.0	-1.2	-7.0	-4.7
68	AF009603_at	<i>SH3p4(SH3-domain GRB2-like 2</i>	3	-1.3	-1.8	-3.2	-6.3
69	²³ rc_AA997651_at	<i>KIAA1796 protein</i>	3	-1.6	1.0	-4.1	-5.4
70	²³ rc_AI180306_at	<i>KIAA1796 protein</i>	3	-1.0	-1.2	-3.9	-8.7

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/ SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
71	rc_AI030734_at	cask-interacting protein 1	3	-1.1	1.1	-5.2	-3.4
72	rc_AI059035_f_at	hypothetical protein FLJ14594	3	-1.3	-1.3	-3.3	-5.1
73	rc_AA955960_at	SH3GL2	3	-1.5	-1.3	-3.8	-4.3
74	AF091834_at	<i>N-ethylmaleimide sensitive factor NSF</i>	3	1.2	-1.8	-3.5	-5.7
75	M25638_s_at	<i>smallest neurofilament protein (NF-L)</i>	3	-1.1	-1.2	-4.8	-6.6
76	rc_AA964581_at	KIAA0363 protein	3	-1.4	1.2	-3.0	-4.1
77	rc_AI009372_at	fibroblast growth factor 12	3	-1.0	-1.3	-4.7	-4.6
78	⁵ rc_AI229196_at	vesicle-associated membrane protein 1 (synaptobrevin 1)	3	-2.0	-1.5	-4.7	-6.6
79	⁵ rc_AI229196_g_at	vesicle-associated membrane protein 1 (synaptobrevin 1)	3	-1.3	-1.3	-4.0	-5.5
80	rc_AA943601_at	protein kinase C and casein kinase substrate in neurons 1	3	-1.5	-1.0	-3.8	-23.1
81	rc_AI235426_at	isoprenylcysteine carboxyl methyltransferase	3	-1.1	-1.1	-10.3	-11.6
82	D10666_at	<i>neural visinin-like protein (NVP)</i>	3	1.2	-1.6	-5.0	-9.2
83	rc_AI227991_at	visinin-like 1	3	-1.2	-1.2	-4.3	-11.6
84	rc_AI228540_at	like-glycosyltransferase	3	-1.2	-1.2	-3.7	-3.5
85	rc_AI172335_at	<i>transcription factor 15 (basic helix-loop-helix)</i>	3	-2.4	-1.6	-4.8	-3.6
86	rc_AA944398_at	<i>fibulin-2 precursor</i>	3	-1.3	1.1	-4.5	-4.3
87	rc_AI059141_at	<i>DOK4, downstream of tyrosine kinase 4</i>	3	-1.3	-1.3	-3.4	-4.5
88	rc_AA943348_at	<i>LFG</i>	3	-1.4	1.1	-3.5	-3.9
89	rc_AI232700_at	<i>LOC157919</i>	3	-1.2	-1.3	-3.2	-4.6
90	rc_AI009884_at	<i>nexin 19</i>	3	1.2	1.9	-4.1	-4.6
91	M27726_at	<i>phosphorylase (B-GPI)</i>	3	-1.4	-1.4	-4.6	-6.0
92	¹⁶ X59737mRNA_at	<i>ubiquitous mitochondrial creatine kinase</i>	3	-1.7	-1.5	-4.6	-4.0
93	¹⁶ X59737mRNA_g_at	<i>ubiquitous mitochondrial creatine kinase</i>	3	-1.8	-1.5	-3.3	-3.1
94	rc_AI012433_at	<i>LOC197287</i>	3	-1.4	-2.2	-3.6	-3.3
95	² rc_AI072060_s_at	<i>ATPase, Na⁺/K⁺ transporting, alpha 1 polypeptide</i>	3	-2.3	-1.3	-3.1	-6.5
96	rc_AA999186_at	hypothetical protein MGC14480	4	-3.3	-3.4	1.3	-2.7
97	rc_AI013253_i_at	<i>carcinoembryonic antigen-related protein</i>	4	-4.6	-3.1	1.1	1.1

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
98	rc_AA818790_at	<i>novel SH2-containing protein 3; Cas/HEF1-associated signal transducer; SH2-containing Eph receptor-binding protein 1</i>	4	-3.9	-3.4	-1.5	-1.2
99	rc_AA926175_f_at	<i>midkine (neurite growth-promoting factor 2)</i>	4	-4.4	-4.2	4.0	1.2
100	rc_AI235753_at	<i>mitogen-activated protein kinase 3, ERK1, p44MAPK</i>	4	3.2	3.5	1.5	-1.2
101	rc_AI230216_at	<i>dihydroorotate oxidase</i>	4	1.0	2.8	3.4	5.1
102	rc_AI058975_at	<i>SRY (sex determining region Y)-box 11</i>	4	2.3	3.4	3.4	3.5
103	U23407_at	<i>cellular retinoic acid-binding protein II (CRABP II)</i>	4	1.5	2.4	3.1	4.8
104	M22400_at	<i>developmentally regulated intestinal protein (OCI-5)</i>	4	1.7	2.2	4.1	3.9
105	rc_AA925256_at	<i>hypothetical protein FLJ14054</i>	4	1.3	-1.4	3.1	3.4
106	rc_AA817875_at	<i>sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C</i>	4	1.7	1.2	4.6	3.9
107	rc_AA964755_at	<i>thrombospondin 1</i>	4	1.9	1.1	3.7	6.8
108	rc_AI177099_at	<i>protease, serine, 23; serine protease</i>	4	1.5	1.2	3.7	3.1
109	R47042_at	<i>decorin</i>	4	1.8	1.8	3.4	3.0
110	⁴ rc_AA997590_at	<i>osteoglycin (osteoinductive factor, mimecan)</i>	4	1.3	1.5	4.8	3.7
111	⁴ rc_AI171281_at	<i>Fibromodulin precursor</i>	4	2.2	1.8	4.7	3.7
112	rc_AI072183_at	<i>v-fos FBJ murine osteosarcoma viral oncogene homolog</i>	4	4.0	2.2	3.0	3.0
113	rc_AA859389_at	<i>HT018 protein</i>	4	2.1	1.2	3.7	25.0
114	rc_AA923946_at	<i>Having Semaphorin domain protein (Dan)</i>	4	3.3	3.2	6.0	7.2
115	rc_AA900699_at	<i>CUG triplet repeat, RNA binding protein 2</i>	4	1.9	1.3	6.3	4.5
116	rc_AA944384_at	<i>eyes absent homolog 1 (Drosophila)</i>	4	3.0	-1.2	3.5	3.9
117	rc_AA946142_at	<i>bone morphogenetic protein 5</i>	4	1.0	-1.5	8.2	3.5
117	rc_AI070342_at	<i>phosphodiesterase 6B, cGMP-specific, rod, beta</i>	4	2.1	-1.5	16.1	3.8
119	rc_AI112057_at	<i>solute carrier family 26, member 7</i>	4	2.1	1.7	17.2	5.1
120	rc_AI170314_at	<i>Cfh Complement component factor h (unigene)</i>	4	3.4	2.4	5.4	6.1
121	rc_AI176592_at	<i>interleukin 1 receptor, type I</i>	5	1.3	2.1	8.2	5.0

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
122	rc_AI172290_i_at	<i>wingless-type MMTV integration site family, member 4</i>	5	6.7	4.0	-1.5	6.1
123	J03624_at	galanin	5	3.2	9.5	6.0	13.6
124	M98049_s_at	pancreatitis-associated protein (pap)	5	11.1	27.8	10.0	2.5
125	rc_AI101739_at	vasoactive intestinal peptide	5	4.6	6.9	11.3	63.4
126	X02341cds_at	vasoactive intestinal polypeptide (VIP) precursor	5	2.9	8.3	10.9	23.1
127	M15880_at	neuropeptide Y	5	6.4	9.7	9.3	29.0
128	rc_AA851392_g_at	<i>kinesin-like 4</i>	5	1.9	2.6	5.8	11.2
129	rc_AA945867_at	<i>Jun, v-jun sarcoma virus 17 oncogene homolog</i>	5	1.8	2.9	3.1	4.8
130	rc_AI012030_at	<i>matrix Gla protein</i>	6	1.7	1.5	4.2	4.5
131	M86621_at	dihydropyridine-sensitive L-type calcium channel alpha-2 subunit (CCHL2A)(alpha2delta)	6	2.3	2.4	3.5	8.1
132	rc_AA963716_at	Weak similar to mouse myomesin 2	6	9.9	4.5	3.1	2.5
133	rc_AA900468_at	<i>small nuclear ribonucleoprotein polypeptide B"</i>	6	1.3	4.4	3.3	17.2
134	rc_AA801395_s_at	TNF-induced protein (unigene)	6	2.5	5.4	3.4	3.8
135	rc_AI146189_s_at	<i>annexin A3</i>	6	2.0	4.4	3.8	4.2
136	rc_AI012611_at	<i>RAS guanyl releasing protein 2 (calcium and DAG-regulated)</i>	6	7.7	4.7	2.8	1.0
137	rc_AA819081_at	<i>glioma pathogenesis-related protein</i>	6	1.6	-1.0	3.1	6.3
138	rc_AA901350_at	<i>lysophospholipase I</i>	6	2.0	1.4	3.1	3.1
139	rc_AI010470_at	<i>ceruloplasmin (ferroxidase)</i>	6	1.8	1.3	4.0	4.7
140	rc_AA996499_at	<i>complement component 1, q subcomponent, alpha polypeptide</i>	6	1.5	1.9	3.5	3.8
141	rc_AI177119_at	<i>complement component 1, q subcomponent, gamma polypeptide</i>	6	1.4	1.7	3.6	3.2
142	rc_AI170394_at	MPS1 gene (RR)	6	2.1	8.2	4.6	5.2
143	rc_AA851961_at	<i>hypothetical protein from clone 643</i>	6	1.4	2.9	3.2	11.2
144	rc_AI179953_at	<i>gap junction protein Cx26</i>	6	1.6	-1.3	12.2	10.6
145	rc_AI029920_s_at	insulin-like growth factor binding protein 5	6	2.6	2.1	3.8	3.3
146	AF104362_at	<i>osteoaderin</i>	6	1.9	1.5	3.8	5.9
147	S66184_s_at	lysyl oxidase	6	2.5	2.4	4.2	6.3

