

**(12) PATENT
(19) AUSTRALIAN PATENT OFFICE**

**(11) Application No. AU 199952657 B2
(10) Patent No. 765918**

(54) Title
Vertebrate patched-2 protein

(51)⁷ International Patent Classification(s)
**C12N 015/12 C12N 005/10
C07K 014/705 C12N 015/11
C07K 019/00 C12N 015/62
C12N 001/19 G01N 033/68
C12N 001/21**

(21) Application No: **199952657** (22) Application Date: **1999.04.02**

(87) WIPO No: **WO99/53058**

(30) Priority Data

(31) Number **09/060939** (32) Date **1998.04.15** (33) Country **US**

(43) Publication Date : **1999.11.01**

(43) Publication Journal Date : **2000.01.06**

(44) Accepted Journal Date : **2003.10.02**

(71) Applicant(s)
Genentech, Inc.

(72) Inventor(s)
Frederic J De Sauvage; David A Carpenter

(74) Agent/Attorney
Griffith Hack, GPO Box 1285K, MELBOURNE VIC 3001

(56) Related Art
**ACCESSION 060589
TAKABATAKE ET AL FEBS LETTERS VOL 410:485-489**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

52657/99



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ :	C12N 15/12, C07K 14/705, C12N 1/19, 1/21, 5/10, 15/62, C07K 19/00, C12N 15/11, G01N 33/68	A1	(11) International Publication Number: WO 99/53058
			(43) International Publication Date: 21 October 1999 (21.10.99)

(21) International Application Number: PCT/US99/07417

(22) International Filing Date: 2 April 1999 (02.04.99)

(30) Priority Data:
09/060,939 15 April 1998 (15.04.98) US

(71) Applicant: GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors: DE SAUVAGE, Frederic, J.; 187 Shooting Star Isle, Foster City, CA 94404 (US). CARPENTER, David, A.; 1582 22nd Avenue, San Francisco, CA 94122 (US).

(74) Agents: SVOBODA, Craig, G. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

IP AUSTRALIA

1 NOV 1999

R E C E I V E D

(54) Title: VERTEBRATE PATCHED-2 PROTEIN

(57) Abstract

The present invention relates to nucleotide sequences, including expressed sequence tags (ESTs), oligonucleotide probes, polypeptides, antibodies, vectors and host cells expressing, immunoadhesins, agonists and antagonists to *patched-2*.

Patched-2

FIELD OF THE INVENTION

- 5 The present invention relates generally to signaling molecules, specifically to signaling and mediator molecules in the hedgehog (hh) cascade which are involved in cell proliferation and differentiation.

BACKGROUND OF THE INVENTION

10 All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

15 Development of multicellular organisms depends, at least in part, on mechanisms which specify, direct or maintain positional information to pattern cells, tissues, or organs. Various secreted signaling molecules, such as members of the transforming growth factor-beta (TGF- β), Wnt, fibroblast growth factors and hedgehog families have been associated with patterning activity of different cells and structures in Drosophila as well as in vertebrates. Perrimon, Cell: 80: 517-520 (1995).

20 Segment polarity genes were first discovered in Drosophila, which when mutated caused a change in the pattern of structures of the body segments. These changes affected the pattern along the head to tail axis. Hedgehog (Hh) was first identified as a segment-polarity gene by a genetic screen in Drosophila melanogaster, Nusslein-Volhard et al., Roux. Arch. Dev. Biol. 193: 267-282 (1984), that plays a wide variety of developmental functions. Perrimon, *supra*. Although only one Drosophila Hh gene has been 25 identified, three mammalian Hh homologues have been isolated: Sonic Hh (Shh), Desert Hh (Dhh) and Indian Hh (Ihh), Echelard et al., Cell 75: 1417-30 (1993); Riddle et al., Cell 75: 1401-16 (1993). Shh is expressed at high level in the notochord and floor plate of developing vertebrate embryos, and acts to establish cell fate in the developing limb, somites and neural tube. In vitro explant assays as well as ectopic expression of Shh in transgenic animals show that Shh plays a key role in neural tube patterning,

30 Echelard et al. (1993), *supra*; Ericson et al., Cell 81: 747-56 (1995); Marti et al., Nature 375: 322-5 (1995); Roelink et al. (1995), *supra*; Hynes et al., Neuron 19: 15-26 (1997). Hh also plays a role in the development of limbs (Krauss et al., Cell 75: 1431-44 (1993); Laufer et al., Cell 79, 993-1003 (1994)), somites (Fan and Tessier-Lavigne, Cell 79, 1175-86 (1994); Johnson et al., Cell 79: 1165-73 (1994)), lungs (Bellusci et al., Develop. 124: 53-63 (1997) and skin (Oro et al., Science 276: 817-21 (1997)).

35 Likewise, Ihh and Dhh are involved in bone, gut and germinal cell development, Apelqvist et al., Curr. Biol. 7: 801-4 (1997); Bellusci et al., Development 124: 53-63 (1997); Bitgood et al., Curr. Biol. 6: 298-304 (1996); Roberts et al., Development 121: 3163-74 (1995). Specifically, Ihh has been implicated in chondrocyte development [Vortkamp, A. et al., Science 273: 613-22 (1996)] while Dhh plays a key role

in testis development. Bitgood et al., *supra*. With the exception of the gut, in which both Ihh and Shh are expressed, the expression patterns of the hedgehog family members do not overlap. Bitgood et al., *supra*.

At the cell surface, Hh function appears to be mediated by a multicomponent receptor complex involving patched (ptch) and smoothened (smo), two multi-transmembrane proteins initially identified as 5 segment polarity genes in *Drosophila* and later characterized in vertebrates. Nakano et al., *Nature* 341: 508-513 (1989); Goodrich et al., *Genes Dev.* 10: 301-312 (1996); Marigo et al., *Develop.* 122: 1225-1233 (1996); van den Heuvel, M. & Ingham, P.W., *Nature* 382: 547-551 (1996); Alcedo, J. et al., *Cell* 86: 221-232 (1996); Stone, D.M. et al., *Nature* 384: 129-34 (1996). Upon binding of Hh to Ptch, the normal inhibitory effect of Ptch on Smo is relieved, allowing Smo to transduce the Hh signal across the plasma 10 membrane. It

remains to be established if the *Ptch/Smo* receptor complex mediates the action of all 3 mammalian hedgehogs or if specific components exist. Interestingly, a second murine *Ptch* gene, *Ptch-2* was recently isolated [Motoyama, J. et al., *Nature Genetics* 18: 104-106 (1998)], but its function as a *Hh* receptor has not been established. In order to characterize *Ptch-2* and compare it to *Ptch* with respect to the biological 5 function of the various *Hh* family members, Applicants have isolated the human *Ptch-2* gene. Biochemical analysis of *Ptch* and *Ptch-2* show that both bind to all members of the *Hh* family with similar affinity and that both molecules can form a complex with *Smo*. However, the expression patterns of *Ptch-2* and *Ptch* do not overlap. While *Ptch* is expressed throughout the mouse embryo, *Ptch-2* is found mainly in 10 spermatocytes which require Desert Hedgehog (*Dhh*) for proper development suggesting that *Ptch-2* mediates *Dhh*'s activity in the testis. Chromosomal localization of *Ptch-2* places it on chromosome 1p33-34, a region deleted in some germ cell tumors, raising the possibility that *Ptch-2* may be a tumor suppressor in 15 *Dhh* target cells.

SUMMARY OF THE INVENTION

In one embodiment, the invention provides an isolated nucleic acid molecule having at least about 15 80% sequence identity to (a) a DNA molecule encoding a patched-2 polypeptide comprising the sequence of amino acids 1 to 1203 of Fig. 1, or (b) the complement of the DNA molecule of (a); and encoding a polypeptide having *patched-2* biological activity. The sequence identity preferably is > 91%, more preferably about 92%, most preferably about 95%. In one aspect, the isolated nucleic acid has at least > 91%, 20 preferably at least about 92%, and even more preferably at least about 95% sequence identity with a polypeptide having amino acid residues 1 to about 1203 of Fig. 1. In a further aspect, the isolated nucleic acid molecule comprises DNA encoding a human *patched-2* polypeptide having amino acid residues 1 to about 1203 of Fig. 1. In yet another aspect, the invention provides for an isolated nucleic acid comprising DNA having at least a 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the cDNA in ATCC Deposit No. 209778 (designation: pRK7.hptc2.Flag-1405), alternatively the 25 coding sequence of clone pRK7.hptc2.Flag-1405, deposited under accession number ATCC 209778. In a still further aspect, the invention provides for a nucleic acid comprising human *patched-2* encoding sequence of the cDNA in ATCC deposit No. 209778 (designation: pRK7.hptc2.Flag-1405) or a sequence which hybridizes thereto under stringent conditions.

In another embodiment, the invention provides a vector comprising DNA encoding a human 30 *patched-2* polypeptide. A host cell comprising such a vector is also provided. By way of example, the host cells may be mammalian cells, (e.g. CHO cells), prokaryotic cells (e.g., *E. coli*) or yeast cells (e.g., *Saccharomyces cerevisiae*). A process for producing *patched-2* polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of *patched-2* and recovering the same from the cell culture.

35 In yet another embodiment, the invention provides an isolated *patched-2* polypeptide. In particular, the invention provides isolated native sequence *patched-2* polypeptide, which in one embodiment is a human *patched-2* including an amino acid sequence comprising residues 1 to about 1203 of Figure 1. Human *patched-2* polypeptides with or without the initiating methionine are specifically included. Alternatively, the invention provides a human *patched-2* polypeptide encoded by the nucleic acid deposited under accession

number ATCC Deposit No. 209778.

In yet another embodiment, the invention provides chimeric molecules comprising a *patched-2* polypeptide *patched-2* to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises a *patched-2* polypeptide *patched-2* to an epitope tag sequence or a constant region of an immunoglobulin.

In yet another embodiment, the invention provides expressed sequence tag (EST) comprising the nucleotide sequences identified in Fig. 2A (905531) (SEQ ID NO:3) and Fig. 2B (1326258) (SEQ ID NO:5).

In yet another embodiment, the invention provides for alternatively spliced variants of human *patched-2* having *patched-2* biological activity.

In yet another embodiment, the invention provides for method of using *patched-2* for the treatment of disorders which are mediated at least in part by Hedgehog (*Hh*), especially Desert hedgehog (*Dhh*). In particular, testicular cancer. In yet another embodiment, the invention provides a method of using antagonists or agonists of *patched-2* for treating disorders or creating a desirable physiological condition effected by blocking *Hh* signaling, especially *Dhh* signaling. (E.g, contraception).

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide (SEQ ID NO:1) and derived amino acid (SEQ ID NO:2) sequence of a native sequence of human *Ptch-2*.

Figure 2A shows EST 905531 (SEQ ID NO:3) and Fig. 2B shows EST 1326258 (SEQ ID NO:5) in alignment with human *Ptch* (SEQ ID NO:18). These ESTs were used in the cloning of human full-length *Ptch-2* (SEQ ID NO:1).

Figure 3 shows a comparison between human *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2). Gaps introduced for optimal alignment are indicated by dashes. Identical amino acids are boxed. The 12 transmembrane domains are indicated by the gray boxes, all of which are conserved between the two sequences. Alignment results between the two sequences indicate 53% identity. The most significant difference is a shorter C-terminal intracellular domain in human *Ptch-2* (SEQ ID NO:2) in comparison with human *Ptch* (SEQ ID NO:4).

Figure 4 shows a northern blot of *Ptch-2* (SEQ ID NO:2) which indicates expression is limited to the testis. Multiple human fetal and adult tissue northern blots were probe fragments corresponding to the 3'-untranslated region of murine *Ptch-2*.

Figure 5 shows a chromosomal localization of two BAC clones which were isolated by PCR screening with human *patched-2* derived probes. Both probes were mapped by FISH to human chromosome 1p33-34.

Figure 6 is an *in situ* hybridization comparing *Ptch* (SEQ ID NO:4), *Ptch-2* (SEQ ID NO:2) and *Fused* (FuRK) (SEQ ID NO:10) expression. High magnification of mouse testis showing expression of (a) *Ptch*, *Ptch-2* (SEQ ID NO:2) (b) and FuRK (SEQ ID NO:10) (c). Low magnification of testis section hybridized with *Ptch-2* sense (SEQ ID NO:11) (d) and anti-sense probe (SEQ ID NO:12) (e) respectively. Fig. 6(f) shows low magnification of testis section hybridized with FuRK (SEQ ID NO:10 encoding nucleic acid). Scale bar: a, b, c: 0.05 mm; d, e, f: 0.33 mm.

Figure 7A is logarithmic plot comparing the binding *Ptch-2* (SEQ ID NO:2) to *Dhh* (SEQ ID

NO:13) and Shh (SEQ ID NO:14). Competitive binding of recombinant murine 125I-Shh to 293 cells overexpressing Ptch (SEQ ID NO:4) or Ptch-2 (SEQ ID NO:2). There was no detectable binding to mock transfected cells (data not shown). Figure 7B is a western blot illustrating co-immunoprecipitation of epitope tagged Ptch (SEQ ID NO:4) or Ptch-2 (SEQ ID NO:2) with epitope tagged Smo (SEQ ID NO:15). Immunoprecipitation was performed with antibodies to the Flag tagged Ptch (SEQ ID NO:4) and analyzed on a 6% acrylamide gel with antibodies to the Myc tagged Smo (SEQ ID NO:15). Protein complexes can be detected for both Ptch (SEQ ID NO:4) and Ptch-2 (SEQ ID NO:2) with Smo (SEQ ID NO:15). Ptch (SEQ ID NO:4) and Ptch-2 (SEQ ID NO:2) express at similar levels as shown by immunoprecipitation using antibodies to the Flag-tag and western blot using the same anti-Flag antibody.