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/ SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
148	D84418_s_at	<i>chromosomal protein HMG2,</i>	6	-1.4	1.3	3.0	8.7
149	rc_AA891302_g_at	<i>MAP4K1</i>	6	1.6	5.1	3.0	26.4
150	rc_AA866404_at	<i>estradiol 17 beta-dehydrogenase 8</i>	6	1.5	3.6	3.8	6.3
151	rc_AI230572_at	<i>nuclease sensitive element binding protein 1</i>	6	1.9	3.4	5.1	5.2
152	C06892_at	<i>Msln, mesothelin</i>	6	1.9	-1.1	5.8	-4.1
153	²⁷ , ²⁸ L32591mRNA_at	<i>growth arrest and DNA-damage-inducible, alpha</i>	6	1.3	2.6	3.4	6.5
154	²⁷ , ³⁰ rc_AI070295_g_at	<i>GADD45A, growth arrest and DNA-damage-inducible, alpha</i>	6	2.2	2.6	3.1	5.7
155	²⁸ , ³⁰ rc_AI070295_at	<i>growth arrest and DNA-damage-inducible, alpha</i>	6	2.7	1.7	3.9	4.8
156	rc_AI229785_at	<i>similar to Keratin, type I cytoskeletal 19 (Cytokeratin 19) (K19) (CK 19)</i>	6	1.2	3.1	4.6	8.6
157	³ M32062_at	<i>Fc-gamma receptor</i>	6	1.2	1.3	3.2	6.3
158	³ M32062_g_at	<i>Fc-gamma receptor</i>	6	1.6	1.4	3.3	3.5
159	M57263_at	<i>protein-glutamine gamma-glutamyltransferase</i>	6	31.5	7.5	15.5	9.5
160	M63282_at	<i>leucine zipper protein</i>	6	2.9	5.8	4.5	11.1
161	rc_AI045030_s_at	<i>CCAAT/enhancer binding protein (C/EBP), delta</i>	6	2.7	1.9	4.7	12.7
162	U33935_at	<i>corticotensin</i>	6	-1.7	-1.2	7.1	47.2
163	rc_AA891911_at	<i>small proline-rich protein 1B (cornifin)</i>	6	1.9	1.6	3.3	6.2
164	X81193_at	<i>muscle LIM protein</i>	6	2.0	1.9	3.6	13.8
165	rc_AA859447_at	<i>chromosome 6 open reading frame 37</i>	6	1.7	3.0	8.8	6.6
166	rc_AI060313_at	<i>glyceraldehyde-3-phosphate dehydrogenase type 2</i>	6	-1.3	-1.5	4.3	4.1
167	D88250_at	<i>serine protease</i>	6	1.7	1.9	4.9	3.6
168	rc_AI228153_s_at	<i>similar to HLA class II histocompatibility antigen</i>	7	1.4	12.7	6.2	7.5
169	¹⁵ X53054_at	<i>RT1.D beta chain</i>	7	2.5	4.2	5.0	7.1
170	K02815_s_at	<i>MHC RT1-B region class II</i>	7	1.6	1.9	3.7	7.2
171	¹⁵ X53054_g_at	<i>RT1.D beta chain</i>	7	1.9	3.2	6.1	6.7

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
172	M36151cds_s_at	MHC class II A-beta RT1.B-b-beta	7	2.4	2.3	3.9	7.5
173	U65217_i_at	MHC class II antigen RT1.B beta chain	7	2.7	2.9	5.4	9.4
174	rc_AI071264_at	<i>BCL2-related protein A1</i>	7	-1.7	-1.2	16.2	24.4
175	rc_AI136662_at	<i>secretory leukocyte protease inhibitor</i>	7	-1.2	-1.6	16.3	37.0
176	rc_AI639103_s_at	<i>actin, alpha 1, skeletal muscle</i>	7	2.0	1.6	37.3	41.2
177	U18729_at	<i>cytochrome b558 alpha-subunit</i>	7	1.3	2.3	3.2	4.2
178	^{8,9,10} X13044_at	MHC-associated invariant chain gamma	7	2.3	3.8	4.7	7.1
179	^{8,11,12} X13044_g_at	MHC-associated invariant chain gamma	7	2.7	3.7	5.6	10.0
180	^{9,11,13} X14254cds_g_at	MHC class II-associated invariant chain	7	3.2	3.2	6.2	8.5
181	^{10,12,13} X14254cds_at	MHC class II-associated invariant chain	7	2.6	6.7	4.8	11.8
182	²⁹ rc_AI171966_at	<i>major histocompatibility complex, class II, DM beta</i>	7	1.5	1.5	3.6	7.3
183	²⁹ U31599_g_at	<i>MHC class II-like beta chain (RT1.DMb)</i>	7	4.4	4.8	11.0	10.3
184	rc_AA799323_at	<i>Pleckstrin (Platelet p47 protein)</i>	7	7.9	3.7	3.7	14.4
185	Z49761_at	<i>RT1.Ma</i>	7	3.2	5.4	2.9	2.6
186	¹⁸ X71127_at	complement protein C1q beta chain	7	2.1	7.3	6.6	7.0
187	¹⁸ X71127_g_at	complement protein C1q beta chain	7	2.5	11.4	6.3	6.5
188	X73371_at	<i>Fc gamma receptor</i>	7	2.2	1.0	6.0	4.1
189	rc_AI113008_at	<i>proteoglycan 4</i>	7	1.3	-1.2	11.4	15.3
190	rc_AI011203_at	<i>hypothetical protein FLJ22662</i>	7	6.9	4.5	3.5	2.3
191	E12286cds_at	<i>GM2 activator protein</i>	7	1.4	-1.3	4.2	5.3
192	Y07534cds_s_at	<i>mitochondrial vitamin D(3) 25-hydroxylase</i>	7	-1.0	-1.4	3.1	4.1
193	M58364_at	GTP cyclohydrolase I	7	2.4	4.5	3.4	4.3
194	J02720_at	<i>liver arginase</i>	7	2.8	2.7	13.0	3.8
195	¹⁹ M29866_s_at	<i>complement component C3</i>	7	-1.1	1.4	14.5	52.5
196	¹⁹ X52477_at	<i>pre-pro-complement C3</i>	7	1.6	4.6	4.4	27.3

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/ SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
197	rc_AI639117_s_at	<i>complement factor B preproprotein</i>	7	-2.2	1.5	5.2	3.1
198	S61865_s_at	<i>syndecan=heparan sulfate proteoglycan core protein</i>	8	3.2	15.1	4.0	3.6
199	D28561_s_at	<i>glucose transporter, GLUT4</i>	8	3.1	4.6	2.8	1.2
200	rc_AA800206_at	actinin, alpha 2	8	15.3	7.6	7.7	7.7
201	X14812_at	ventricular myosin light chain1	8	7.1	22.7	2.7	1.8
202	X59736mRNA_g_at	sarcomeric mitochondrial creatine kinase	8	6.1	3.4	1.6	1.7
203	^{24, 25} rc_AA891522_f_at	myosin, heavy polypeptide 7, cardiac muscle, beta	8	20.6	13.1	10.9	-1.3
204	^{14, 24} X15939_f_at	beta cardiac myosin heavy chain	8	9.8	5.3	2.2	1.2
205	J00692_at	skeletal muscle alpha-actin gene	8	24.7	13.5	9.4	3.6
206	rc_AI010605_at	titin	8	78.2	49.0	16.9	7.0
207	rc_AA946094_at	Mb myoglobin (unigene)	8	49.6	25.9	4.0	9.6
208	X02412_at	striated muscle alpha-tropomyosin	8	5.1	5.6	3.8	1.3
209	rc_AI010583_at	actinin, alpha 3	8	41.1	17.6	5.4	-1.1
210	K02423cds_s_at	fast myosin alkali light chain exon 1	8	9.7	6.8	5.9	1.9
211	⁶ X00975_at	muscle myosin light chain 2 (Ruiru)	8	15.0	13.8	11.3	2.7
212	rc_AI136540_at	tropoin T3, skeletal, fast	8	34.2	13.6	10.6	4.9
213	M10140_at	skeletal muscle creatine kinase	8	35.1	19.6	11.6	3.0
214	M99223_at	calcium transporting ATPase	8	84.5	16.9	12.7	1.8
215	S68736_at	myosin heavy chain	8	47.7	13.6	6.0	5.0
216	rc_AI639532_at	human troponin fast, slow (Dan)	8	107.6	21.3	14.8	5.9
217	L24897_s_at	myosin heavy chain	8	74.8	39.0	20.5	-1.4
218	M73701_at	tropoin I	8	55.0	31.4	7.0	7.4
219	rc_AA851223_at	enolase 3, (beta, muscle)	8	70.1	96.4	31.3	1.5
220	rc_AI639096_at	myosin binding protein C, fast type	8	19.0	21.4	22.4	4.4
221	L00088expanded_cds#2_at	fast myosin alkali light chain	8	551.3	21.9	30.6	96.3
222	⁶ X00975_g_at	muscle myosin light chain 2	8	85.6	33.4	16.9	44.5
223	rc_AI059389_at	similar to Adenysuccinate synthetase, muscle isozyme (IMP--Aspartate ligase) (ADSS) (AMPSASE)	8	28.7	7.5	2.8	2.0

TABLE 2 (continued)

Sequence ID	Probeset ID	Short annotation	Transcript class	Fold change L4 SNL ipsi/sham ipsi	Fold change L4 SNL ipsi/ SNL contra	Fold change L5 SNL ipsi/sham ipsi	Fold change L6 SNL ipsi/sham ipsi
224	rc_AA963366_at	Tropomyosin beta chain (unigene)	8	14.7	4.8	10.0	5.6
225	rc_AI044544_at	nebulin-related anchoring protein (unigene)	8	34.3	23.9	7.2	1.1
226	²⁰ L10669_at	glycogen phosphorylase muscle isozyme	8	5.7	4.1	4.3	-1.1
227	X12554cds_s_at	heart cytochrome c oxidase subunit Via	8	4.4	4.0	1.8	-1.1
228	²⁰ L10669_g_at	glycogen phosphorylase muscle isozyme	8	5.7	9.8	3.0	1.2
229	rc_AA945951_at	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 2	8	16.0	7.0	11.7	-1.3
230	rc_AI171640_at	<i>Cacng1</i> , calcium channel, voltage-dependent, gamma submit 1	8	8.5	4.1	1.5	2.8
231	Z17319_at	phosphoglyceromutase	8	37.1	8.0	3.0	-1.1
232	¹⁷ X59864mRNA_at	ASM 15 (Airway Smooth Muscle?)	8	56.3	30.5	20.3	11.0
233	¹⁷ X59864mRNA_g_at	ASM15	8	8.6	4.9	3.2	1.7
234	rc_AA819140_at	Ca3 Carbonic anhydrase 3 (unigene)	8	17.4	5.3	6.4	3.4
235	J04993_at	slow fiber troponin I	8	3.2	3.2	3.1	-1.4
236	⁷ X07314cds_at	heart myosin light chain 2 (MLC2)	8	113.8	32.9	5.5	3.4
237	⁷ X07314cds_g_at	heart myosin light chain 2 (MLC2)	8	30.6	23.7	4.3	2.8
238	^{14, 25} X15939_i_at	beta cardiac myosin heavy chain	8	72.0	43.6	40.6	2.6
239	D86556_i_at	Protein Kinase	9	1.4	-1.2	-4.4	-3.9
240	E00903cds_s_at	rat atrial natriuretic factor(ANF)	9	-1.0	1.6	-4.1	-5.1
241	rc_AA946199_at	snail homolog 1 (<i>Drosophila</i>)	9	1.3	-1.7	4.1	-3.2
242	S60953_s_at	<i>trkC(ki39)</i> =receptor tyrosine kinase	9	-3.1	-3.6	1.2	-1.5
243	rc_AI012505_at	diacylglycerol O-acyltransferase homolog 2 (mouse)	9	-1.7	-1.0	-4.1	-6.2
244	D84336_at	ZOG	9	-3.3	-4.9	-1.2	2.0
245	L19180_at	receptor-linked protein tyrosine phosphatase (PTP-P1)	9	-3.4	-2.6	-8.8	-4.9
246	M90514mRNA_at	<i>Drosophila pecanex</i> locus	9	1.0	-1.5	3.6	-4.4
247	rc_AA866439_at	Hypothetical protien XP_086647 (104 aa)	9	-3.1	-4.0	-1.2	-1.4
248	X59608exon_at	RNA PolIII-c	9	-2.8	-3.3	-4.7	-6.6
249	rc_AA859983_at	cyclin M4	9	-3.3	-6.1	-2.6	-4.4

Footnotes:

- ¹ By bioinformatic analysis these two probesets represent probable splice variants of the same gene.
- ² By bioinformatic analysis these two probesets represent the same gene.
- ³ By bioinformatic analysis these two probesets represent the same gene.
- ⁴ By bioinformatic analysis these two probesets represent the same gene.
- ⁵ By bioinformatic analysis these two probesets represent the same gene.
- ⁶ By bioinformatic analysis these two probesets represent the same gene.
- ⁷ By bioinformatic analysis these two probesets represent the same gene.
- ⁸ By bioinformatic analysis these two probesets represent the same gene.
- ⁹ By bioinformatic analysis these two probesets represent splice variants of the same gene.
- ¹⁰ By bioinformatic analysis these two probesets represent splice variants of the same gene.
- ¹¹ By bioinformatic analysis these two probesets represent splice variants of the same gene.
- ¹² By bioinformatic analysis these two probesets represent splice variants of the same gene.
- ¹³ By bioinformatic analysis these two probesets represent the same gene.
- ¹⁴ By bioinformatic analysis these two probesets represent the same gene.
- ¹⁵ By bioinformatic analysis these two probesets represent the same gene.
- ¹⁶ By bioinformatic analysis these two probesets represent the same gene.
- ¹⁷ By bioinformatic analysis these two probesets represent the same gene.
- ¹⁸ By bioinformatic analysis these two probesets represent the same gene.
- ¹⁹ By bioinformatic analysis these two probesets represent the same gene.
- ²⁰ By bioinformatic analysis these two probesets represent the same gene.
- ²¹ By bioinformatic analysis these two probesets represent the same gene.
- ²² By bioinformatic analysis these two probesets represent the same gene.
- ²³ By bioinformatic analysis these two probesets represent the same gene.
- ²⁴ By bioinformatic analysis these two probesets represent the same gene.
- ²⁵ By bioinformatic analysis these two probesets represent the same gene.
- ²⁶ By bioinformatic analysis these two probesets represent the same gene.
- ²⁷ By bioinformatic analysis these two probesets represent the same gene.
- ²⁸ By bioinformatic analysis these two probesets represent the same gene.
- ²⁹ By bioinformatic analysis these two probesets represent the same gene.
- ³⁰ By bioinformatic analysis these two probesets represent the same gene.
- ³¹ By bioinformatic analysis these two probesets represent the same gene.
- ³² By bioinformatic analysis the probesets identified in footnotes 2 and 26 above represent the same gene.
- ³³ By bioinformatic analysis the probesets identified in footnotes 8 to 13 above represent the same gene.
- ³⁴ By bioinformatic analysis the probesets identified in footnotes 14, 24 and 25 above represent the same gene.
- ³⁵ By bioinformatic analysis the probesets identified in footnotes 27, 28 and 30 above represent the same gene.

TABLE 3: Definition of Transcript Class

Transcript class	Description	Expression summary
1	known and novel DRG-specific pain targets	High level in DRG with low levels everywhere else; expression decreased in injured DRG's (L5 and L6 in SNL animals)
2	Neuronal and cellular signal transduction proteins	Highly expressed in DRG, brain and spinal cord; expression decreased in injured DRG's
3	Neuronal markers	Highly expressed in DRG, brain and spinal cord; decreased in injured DRG's
4	Cellular signal transduction proteins	Expression in DRG; expression increased in SNL animals
5	known and novel neuropeptides or secreted molecules	Expression increased in SNL animals
6	Inflammatory response genes A	Expression in DRG (low level); expression increased in SNL animals
7	Inflammatory response genes B	Expression increased in SNL animals; higher expression in spleen
8	Markers of muscle tissue	Expression increased in SNL animals; higher expression in muscle
9	Unknown	Expression in DRG; decreased in SNL animals

TABLE 4: TaqMan Validation

TaqMan Validation refers to the confirmation of gene expression changes detected by Affymetrix GeneChip hybridization experiments through the use of a second independent assay, quantitative reverse transcription-PCR ("TaqMan assay"). As the TaqMan assay detects expression of a particular gene using three different oligonucleotide sequences, it is more sensitive and specific than the GeneChip assay. Gene-specific TaqMan assays were designed for each gene in the table, and used to independently confirm the changes in mRNA expression detected by the GeneChip assay. The tissue RNA samples assayed were extracted from a separate pool of tissue samples (Pool 2) than those used for the GeneChip assays (Pool 1). The TaqMan validation was performed on ten of the tissue samples: naïve "ipsilateral" (left) L4 DRG; naïve "contralateral" (right) L4 DRG; naïve "ipsilateral" (left) L5 DRG; sham surgery ipsilateral L4 DRG; sham surgery ipsilateral L5 DRG; Chung surgery ipsilateral L4 DRG; Chung surgery contralateral L4 DRG; Chung surgery plus gabapentin treatment ipsilateral L4 DRG; Chung surgery ipsilateral L5 DRG; Chung surgery plus gabapentin treatment ipsilateral L5 DRG. Changes in mRNA expression detected by GeneChip experiments for a particular gene were said to be "validated" ("Y") by TaqMan assays if the TaqMan assay found the same direction of change (increased or decreased) in gene expression found in the GeneChip experiments between Chung surgery RNA and naïve, sham, and contralateral tissue RNA, beyond the 2-fold limit of difference detection that is statistically significant for the assay. Gene expression changes were said to be "not validated" ("N") if mRNA expression in the TaqMan assays was not changed in the same direction, or were found to be changed by less than 2-fold.

Sequence ID	Probeset ID	Annotation	Transcript class	Taqman confirmation	TaqMan forward primer sequence	SEQ ID NO:	TaqMan reverse primer sequence	SEQ ID NO:	TaqMan probe sequence	SEQ ID NO:
22	rc_AA90019_8_at	poliovirus receptor-related 2 (herpesvirus entry mediator B)	1	N	GGCGGTGCCCTT CCA	803	ATGCCAGTCAGCAACA ATGG	804	CCCAGAAGAACCAG TCCAACCGGT	805
24	rc_AI011627_at	hypothetical protein MGC2628	1	Y	AAGTTCTACAGG CGCTGTACACAA	806	AGGAACCGCAGAGAA CTGACA	807	CCGCCAGGGCACC CCAT	808
16	rc_AI058904_at	hypothetical protein FLJ14735	1	N	TCACTGCTTGT TACTTCTCATGG T	809	TGGGACGGGCTTCTT AAGTT	810	TGCCGACTCCGACT GGTGAAGCA	811
2	AF029310_at	VR1 (vanilloid receptor, capsaicin receptor)	1	Y	TGGACTACCTGG AACACCAATGT	812	CGCTTGACGCCCTCAC A	813	TATCATCAACGAGG ACCCAGGCAA	814

TABLE 4: TaqMan Validation

Sequence ID	Probeset ID	Annotation	Transcript class	Taqman confirmation	TaqMan forward primer sequence	SEQ ID NO:	TaqMan reverse primer sequence	SEQ ID NO:	TaqMan probe sequence	SEQ ID NO:
3	AF039583_s_at	<i>decay accelerating factor GPI-form precursor (DAF)</i>	1	Y	GTTGTGGTCT GTATGCTGTCAT C	815	CCATTCCAGACAACCT CCTTTC	816	CTTGAAGGTGTGCT AGAAATGATAACAA AG	817
5	AF059030_at	Na channel alpha subunit NaN, voltage-gated	1	Y	CTACCTCACTGC CTCACAGCTTAG	818	AACGGCCTGTGTTTCAG TGAGT	819	CCAGCCTCTGGCGA GCAGGC	820
10	L31621_s_at	<i>nicotinic acetylcholine receptor alpha 3 subunit</i>	1	Y	TCATCCTGGTGT GCATTTTAGG	821	CTATGTGTCATCTCTG GCCATCA	822	ACGGCGGGATTATT TCTGCAACCC	823
9	M83561_s_at	glutamate receptor subunit 5-2 (GluR5-2), kainate subtype	1	Y	GATCAAAAGAAC ACACCTGTAAG AAGAA	824	TCCTCTGAAATCTGTC CTCCAAA	825	AAGCCACACGTCC GCTGCAC	826
124	M98049_s_at	pancreatitis-associated protein (pap)	8	Y	ATACCCCTCTGCA CGCATTAGTTG	827	TGAAACAGGGCATAG CAGTAGGA	828	CCCAAAGGCTCCCA GGCATATGG	829
20	rc_AA4886_8_at	heparanase	1	Y	CCAGCAAGCCTT TCCTCAAG	830	TGAAGACAGTTATGGG CATTGAGA	831	CAAGTCTTACTGGCT AGGAATAGGTCTGT GGTCA	832
128	rc_AA85139_2_g_at	<i>mouse kinesin family member 22B (Kif22b) / mouse kinesin-like 4</i>	9	Y	GAGGTGGAGGA CCTGGAACAG	833	GATTCGCCCTTCAGGAA AGACTCT	834	TGGAAGGCATCTCT GGGAAGCAGG	835
11	rc_AA89163_1_at	<i>KIAA1163 protein</i>	1	Y	TCCGGGTGTGTA TGTGTCITTG	836	GGTTGCCAGCCTGGT GTAC	837	TCTCTTCTGTGTCT AGGACTCTTCC	838