10 Figure 8 is a sequence comparison between human Ptch-2 (SEQ ID NO:2) and murine Ptch-2 (SEQ ID NO:7), which indicates that there is about 91% identity between the two sequences.

Figure 9 is an in situ hybridization which demonstrates the accumulation of Ptch (SEQ ID NO:4) and Ptch-2 (SEQ ID NO:2) mRNA detected by in situ hybridization in basal cells of E18 transgenic mice overexpressing SMO-M2 (SEQ ID NO:16) (Xie et al., Nature 391: 90-92 (1998)).

15 Figure 10 is a partial sequence representing clone 3A (SEQ ID NO:8), a partial patched-2 fragment which was initially isolated from a fetal brain library.

Figure 11 is a partial sequence representing clone 16.1 (SEQ ID NO:9), a partial patched-2 fragment which isolated from a testis library.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20 I. Definitions

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

The terms "patched-2" and "patched-2 polypeptide" when used herein encompass native sequence patched-2 and patched-2 variants (which are further defined herein) having patched-2 biological activity.

25 Patched-2 may be isolated from a variety of sources, such as from testes tissue types or from another source, or prepared by recombinant or synthetic methods.

30 A "native sequence patched-2" comprises a polypeptide having the same amino acid sequence as a human patched-2 derived from nature. Such native sequence patched-2 can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence vertebrate patched-2" specifically encompasses naturally occurring truncated forms of human patched-2, naturally occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of human patched-2. Thus, one embodiment of the invention, the native sequence patched-2 is a mature or full-length native Ptch-2 comprising amino acids 1 to 1203 of Fig. 1 (SEQ ID NO:2) with or without the initiating methionine at position 1.

35 "Patched-2 variant" means an active human patched-2 as defined below having at least > 91% amino acid sequence identity to (a) a DNA molecule encoding a patched-2 polypeptide, or (b) the complement of the DNA molecule of (a). In a particular embodiment, the patched-2 variant has at least > 91% amino acid sequence homology with the human Ptch-2 (SEQ ID NO:2) having the deduced amino acid sequence shown in Fig. 1 for a full-length native sequence human patched-2. Such patched-2 variants

include, without limitation, *patched-2* polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the sequence of Fig. 1 (SEQ ID NO:2). Preferably, the nucleic acid or amino acid sequence identity is at least about 92%, more preferably at least about 93%, and even more preferably at least about 95%.

5 "Percent (%) amino acid sequence identity" with respect to the *patched-2* sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the *patched-2* sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using 10 publicly available computer software such as BLAST-2 software that are set to their default parameters. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

15 "Percent (%) nucleic acid sequence identity" with respect to the *patched-2* sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the *patched-2* sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using 20 publicly available computer software such as BLAST-2 software that are set to their default parameters. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising *patched-2* polypeptide, or a portion thereof, *patched-2* to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not 25 interfere with activity of the *patched-2* polypeptide. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 to about 50 amino acid residues (preferably, between about 10 to about 20 residues).

30 As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesin comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a 35 ligand. The immunoglobulin constant domain sequence in the immunoadhesins may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3 or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM. Immunoadhesion reported in the literature include fusions of the T cell receptor [Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 2936-2940 (1987)]; CD4⁺ [Capron *et al.*, *Nature* 337: 525-531 (1989); Traunecker *et al.*, *Nature* 339: 68-70 (1989); Zettmeissl *et al.*, *DNA Cell Biol. USA* 9: 347-353 (1990); Byrn

et al., *Nature* 344, 667-670 (1990)]; L-selectin (homing receptor) [Watson et al., *J. Cell. Biol.* 110, 2221-2229 (1990); Watson et al., *Nature* 349, 164-167 (1991)]; CD44* [Aruffo et al., *Cell* 61, 1303-1313 (1990)]; CD28* and B7* [Linsley et al., *J. Exp. Med.* 173, 721-730 (1991)]; CTLA-4* [Lisley et al., *J. Exp. Med.* 174, 561-569 (1991)]; CD22* [Stamenkovic et al., *Cell* 66, 1133-1144 (1991)]; TNF receptor [Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88, 10535-10539 (1991); Lesslauer et al., *Eur. J. Immunol.* 27, 2883-2886 (1991); Peppel et al., *J. Exp. Med.* 174, 1483-1489 (1991)]; NP receptors [Bennett et al., *J. Biol. Chem.* 266, 23060-23067 (1991)]; IgE receptor α -chain* [Ridgway and Gorman, *J. Cell. Biol.* 115, abstr. 1448 (1991)]; HGF receptor [Mark, M.R. et al., *J. Biol. Chem.*, 267(36): 26166-26171 (1992)], where the asterisk (*) indicates that the receptor is a member of the immunoglobulin superfamily.

10 "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends upon the ability of denatured DNA to reanneal when complementary strands are present in an environment near but below their T_m (melting temperature). The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. Moreover, stringency is also inversely proportional to salt concentrations. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., *Current Protocols in Molecular Biology* (1995).

20 "Stringent conditions," as defined herein may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μ g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

30 "Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the vertebrate *patched-2* natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" *patched-2* nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the *patched-2* nucleic acid. An isolated *patched-2* nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated *patched-2* nucleic acid molecules therefore are 5 distinguished from the corresponding native *patched-2* nucleic acid molecule as it exists in natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

10 Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate 15 translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

20 The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies), antibody compositions with polyepitopic specificity, as well as antibody fragments (e.g., Fab, F(ab')₂ and Fv), so long as they exhibit the desired biological 25 activity.

25 The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are 30 synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler & Milstein, Nature 256:495 (1975), or may be 35 made by recombinant DNA methods [see, e.g. U.S. Patent No. 4,816,567 (Cabilly *et al.*)].

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while

the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity [U.S. Patent No. 4,816,567; Cabilly *et al.*; Morrison *et al.*, Proc. Natl. Acad. Sci. USA **81**, 6851-6855 (1984)].

5 "Humanized" forms of non-human (e.g. murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a 10 non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, corresponding non-human residues replace Fv framework residues of the human immunoglobulin. Furthermore, humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise 15 substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see: Jones *et al.*, Nature **321**, 522-525 (1986); Riechmann *et al.*, Nature 20 **332**, 323-327 (1988); Presta, Curr. Op. Struct. Biol. **2** 593-596 (1992) and U.S. Patent No. 5,225,539 (Winter) issued July 6, 1993.

"Active" or "activity" for the purposes herein refers to form(s) of *patched-2* which retain the biologic and/or immunologic activities of native or naturally occurring *patched-2*. A preferred activity is the ability to bind to and affect, e.g., block or otherwise modulate, *hedgehog* (*Hh*), especially *desert hedgehog* (*Dhh*) signaling. For example, the regulation of the pathogenesis of testicular cancer, male spermatocyte formation and basal cell carcinoma.

The term "antagonist" is used herein in the broadest sense to include any molecule which blocks, prevents, inhibits, neutralizes the normal functioning of *patched-2* in the *hedgehog* (*Hh*) signaling pathway. One particular form of antagonist includes a molecule that interferes with the interaction between *Dhh* and *patched-2*. Alternatively, an antagonist could also be a molecule which increases the levels of *patched-2*. In a similar manner, the term "agonist" is used herein to include any molecule which promotes, enhances or stimulates the binding of a *Hh* to *patched-2* in the *Hh* signaling pathway or otherwise upregulates it (e.g., blocking binding of *Ptch-2* (SEQ ID NO:2) to *Smo* (SEQ ID NO:17)). Suitable molecules that affect the protein-protein interaction of *Hh* and *patched-2* and its binding proteins include fragments of the latter or 30 small bioorganic molecules, e.g., peptidomimetics, which will prevent or enhance, as the case may be, the binding of *Hh* to *patched-2*. Non-limiting examples include proteins, peptides, glycoproteins, glycopeptides, glycolipids, polysaccharides, oligosaccharides, nucleic acids, bioorganic molecules, peptidomimetics, pharmacological agents and their metabolites, transcriptional and translation control sequences, and the like.

Another preferred form of antagonist includes antisense oligonucleotides that inhibit proper transcription of wild type *patched-2*.

The term "modulation" or "modulating" means upregulation or downregulation of a signaling pathway. Cellular processes under the control of signal transduction may include, but are not limited to, transcription of specific genes; normal cellular functions, such as metabolism, proliferation, differentiation, adhesion, apoptosis and survival, as well as abnormal processes, such as transformation, blocking of differentiation and metastasis.

The techniques of "polymerase chain reaction," or "PCR", as used herein generally refers to a procedure wherein minute amounts of a specific piece of nucleic acid, RNA and/or DNA are amplified as described in U.S. Pat. No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers may coincide with the ends of the amplified material. PCR sequences form total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage, or plasmid sequences, etc. See generally Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 51: 263 (1987); Erlich, Ed., *PCR Technology*, (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

II. Compositions and Methods of the Invention

20 A. Full-length *patched-2*

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as *patched-2*. In particular, Applicants have identified and isolated cDNA encoding a human *patched-2* polypeptide, as disclosed in further detail in the Examples below. Using BLAST, BLAST-2 and FastA sequence alignment computer programs (set to the default parameters). Applicants found that a full-length native sequence human *patched-2* (i.e., *Ptch-2* in Figure 3, SEQ ID NO:2) has 53% amino acid sequence identity with a human *patched* (i.e., *Ptch*, SEQ ID NO:4). Moreover a human full-length *patched-2* (i.e., *Ptch-2*, SEQ ID NO:2) has about a 91% sequence identity with murine *Ptch-2* (SEQ ID NO:7) (Fig. 8). Accordingly, it is presently believed that the human *patched-2* (i.e., *Ptch-2*, SEQ ID NO:2) disclosed in the present application is a newly identified member of the 30 mammalian hedgehog signaling cascade, specifically *Desert hedgehog*.

The full-length native sequence of human *patched-2* gene, or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length gene or to isolate still other vertebrate homolog genes (for instance, those encoding naturally-occurring variants of *patched-2* or *patched-2* from other species) which have a desired sequence identity to the human *patched-2* sequence disclosed in Fig. 1 (SEQ ID NO:2). Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from the nucleotide sequence of Fig. 1 (SEQ ID NO:1) or from genomic sequences including promoters, enhancer elements and introns of native sequence vertebrate *patched-2*. By way of example, a screening method will comprise isolating the coding region of the vertebrate *patched-2* gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may

be labeled by a variety of labels, including radionucleotides such as ^{32}P or ^{35}S , or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the vertebrate *patched-2* gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine which members of such libraries the probe hybridizes to.

5 B. *Patched-2* Variants

In addition to the full-length native sequence *patched-2* described herein, it is contemplated that *patched-2* variants can be prepared. *Patched-2* variants can be prepared by introducing appropriate nucleotide changes into a known *patched-2* DNA, or by synthesis of the desired *patched-2* polypeptides. 10 Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of *patched-2*.

Variations in the native full-length sequence *patched-2* or in various domains of the *patched-2* described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a 15 substitution, deletion or insertion of one or more codons encoding the *patched-2* that results in a change in the amino acid sequence of *patched-2* as compared with the native sequence *patched-2*. Optionally, the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of *patched-2*. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the 20 *patched-2* with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of 1 to 5 amino acids. The variation allowed may be determined by 25 systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity in the *in vitro* assay described in the Examples below.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10: 6487 (1987)], cassette mutagenesis 30 [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the vertebrate *patched-2* variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. 35 Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The

Proteins. (W.H. Freeman & Co., N.Y.); Chothia. *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

In the comparison between human *patched* and *patched-2* sequences depicted in Figure 3 (e.g., *Ptch*, SEQ ID NO:4 and *Ptch-2*, SEQ ID NO:2), the 12 transmembrane domains are identified in gray, while identical residues are boxed. Gaps are indicated by dashes (-) and are inserted to maximize the total identity score between the two sequences.

5 C. Modifications of *patched-2*

Covalent modifications of *patched-2* are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of *patched-2* with an organic 10 derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the *patched-2*. Derivatization with bifunctional agents is useful, for instance, for crosslinking *patched-2* to a water-insoluble support matrix or surface for use in the method for purifying anti-*patched-2* antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazo-acetyl)-2-phenylethane, glutaraldehyde, N-hydroxy-succinimide esters, for example, esters with 4-azido-salicylic acid, 15 homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidyl-propionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)-dithio]propioimidate.

Other modifications include deamidation of glutamyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, 20 phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation 25 of any C-terminal carboxyl group.

Another type of covalent modification of *patched-2* comprises linking the *patched-2* polypeptide to 25 one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. Such modifications would be expected to increase the half-life of the molecules in circulation in a mammalian system; Extended half-life of *patched-2* molecules might be useful under certain 30 circumstances, such as where the *patched-2* variant is administered as a therapeutic agent.

The *patched-2* of the present invention may also be modified in a way to form a chimeric molecule 35 comprising *patched-2* bonded to another, heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of *patched-2* with a tag polypeptide, which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the *patched-2*. The presence of such epitope-tagged forms of the *patched-2* can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag 40 enables the *patched-2* to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. In an alternative embodiment, the chimeric molecule may comprise a fusion of the *patched-2* with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule.