TABLE 4: TaqMan Validation

Sequence ID	Probeset ID	Annotation	Transcript class	Taqman confirmation	TaqMan forward primer sequence	SEQ ID NO:	TaqMan reverse primer sequence	SEQ ID NO:	TaqMan probe sequence	SEQ ID NO:
23	rc_AA95779_2_s_at	low density lipoprotein receptor-related protein 3	1	N	TGCCAGTGGCAT CCAAGAT	839	TCATCATCACTAGCCT CCAATATTG	840	AGCCCTCCACCAAC CTGCTCC	841
12	rc_AA96527_2_at	<i>calsenilin</i> , <i>presenilin binding protein</i> , <i>EF hand transcription factor</i> , <i>Kv channel interacting protein 1</i> , DREAM	1	Y	AAGGCTCAGAC AGCAGTGACAG T	842	GGGCTGTAGCTGGTC CAA	843	ACGGTGGCCATCA GCCAG	844
46	S80376_AT	<i>Rat G olf-alpha cDNA sequence</i>	1	Y	ACGGGTGATGG CAAAACATTAC	845	GTTGAACACTCTGCGG ATGTTC	846	CTACCCCTCACTTCAC CTGCGCC	847
18	rc_AI072454_at	mouse advillin / rat pervin (regulating neurite outgrowth)	1	Y	CAGCAAAGGTT CATCTACGACTG A	848	GACAGAATACACGAC CCTGAGATCT	849	CCCGACACACGCTG CCCTAGATACG	850
4	rc_AI639123_at	Similar to Mouse CIPP (channel-interacting PDZ domain protein)	1	Y	AGTCTCCTCCAG CTCACAAAGAGA	851	GACGTCATGATGTGT GACTGA	852	CCCGTCAGCACCAG CTCCCTTG	853
6	U01227_s_at	<i>5HT3 receptor</i>	1	Y	TCCTGTTTCCAA CACCAATTCA	854	CCGGGTCTTCAGTGCT AAGTTT	855	TGAGCAACCCCAAGT CCCCCTGTCC	856
14	U27767_at	<i>RGS4</i>	1	Y	TGTCTGAACAGG GCAAACTAAATT A	857	TGGCTTGACGGAACCTC ACTTT	858	CTGCCATGCAGGCT AAGAAAGGAACACT AACT	859

TABLE 4: TaqMan Validation

Sequence ID	Probeset ID	Annotation	Transcript class	Taqman confirmation	TaqMan forward primer sequence	SEQ ID NO:	TaqMan reverse primer sequence	SEQ ID NO:	TaqMan probe sequence	SEQ ID NO:
21	X74978exon_at	<i>prostatic acid phosphatase</i>	1	Y	TCCGGGTGTGTA TGTGCTTTG	860	GGTTGGCAGCCTGGT GTAC	861	TCTCTTCTGTGTCT AGGACTCTTCC	862
19	X92184_at	voltage-gated sodium channel (SNS/PN3/Nav1.8))	1	Y	TCCCGGACAAAT CAGAAACTG	863	CCCTGGTGACACTGTC ATAGGA	864	CTCTGCTACGTCTTT CCCCGC	865
7	Y17606_at	<i>potassium channel, alpha subunit (Kv9.1)</i>	1	Y	GGACCCCAATCCC AGCTCTACA	866	TGACAGATCCGCTAGA ACCTAGTTC	867	CACGGGAAAGGTGG CCAAACCC	868

TABLE 5: Affymetrix probeset sequences were blasted against the NCBI RefSeq DNA database (June 11, 2003) with the program 'blastn'. For each probeset, the top hits against human, mouse, and rat and with a score of 300 or higher are presented. See Table 8 for the Genbank index identifier (GI) and actual sequence corresponding to the human, mouse and rat Refseq top hit sequence ID's.

Patent Sequence ID	Probeset	Human RefSeq top hit Sequence ID	Human top hit Score	Mouse RefSeq top hit Sequence ID	Mouse top hit Score	Rat RefSeq top hit Sequence ID	Rat top hit Score
2	AF029310_at	250	2194			486	5644
3	AF039583_s_at			344	478	487	1750
5	AF059030_at	251	388	345	3814	488	11630
6	U01227_s_at	252	979	346	1524	489	3033
7	Y17606_at	253	1082	347	2480	490	5356
8	M24104_g_at	254	551	348	948	491	829
9	M83561_s_at	255	3455	349	3990	492	3558
10	L31621_s_at	256	1548	350	2524	493	3683
12	rc_AA965272_at			351	325		
13	U59672_at	252	1003	346	1576	489	4367
14	U27767_at	257	775	352	1415	494	2056
15	rc_AI227592_at			351	414		
16	rc_AI058904_at			353	646		
17	X13804cds_at	258	787			495	3150
18	rc_AI072454_at					496	963
19	X92184_at	259	848	354	8429	497	12810
20	rc_AA848868_at					498	894
21	X74978exon_at					499	848
23	rc_AA957792_s_at					500	624
24	rc_AI011627_at			355	438		
25	rc_AA955302_at	260	474	356	660	501	829
26	U10354_at	261	3114	357	4678	502	6732
27	AB017544_g_at	262	1074	358	1362	503	1846
29	M28647_g_at	263	3390	359	5071	504	6340
30	M74494_g_at	263	684	359	1001	504	1285
31	rc_AA955164_at			360	321		
32	rc_AA893158_at					505	620
33	rc_AI010339_r_at			361	710		
34	AA933181_at	264	480	362	468		
36	AF060173_at	265	1760			506	4799
37	J05087_at	266	3188	363	4080	507	718
38	S56508_s_at	267	2315	364	3364	508	4639
39	K02248cds_s_at	268	418	365	640	509	688
40	M25890_at	268	426	365	805	509	1059

TABLE 5 (continued)

Patent Sequence ID	Probeset	Human RefSeq top hit Sequence ID	Human top hit Score	Mouse RefSeq top hit Sequence ID	Mouse top hit Score	Rat RefSeq top hit Sequence ID	Rat top hit Score
41	rc_AI230211_s_at					510	1023
44	M76426_at	269	2123	366	3679	511	5487
45	U52103_at	270	1497	367	2884		
46	S80376_at	271	1505	368	613		
47	X70662_at	272	1780	369	2783	512	3382
49	rc_AI171087_at	273	406				
50	rc_AA818595_at			370	345		
53	rc_AA997117_at	274	609	371	807		
54	rc_AI044249_at			372	385	513	789
55	rc_AI029180_at					514	307
56	rc_AI029290_at			373	406		
57	rc_AA963649_at			374	585		
58	rc_AI175539_at	275	452	375	902	515	1168
59	AF054586_at	276	1003	376	3029	516	5783
60	AF090133_at	277	626	377	1070	517	1237
61	U31554_at	278	1532	378	1731	518	2101
63	U94322_s_at	279	630	379	1076	519	3148
64	AA686952_f_at			380	371		
65	AB003991_g_at	280	954	381	854	520	870
66	AB003992_s_at	281	969	381	1231	520	1231
67	M22253_at	283	4734	382	2389	521	13570
68	AF009603_at	284	940	383	1630		
69	rc_AA997651_at			384	460		
70	rc_AI180306_at			385	799		
71	rc_AI030734_at					522	404
73	rc_AA955960_at			383	482		
74	AF091834_at	284	545	385	775	523	1057
75	M25638_s_at	285	775			524	1616
78	rc_AI229196_at			348	577		
79	rc_AI229196_g_at			348	577		
82	D10666_at	286	1009	386	1086	525	1689
83	rc_AI227991_at			385	480		
84	rc_AI228540_at			387	585		
86	rc_AA944398_at			388	371		
87	rc_AI059141_at			389	523		
91	M27726_at	287	545	390	775		
92	X59737mRNA_at	288	1661	391	2226		
93	X59737mRNA_g_at	288	1661	391	2226		
94	rc_AI012433_at			392	678	526	1072

TABLE 5 (continued)

Patent Sequence ID	Probeset	Human RefSeq top hit Sequence ID	Human top hit Score	Mouse RefSeq top hit Sequence ID	Mouse top hit Score	Rat RefSeq top hit Sequence ID	Rat top hit Score
98	rc_AA818790_at			393	450		
99	rc_AA926175_f_at					527	317
100	rc_AI235753_at			394	315	528	355
102	rc_AI058975_at	289	325				
103	U23407_at	290	450			529	1564
104	M22400_at	291	2181	395	3142	530	3770
107	rc_AA964755_at	292	321				
108	rc_AI177099_at			396	490		
109	R47042_at			397	414		
110	rc_AA997590_at			398	317		
111	rc_AI171281_at			398	480		
112	rc_AI072183_at	293	557	399	444		
113	rc_AA900699_at	294	468	400	599		
114	rc_AA946142_at			401	343		
120	rc_AI170314_at					531	1166
121	rc_AI176592_at			402	398		
122	rc_AI172290_i_at	295	654	403	938	532	1047
123	J03624_at			404	609	533	1386
124	M98049_s_at			405	684	534	1443
125	rc_AI101739_at			406	613		
126	X02341cds_at	296	337	406	676		
127	M15880_at	297	359	407	803	535	1068
128	rc_AA851392_g_at			408	537		
129	rc_AA945867_at			409	337	536	914
130	rc_AI012030_at			410	488	537	886
131	M86621_at	298	3689	411	2966	538	7265
132	rc_AA900468_at	299	454	412	622		
135	rc_AI146189_s_at			413	307	539	480
136	rc_AI012611_at	300	329	414	585		
137	rc_AA819081_at			415	353		
140	rc_AA996499_at			416	305		
141	rc_AI177119_at			417	468		
144	rc_AI179953_at			418	664		
145	rc_AI029920_s_at					540	500
146	AF104362_at	301	577	419	2014	541	3025
147	S66184_s_at					542	406
148	D84418_s_at	302	674	420	952	543	1223
150	rc_AA866404_at			421	817		
152	C06892_at			422	480	544	795

TABLE 5 (continued)

Patent Sequence ID	Probeset	Human RefSeq top hit Sequence ID	Human top hit Score	Mouse RefSeq top hit Sequence ID	Mouse top hit Score	Rat RefSeq top hit Sequence ID	Rat top hit Score
153	L32591mRNA_at	303	511	423	787	545	1409
154	rc_AI070295_g_at			423	373		
155	rc_AI070295_at			423	373		
156	rc_AI229785_at			424	381		
157	M32062_at			425	821	546	1197
158	M32062_g_at			425	821	546	1197
159	M57263_at	304	2151	426	3618	547	5366
160	M63282_at	305	708	427	1794	548	3711
161	rc_AI045030_s_at			428	680	549	886
162	U33935_at			429	821	550	1148
163	rc_AA891911_at			430	394		
164	X81193_at	306	700	431	999	551	1691
166	rc_AI060313_at			432	375		
167	D88250_at	307	930	433	1707	552	5765
169	X53054_at	308	444	434	793		
170	K02815_s_at	309	357	435	632		
171	X53054_g_at	309	444	435	793		
172	M36151cds_s_at			437	406		
173	U65217_i_at	310	347	437	535		
174	rc_AI071264_at			438	525	553	882
175	rc_AI136662_at					554	567
177	U18729_at	311	432	438	886	555	1405
178	X13044_at			439	827	556	2232
179	X13044_g_at			439	827	556	2232
180	X14254cds_g_at			439	696	556	1144
181	X14254cds_at			439	696	556	1144
182	rc_AI171966_at			440	601		
183	U31599_g_at			441	672		
185	Z49761_at			442	928		
186	X71127_at			443	1102	557	2171
187	X71127_g_at			443	1102	557	2171
188	X73371_at			444	1271	558	2767
189	rc_AI113008_at			445	597		
190	rc_AI011203_at			446	535		
191	E12286cds_at			447	741	559	1170
192	Y07534cds_s_at	312	573	448	2042	560	3144
193	M58364_at	313	882	449	1364	561	2014
194	J02720_at	314	601	450	1405	562	2748
195	M29866_s_at	315	369	451	1029	563	1661
196	X52477_at	315	698	451	6536	563	10040

TABLE 5 (continued)

Patent Sequence ID	Probeset	Human RefSeq top hit Sequence ID	Human top hit Score	Mouse RefSeq top hit Sequence ID	Mouse top hit Score	Rat RefSeq top hit Sequence ID	Rat top hit Score
197	rc_AI639117_s_at			452	543		
198	S61865_s_at	316	317	453	2056	564	3558
199	D28561_s_at	317	1572	454	2771	565	4924
200	rc_AA800206_at	318	666	455	989		
201	X14812_at	319	595			566	1754
202	X59736mRNA_g_at	320	1265				
203	rc_AA891522_f_at	321	355	456	416	567	471
204	X15939_f_at	321	7644	456	9501	568	11750
205	J00692_at	322	1372	457	957	569	2206
207	rc_AA946094_at			458	367		
208	X02412_at	323	428	459	914	570	1025
209	rc_AI010583_at	324	387	460	607	571	981
210	K02423cds_s_at	325	402	461	720	572	842
211	X00975_at	326	535	462	936	573	1011
212	rc_AI136540_at			463	311		
213	M10140_at	327	1339	464	2136	574	2792
214	M99223_at	328	3663	465	1281	575	6805
215	S68736_at	329	3261	466	1015	576	341
216	rc_AI639532_at	330	496	467	763		
217	L24897_s_at	331	660	466	551	576	327
218	M73701_at	332	718	468	1114	577	1346
219	rc_AA851223_at	333	595	469	813	578	965
220	rc_AI639096_at			470	545		
221	L00088expanded_cds#2_at	325	488	461	1047	572	844
222	X00975_g_at	326	535	462	936	573	1011
224	rc_AA963366_at			471	309		
225	rc_AI044544_at	334	383				
226	L10669_at	335	2714	472	4044	579	347
227	X12554cds_s_at			473	391	580	517
228	L10669_g_at	336	2714	472	4044	579	347
231	Z17319_at	336	700	474	1213	581	1576
232	X59864mRNA_at			475	1949		
233	X59864mRNA_g_at			475	1949		
234	rc_AA819140_at					582	692

TABLE 5 (continued)

Patent Sequence ID	Probeset	Human RefSeq top hit Sequence ID	Human top hit Score	Mouse RefSeq top hit Sequence ID	Mouse top hit Score	Rat RefSeq top hit Sequence ID	Rat top hit Score
235	J04993_at	337	684	476	936	583	1586
236	X07314cds_at	338	448	477	726		
237	X07314cds_g_at	338	448	477	726		
238	X15939_i_at	321	7644	456	9501	568	11750
239	D86556_i_at			478	1681	584	1863
240	E00903cds_s_at					585	672
241	rc_AA946199_at			479	515		
242	S60953_s_at	339	1584	480	2422	586	4230
243	rc_AI012505_at	340	396	481	624		
244	D84336_at	341	1150	482	2000	587	2567
245	L19180_at	342	3941	483	4000	588	5586
246	M90514mRNA_at			484	858		
248	X59608exon_at	343	983	485	1522		

TABLE 6: Affymetrix probeset sequences were blasted against the NCBI RefSeq protein database (June 11, 2003) with the program 'blastx'. For each probeset, the top hits against human, mouse, and rat and with a score of 150 or higher are presented. See Table 8 for the Genbank index identifier (GI) and actual sequence corresponding to the human, mouse and rat Refseq top hit sequence ID's.

Patent Sequence ID	Probeset	Human RefSeq Sequence ID	Human Score	Mouse RefSeq Sequence ID	Mouse Score	Rat RefSeq Sequence ID	Rat Score
2	AF029310_at	589	1414	661	690	733	1620
3	AF039583_s_at	590	348	662	537	734	717
5	AF059030_at	591	2434	663	2978	735	3362
6	U01227_s_at	592	779	664	853	736	904
7	Y17606_at	593	771	665	837	737	857
9	M83561_s_at	594	1661	666	1520	738	1686
10	L31621_s_at	596	850	667	894	739	907
13	U59672_at	592	790	664	869	736	936
14	U27767_at	596	337	668	339	740	345
17	X13804cds_at	597	345			741	775
19	X92184_at	598	2910	669	3379	742	3552
26	U10354_at	599	1783	670	1864	743	1887
27	AB017544_g_at	600	474	671	485	744	504
29	M28647_g_at	601	1889	672	1937	745	1941
30	M74494_g_at	601	399	672	410	745	411
36	AF060173_at	602	1014			746	1043
37	J05087_at	603	2037	673	1959	747	1725
38	S56508_s_at	604	1308	674	1387	748	1427
44	M76426_at	605	1558	675	1523	749	1664
45	U52103_at	606	1063	676	1107	750	813
46	S80376_at	607	766	677	623	751	622
47	X70662_at	608	800	678	798	752	800
59	AF054586_at	609	924	679	1018	753	1054
60	AF090133_at	610	370	680	375	754	376
61	U31554_at	611	567	681	537	755	569
63	U94322_s_at	612	382	682	431	756	450
65	AB003991_g_at	613	409	683	391	757	391
66	AB003992_s_at	614	408	684	408	758	408
67	M22253_at	615	3359	685	2491	758	3442
68	AF009603_at	616	495	686	500	759	362
74	AF091834_at	617	344	687	344	760	344
75	M25638_s_at	618	405			761	421
82	D10666_at	619	389	687	389	762	389
91	M27726_at	620	303	688	312		

TABLE 6 (continued)

Patent Sequence ID	Probeset	Human RefSeq Sequence ID	Human Score	Mouse RefSeq Sequence ID	Mouse Score	Rat RefSeq Sequence ID	Rat Score
92	X59737mRNA_at	621	762	689	773	763	493
93	X59737mRNA_g_at	621	762	689	773	763	493
104	M22400_at	622	1028	690	1072	764	1094
122	rc_A1172290_i_at	623	327	691	327	765	326
124	M98049_s_at			692	306	766	351
131	M86621_at	624	2079	693	2118	767	2157
146	AF104362_at	625	654	694	752	768	826
148	D84418_s_at	626	354	695	350	769	357
153	L32591mRNA_at	627	305	696	325	770	333
157	M32062_at			697	394	771	478
158	M32062_g_at			698	394	772	478
159	M57263_at	628	1398	698	1475	773	1524
160	M63282_at	629	319	699	329	774	331
161	rc_A1045030_s_at					775	310
164	X81193_at	630	412	700	417	775	426
167	D88250_at	631	1068	701	1161	776	1385
169	X53054_at	632	355	702	399		
171	X53054_g_at	632	355	702	399		
172	M36151cds_s_at			703	324		
177	U18729_at	633	313	704	328	777	337
178	X13044_at			705	339	778	394
179	X13044_g_at			705	339	778	394
180	X14254cds_g_at			705	303	778	346
181	X14254cds_at			705	303	778	346
183	U31599_g_at	634	313	706	352		
185	Z49761_at	635	360	707	416		
186	X71127_at			708	339	779	365
187	X71127_g_at			708	339	779	365
188	X73371_at			709	419	780	539
191	E12286cds_at			710	322	781	348
192	Y07534cds_s_at	636	775	711	931	782	1056
193	M58364_at	637	446	712	463	783	483
194	J02720_at	638	572	713	618	784	657
195	M29866_s_at	639	464	714	507	785	568
196	X52477_at	639	2573	714	2952	785	3242
198	S61865_s_at	640	314	715	394	786	451
199	D28561_s_at	641	803	716	810	787	840
200	rc_AA800206_at	642	352	717	354		
201	X14812_at	643	312			788	321

TABLE 6 (continued)