Ordinarily, the C-terminus of a contiguous amino acid sequence of a *patched-2* receptor is fused to the N-terminus of a contiguous amino acid sequence of an immunoglobulin constant region, in place of the variable region(s), however N-terminal fusions are also possible.

Typically, such fusions retain at least functionally active hinge, CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the CH1 of the heavy chain or the corresponding region of the light chain. This ordinarily is accomplished by constructing the appropriate DNA sequence and expressing it in recombinant cell culture. Alternatively, immunoadhesins may be synthesized according to known methods.

The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion or binding characteristics of the immunoadhesins.

In a preferred embodiment, the C-terminus of a contiguous amino acid sequence which comprises the binding site(s) of *patched-2*, at the N-terminal end, to the C-terminal portion of an antibody (in particular the Fc domain), containing the effector functions of an immunoglobulin, e.g. immunoglobulin G₁ (IgG-1). As herein above mentioned, it is possible to fuse the entire heavy chain constant region to the sequence containing the binding site(s). However, more preferably, a sequence beginning in the hinge region just upstream of the papain cleavage site (which defines IgG Fc chemically; residue 216, taking the first residue of heavy chain constant region to be 114 [Kobat *et al.*, *supra*], or analogous sites of other immunoglobulins) is used in the fusion. Although it was earlier thought that in immunoadhesins the immunoglobulin light chain would be required for efficient secretion of the heterologous protein-heavy chain fusion proteins, it has been found that even the immunoadhesins containing the whole IgG1 heavy chain are efficiently secreted in the absence of light chain. Since the light chain is unnecessary, the immunoglobulin heavy chain constant domain sequence used in the construction of the immunoadhesins of the present invention may be devoid of a light chain binding site. This can be achieved by removing or sufficiently altering immunoglobulin heavy chain sequence elements to which the light chain is ordinarily linked so that such binding is no longer possible. Thus, the CH1 domain can be entirely removed in certain embodiments of the *patched-2*/immunoglobulin chimeras.

In a particularly preferred embodiment, the amino acid sequence containing the extracellular domain(s) of *patched-2* is fused to the hinge region and CH2, CH3; or CH1, hinge, CH2 and CH3 domains of an IgG-1, IgG-2, IgG-3, or IgG-4 heavy chain.

In some embodiments, the *patched-2*/immunoglobulin molecules (immunoadhesins) are assembled as monomers, dimers or multimers, and particularly as dimers or tetramers. Generally, these assembled immunoadhesins will have known unit structures similar to those of the corresponding immunoglobulins. A basic four chain structural unit (a dimer of two immunoglobulin heavy chain-light chain pairs) is the form in which IgG, IgA and IgE exist. A four chain unit is repeated in the high molecular weight immunoglobulins; IgM generally exists as a pentamer of basic four-chain units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in a multimeric form in serum. In the case of multimers, each four chain unit may be the same or different.

It is not necessary that the entire immunoglobulin portion of the *patched-2*/immunoglobulin chimeras be from the same immunoglobulin. Various portions of different immunoglobulins may be combined, and

variants and derivatives of native immunoglobulins can be made as herein above described with respect to *patched-2*, in order to optimize the properties of the immunoadhesin molecules. For example, immunoadhesin constructs in which the hinge of IgG-1 was replaced with that of IgG-3 were found to be functional and showed pharmacokinetics comparable to those of immunoadhesins comprising the entire IgG-1 heavy chain.

5 Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8: 2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein 10 Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an α -tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)]. A preferred tag is the influenza HA tag.

15 D. Preparation of *patched-2*

The description below relates primarily to production of a particular *patched-2* by culturing cells transformed or transfected with a vector containing *patched-2* nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare *patched-2*. For instance, the *patched-2* sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the vertebrate *patched-2* may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length *patched-2*.

20 1. Isolation of DNA encoding vertebrate *patched-2*

DNA encoding *patched-2* may be obtained from a cDNA library prepared from tissue believed to possess the *patched-2* mRNA and to express it at a detectable level. Accordingly, human *patched-2* DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as 25 described in the Examples. The vertebrate *patched-2*-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

30 Libraries can be screened with probes (such as antibodies to the *patched-2* or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as 35 described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding vertebrate *patched-2* is to use PCR methodology [Sambrook et al., supra; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide

sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ³²P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including 5 moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer 10 software programs such as BLAST, BLAST-2, ALIGN, DNAstar, and INHERIT which employ various algorithms to measure homology.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect 15 precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for *patched-2* production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired 20 sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example, CaPO₄ and 25 electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian 30 cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology* 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*, 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by 35 nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology*, 185:527-537 (1990) and Mansour *et al.*, *Nature*, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote,

yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635).

5 In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for vertebrate *patched-2*-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism.

10 Suitable host cells for the expression of vertebrate *patched-2* are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen. Virol.*, 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

3. Selection and Use of a Replicable Vector

20 The nucleic acid (e.g., cDNA or genomic DNA) encoding *patched-2* may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin 25 of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques, which are known to the skilled artisan.

30 Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. A preferred replicable expression vector is the plasmid pRK5. Holmes *et al.*, *Science*, 253:1278-1280 (1991).

35 Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the *patched-2* nucleic acid, such as DHFR or thymidine kinase.

An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.* *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb *et al.* *Nature*, 282:39 (1979); Kingsman *et al.* *Gene*, 7:141 (1979); Tschemper *et al.* *Gene*, 10:157 (1980)]. The 5 *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the *patched-2* nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β -lactamase and lactose promoter 10 systems [Chang *et al.* *Nature*, 275:615 (1978); Goeddel *et al.* *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer *et al.* *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding *patched-2*.

15 Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.* *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.* *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-fructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, 20 phosphoglucose isomerase, and glucokinase.

25 Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

30 *Patched-2* transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

35 Inserting an enhancer sequence into the vector may increase transcription of a DNA encoding the *patched-2* by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at

a position 5' or 3' to the *patched-2* coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding *patched-2*.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of *patched-2* in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence *patched-2* polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence to *patched-2* DNA and encoding a specific antibody epitope.

5. Purification of Polypeptide

Forms of *patched-2* may be recovered from host cell lysates. Since *patched-2* is membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of *patched-2* can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify *patched-2* from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the *patched-2*. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods

in *Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*. Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular *patched-2* produced.

E. Uses for *patched-2*

5 (1) *Patched-2 is a specific receptor for desert hedgehog (Dhh)*

The *hedgehog (Hh)* signaling pathway has been implicated in the formation of embryonic structures in mammals and invertebrates. The multi-pass transmembrane receptor *patched*, is a negative regulator of the *Hh* pathway, repressing the serpentine signaling molecule *smoothened (Smo)*. Data have shown that loss of *Patched* leads to deregulation of the *Hh* pathway leading to formation of aberrant structures in the 10 embryos and carcinoma in the adult.

Applicants' newly identified second human *patched* gene, termed *patched-2* (e.g., *Ptch-2*, SEQ ID NO:2), has a similar 12 transmembrane domain topology as does *patched*, and can bind to all the members of the *Hh* family and can complex with *Smo* (e.g., SEQ ID NO:17). However, the expression patterns of *patched-2* and *patched* do not overlap. *Patched-2* is expressed mainly in the developing spermatocytes, 15 which are supported directly by the *Desert hedgehog* producing Sertoli cells, which suggests that *patched-2* is a receptor for *Desert hedgehog*.

In the adult tubule, Sertoli cells, which are unusually large secretory cells, traverse the seminiferous tubule from the basal lamina to the luminal aspect, sending out cytoplasmic protrusions that engulf the germ cells. These contacts are particularly close during spermiogenesis, in which the haploid round spermatids undergo differentiation to produce the highly specialized, motile sperm. Tight junctions between adjacent Sertoli cells compartmentalize the tubule into a basal region, which contains mitotic spermatogonia and early spermatocytes, and an adluminal compartment, which contains meiotic spermatocytes and maturing spermatids. In fact, a Sertoli-derived cell line supports the meiotic progression of germ cells in culture, consistent with the view that factors derived from Sertoli cells contribute to germ cell maturation, 25 Rassoulzadegan, M., *et al.*, *Cell* 1993, 75: 997-1006. Loss of *Dhh* activity results in a recessive, sex-specific phenotype. Female mice homozygous for the mutation were fully viable and fertile, whereas male mice were viable but infertile. A gross examination indicated that, as early as 18.5 dpc, the testes of mutant males were noticeably smaller than those of heterozygous littermates. Bitgood *et al.*, *Curr. Biol.*, 1996 6(3): 298-304. Thus, Sertoli cells likely independently regulate mitotic and meiotic stages of germ cell development 30 during postnatal development. Therefore, since *patched-2* appears to be the receptor for *Dhh* (SEQ ID NO:13), molecules which modulate the binding of *Dhh* (SEQ ID NO:13) to *patched-2* would affect the activation of *Dhh* (SEQ ID NO:13) signaling, and thereby would have utility in the treatment of conditions which are modulated by *Dhh* (SEQ ID NO:13). (For example, testicular cancer). Alternatively, it is also provided that antagonists or agonists of *patched-2* may be used for treating disorders or creating a desirable 35 physiological condition effected by blocking *Dhh* signaling. (E.g, contraception, infertility treatment).

(2) *General uses for *patched-2**

Nucleotide sequences (or their complement) encoding *patched-2* have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. *Patched-2* nucleic acid will also be useful for the preparation of

patched-2 polypeptides by the recombinant techniques described herein.

The full-length native sequence *patched-2* gene, or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length gene or to isolate still other genes (for instance, those encoding naturally-occurring variants of *patched-2*) which have a desired sequence identity to the *patched-2* sequence disclosed in Fig. 1 (SEQ ID NO:1). Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from the nucleotide sequence of Fig. 1 (SEQ ID NO:1) or from genomic sequences including promoters, enhancer elements and introns of native sequence *patched-2*. By way of example, a screening method will comprise isolating the coding region of the *patched-2* gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as ^{32}P or ^{35}S , or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the *patched-2* gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine to which members of such libraries the probe hybridizes. Hybridization techniques are described in further detail in the Examples below.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related *patched-2* sequences.

Nucleotide sequences encoding *patched-2* can also be used to construct hybridization probes for mapping the gene, which encodes *patched-2* and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

Patched-2 polypeptides can be used in assays to identify the other proteins or molecules involved in complexing with *patched-2* which ultimately results in the modulation of *hedgehog* signaling. Alternatively, these molecules can modulate the binding of *patched-2* to *Dhh* (SEQ ID NO:13). By such methods, inhibitors of the binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the substrate of *patched-2* can be used to isolate correlative complexing proteins. Screening assays can be designed to find lead compounds that mimic the biological activity of a native *patched-2* or to find those that act as a substrate for *patched-2*. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Such small molecule inhibitors could block the enzymatic action of *patched-2*, and thereby inhibit *hedgehog* signaling. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode *patched-2* or its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (e.g., a mouse or rat) is an animal having cells that contain a transgene, was introduced into the animal or an ancestor of the animal at a prenatal, e.g., an embryonic stage. A transgene is a DNA sequence that is integrated into the genome of a cell from which a

transgenic animal develops. In one embodiment, cDNA encoding *patched-2* can be used to clone genomic DNA encoding *patched-2* in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding *patched-2*. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for *patched-2* transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding *patched-2* introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding *patched-2*. Such animals can be used as tester animals for reagents thought to confer protection from, for example, 10 pathological conditions associated with its overexpression.

Non-human homologues of vertebrate *patched-2* can be used to construct a *patched-2* "knock out" animal which has a defective or altered gene encoding *patched-2* as a result of homologous recombination between the endogenous gene encoding *patched-2* and altered genomic DNA encoding *patched-2* introduced into an embryonic cell of the animal. For example, cDNA encoding *patched-2* can be used to clone genomic 15 DNA encoding *patched-2* in accordance with established techniques. A portion of the genomic DNA encoding *patched-2* can be deleted or replaced with another gene, such as a gene encoding a selectable marker that can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line 20 (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li *et al.*, *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster 25 animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the *patched-2* polypeptide.

30 Suppression or inhibition (antagonism) of *Dhh* signaling is also an objective of therapeutic strategies. Since *patched-2* can combine with all members of the hedgehog family (*i.e.*, *Shh*, *Dhh*, *Ihh*), antagonist molecules which prevent the binding of hedgehog molecules to *Ptch-2* (SEQ ID NO:2) have therapeutic utility. For example, *SHh* signaling is known to be activated in Basal Cell Carcinoma: *Dhh* (SEQ ID NO:13) is known to be involved in the regulation of spermatogenesis. Inhibitor or antagonist of *Hh* 35 signaling would be effective therapeutics in the treatment of Basal Cell Carcinoma or male contraception, respectively.

The stimulation of *Dhh* signaling (agonism) is also an objective of therapeutic strategies. Since *Ptch-2* (SEQ ID NO:2) also binds to the other members of the *Hh* family, *Ihh* and *Shh*, activating *Dhh* signaling would be useful in disease states or disorders characterized by inactive or insufficient *Hh* signaling.

For example, degenerative disorders of the nervous system, e.g., Parkinson's disease, memory deficits, Alzheimer's disease, Lou Gehrig's disease, Huntington's disease, schizophrenia, stroke and drug addiction. Additionally, *patched-2* agonists could be used to treat gut diseases, bone diseases, skin diseases, diseases of the testis (including infertility), ulcers, lung diseases, diseases of the pancreas, diabetes, osteoporosis.