Patent Sequence ID	Probeset	Human RefSeq Sequence ID	Human Score	Mouse RefSeq Sequence ID	Mouse Score	Rat RefSeq Sequence ID	Rat Score
202	X59736mRNA_g_at	644	818	689	689	763	507
204	X15939_f_at	645	3299	718	3362	789	3389
205	J00692_at	646	765	719	759	790	765
211	X00975_at	647	315	720	320	791	320
213	M10140_at	648	706	721	726	792	729
214	M99223_at	649	1823	722	1610	793	1869
215	S68736_at	650	1322	718	1122	794	1132
217	L24897_s_at	650	306				
218	M73701_at	651	335	723	335	795	338
222	X00975_g_at	647	315	720	320	791	320
226	L10669_at	652	1600	724	1628	796	1348
228	L10669_g_at	652	1600	724	1628	796	1348
231	Z17319_at	653	481	725	508	797	519
235	J04993_at	654	333	726	338	798	341
236	X07314cds_at	655	320	727	325		
237	X07314cds_g_at	655	320	727	325		
238	X15939_i_at	645	3299	718	3362	789	3389
239	D86556_i_at	656	457	728	629	799	630
242	S60953_s_at	657	1580	729	906	800	1623
244	D84336_at	658	681	730	640	801	793
245	L19180_at	659	2733	731	1747	802	1761
246	M90514mRNA_at	660	332	732	384		

TABLE 7: CORRELATION OF SEQ ID NOS WITH GENBANK IDENTIFIERS

SEQ ID NO:	GI NUMBER
250	18375664
251	7657541
252	4504542
253	25777652
254	9257249
255	28416443
256	19923121
257	11184227
258	10835088
259	5730032
260	4826843
261	4557410
262	4758895
263	31541982
264	22095346
265	24308166
266	11386154
267	17505197
268	4507242
269	18765697
270	11321616
271	4504042
272	27436963
273	11545772
274	7661941
275	4506334
276	23199981
277	11545919
278	4505024
279	8051600
280	18765732
281	18765734
282	29893558
283	4506930
284	11079227
285	5453761
286	21361558
287	21361369
288	11641403
289	30581115
290	6382069
291	5360213
292	4507484
293	6552332

294	5729815
295	17402921
296	4507896
297	31542152
298	4757893
299	21359843
300	20149569
301	4826875
302	14141173
303	9790904
304	4507474
305	4502262
306	4502892
307	4502494
308	4504410
309	18426974
310	24797068
311	4557504
312	13904863
313	4503948
314	10947138
315	4557384
316	29568085
317	4507010
318	4501892
319	4557776
320	4502854
321	4557772
322	5016087
323	27597084
324	4557240
325	17986272
326	28372498
327	21536287
328	27886527
329	7669505
330	4507616
331	11024711
332	4507620
333	16554590
334	8400716
335	5032008
336	4505754
337	21361554
338	4557774
339	4505474
340	26024196

341	21361079
342	19743920
343	14589948
344	6753599
345	6755405
346	7305174
347	6680547
348	31543936
349	22164777
350	21553070
351	9790064
352	31543587
353	31542054
354	6677874
355	22122736
356	31341473
357	7305100
358	9790152
359	21450276
360	11528519
361	31341613
362	9055173
363	31343139
364	21450202
365	6678034
366	24429567
367	6753677
368	31342887
369	31543027
370	7710021
371	23943917
372	19387849
373	27229183
374	28076914
375	7949118
376	20127401
377	6755970
378	31342150
379	31560583
380	6677720
381	24475932
382	9055327
383	31560791
384	31340870
385	31543348
386	31560507
387	6754505

388	6679756
389	16716572
390	24418918
391	6753427
392	31340641
393	7305328
394	21489932
395	7710029
396	31541881
397	6681142
398	31543365
399	31560587
400	6753779
401	6671641
402	6680416
403	6678594
404	6753939
405	6754979
406	30794515
407	27754168
408	21704181
409	6754401
410	6678875
411	6753233
412	23956109
413	7304886
414	6755289
415	21312071
416	6671649
417	6671651
418	31542892
419	6754933
420	6680228
421	7305124
422	9256567
423	6681148
424	6680605
425	24475601
426	9910583
427	31542153
428	31560717
429	12025527
430	6678114
431	7304986
432	26251306
433	21450096
434	6912403

435	13540710
436	6754115
437	6671629
438	22094076
439	29789019
440	6754121
441	6754123
442	6754119
443	6753219
444	6857782
445	10946747
446	13385273
447	31560810
448	31340947
449	31542888
450	7106254
451	23956043
452	6996918
453	6755437
454	6678014
455	15149486
456	18859640
457	14192921
458	21359819
459	31560029
460	7304854
461	29789015
462	7949077
463	6755844
464	6671761
465	6671588
466	21489940
467	6678370
468	6678390
469	6679650
470	22122730
471	11875202
472	6755255
473	6753501
474	9256623
475	12963478
476	31343605
477	6754777
478	31340562
479	6755585
480	24475663
481	31560169

482	6753641
483	25092608
484	21450094
485	6677794
486	14010882
487	29789131
488	15011860
489	13242305
490	16758837
491	6981611
492	8393480
493	16258818
494	8394182
495	29789025
496	13242317
497	8394241
498	12018275
499	9910501
500	16758309
501	27465532
502	8393052
503	25453373
504	6978542
505	18426843
506	19705530
507	24475940
508	18543340
509	6981581
510	13929039
511	12408297
512	8393642
513	13540625
514	6981709
515	11968063
516	20127389
517	11120707
518	25742795
519	31542883
520	13591881
521	13540708
522	18093103
523	13489066
524	13929097
525	6981707
526	21426812
527	13540680
528	8393330

529	25453403
530	6980975
531	18426829
532	16758129
533	15147220
534	16757981
535	6981285
536	19923684
537	6981203
538	31542334
539	6978502
540	6981087
541	13929163
542	8393732
543	8393535
544	14389304
545	13162297
546	31542803
547	13928911
548	6978540
549	6978642
550	9507228
551	16924003
552	20302094
553	19173783
554	16758101
555	13162358
556	6978636
557	9506432
558	28461146
559	27229315
560	30578395
561	13242244
562	8392919
563	8393023
564	6981517
565	6980957
566	6981239
567	8393803
568	8393806
569	9506370
570	14192924
571	19173799
572	13487932
573	6981237
574	6978660
575	17157986

576	6981233
577	8394465
578	6978810
579	11560086
580	6978690
581	8393947
582	31377483
583	8394462
584	8393034
585	6981277
586	9506938
587	16758571
588	9507010
589	18375665
590	10835143
591	7657542
592	4504543
593	13492973
594	28416444
595	19923122
596	5032039
597	10835089
598	5730033
599	4557411
600	4758896
601	21361181
602	24308167
603	11386155
604	17505198
605	18765698
606	11321617
607	4504043
608	27436964
609	14149631
610	11545920
611	4505025
612	4503905
613	18765733
614	18765735
615	29893559
616	4506931
617	11079228
618	5453762
619	21361559
620	21361370
621	10334859
622	4758462

623	17402922
624	4757894
625	4826876
626	11321591
627	4503287
628	4507475
629	4502263
630	4502893
631	4502495
632	4504411
633	4557505
634	4504399
635	18765715
636	4503211
637	4503949
638	10947139
639	4557385
640	29568086
641	4507011
642	4501893
643	4557777
644	4502855
645	4557773
646	4501881
647	28372499
648	21536288
649	27886529
650	7669506
651	4507621
652	5032009
653	4505755
654	21361555
655	4557775
656	4502553
657	4505475
658	21361080
659	19743921
660	21902519
661	11528502
662	6753600
663	6755406
664	7305175
665	6680548
666	22164778
667	21553071
668	31543588
669	6677875

TABLE 7

670	7305101
671	9790153
672	21450277
673	28893283
674	21450203
675	24429568
676	6753678
677	6754014
678	31543028
679	20127402
680	6755971
681	30425330
682	31560584
683	6755588
684	6755410
685	31560792
686	31543349
687	6755983
688	24418919
689	6753428
690	7710030
691	6678595
692	6754980
693	6753234
694	6754934
695	6680229
696	6681149
697	6753834
698	9910584
699	31542154
700	7304987
701	21450097
702	6912404
703	6754116
704	22094077
705	29789020
706	6754124
707	6754120
708	6753220
709	6857783
710	6806917
711	30578401
712	6679967
713	7106255
714	23956044
715	6755438
716	6678015

717	15149487
718	18859641
719	14192922
720	7949078
721	6671762
722	6806903
723	6678391
724	6755256
725	9256624
726	10946862
727	6754778
728	6753248
729	11993956
730	6753642
731	25092609
732	21450095
733	14010883
734	29789132
735	9507065
736	13242306
737	16758838
738	8393481
739	16258819
740	8394183
741	29789026
742	8394242
743	8393053
744	25453374
745	6978543
746	19705531
747	16758008
748	18543341
749	12408298
750	25742568
751	9506737
752	8393643
753	20127390
754	11120708
755	25742796
756	9506709
757	13591882
758	13540709
759	13676847
760	13489067
761	13929098
762	6981708
763	31542401

764	6980976
765	16758130
766	16757982
767	31542335
768	13929164
769	8393536
770	13162298
771	31542804
772	13928912
773	6978541
774	6978643
775	16924004
776	20302095
777	13162359
778	6978637
779	9506433
780	28461147
781	27229316
782	30578396
783	13242245
784	8392920
785	8393024
786	6981518
787	6980958
788	6981240
789	8393807
790	9506371
791	6981238
792	6978661
793	17157987
794	6981234
795	8394466
796	11560087
797	8393948
798	8394463
799	8393035
800	9506939
801	16758572
802	9507011

Discussion

These data demonstrate that each of the genes listed in Tables 1 and 2, above (and their species orthologs listed in Tables 5 and 6), are useful not only for identifying (*e.g.*, diagnosing) individuals having neuropathic pain, but also for monitoring a therapy (for example a drug treatment) or treatment for such pain. Each of these genes or their gene products can also be used to screen for novel therapies for the treatment of pain, as well as other indications, including addiction, seizure and epilepsy, neurodegenerative disorders, anxiety, depression, headache, and inflammatory bowel disease, among others.