5 F. Anti-*patched-2* Antibodies

The present invention further provides anti- vertebrate *patched-2* antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

10 1. Polyclonal Antibodies

The anti-*patched-2* antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the *patched-2* polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants that may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

20 2. Monoclonal Antibodies

The anti-*patched-2* antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the *patched-2* polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression

of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Rockville, Maryland. Human myeloma and mouse-human heteromyeloma cell lines also have been 5 described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence 10 of monoclonal antibodies directed against *patched-2*. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution 15 procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture 20 medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be 25 readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be 30 modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another

amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

5 3. Humanized Antibodies

The anti-*patched-2* antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.*, 147(1):86-95 (1991)].

4. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the vertebrate *patched-2*, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

5 Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature*, **305**:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one
10 has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, **10**:3655-3659 (1991).

15 Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for
20 example, Suresh *et al.*, *Methods in Enzymology*, **121**:210 (1986).

5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and
25 for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptoputyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

30 G. Uses for anti-*patched-2* Antibodies

The anti-*patched-2* antibodies of the invention have various utilities. For example, anti-*patched-2* antibodies may be used in diagnostic assays for *patched-2*, e.g., detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either
35 heterogeneous or homogeneous phases [Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I , a

5 fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature*, 144: 945 (1962); David *et al.*, *Biochemistry*, 13:1014 (1974); Pain *et al.*, *J. Immunol. Meth.*, 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.*, 30:407 (1982).

10 Anti-*patched-2* antibodies also are useful for the affinity purification of *patched-2* from recombinant cell culture or natural sources. In this process, the antibodies against *patched-2* are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the *patched-2* to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the *patched-2*, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the *patched-2* from the antibody.

15 Basal cell carcinoma (BCC) is the most common human cancer. The *Hh* signaling pathway was found to be activated in all BCCs. Loss of *patched* function is thought to lead to unregulated *Smo* activity and is responsible for about half of all BCCs. *Patched* being a target of the *Hh* pathway itself, increases in *patched* mRNA levels have been detected in BCC [Gailani, *et al.*, *Nature Genet.* 14: 78-81 (1996)] as well as in animal models of BCC. Oro *et al.*, *Science* 276: 817-821 (1997); Xie *et al.*, *Nature* 391: 90-92 (1998). Abnormal activation of *Sh* signaling, such as that which occurs in BCC, was examined to confirm whether *patched-2* expression was increased. As shown in Fig. 9, an *in situ* hybridization for *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2) in *Smo*-M2 (SEQ ID NO:16) transgenic mice (Xie *et al.*, *supra*), while lower than *Ptch*, was still high in tumor cells. This suggests that therapeutic antibodies directed toward *Ptch-2* (SEQ ID NO:2) may be useful for the treatment of BCC.

20 Anti-*patched-2* antibodies also have utilities similar to those articulated for under the previous section "E. Uses of Patched-2". Depending on whether anti-*patched-2* antibodies will bind *patched-2* receptors so as to either inhibit *Hh* signaling (antagonist) or inhibit *patched-2* complexing with *Smo* (SEQ ID NO:17) and thereby remove the normal inhibitory effect of *Smo* (SEQ ID NO:17) on *Hh* signaling (agonist) the antibody will have utilities corresponding to those articulated previously for *patched-2*.

H. *Patched-2* Antagonists

25 Several approaches may be suitably employed to create the *patched-2* antagonist and agonist compounds of the present invention. Any approach where the antagonist molecule can be targeted to the interior of the cell, which interferes or prevents wild type *patched-2* from normal operation is suitable. For example, competitive inhibitors, including mutant *patched-2* receptors which prevent wild type *patched-2* from properly binding with other proteins necessary for *Dhh* and *Hh* signaling. Additional properties of such antagonist or agonist molecules are readily determinable by one of ordinary skill, such as size, charge and 30 hydrophobicity suitable for transmembrane transport.

35 Where mimics or other mammalian homologues of *patched-2* are to be identified or evaluated, the cells are exposed to the test compound and compared to positive controls which are exposed only to human *patched-2*, and to negative controls which were not exposed to either the compound or the natural ligand. Where antagonists or agonists of *patched-2* signal modulation are to be identified or evaluated, the cells are

exposed to the compound of the invention in the presence of the natural ligand and compared to controls which are not exposed to the test compound.

5 Detection assays may be employed as a primary screen to evaluate the *Hh* signaling inhibition/enhancing activity of the antagonist/agonist compounds of the invention. The assays may also be used to assess the relative potency of a compound by testing a range of concentrations, in a range from 100 mM to 1 pM, for example, and computing the concentration at which the amount of phosphorylation or signal transduction is reduced or increased by 50% (IC₅₀) compared to controls.

10 Assays can be performed to identify compounds that affect *Hh* signaling of *patched-2* substrates. Specifically, assays can be performed to identify compounds that increase the phosphorylation activity of *patched-2* or assays can be performed to identify compounds that decrease the *Hh* signaling of *patched-2* substrates. These assays can be performed either on whole cells themselves or on cell extracts. The assays 15 can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, cell based assays, etc. Such assay formats are well known in the art.

15 The screening assays of the present invention are amenable to high-throughput screening of chemical libraries, and are particularly suitable for identifying small molecule drug candidates.

(1) *Antagonist and agonist molecules*

20 To screen for antagonists and/or agonists of *patched-2* signaling, the assay mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, *patched-2* induces hedgehog signaling with a reference activity. The mixture components can be added in any order that provides for the requisite hedgehog activity. Incubation may be performed at any temperature 25 that facilitates optimal binding, typically between about 4° and 40°C, more commonly between about 15° and 40°C. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening, and are typically between about 0.1 and 10 hours, preferably less than 5 hours, more preferably less than 2 hours. After incubation, the effect of the candidate pharmacological agent on the *patched-2* signaling is determined in any convenient way. For cell-free binding-type assays, a separation 30 step is often used to separate bound and unbound components. Separation may, for example, be effected by precipitation (e.g. TCA precipitation, immunoprecipitation, etc.), immobilization (e.g. on a solid substrate), followed by washing. The bound protein is conveniently detected by taking advantage of a detectable label attached to it, e.g. by measuring radioactive emission, optical or electron density, or by indirect detection using, e.g. antibody conjugates.

35 For example, a method of screening for suitable *patched-2* antagonists and/or agonists could involve the application of *Dhh* and other hedgehog ligands. Such a screening assay could compare *in situ* hybridization in the presence and absence of the candidate antagonist and/or agonist in a *patched-2* expressing tissue as well as confirmation or absence of *patched-2* modulated cellular development. Typically these methods involve exposing an immobilized *patched-2* to a molecule suspected of binding thereto and determining the level of ligand binding downstream activation of reporter constructs and/or evaluating whether or not the molecule activates (or blocks activation of) *patched-2*. In order to identify such *patched-2* binding ligands, *patched-2* can be expressed on the surface of a cell and used to screen

libraries of synthetic candidate compounds or naturally-occurring compounds (e.g., from endogenous sources such as serum or cells).

Suitable molecules that affect the protein-protein interaction of *patched-2* and its binding proteins include fragments of the latter or small molecules, e.g., peptidomimetics, which will inhibit ligand-receptor interaction. Such small molecules, which are usually less than 10 K molecular weight, are preferable as therapeutics since they are more likely to be permeable to cells, are less susceptible to degradation by various cellular mechanisms, and are not as apt to elicit an immune response as proteins. Small molecules include but are not limited to synthetic organic or inorganic compounds. Many pharmaceutical companies have extensive libraries of such molecules, which can be conveniently screened by using the assays of the present invention. Non-limiting examples include proteins, peptides, glycoproteins, glycopeptides, glycolipids, polysaccharides, oligosaccharides, nucleic acids, bioorganic molecules, peptidomimetics, pharmacological agents and their metabolites, transcriptional and translation control sequences, and the like.

A preferred technique for identifying molecules which bind to *patched-2* utilizes a chimeric substrate (e.g., epitope-tagged *patched-2* or *patched-2* immunoadhesin) attached to a solid phase, such as the well of an assay plate. The binding of the candidate molecules, which are optionally labeled (e.g., radiolabeled), to the immobilized receptor can be measured. Alternatively, competition for various *Hh* pathways, especially *Dhh* (SEQ ID NO:13) can be measured. In screening for antagonists and/or agonists, *patched-2* can be exposed to a *patched-2* substrate followed by the putative antagonist and/or agonist, or the *patched-2* binding protein and antagonist and/or agonist can be added simultaneously, and the ability of the antagonist and/or agonist to block *patched-2* activation can be evaluated.

(2) *Detection assays*

The *patched-2* polypeptides are useful in assays for identifying lead compounds for therapeutically active agents that modulate *patched-2* receptor/ligand hedgehog signaling. Specifically, lead compounds that either prevent the formation of *patched-2* signaling complexes or prevent or attenuate *patched-2* modulated hedgehog signaling (e.g., binding to *patched-2*) can be conveniently identified.

Various procedures known in the art may be used for identifying, evaluating or assaying the inhibition of activity of the *patched-2* proteins of the invention. As *patched-2* is believed to be a receptor for *Dhh* (SEQ ID NO:13), but also binds *Shh* (SEQ ID NO:14) and *Ihh* (SEQ ID NO:29), techniques known for use with identifying ligand/receptor modulators may also be employed with the present invention. In general, such assays involve exposing target cells in culture to the compounds and a) biochemically analyzing cell lysates to assess the level and/or identity of binding; or (b) scoring phenotypic or functional changes in treated cells as compared to control cells that were not exposed to the test substance. Such screening assays are described in U.S.P. 5,602171, U.S.P. 5,710,173, WO 96/35124 and WO 96/40276.

(a) *Biochemical detection techniques*

Biochemical analysis can be evaluated by a variety of techniques. One typical assay mixture which can be used with the present invention contains *patched-2* and a ligand protein with which *patched-2* is normally associated (e.g., *Dhh* (SEQ ID NO:13)) usually in an isolated, partially pure or pure form. One or both of these components may be *patched-2* to another peptide or polypeptide, which may, for example, provide or enhance protein-protein binding, improve stability under assay conditions, etc.

In addition, one of the components usually comprises or is coupled to a detectable label. The label may provide for direct detection by measuring radioactivity, luminescence, optical or electron density, etc., or indirect detection such as an epitope tag, an enzyme, etc. The assay mixture can additionally comprise a candidate pharmacological agent, and optionally a variety of other components, such as salts, buffers, carrier proteins, e.g. albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc., which facilitate binding, increase stability, reduce non-specific or background interactions, or otherwise improve the efficiency or sensitivity of the assay.

The following detection methods may also be used in a cell-free system wherein cell lysate containing the signal transducing substrate molecule and *patched-2* is mixed with a compound of the invention. To assess the activity of the compound, the reaction mixture may be analyzed by the SDS-PAGE technique or it may be added to substrate-specific anchoring antibody bound to a solid support, and a detection procedure as described above is performed on the separated or captured substrate to assess the presence or absence of a *patched-2* binding ligand. The results are compared to those obtained with reaction mixtures to which the compound is not added. The cell-free system does not require the natural ligand or knowledge of its identity. For example, Posner *et al.* (U.S.P. 5,155,031 describes the use of insulin receptor as a substrate and rat adipocytes as target cells to demonstrate the ability of pervanadate to inhibit PTP activity. Another example, Burke *et al.*, *Biochem. Biophys. Res. Comm.* 204: 129-134 (1994) describes the use of autophosphorylated insulin receptor and recombinant PTP1B in assessing the inhibitory activity of a phosphoryrosyl mimetic.

20 (i) *Whole cell detection*

A common technique involves incubating cells with *patched-2* and radiolabeled ligand, lysing the cells, separating cellular protein components of the lysate using an SDS-polyacrylamide gel (SDS-PAGE) technique, in either one or two dimensions, and detecting the presence of labeled proteins by exposing X-ray film. Detection can also be effected without using radioactive labeling. In such a technique, the protein components (e.g., separated by SDS-PAGE) are transferred to a nitrocellulose membrane where the presence of *patched*-ligand complexes is detected using an anti-ligand antibody.

Alternatively, the anti-*patched-2* ligand antibody can be conjugated with an enzyme, such as horseradish peroxidase, and detected by subsequent addition of a colorimetric substrate for the enzyme. A further alternative involves detecting the anti-*patched-2* ligand by reacting with a second antibody that recognizes anti-*patched-2* ligand, this second antibody being labeled with either a radioactive moiety or an enzyme as previously described. Examples of these and similar techniques are described in Hansen *et al.*, *Electrophoresis* 14: 112-126 (1993); Campbell *et al.*, *J. Biol. Chem.* 268: 7427-7434 (1993); Donato *et al.*, *Cell Growth Diff.* 3: 258-268 (1992); Katagiri *et al.*, *J. Immunol.* 150: 585-593 (1993). Additionally, the anti-*patched-2* ligand can be detected by labeling it with a radioactive substance, followed by scanning the labeled nitrocellulose to detect radioactivity or exposure of X-ray film.

Further detection methods may be developed which are preferred to those described above. Especially for use in connection with high-throughput screening, it is expected that such methods would

exhibit good sensitivity and specificity, extended linear range, low background signal, minimal fluctuation, compatibility with other reagents, and compatibility with automated handling systems.

The *in vivo* efficacy of the treatment of the present invention can be studied against chemically induced tumors in various rodent models. Tumor cell lines propagated in *in vitro* cell cultures can be introduced in experimental rodents, e.g. mice by injection, for example by the subcutaneous route. Techniques for chemical induction of tumors in experimental animals are well known in the art.

(ii) *Kinase assays*

Because *patched-2* is a negative regulator of *Hh* signaling, which when activated by *Hh* releases the normal inhibitory effect on *Smo*, the inhibition of *patched-2* binding to *Smo* can be measured by activation of various kinase substrate associated with *Hh* signaling. When the screening methods of the present invention for *patched-2* antagonists/agonists are carried out as an *ex vivo* assay, the target kinase (e.g. *fused*) can be a substantially purified polypeptide. The kinase substrate (e.g., MBP, *Gli*) is a substantially purified substrate, which in the assay is phosphorylated in a reaction with a substantially purified phosphate source that is catalyzed by the kinase. The extent of phosphorylation is determined by measuring the amount of substrate phosphorylated in the reaction. A variety of possible substrates may be used, including the kinase itself in which instance the phosphorylation reaction measured in the assay is autophosphorylation. Exogenous substrates may also be used, including standard protein substrates such as myelin basic protein (MBP); yeast protein substrates; synthetic peptide substrates, and polymer substrates. Of these, MBP and other standard protein substrates may be regarded as preferred. Other substrates may be identified, however, which are superior by way of affinity for the kinase, minimal perturbation of reaction kinetics, possession of single or homogenous reaction sites, ease of handling and post-reaction recover, potential for strong signal generation, and resistance or inertness to test compounds.

Measurement of the amount of substrate phosphorylated in the *ex vivo* assay of the invention may be carried out by means of immunoassay, radioassay or other well-known methods. In an immunoassay measurement, an antibody (such as a goat or mouse anti-phosphoserine/threonine antibody) may be used which is specific for phosphorylated moieties formed during the reaction. Using well-known ELISA techniques, the phosphoserine/threonine antibody complex would itself be detected by a further antibody linked to a label capable of developing a measurable signal (as for example a fluorescent or radioactive label). Additionally, ELISA-type assays in microtitre plates may be used to test purified substrates. Peraldi *et al.*, *J. Biochem.* 285: 71-78 (1992); Schraag *et al.*, *Anal. Biochem.* 211: 233-239 (1993); Cleavland, *Anal. Biochem.* 190: 249-253 (1990); Farley, *Anal. Biochem.* 203: 151-157 (1992) and Lozano, *Anal. Biochem.* 192: 257-261 (1991).

For example, detection schemes can measure substrate depletion during the kinase reaction. Initially, the phosphate source may be radiolabeled with an isotope such as ^{32}P or ^{33}P , and the amount of substrate phosphorylation may be measured by determining the amount of radiolabel incorporated into the substrate during the reaction. Detection may be accomplished by: (a) commercially available scintillant-containing plates and beads using a beta-counter, after adsorption to a filter or a microtitre well surface, or (b) photometric means after binding to a scintillation proximity assay bead or scintillant plate. Weernink and

Kijken. *J. Biochem. Biophys. Methods* 31: 49, 1996; Braunwalder *et al.*, *Anal. Biochem.* 234: 23 (1996); Kentrup *et al.*, *J. Biol. Chem.* 271: 3488 (1996) and Rusken *et al.*, *Meth. Enzymol.* 200: 98 (1991).

Preferably, the substrate is attached to a solid support surface by means of non-specific or, preferably, specific binding. Such attachment permits separation of the phosphorylated substrate from unincorporated, labeled phosphate source (such as adenosine triphosphate prior to signal detection. In one embodiment, the substrate may be physically immobilized prior to reaction, as through the use of NuncTM high protein binding plate (Hanke *et al.*, *J. Biol. Chem.* 271: 695 (1996)) or Wallac ScintiStripTM plates (Braunwalder *et al.*, *Anal. Biochem.* 234: 23 (1996). Substrate may also be immobilized after reaction by capture on, for example, P81 phosphocellulose (for basic peptides), PEI/acidic molybdate resin or DEAE, or 10 TCA precipitation onto WhatmanTM 3MM paper, Tiganis *et al.*, *Arch. Biochem. Biophys.* 325: 289 (1996); Morawetz *et al.*, *Mol. Gen. Genet.* 250: 17 (1996); Budde *et al.*, *Int J. Pharmacognosy* 33: 27 (1995) and Casnelli, *Meth. Enz.* 200: 115 (1991). Yet another possibility is the attachment of the substrate to the support surface, as by conjugation with binding partners such as glutathione and streptavidin (in the case of GST and biotin), respectively which have been attached to the support, or via antibodies specific for the tags 15 which are likewise attached to the support.

Further detection methods may be developed which are preferred to those described above. Especially for use in connection with high-throughput screening, it is expected that such methods would exhibit good sensitivity and specificity, extended linear range, low background signal, minimal fluctuation, compatibility with other reagents, and compatibility with automated handling systems.

20 The *in vivo* efficacy of the treatment of the present invention can be studied against chemically induced tumors in various rodent models. Tumor cell lines propagated in *in vitro* cell cultures can be introduced in experimental rodents, e.g. mice by injection, for example by the subcutaneous route. Techniques for chemical inducement of tumors in experimental animals are well known in the art.

(b) *Biological detection techniques:*

25 The ability of the antagonist/agonist compounds of the invention to modulate the activity of *patched-2*, which itself modulates *hedgehog* signaling, may also be measured by scoring for morphological or functional changes associated with ligand binding. Any qualitative or quantitative technique known in the art may be applied for observing and measuring cellular processes which comes under the control of *patched-2*. The activity of the compounds of the invention can also be assessed in 30 animals using experimental models of disorders caused by or related to dysfunctional *hedgehog* signaling. For example, ineffective *Dhh* *hedgehog* signaling in mice leads to viable but sterile mice. Additionally, proper *Shh* signaling is critical to murine embryonic development at the notochord and floor plate, neural tube, distal limb structures, spinal column and ribs. Improper *Shh* signaling, is also correlative with cyclopia. Any of these phenotypic properties could be evaluated and quantified in a screening assay for *patched-2* 35 antagonists and/or agonist. Disease states associated with overexpression of *hedgehog* is associated with basal cell carcinoma while inactive *Shh* signaling leads to improper neural development.

The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. The dosage of the compounds of the invention should lie within a range

of circulating concentrations with little or no toxicity. The dosage may vary within this range depending on the dosage form employed and the route of administration.

(2) *Antisense oligonucleotides*

Another preferred class of antagonists involves the use of gene therapy techniques, include the administration of antisense oligonucleotides. Applicable gene therapy techniques include single or multiple administrations of therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. Reference short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by restricted uptake by the cell membrane, Zamecnik *et al.*, *Proc. Natl. Acad. Sci. USA* 83: 4143-4146 (1986). The anti-sense oligonucleotides can be modified to enhance their uptake, e.g., by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques known for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, *ex vivo*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection, Dzra *et al.*, *Trends Biotech.* 11: 205-210 (1993). In some situations it is desirable to provide the nucleic acid source with an agent that targets the cells, such as an antibody specific for a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g., capsid proteins or fragments thereof specific for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262: 4429-4432 (1987); Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 3410-3414 (1990). For a review of known gene targeting and gene therapy protocols, see Anderson *et al.*, *Science* 256: 808-813 (1992).

In one embodiment of the invention, *patched-2* expression may be reduced by providing *patched-2*-expressing cells with an amount of *patched-2* antisense RNA or DNA effective to reduce expression of the *patched-2* protein.

I. Diagnostic Uses

Another use of the compounds of the invention (e.g., *patched-2*, *patched-2* and anti-*patched-2* antibodies) described herein is to help diagnose whether a disorder is driven, to some extent, by *patched-2* or hedgehog signaling. For example, basal cell carcinoma cells are associated with active hedgehog signaling, spermatocyte formation is associated with *Dhh* signaling, and defective *patched* and *patched-2* suppression may be associated with testicular carcinomas.

A diagnostic assay to determine whether a particular disorder is driven by *patched-2* modulated hedgehog signaling, can be carried out using the following steps: (1) culturing test cells or tissues; (2) administering a compound which can prevent *patched-2* binding with *Smo* (SEQ ID NO:17), thereby activating the *Hh* signaling pathway; and (3) measuring the amount of *Hh* signaling. The steps can be carried

out using standard techniques in light of the present disclosure. For example, standard techniques can be used to isolate cells or tissues and culturing or *in vivo*.

Compounds of varying degree of selectivity are useful for diagnosing the role of *patched-2*. For example, compounds which inhibit *patched-2* in addition to another form of kinase can be used as an initial test compound to determine if one of several signaling ligands drive the disorder. The selective compounds can then be used to further eliminate the possible role of the other ligands in driving the disorder. Test compounds should be more potent in inhibiting ligand-*patched-2* binding activity than in exerting a cytotoxic effect (e.g., an IC₅₀/LD₅₀ of greater than one). The IC₅₀ and LD₅₀ can be measured by standard techniques, such as an MTT assay, or by measuring the amount of LDH released. The degree of IC₅₀/LD₅₀ of a compound should be taken into account in evaluating the diagnostic assay. For example, the larger the IC₅₀/LD₅₀ ratio the more relative the information. Appropriate controls take into account the possible cytotoxic effect of a compound of a compound, such as treating cells not associated with a cell proliferative disorder (e.g., control cells) with a test compound, can also be used as part of the diagnostic assay. The diagnostic methods of the invention involve the screening for agents that modulate the effects of *patched-2* upon *hedgehog* signaling. Exemplary detection techniques include radioactive labeling and immunoprecipitating (U.S.P. 5,385,915).

J. Pharmaceutical Compositions and Dosages

Therapeutic formulations of the compositions of the invention are prepared for storage as lyophilized formulations or aqueous solutions by mixing the *patched-2* molecule, agonist and/or antagonist having the desired degree of purity with optional "pharmaceutically-acceptable" or "physiologically-acceptable" carriers, excipients or stabilizers typically employed in the art (all of which are termed "excipients"). For example, buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants and other miscellaneous additives. (See Remington's Pharmaceutical Sciences, 16th Ed. . A. Osol. Ed. (1980)). Such additives must be nontoxic to the recipients at the dosages and concentrations employed.

Buffering agents help to maintain the pH in the range which approximates physiological conditions. They are preferably present at concentration ranging from about 2mM to about 50 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-

sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, there may be mentioned phosphate buffers, histidine buffers and trimethylamine salts such as Tris.

Preservatives are added to retard microbial growth, and are added in amounts ranging from 0.2% - 5 1% (w/v). Suitable preservatives for use with the present invention include phenol, benzyl alcohol, *meta*-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, iodide), hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

10 Isotonifiers sometimes known as "stabilizers" are present to ensure isotonicity of liquid compositions of the present invention and include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabinol, xylitol, sorbitol and mannitol. Polyhydric alcohols can be present in an amount between 0.1% to 25% by weight, preferably 1% to 5% taking into account the relative amounts of the other ingredients.

15 Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as 20 inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocitic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (i.e. < 10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and 25 trisaccharides such as raffinose; polysaccharides such as dextran. Stabilizers can be present in the range from 0.1 to 10.000 weights per part of weight active protein.

30 Non-ionic surfactants or detergents (also known as "wetting agents") are present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic® polyols, polyoxyethylene sorbitan monoethers (Tween®-20, Tween®-80, etc.). Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

35 Additional miscellaneous excipients include bulking agents, (e.g. starch), chelating agents (e.g. EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), and cosolvents.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely

affect each other. For example, it may be desirable to further provide an immunosuppressive agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsule prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th edition, A. Osol, Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished, 10 for example, by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the compounds of the invention, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or 15 poly(vinylalcohol)), polylactides (U.S. Pat. No.3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enaole release of molecules for over 100 days, certain hydrogels release proteins for 20 shorter time periods. When encapsulated compounds of the invention remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by 25 modifying sulphydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

The amount of therapeutic polypeptide, antibody or fragment thereof which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the dose-response 30 curve and the pharmaceutical compositions of the invention first *in vitro*, and then in useful animal model systems prior to testing in humans. However, based on common knowledge of the art, a pharmaceutical composition effective in modulating *Dhh* and *Hh* signaling may provide a local *patched-2* protein concentration of between about 10 and 1000 ng/ml, preferably between 100 and 800 ng/ml and most preferably between about 200 ng/ml and 600 ng/ml of *Ptch-2* (SEQ ID NO:2).

35 In a preferred embodiment, an aqueous solution of therapeutic polypeptide, antibody or fragment thereof is administered by subcutaneous injection. Each dose may range from about 0.5 µg to about 50 µg per kilogram of body weight, or more preferably, from about 3 µg to about 30 µg per kilogram body weight.

The dosing schedule for subcutaneous administration may vary from once a week to daily depending on a number of clinical factors, including the type of disease, severity of disease, and the subject's sensitivity to the therapeutic agent.

5 *Patched-2* polypeptide may comprise an amino acid sequence or subsequence thereof as indicated in Fig. 1, active amino acid sequence derived therefrom, or functionally equivalent sequence as this subsequence is believed to comprise the functional portion of the *patched-2* polypeptide.