WHAT IS CLAIMED:

1. A method of detecting a pain response in a cell, which method comprises
 - (a) determining the expression level in a test cell of at least one nucleic acid molecule which hybridizes under standard conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of **SEQ ID NOS: 1-249**; and
 - (b) comparing the expression level determined in step (a) to the expression level of the at least one nucleic acid molecule in a control cell from an animal model of pain;
wherein an expression level of the at least one nucleic acid molecule from the test cell which is similar or identical to the expression level of the at least one nucleic acid molecule from the control cell is indicative of a pain response in the test cell.
2. The method of claim 1, wherein the animal model is a model of neuropathic pain.
3. The method of claim 1, wherein the test and control cells naturally express at least one nucleic acid molecule comprising a nucleotide sequence selected from any one of **SEQ ID NOS: 1-249**.
4. The method of claim 1, wherein the test and control cells have been genetically modified to express at least one nucleic acid molecule comprising a nucleotide sequence selected from any one of **SEQ ID NOS: 1-249**.
5. The method of claim 1, wherein the test and control cells are both from the central nervous system.
6. The method of claim 1, wherein the test and control cells are both from the peripheral nervous system.
7. The method of claim 1, wherein the test and control cells are both from the dorsal root ganglion.
8. The method of claim 1, wherein the test and control cells are both neuronal cells.

9. The method of claim 1, wherein the nucleic acid molecule encodes a mammalian protein

10. The method of claim 1, wherein the nucleic acid molecule encodes a rat, mouse or human protein.

11. The method of claim 1, wherein the expression level of the nucleic acid molecule in each of the test and control cells is determined by quantifying the amount of mRNA.

12. The method of claim 1, wherein the expression level of the nucleic acid molecule in each of the test and control cells is determined by quantifying the amount protein encoded by said nucleic acid molecule in each of the two cells.

13. A method for identifying a candidate compound for the treatment of pain, which method comprises:

(a) contacting a test cell with a test compound for a time sufficient for the test cell to respond to the test compound;

(b) determining the expression level in the test cell of at least one nucleic acid molecule which hybridizes under standard conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of **SEQ ID NOS:1-249**; and

(c) comparing the expression level or levels determined in step (b) to the expression level or levels of the at least one nucleic acid molecule in a control cell that is not contacted with the test compound,

wherein a change in expression of the at least one nucleic acid molecule when the test cell is contacted with the test compound indicates that the test compound is a candidate compound useful to treat pain.

14. The method of claim 13, wherein the test and control cells naturally express at least one nucleic acid molecule comprising a nucleotide sequence selected from any one of **SEQ ID NOS: 1-249**.

15. The method of claim 13, wherein the test and control cells have been genetically modified to express at least one nucleic acid molecule comprising a nucleotide sequence selected from any one of **SEQ ID NOs: 1-249**.

16. The method of claim 13, wherein the test and control cells are both from the central nervous system.

17. The method of claim 13, wherein the test and control cells are both from the peripheral nervous system.

18. The method of claim 13, wherein the test and control cells are both from the dorsal root ganglion.

19. The method of claim 13, wherein the test and control cells are both neuronal cells.

20. The method of claim 13, wherein the nucleic acid molecule encodes a mammalian protein.

21. The method of claim 13, wherein the nucleic acid molecule encodes a rat, mouse or human protein.

22. The method of claim 13, wherein the expression level of the nucleic acid molecule in each of the test and control cells is determined by quantifying the amount of mRNA corresponding to any one of SEQ ID NOs: 1-249 in each of the two cells.

23. The method of claim 13, wherein the expression level of the nucleic acid molecule in each of the test and control cells is determined by quantifying the amount of protein encoded by said nucleic acid molecule in each of the two cells.

24. The method of claim 13, wherein the test compound decreases the expression level of the nucleic acid molecule.

25. The method of claim 13, wherein the test compound increases the expression level of the nucleic acid molecule.

26. The method of claim 13, wherein the test compound is a small inorganic molecule, a small organic molecule, a polypeptide, a nucleic acid molecule, or a chimera or derivative thereof.

27. The method of claim 26, wherein the polypeptide is an antibody or an immunospecific fragment thereof.

28. The method of claim 13, wherein the pain to be treated by the candidate compound is neuropathic pain.

29. A method for identifying a candidate compound for the treatment of pain, which method comprises:

- (a) contacting a first cell with a known analgesic compound;
- (b) contacting a second cell with a test compound;
- (c) determining the expression level in the first and second cells of one or more nucleic acid molecules which hybridize under standard conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of **SEQ ID NOS:1-249**; and

- (d) comparing the expression level of the one or more nucleic acid molecules in said second cell to the expression level of the one or more nucleic acids in said first cell

wherein a change in the expression level of the one or more nucleic acid molecules in said second cell that is similar to or identical to the expression level of the one or more nucleic acid molecules in the first cell indicates that the test compound is a candidate compound useful to treat pain.

30. The method of claim 29, wherein the expression levels of the one or more nucleic acid molecules in the first and second cells are compared to the expression level of the one or more nucleic acids in a third control cell that has not been contacted with the analgesic or test compound, such that a change in the expression level of the one or more nucleic acid molecules in said first and second cells that is different from the expression level

of the one or more nucleic acid molecules in the third cell serves to further indicate that the test compound is a candidate compound useful to treat pain.

31. The method of claim 29, wherein the first and second cells naturally express a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**.

32. The method of claim 29, wherein the first and second cells have been genetically modified to express a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**.

33. The method of claim 29, wherein the first and second cells are both from the central nervous system.

34. The method of claim 29, wherein the first and second cells are both from the peripheral nervous system.

35. The method of claim 29, wherein, the first and second cells are both from the dorsal root ganglion.

36. The method of claim 29, wherein the first and second cells are each neuronal cells.

37. The method of claim 29, wherein the nucleic acid molecule encodes a mammalian protein.

38. The method of claim 29, wherein the nucleic acid molecule encodes a rat, mouse or human protein.

39. The method of claim 29, wherein the expression level of the nucleic acid molecule in each of the first and second cells is determined by quantifying the amount of mRNA corresponding to any one of **SEQ ID NOs: 1-249** in each of the two cells.

40. The method of claim 29, wherein the expression level of the nucleic acid molecule in each of the first and second cells is determined by quantifying the amount protein encoded by said nucleic acid molecule in each of the two cells.

41. The method of claim 29, wherein the test compound is a small inorganic molecule, a small organic molecule, a polypeptide, a nucleic acid molecule, or a chimera or derivative thereof.

42. The method of claim 41, wherein the polypeptide is an antibody or an immunospecific fragment thereof.

43. The method of claim 29, wherein the pain to be treated by the candidate compound is neuropathic pain.

44. The method of claim 30, wherein the first, second and third cells naturally express a nucleic acid molecule comprising a nucleotide sequence selected from SEQ ID NOs: 1-249.

45. The method of claim 30, wherein the first, second and third cells have been genetically modified to express a nucleic acid molecule comprising a nucleotide sequence selected from SEQ ID NOs: 1-249.

46. The method of claim 30, wherein the first, second and third cells are from the central nervous system.

47. The method of claim 30, wherein the first, second and third cells are from the peripheral nervous system.

48. The method of claim 30, wherein, the first and second cells are both from the dorsal root ganglion.

49. The method of claim 30, wherein the first, second and third cells are neuronal cells.

50. A method of diagnosing pain in an animal comprising
(a) determining in a first cell from an animal suspected of having pain the expression level of a nucleic acid molecule which hybridizes under standard conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of **SEQ ID NOS: 1-249**; and

(b) comparing the expression level determined in step (a) to the expression level of the nucleic acid molecule in a second cell from an animal model of pain;

wherein an expression level of the nucleic acid molecule from the first cell which is similar or identical to the expression level of the nucleic acid molecule in the second cell is indicative of pain in the animal.

51. The method of claim 50, wherein the first and second cells naturally express a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOS: 1-249**.

52. The method of claim 50, wherein the first and second cells have been genetically modified to express a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOS: 1-249**.

53. The method of claim 50, wherein the first and second cells are both from the central nervous system.

54. The method of claim 50, wherein the first and second cells are both from the peripheral nervous system.

55. The method of claim 50, wherein the first and second cells are both from the dorsal root ganglion.

56. The method of claim 50, wherein the first and second cells are neuronal cells.

57. The method of claim 50, wherein the nucleic acid molecule encodes a mammalian protein.

58. The method of claim 50, wherein the nucleic acid molecule encodes a rat, mouse or human protein.

59. The method of claim 50, wherein the expression level of the nucleic acid molecule in each of the first and second cells is determined by quantifying the amount of mRNA corresponding to any one of **SEQ ID NOs: 1-249** in each of the two cells.

60. The method of claim 50, wherein the expression level of the nucleic acid molecule in each of the first and second cells is determined by quantifying the amount protein encoded by said nucleic acid molecule in each of the two cells.

61. A method for monitoring the efficacy of an analgesic treatment in a cell comprising:

(a) contacting a test cell with an analgesic compound for a time period sufficient to allow the test cell to respond to said compound;

(b) determining in the test cell the expression level of a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**; and

(c) comparing the expression level of the nucleic acid molecule in step (b) to the expression level of the nucleic acid molecule in a control cell that has not been contacted with the analgesic compound;

wherein a detectable change in the expression level of the nucleic acid molecule in the test cell in response to contact with the analgesic compound compared to the expression of the nucleic acid molecule in the control cell that has not been contacted with the analgesic compound is indicative of the efficacy of the analgesic compound.

62. The method of claim 61, wherein the test and control cells naturally express a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**.

63. The method of claim 61, wherein the test and control cells have been genetically modified to express a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**.

64. The method of claim 61, wherein the test and control cells are both from the central nervous system.

65. The method of claim 61, wherein the test and control cells are both from the peripheral nervous system.

66. The method of claim 61, wherein, the test and control cells are both from the dorsal root ganglion.

67. The method of claim 61, wherein the test and control cells are neuronal cells.

68. The method of claim 61, wherein the nucleic acid molecule encodes a mammalian protein.

69. The method of claim 61, wherein the nucleic acid molecule encodes a rat, mouse or human protein.

70. The method of claim 61, wherein the expression level of the nucleic acid molecule in each of the test and control cells is determined by quantifying the amount of mRNA corresponding to any one of **SEQ ID NOs: 1-249** in each of the two cells.

71. The method of claim 61, wherein the expression level of the nucleic acid molecule in each of the test and control cells is determined by quantifying the amount of protein encoded by said nucleic acid molecule in each of the two cells.