10 If the subject manifests undesired side effects such as temperature elevation, cold or flu-like symptoms, fatigue, etc., it may be desirable to administer a lower dose at more frequent intervals. One or more additional drugs may be administered in combination with *patched-2* to alleviate such undesired side effects, for example, an anti-pyretic, anti-inflammatory or analgesic agent.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

15 EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Rockville, Maryland.

20 EXAMPLE 1

Introduction

At the cell surface, *Hh* function appears to be mediated by a multicomponent receptor complex involving *Ptch* and *Smo* (SEQ ID NO:17), two multi-transmembrane proteins initially identified as segment polarity genes in *Drosophila* and later characterized in vertebrates. Nakano, Y. *et al.*, *Nature* 341: 508-513 (1989); Goodrich *et al.*, *Gene Dev.* 10: 301-312 (1996); Marigo *et al.*, *Develop.* 122: 1225-1233 (1996); van den Heuvel *et al.*, *Nature* 382: 547-551 (1996); Alcedo *et al.*, *Cell* 86: 221-232 (1996); Stone *et al.*, *Nature* 384: 129-134 (1996). Both genetic and biochemical evidence support the existence of a receptor complex where *Ptch* (SEQ ID NO:4) is the ligand binding subunit, and where *Smo* (SEQ ID NO:17), a G-protein coupled receptor-like molecule, is the signaling component. Stone *et al.*, *Nature* 384: 129-134 (1996), Marigo *et al.*, *Nature* 384: 176-179 (1996), Chen *et al.*, *Cell* 87: 553-63 (1996). Upon binding of *Hh* to *Ptch* (SEQ ID NO:4), the normal inhibitory effect of *Ptch* (SEQ ID NO:4) on *Smo* (SEQ ID NO:17) is relieved, allowing *Smo* (SEQ ID NO:17) to transduce the *Hh* signal across the plasma membrane.

Results

It remains to be established if the *PTCH/SMO* receptor complex mediates the action of all 3 mammalian *Hhs* or if specific components exist. Recently, a second murine *Patched* gene, *mPatched-2* (SEQ ID NO:7) was recently isolated [Motoyama *et al.*, *Nature Genet.* 18: 104-106 (1998)] but its function as a *Hh* receptor has not been established. In order to characterize *patched-2* (SEQ ID NO:2) and compare it to *Patched* (SEQ ID NO:4) with respect to the biological function of the various *Hh* family members, we have screened EST databases with the *Patched* (SEQ ID NO:4) protein and identified 2 EST candidates for a

novel human *Patched* gene. A full length cDNA encoding human *Ptch-2* (SEQ ID NO:2) was cloned from a testis library. The initiation ATG defines a 3612 nucleotide open reading frame encoding a 1204 amino acid long protein with a predicted molecular weight of approximately 131 kDa. The overall identity between human *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2) is 54% (Fig. 1), while the identity between human 5 *PTCH-2* and the recently described mouse *Ptch-2* (SEQ ID NO:7) is 90% (Fig. 8). The most obvious structural difference between the two human *Patched* proteins is a truncated C-terminal cytoplasmic domain in *Ptch-2* (SEQ ID NO:2). In addition, only one of the two glycosylation sites present in *Ptch* (SEQ ID NO:4) is conserved in *Ptch-2* (SEQ ID NO:2).

To determine if *Patched-2* is a *Hh* receptor and if the two *Patched* molecules are capable of 10 discriminating between the various *Hh* ligands through specific binding, Applicants transfected human 293 embryonic kidney cells with *Ptch* (SEQ ID NO:4) or *Ptch-2* (SEQ ID NO:2) expression constructs and analyzed the cells for binding of *Shh*, *Dhh* and *Ihh*. As shown on Figure 7A, binding of ^{125}I -*Shh* can be 15 competed with an excess of *Shh*, *Dhh* or *Ihh* (SEQ ID NOS: 14, 13 and 29), respectively. Scatchard analysis of the displacement curves indicates that all *Hhs* have similar affinity for *Ptch* (SEQ ID NO:4) (*Shh*, 1.0nM (SEQ ID NO:14); *Dhh*, 2.6nM (SEQ ID NO:13); *Ihh*, 1.0nM (SEQ ID NO:29) and *Ptch-2* (SEQ ID NO:2) (Shh, 1.8nM (SEQ ID NO:14); *Dhh*, 0.6nM (SEQ ID NO:13); *Ihh*, 0.4nM (SEQ ID NO:29) indicating that both *PTCH* (SEQ ID NO:4) and *PTCH-2* (SEQ ID NO:2) can serve as physiological receptors for the 3 mammalian *Hh* proteins.

Applicants next determined whether, like *Patched*, *Patched-2* forms a physical complex with *Smo* 20 (SEQ ID NO:17). Expression constructs for Flag-tagged *Ptch* (SEQ ID NO:4) or *Ptch-2* (SEQ ID NO:2) were transiently co-transfected in 293 cells with Myc-tagged *Smo* (SEQ ID NO:17). As described previously [Stone *et al.*, *Nature* 384: 129-34 (1996)], in cells expressing *Ptch* (SEQ ID NO:4) and *Smo* (SEQ ID NO:15), *Ptch* (SEQ ID NO:4) can be immunoprecipitated with antibodies against the epitope-tagged *Smo* (SEQ ID NO:15) (Fig. 7B). Similarly, *Patched-2* can be immunoprecipitated with antibodies against the 25 epitope-tagged *Smo* (SEQ ID NO:15) when the two proteins are co-expressed in 293 cells. Together, these results suggest a model where *Patched-2* forms a multicomponent *Hh* receptor complex with *Smo* (SEQ ID NO:17) similar to the one described for *PTCH* (Stone *et al.*, *supra*). Interestingly, these results also demonstrate that the long C-terminal tail which is missing in *Patched-2* is not required for the interaction with *Smo* (SEQ ID NO:17) as was already suggested by the analysis of truncated *Patched* (Stone *et al.*, *supra*). However, it remains possible that the absence of a C-terminal domain affects the capacity of 30 *Patched-2* to block signaling by *Smo* (SEQ ID NO:17) or leads to difference in signaling by *Patched* compared to *Patched-2*.

To further investigate whether *Patched-2* could mediate the action of a specific *Hh* molecule based 35 on its expression profile, Applicants have compared the expression pattern of *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2). First, Northern blot analysis using a probe specific for *Ptch-2* (SEQ ID NO:1) revealed high levels of *PTCH2* mRNA in the testis (Fig. 4). By this method, *Ptch-2* (SEQ ID NO:1) expression was not detected in any other tissue analyzed including embryonic tissues (data not shown). This profile is very different from the one observed for *Ptch* (SEQ ID NO:18) which was not found in testis by Northern blot but in a large number of adult and embryonic tissues [Goodrich *et al.*, *Genes Dev.* 10: 301-312

(1996)]. More detailed analysis of the expression pattern of *Ptch* (SEQ ID NO:18) and *Ptch*-2 (SEQ ID NO:1) was performed by *in situ* hybridization with particular attention to testis. As previously described (Motoyama *et al.*, *supra*), low levels of *Ptch*-2 (SEQ ID NO:1) expression were detected in epithelial cells of the developing tooth and skin (data not shown). High levels of *Ptch*-2 (SEQ ID NO:2) encoding mRNA are expressed inside the seminiferous tubule, on the primary and secondary spermatocytes (Fig. 6B,6E) while only low levels of *Ptch* (SEQ ID NO:4) encoding mRNA can be detected on the Leydig cells located in the interstitium of the seminiferous tubules (Fig. 6A). The primary and secondary spermatocytes are in close contact with the supporting Sertoli cells, the source of *Dhh* (SEQ ID NO:13) in the testis [Bitgood *et al.*, *Curr. Biol.* 6: 298-304 (1996)]. To determine which one of the 2 receptors is the most relevant mediator of *Dhh* (SEQ ID NO:13) activity in the testis, we have analyzed the expression profile of FuRK (SEQ ID NO:10), a Fused Related Kinase that is believed to be a component of the *Hh* signaling pathway. Consistent with the idea that *Patched*-2 is the target of *Dhh* in the testis, we found that FuRK (SEQ ID NO:10) is expressed only in germ cells where it colocalizes with *Ptch*-2 (SEQ ID NO:2) (Figure 4c,f). *Dhh* (SEQ ID NO:13) is required for proper differentiation of germ cells since male *Dhh*-deficient mice are sterile due to lack of mature sperm (Bitgood *et al.*, *supra*). Our data suggest that *Dhh* (SEQ ID NO:13) acts directly on germ cells through *Ptch*-2 (SEQ ID NO:2) while the function of *Ptch* (SEQ ID NO:4) expressed at low levels on testosterone producing Leydig cells is unclear.

Discussion

Loss of heterozygosity (LOH) for *Patched* was reported to occur with high frequency in familial as well as sporadic basal cell carcinoma [Johnson *et al.*, *Science* 272: 1668-71 (1996); Hahn *et al.*, *Cell* 85: 841-51 (1996); Gailani *et al.*, *Nature Genetics* 14: 78-81; Xie *et al.*, *Cancer Res.* 57: 2369-72 (1997)], suggesting that it functions as a tumor suppressor. According to the receptor model described above, loss of *Patched* function may result in aberrant signaling by *Smo* (SEQ ID NO:17), leading to hyperproliferation of the skin basal cell layer. If, as suggested above, *Patched*-2 mediates the function of *Dhh*, loss of *Patched*-2 may lead to tumor formation in tissues where *Smo* (SEQ ID NO:17) activity is controlled by *Patched*-2. The gene encoding *Ptch*-2 (SEQ ID NO:2) was mapped by fluorescence *in situ* hybridization and by PCR using a radiation hybrid panel to human chromosome 1p33-34 (data not shown). Interestingly, recent analysis of recurrent chromosomal abnormalities in testicular tumors, including seminomas, revealed a deletion of the region 1p32-36 [Summersgill *et al.*, *B. J. Cancer* 77: 305-313 (1998)]. Loss of this region encompassing the *Patched*-2 locus was consistent in 36% of the germ cell tumor cases. These data raise the possibility that, like *Patched* in basal cell carcinoma and medulloblastoma, *Patched*-2 may be a tumor suppressor in *Dhh* (SEQ ID NO: 13) target cells such as spermatocytes, further implicating *Hh* signaling in cancer.

In summary, our data demonstrate that both *Patched* and *Patched*-2 are genuine *Hh* receptors and that they are both capable of forming a complex with *Smo* (SEQ ID NO:17). Although binding data indicate that *Patched* and *Patched*-2 do not discriminate between the various *Hh* ligands through affinity differences, the distinct tissue distribution of these 2 receptors suggests that *in vivo*, *Patched* may be the primary receptor for *Shh* whereas *Patched*-2 will mediate mainly *Dhh* signaling. The function of *Patched* expression in Leydig cells in the absence of some of the *Hh* signaling components remain to be explained. Similarly, it will be of interest to determine if *Patched*-2 plays a role when expressed in *Shh* expressing cells present in the

developing tooth and skin Motoyama *et al.*, *Nature Genet.* **18**: 104-106 (1998). Finally, the existence of *Patched-2* raises the question of whether additional *patched* receptors exist, in particular one that mediates the function of *Ihh* (SEQ ID NO: 29).

Material and Methods

5 *1. Isolation of human patched-2 cDNA clones*

An expressed sequence tag (EST) DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) was searched for a human homologue of the *Drosophila* segment polarity gene *patched-2*. Two ESTs (Incyte #905531 and 1326258) (Fig. 2) were identified as a potential candidates. In order to identify human cDNA libraries containing human *patched-2* clones, human cDNA libraries in pRK5 were first screened by

10 PCR using the following primers:

5'-905531(A): 5'-AGGCAGGGGATCACAGCA-3' (SEQ ID NO:19)

3'-905531(A): 5'-ATACAAAGAGTTCCACT-3' (SEQ ID NO:20)

A fetal lung library was selected and enriched for *patched-2* cDNA clones by extension of single stranded DNA from plasmid libraries grown in dut/ung⁻ host using the 3'-905531(A) primer in a reaction containing

15 10µl of 10x PCR Buffer (Klentaq®), 1µl dNTP (200 µM), 1 µl library DNA (200 ng), 0.5 µl primer, 86.5 µl H₂O and 1 µl of Klentaq® (Clontech) added after a hot start. The reaction was denatured for 1 min. at 95°C, annealed for 1 min. at 60°C then extended for 20 min. at 72°C. DNA was extracted with phenol/CHCl₃, ethanol precipitated, then transformed by electroporation into DH10B (Invibco/BRL) host bacteria. Colonies from each transformation were replica plated on nylon membranes and screened with an overlapping oligo

20 probe derived from the EST sequence (#905531) of the following sequence:

5'-ptch2 probe: 5'-CTGCGGCCCTGCTTCCTGCTGGCCGTGCATCCTGCTGGTGTGC-3'

(SEQ ID NO:21)

3'-ptch2probe: 5'-AGAGCACAGACGAGGAAAGTGCACACCAGCAGGATGCAGACGGCC-3'

(SEQ ID NO:22)

25 The oligo probe was labeled with [γ -³²P]-ATP and T4 polynucleotide kinase. Filters were hybridized overnight at 42°C in 50% formamide, 5xSSC, 10xDenhardt's, 0.05M sodium phosphate (pH 6.5), 0.1% sodium pyrophosphate, 50 µg/ml of sonicated salmon sperm DNA. The filters were then rinsed in 2x SSC and washed in 0.1x SSC, 0.1% SDS then exposed to Kodak® X Ray films. Using this procedure, a partial clone was isolated from the fetal brain library (clone 3A – Fig. 10) (SEQ ID NO:8). In order to isolate the

30 missing 5'-sequence, a testis library (see northern blot analysis, infra) was screened. The primer set used to amplify a 204 bp probe from clone 3A to probe the testis library was:

RACE 5: 5'-ACTCCTGACTTGTAGCAGATT-3' (SEQ ID NO. 23) and

RACE 6: 5'-AGGCTGCATACACCTCTCAGA-3' (SEQ ID NO:24).

The amplified probe was purified by excision from an agarose gel and labeled with a random primer labeling kit (Boehringer Mannheim). Several clones were isolated, including one (clone 16.1 – Fig. 11) (SEQ ID NO:9) containing a potential initiation methionine. A full length cDNA encoding a *Patched-2* was reconstructed by assembling several of these clones. The full length cDNA encoding human *Pitch-2* (Fig. 1

(SEQ ID NO:1)) has a 3612 nucleotide long open reading frame encoding a 1204 amino acid protein with a 144 kDa predicted molecular weight. Alignment with human *Ptch* (SEQ ID NO:4) reveals a 53% identity between the 2 molecules at the amino acid level (Fig. 3). All 12 transmembrane domains are conserved. The most significant difference is a shorter C-terminal intracellular domain in *Ptch-2* (SEQ ID NO:2) compared to *Ptch* (SEQ ID NO:4).

5 2. *Northern blot analysis:*

In order to determine the best tissue source for isolation of the complete full length *Patched-2* cDNA as well as to determine its expression profile, we probed human multiple tissue northern blots (Clontech) with a 752 bp fragment amplified from the 3' untranslated region of *Patched-2* using the 10 following primers:

TM2: TM2 5-GCTTAGGCCGAGGAGAT-3' (SEQ ID NO:25)

UTR2: 5'-AACTCACAACTTCTCTCCA-3' (SEQ ID NO:26).

The resulting fragment was gel purified and labeled by random priming. The blots were hybridized in ExpressHyb® hybridization solution (Clontech) in the presence of 1×10^6 cpm/ml 32 P-labeled probe at 42°C 15 overnight. The blots were washed in 2x SSC at room temperature for 10 minutes and washed in 0.1 x SSC/0.1 % SDS at 42°C for 30 minutes then exposed to x-ray film overnight. Fig. 4 shows that *Ptch-2* message is expressed at high levels in only the testis.

15 3. *Chromosomal localization:*

The primers TM2 (SEQ ID NO:25) and UTR2 (SEQ ID NO:26) described above were used to 20 screen the Genome Systems (St. Louis, MO) BAC library. Two individual BAC clones were obtained from this library and chromosomal localization of both of the clones by FISH indicated that *Ptch-2* (SEQ ID NO:2) maps to human chromosome 1p33-34 (FIG 5). Loss of heterozyosity (LOH) for *Patched* was reported to occur with high frequency in basal cell carcinoma. Loss of *Patched* function is thought to lead to 25 constitutive signaling by Smoothened (*Smo*) (SEQ ID NO:17), resulting in hyperproliferation of the basal layer of the dermis. A similar mechanism may lead to the formation of germ cell tumors. This model proposes that the first step in the progression of a germ cell tumor is an initial loss of DNA by a germ cell precursor, leading to a neoplastic germ cell which then forms a seminoma [De Jong *et al.*, *Cancer Genet. Cytogenet.* 48: 143-167 (1990)]. From the invasive seminoma, all other forms of germ cell tumor types 30 develop. Approximately 80% of all germ cell tumors correlate with an isochromosome 12p (i12p) and is found at a higher frequency in non-seminomas than seminomas [Rodriguez *et al.*, *Cancer Res.* 52: 2285-2291 (1992)]. However, analysis of recurrent chromosomal abnormalities in testicular tumors including seminomas revealed a deletion of the region 1p32-36. Loss of this region was consistent in 36% of the germ cell tumor cases of in a recent study Summersgill *et al.*, *B. J. Cancer* 57: 305-313 (1998)]. A similar 35 deletion of chromosome 1p32-36 has been reported at a frequency of 28% in oligodendrogiomas; Bello, *et al.*, *Int. J. Cancer* 57: 172-175 (1994). While expression of *Ptch-2* (SEQ ID NO:2) in the brain was not examined here in detail, *Ptch-2* (SEQ ID NO:2) is thought to be the *Dhh* receptor (see below) and expression of *Dhh* by murine Schwann cells was previously reported [Bitgood *et al.*, *Develop. Biol.* 172: 126-138 (1995)]. Since *Ptch-2* (SEQ ID NO:2) localizes to chromosome 1p33-34 it is possible that *Patched-2*

regulates *Smo* (SEQ ID NO:17) signaling in *Dhh* target cells and that loss of *Patched-2* function leads to abnormal *Smo* (SEQ ID NO:17) signaling in these cells and subsequent tumor formation.

4. *In situ hybridization:*

5 Mouse testis sections were cut at 16 μ m, and processed for *in situ* hybridization by the method described in Phillips *et al.*, *Science* 250: 290-294 (1990). 33 P-UTP labeled RNA probes were generated as described in Melton *et al.*, *Nucleic Acids Res.* 12: 7035-7052 (1984). Sense and antisense probes were synthesized from the 3' non-coding region of the mouse *Patched* or *Patched-2* and from a mouse FuRK cDNA fragment corresponding to the region encoding amino acid 317-486 of the human sequence using T3 and T7, respectively.

10 PTCH:

503 (Anti-sense)

5'GGATTCTAATACGACTCACTATAGGGCCCAATGGCCTAAACCGACTGC3' (SEQ ID NO:27)

503 (Sense)

5'CTATGAAATTAAACCCTCACTAAAGGGACCCACGGCCTCTCCTCAC3' (SEQ ID NO:28)

15 PTCH2:

504 (Anti-sense)

5'GGATTCTAATACGACTCACTATAGGGCCCCTAACTCCGCTGCTCCAC3' (SEQ ID NO:12)

504 (Sense)

5'CTATGAAATTAAACCCTCACTAAAGGGAGCTCCCGTAGTCCCTATGTG3' (SEQ ID NO:11)

20

FuRK sense and antisense were synthesized from a mouse fused DNA fragment using T3 and T7, respectively, corresponding to the region encoding amino acid residues 317-486 of the human.

Figure 6 illustrates that, although both *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2) are expressed in testis, their expression pattern does not overlap. *Ptch* (SEQ ID NO:4) is expressed in the Leydig cells of the interstitium while *Ptch-2* (SEQ ID NO:2) is expressed in the primary and secondary spermatocytes.

30 The expression of *Patched-2* specifically in the developing spermatogonia suggest that *Patched-2* is the immediate target of *Dhh* (SEQ ID NO. 13). *Dhh* (SEQ ID NO. 13) is expressed by Sertoli cells and mice deficient in *Dhh* (SEQ ID NO. 13) are sterile because of a defect in sperm production [Bitgood *et al.*, *Curr. Biol.* 6: 298-304 (1996)]. Although this effect on germ cells was thought to be indirect and mediated by *Patched* present on Leydig cells, our data suggest that *Dhh* directly acts on germ cells through *Patched-2*. This is further demonstrated by the localization of FuRK (SEQ ID NO. 10), an intracellular kinase homologous to *Drosophila* Fused and involved in transducing the Hedgehog (*Hh*) signal. As shown in Figure 6, FuRK (SEQ ID NO:10) is colocalizes with *Ptch-2* (SEQ ID NO:2) in germ cells and not with *Ptch* (SEQ ID NO:4) in Leydig cells, suggesting that *Patched-2* and not *Patched* will be able to transduce the *Dhh* signal. These results suggest that *Patched-2* is a *Dhh* receptor.

35 *Ptch-2* mRNA levels in *Smo-M2* (SEQ ID NO. 16) transgenic mice [a *Smo* mutation which results in autonomous phenotypes similar to BCC, Xie *et al.*, *Nature* 391: 90-92 (1998)] can be increased upon

abnormal activation of the *Hh* signaling pathway. As indicated in Fig. 9, *Ptch-2* (SEQ ID NO:2) levels were high in tumor cells (although lower than *Ptch* (SEQ ID NO:4) levels). This suggests that antibodies directed toward *Ptch-2* (SEQ ID NO:2) may be useful in the treatments of BCC.

5. *Immunoprecipitation with Smo:*

5 The binding of *Patched-2* to *Smo* (SEQ ID NO:17) was assessed by cotransfection using a transient transfection system of a myc-epitope tagged *Smo* (SEQ ID NO:15) and a FLAG-epitope tagged *Patched* or *Patched-2* expression construct in 293 cells using standard techniques (Gorman, C., *DNA Cloning: A Practical Approach*, C. C. Thomas, DM ed., Vol. 11, pp. 143-190, IRL Press, Washington, D.C.). 36 hours after transfection, the cells were lysed in 1% NP-40 and immunoprecipitated overnight with the 9E10 anti-myc antibody or with the M2 anti-FLAG antibody (IBI-Kodak) followed by protein A Sepharose, and then separated on a denatured 6% polyacrylamide gel. Proteins were detected by transfer to nitrocellulose and probing with antibodies to Flag or Myc epitopes, using the ECL detection system (Amersham). Figure 7B indicates that both *Ptch* (SEQ ID NO:4) or *Ptch-2* (SEQ ID NO:2) are expressed at the same level (IP Flag, Blot Flag) and that like *Ptch* (SEQ ID NO:4), *Ptch-2* (SEQ ID NO:2) forms a physical complex with *Smo* (SEQ ID NO:17). These results suggest that like *Patched*, *Patched-2* controls *Hh* signaling through its interaction with *Smo* (SEQ ID NO:17).

6. *Hh Binding:*

To determine whether *Patched-2* is able to bind to the various hedgehog ligands, 293 cells were transfected with *Ptch* (SEQ ID NO:4) or *Ptch-2* (SEQ ID NO:2) using standard procedures. Cells were 20 incubated with 100 pM ^{125}I -*Shh* (19kD amino terminal fragment of murine *Shh* (SEQ ID NO:14)) in the presence or absence of excess unlabeled *Shh* (SEQ ID NO:14) or *Dhh* (SEQ ID NO:13) for 2h at room temperature. After equilibrium was reached, the ligand bound cells were centrifuged through a continuous sucrose gradient to separate unincorporated and then counted in a scintillation counter. Figure 7A shows that both *Dhh* (SEQ ID NO:13) and *Shh* (SEQ ID NO:14) bind to *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2). Varying concentrations of cold competitor indicate that the 2 ligands have similar affinity for *Ptch* (SEQ ID NO:4) and *Ptch-2* (SEQ ID NO:2).

Example 2

Expression of *patched-2* in *E. coli*

The DNA sequence encoding human *patched-2* is initially amplified using selected PCR primers. 30 The primers should contain restriction enzyme sites that correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences 35 that encode for an antibiotic resistance gene, a *trp* promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the vertebrate *patched-2* coding region, lambda transcriptional terminator, and an *argU* gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic

resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The 5 cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized vertebrate *patched-2* protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

10

Example 3

Expression of patched-2 in mammalian cells

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the vertebrate *patched-2* DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the vertebrate *patched-2* DNA using ligation methods such as described in Sambrook *et al.*, 15 *supra*. The resulting vector is called pRK5-*patched-2*.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-*patched-2* DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya *et al.*, *Cell*, 31:543 (1982)] and dissolved 20 in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl₂. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO₄, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated 25 for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml ³⁵S-cysteine and 200 µCi/ml ³⁵S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of 30 time to reveal the presence of vertebrate *patched-2* polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, vertebrate *patched-2* may be introduced into 293 cells transiently using the dextran sulfate method described by Sompanyrac *et al.*, *Proc. Natl. Acad. Sci.*, 78:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-*patched-2* DNA is added. The cells are 35 first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the

conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed vertebrate *patched-2* can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, vertebrate *patched-2* can be expressed in CHO cells. The pSVi-*patched-2* 5 can be transfected into CHO cells using known reagents such as CaPO₄ or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as ³⁵S-methionine. After determining the presence of vertebrate *patched-2* polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the 10 expressed vertebrate *patched-2* can then be concentrated and purified by any selected method.

Epitope-tagged vertebrate *patched-2* may also be expressed in host CHO cells. The vertebrate *patched-2* may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into an expression vector. The poly-his tagged vertebrate *patched-2* insert can then be subcloned into a SV40 driven vector containing a selection marker such as 15 DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged vertebrate *patched-2* can then be concentrated and purified by any selected method, such as by Ni²⁺-chelate affinity chromatography.

Example 4

Expression of vertebrate *patched-2* in Yeast

The following method describes recombinant expression of vertebrate *patched-2* in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of vertebrate *patched-2* from the ADH2/GAPDH promoter. DNA encoding vertebrate *patched-2*, a selected signal peptide and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct 25 intracellular expression of vertebrate *patched-2*. For secretion, DNA encoding vertebrate *patched-2* can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, the yeast alpha-factor secretory signal/leader sequence, and linker sequences (if needed) for expression of vertebrate *patched-2*.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids 30 described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant vertebrate *patched-2* can subsequently be isolated and purified by removing the yeast 35 cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing vertebrate *patched-2* may further be purified using selected column chromatography resins.

Example 5

Expression of vertebrate *patched-2* in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of vertebrate *patched-2* in Baculovirus-infected insect cells.