72. The method of claim 61, wherein the test compound is a small inorganic molecule, a small organic molecule, a polypeptide, a nucleic acid molecule, or a chimera or derivative thereof.

73. The method of claim 72, wherein the polypeptide is an antibody or an immunospecific fragment thereof.

74. A method for identifying a candidate compound capable of binding to a protein encoded by a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**, said method comprising;

(a) contacting said protein with a test compound under conditions that permit binding of the test compound to the protein; and

(b) detecting binding of the test compound to the protein.

75. The method of claim 74, wherein the nucleic acid molecule encodes a mammalian protein

76. The method of claim 74, wherein the nucleic acid molecule encodes a rat, mouse or human protein.

77. The method of claim 76, wherein the test compound is a small inorganic molecule, a small organic molecule, a polypeptide, a nucleic acid molecule, or a chimera or derivative thereof.

78. The method of claim 77, wherein the polypeptide is an antibody or an immunospecific fragment thereof.

79. The method of claim 74, wherein said protein is: (i) present in an intact cell that expresses the protein; or (ii) present in a membrane fraction; or (iii) an isolated protein that is unassociated with a cell or membrane fraction.

80. The method of claim 74, wherein the intact cell used is from an animal model of pain.

81. The method of claim 80, wherein the animal model of pain is a model of neuropathic pain.

82. A method for treating a condition that can be treated by modulating expression of a nucleic acid molecule comprising a nucleotide sequence selected from **SEQ ID NOs: 1-249**, or by modulating the activity of the protein encoded by said nucleic acid molecule, said method comprising administering to a subject in need of such treatment a therapeutically effective amount of a compound that modulates expression of said nucleic acid molecule or that modulates activity of said protein.

83. The method of claim 82, wherein the compound decreases the expression of said nucleic acid molecule or decreases the activity of said protein.

84. The method of claim 82, wherein the treated condition is pain.

85. The method of claim 84, wherein the treated condition is selected from the group consisting of neuropathic pain, nociceptive pain, chronic pain, inflammatory pain, pain associated with cancer, and pain associated with rheumatic disease.

86. The method of claim 82, wherein the treated condition is selected from the group consisting of addiction, seizure stroke, ischemia, a neurodegenerative disorder, anxiety, depression, headache, asthma, rheumatic disease, osteoarthritis, retinopathy, inflammatory eye disorders, pruritis, ulcer, gastric lesions, uncontrollable urination, an inflammatory or unstable bladder disorder, inflammatory bowel disease, irritable bowel syndrome (IBS), irritable bowel disease (IBD), gastroesophageal reflux disease (GERD), functional dyspepsia, functional chest pain of presumed oesophageal origin, functional dysphagia, non-cardiac chest pain, symptomatic gastroesophageal disease, gastritis, aerophagia, functional constipation, functional diarrhea, burbulence, chronic functional abdominal pain, recurrent abdominal pain (RAP), function abdominal bloating, functional biliary pain, functional incontinence, functional ano-rectal disorder, cholecystalgia, interstitial dysitis, dysmenorrhea, and dyspareunia.

87. A method for identifying new targets for pain, said method comprising:

(a) providing a first protein, or fragment thereof, encoded by any one of **SEQ ID NOS: 1-249**;

(b) providing a second protein, or fragment thereof, in close proximity to said first protein; and

(c) measuring an interaction between said first and second protein;

wherein a measurable interaction between said first and second proteins indicates that the second protein is a ligand or binding partner for said first protein, and therefore a candidate target for pain.

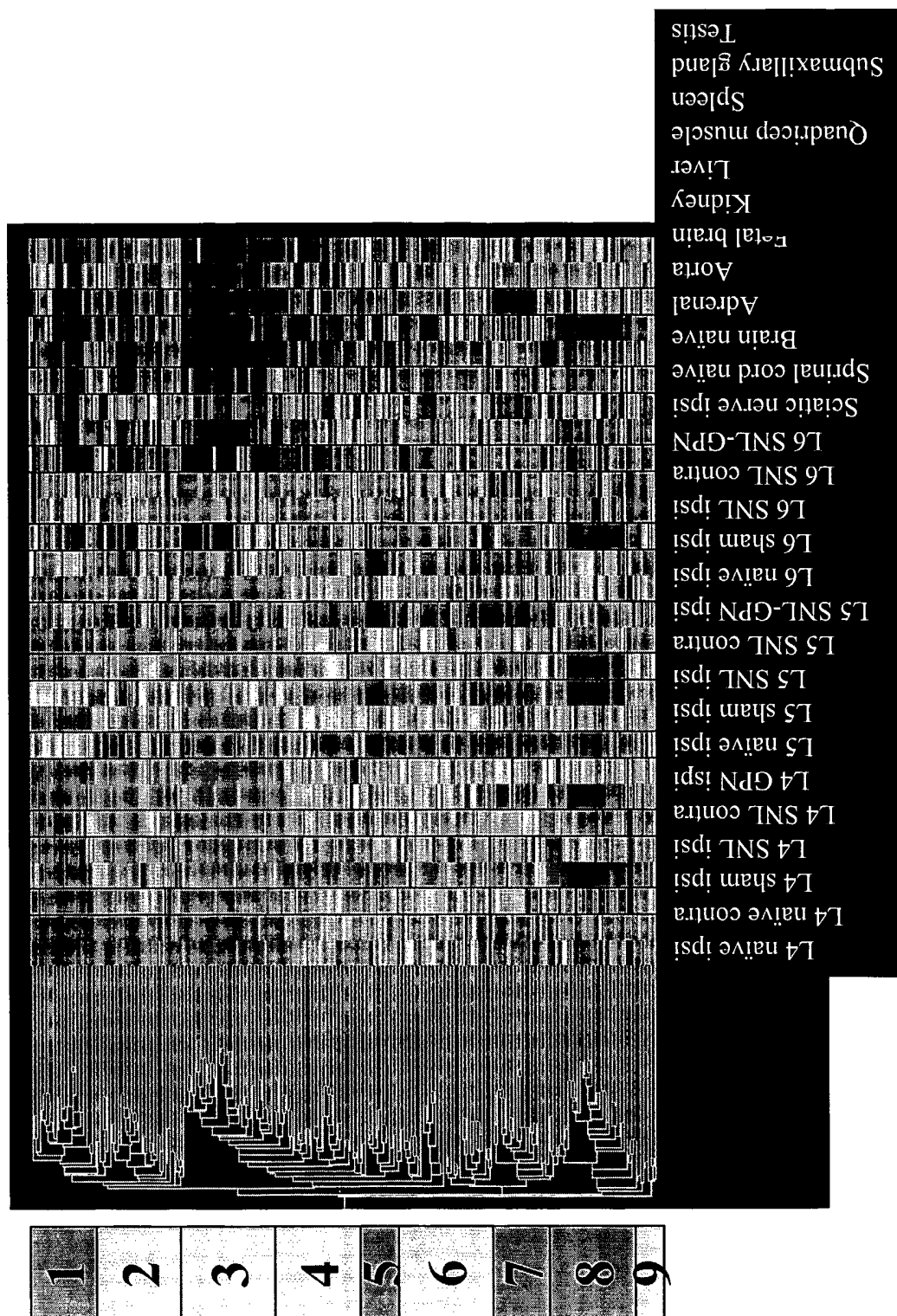
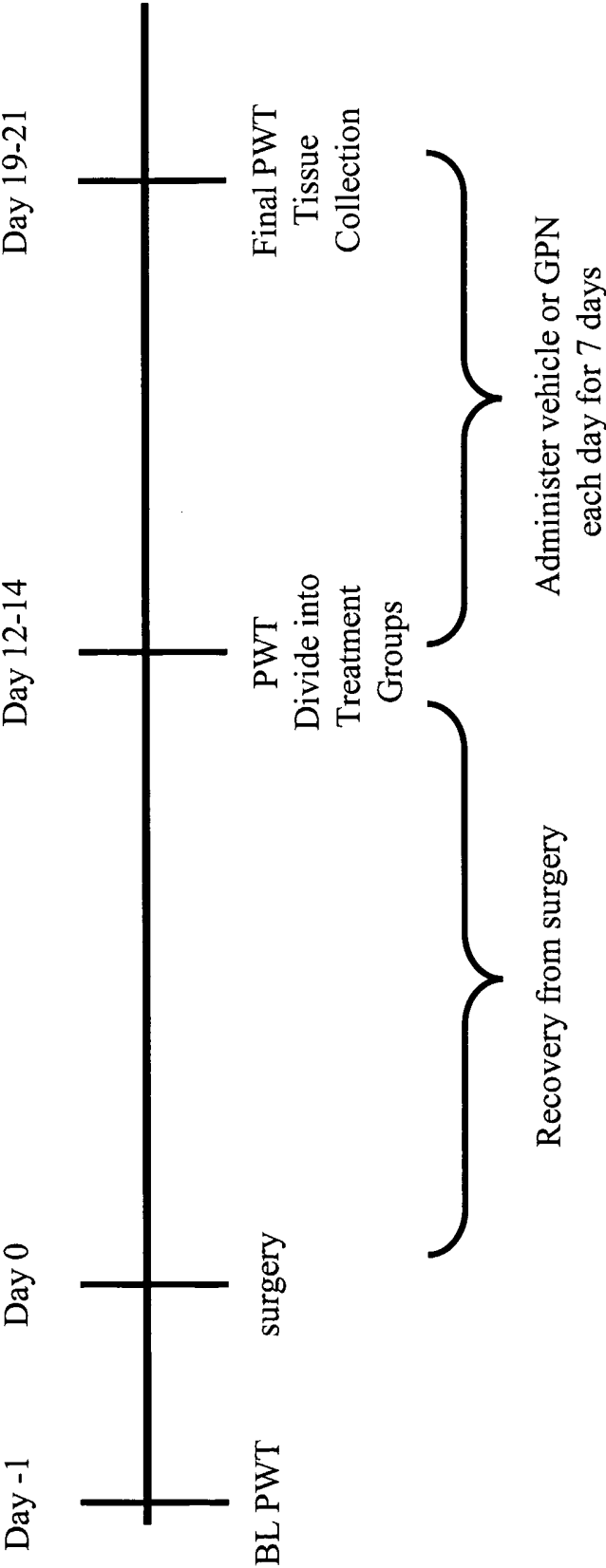


Figure 2



PWT = paw withdrawal threshold (paw pressure test)
BL = baseline
GPN = 100 mg/kg gabapentin i.p.