5 The vertebrate *patched-2* is *patched-2* upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the vertebrate *patched-2* or the desired portion of the vertebrate *patched-2* (such as the sequence encoding the extracellular domain of a transmembrane protein) is
10 amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

15 Recombinant baculovirus is generated by co-transfected the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression is performed as described by O'Reilley et al., *Baculovirus expression vectors: A laboratory Manual*, Oxford: Oxford University Press (1994).

20 Expressed poly-his tagged vertebrate *patched-2* can then be purified, for example, by Ni^{2+} -chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl_2 ; 0.1 mM EDTA; 10% Glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% Glycerol, pH 7.8) and filtered through a 0.45 μm filter. A Ni^{2+} -NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A_{280} with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% Glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A_{280} baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or western blot with Ni^{2+} -NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His₁₀-tagged vertebrate *patched-2* are pooled and dialyzed against loading buffer.

35 Alternatively, purification of the IgG tagged (or Fc tagged) vertebrate *patched-2* can be performed using known chromatography techniques, including for instance, Protein A or protein G column

chromatography

Example 6

Preparation of Antibodies that Bind Vertebrate *patched*-2

This example illustrates preparation of monoclonal antibodies, which can specifically bind vertebrate *patched*-2.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified vertebrate *patched*-2, fusion proteins containing vertebrate *patched*-2, and cells expressing recombinant vertebrate *patched*-2 on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

10 Mice, such as Balb/c, are immunized with the vertebrate *patched*-2 immunogen (e.g., extracellular portions or cells expressed *patched*-2) emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the 15 selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect vertebrate *patched*-2 antibodies.

20 After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of vertebrate *patched*-2. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-patched-2 cells, myeloma hybrids, and spleen cell hybrids.

25 The hybridoma cells will be screened in an ELISA for reactivity against vertebrate *patched*-2. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against vertebrate *patched*-2 is within the skill in the art.

30 The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti- vertebrate *patched*-2 monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

Example 7

Gli Luciferase Assay

35 The following assay may be used to measure the activation of the transcription factor *GLI*, the mammalian homologue of the *Drosophila cubitus interruptus* (Ci). It has been shown that *GLI* is a transcription factor activated upon *SHh* stimulation of cells.

Nine (9) copies of a *GLI* binding site present in the *HNF3β* enhancer. (Sasaki *et al.*, *Development*

124: 1313-1322 (1997)). are introduced in front of a thymidine kinase minimal promoter driving the luciferase reporter gene in the pGL3 plasmid (Promega). The sequence of the *GLI* binding sequence is: TCGACAAGCAGGGAACACCCAAGTAGAAGCTC (p9XGliLuc) (SEQ ID NO:31), while the negative control sequence is: TCGACAAGCAGGGAAAGTGGAGTAGAAGCTC (p9XmGliLuc) (SEQ ID NO:32). These constructs are cotransfected with the full length *Ptch-2* and *Smo* in C3H10T1/2 cells grown in F12. DMEM (50:50), 10% FCS heat inactivated. The day before transfection 1×10^5 cells per well was inoculated in 6 well plates, in 2 ml of media. The following day, 1 μ g of each construct is cotransfected in duplicate with 0.025 mg ptkRenilla luciferase plasmid using lipofectamine (Gibco-BRL) in 100 μ l OptiMem (with GlutaMAX) as per manufacturer's instructions for 3 hours at 37°C. Serum (20%, 1 ml) is then added to each well and the cells were incubated for 3 more hours at 37°C. Cells are then washed twice with PBS, then incubated for 48 hours at 37°C in 2 ml of media. Each well is then washed with PBS, and the cells lysed in 0.5 ml Passive Lysis Buffer (Promega) for 15 min. at room temperature on a shaker. The lysate is transferred in eppendorf tubes on ice, spun in a refrigerated centrifuge for 30 seconds and the supernatant saved on ice. For each measure, 20 μ l of cell lysate is added to 100 μ l of LARII (luciferase assay reagent, Promega) in a polypropylene tube and the luciferase light activity measured. The reaction is stopped by the addition of Stop and Glow buffer (Promega), mixed by pipetting up and down 3 to 5 times and *Renilla* luciferase lights activity is measured on the luminometer.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

<u>Designation:</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
pRK7.hptc2.Flag-1405	209778	4/14/98

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC § 122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to

practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable 5 the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

EDITORIAL NOTE

APPLICATION NUMBER - 52657/99

The following Sequence Listing pages 1 to 40 are part of the description. The claims pages follow on pages "48" to "50".

Sequence Listing

<110> Genentech, Inc.

<120> Patched-2

<130> P1405PCT

5 <141> 1999-04-02

<150> US 09/060,939

<151> 1998-04-15

<160> 32

<210> 1

10 <211> 4030

<212> DNA

<213> Homo sapiens

<400> 1

gttatttcag gccatggtgt tgcgccaat taattccga tccagacatg 50

15 ataagataca ttgatgagtt tggacaaacc acaactagaa tgcagtgaaa 100

aaaatgcttt atttgtgaaa tttgtgatgc tattgctta tttgtAACCA 150

ttataagctg caataaacaa gttgggccc ggcggccaag cttctgcagg 200

tcgactctag aggatccccg gggaaattccg gcatgactcg atcgccgccc 250

ctcagagagc tgcccccgag ttacacaccc ccagctcgaa ccgcagcacc 300

20 ccagatccta gctggagcc tgaaggctcc actctggctt cgtgcttact 350

tccagggcct gctttctct ctggatgcg ggatccagag acattgtggc 400

aaagtgtct ttctggact gttggccttt ggggcctgg cattaggtct 450

ccgcatggcc attattgaga caaacttgg acaagctctgg gtagaagtgg 500

550 gcagccgggt gagccaggag ctgcattaca ccaaggagaa gctggggag

25 gaggctgcat acacctctca gatgctgata cagaccgcac gccaggaggg 600

agagaacatc ctcacacccg aagcaattgg cctccacctc caggcagccc 650

tcactgccag taaagtccaa gtatcactct atggaaagtc ctgggatttg 700

aacaaaatct gctacaagtc aggagttccc cttattgaaa atggaatgat 750

tgagtggatg attgagaagc tggatccgtg cgtgatcctc acccccctcg 800

30 actgcttctg ggagggagcc aaactccaag ggggctccgc ctacctgccc 850

ggccgccccgg atatccagtg gaccaacctg gatccagagc agctgctgga 900

ggagctgggt ccctttgcct cccttgggg cttccgggag ctgctagaca 950
aggcacaggt gggccaggcc tacgtgggc ggccctgtct gcaccctgat 1000
gacctccact gcccacctag tgcccccaac catcacagca ggcaggctcc 1050
caatgtggct cacgagctga gtgggggctg ccatggcttc tcccacaaat 1100
5 tcatgcactg gcaggaggaa ttgctgctgg gaggcatggc cagagacccc 1150
caaggagagc tgctgagggc agaggccctg cagagcacct tcttgctgat 1200
gagtccccgc cagctgtacg agcattccg gggtgactat cagacacatg 1250
acattggctg gagtgaggag caggccagca cagtgtaca agcctggcag 1300
cggcgctttg tgcagctggc ccaggaggcc ctgcctgaga acgcttccca 1350
10 gcagatccat gccttctcct ccaccacccct gnatgacatc ctgcattgcgt 1400
tctctgaagt cagtgtgccc cgtgtggtgg gaggctatct gctcatgctg 1450
gcctatgcct gtgtgaccat gctgcggtgg gactgcgccc agtcccaggg 1500
ttccgtgggc cttgccqggg tactgctggt ggccctggcg gtggcctcag 1550
gccttgggct ctgtgccctg ctcggcatca cttcaatgc tgcactacc 1600
15 caggtgctgc ctttctggc tctggaaatc ggcgtggatg acgtattcct 1650
gctggcgcat gccttcacag aggctctgcc tggcacccct ctccaggagc 1700
gcatgggcga gtgtctgcag cgcacggca ccagtgctgt actcacatcc 1750
atcaacaaca tggccgcctt cctcatggct gccctcggtt ccattccctgc 1800
gctgcgagcc ttctccctac aggccggccat agtgggtggc tgcacccctt 1850
20 tagccgtat gcttgccttc ccagccatcc tcagcctggc cctacggcgg 1900
cgccactgccc agcgccctga tgtgctctgc tgcttctcca gtcctgctc 1950
tgctcaggtg attcagatcc tgccccagga gctgggggac gggacagtac 2000
cagtgggcat tgcccacctc actgccacag ttcaagcctt taccactgt 2050
gaagccagca gccagcatgt ggtcaccatc ctgcctcccc aagcccaccc 2100
25 ggtgccccca cttctgacc cactggctc tgagctcttc agccctggag 2150
ggtccacacg ggaccttcta ggccaggagg aggagacaag gcagaaggca 2200
gcctgcaagt ccctgccctg tgcccgtgg aatcttgccc atttcgcccc 2250
ctatcagttt gccccgttgc tgctccagtc acatgccaag gcatcgtgc 2300

tggtgctttt tggtgctttt ctgggcctga gcctctacgg agccaccc 2350
gtgcaagacg gcctggccct gacggatgtg gtgcctcggg gcaccaagga 2400
gcatgccttc ctgagcgccc agctcaggta cttctccctg tacgagggtgg 2450
ccctggtgac ccagggtggc tttgactacg cccattccca acgccc 2500
5 tttgatctgc accagcgctt cagttccctc aaggcggtgc tgccccacc 2550
ggccacccag gcaccccgca cctggctgca ctattaccgc aactggctac 2600
aggaaatcca ggctgccttt gaccaggact gggcttctgg gcgcatcacc 2650
cgccactcgt accgcaatgg ctctgaggat gggccctgg cctacaagct 2700
gctcatccag actggagacg cccaggagcc tctggatttc agccagctga 2750
10 ccacaaggaa gctggtgac agagagggac tgattccacc cgagctttc 2800
tacatggggc tgaccgtgtg ggtgagcagt gacccctgg gtctggcagc 2850
ctcacaggcc aacttctacc ccccacctcc tgaatggctg cacgacaaat 2900
acgacaccac gggggagaac cttcgcatcc cgccagctca gcccttggag 2950
tttgcggcagt tcccccttcct gctgcgtggc ctccagaaga ctgcagactt 3000
15 tgtggaggcc atcgagggggg cccgggcagc atgcgcagag gccggccagg 3050
ctggggtgca cgcctacccc agcggctccc cttcccttt ctgggaacag 3100
tatctgggcc tgcggcgctg cttcctgctg gccgtctgca tcctgctgg 3150
gtgcactttc ctcgtctgtg ctctgctgct cctcaacccc tggacggctg 3200
gcctcatagt gctggtcctg gcgatgatga cagtggaaact cttggatc 3250
20 atgggtttcc tgggcatcaa gctgagtgcc atccccgtgg tgatccttgc 3300
ggcctctgta ggcattggcg ttgagttcac agtccacgtg gctctgggct 3350
tcctgaccac ccagggcagc cggAACCTGC gggccggccca tgcccttgag 3400
cacacatttg cccccgtgac cgatggggcc atctccacat tgctgggtct 3450
gctcatgctt gctggttccc actttgactt cattgttaagg tacttctttg 3500
25 cggcgctgac agtgctcagc ctccctggcc tcctccatgg actcgtgctg 3550
ctgcctgtgc tgctgtccat cctggggcccg ccggccagagg tgatacagat 3600
gtacaaggaa agcccagaga tcctgagtcc accagctcca cagggaggcg 3650
ggcttaggtg gggggcatcc tcctccctgc cccagagctt tgccagagtg 3700

actacacctca tgaccgtggc catccaccca ccccccctgc ctgggtgccta 3750
 catccatcca gcccctgatg agcccccttg gtccccctgct gccactagct 3800
 ctggcaacct cagttccagg ggaccaggc cagccactgg gtgaaagagc 3850
 agctgaagca cagagaccat gtgtggggcg tgtgggtca ctgggaagca 3900
 5 ctgggtctgg tggtagacgc aggacggacc cctggagggc cctgctgctg 3950
 ctgcaccccc tctcccgacc cagctgtcat gggcctccct gatatcgaat 4000
 tcaatcgata gaaccgaggt gcagttggac 4030

<210> 2
 <211> 1203
 10 <212> PRT
 <213> Homo sapiens

<400> 2
 Met Thr Arg Ser Pro Pro Leu Arg Glu Leu Pro Pro Ser Tyr Thr
 1 5 10 15

15 Pro Pro Ala Arg Thr Ala Ala Pro Gln Ile Leu Ala Gly Ser Leu
 20 25 30

Lys Ala Pro Leu Trp Leu Arg Ala Tyr Phe Gln Gly Leu Leu Phe
 35 40 45

20 Ser Leu Gly Cys Gly Ile Gln Arg His Cys Gly Lys Val Leu Phe
 50 55 60

Leu Gly Leu Leu Ala Phe Gly Ala Leu Ala Leu Gly Leu Arg Met
 65 70 75

Ala Ile Ile Glu Thr Asn Leu Glu Gln Leu Trp Val Glu Val Gly
 80 85 90

25 Ser Arg Val Ser Gln Glu Leu His Tyr Thr Lys Glu Lys Leu Gly
 95 100 105

Glu Glu Ala Ala Tyr Thr Ser Gln Met Leu Ile Gln Thr Ala Arg
 110 115 120

30 Gln Glu Gly Glu Asn Ile Leu Thr Pro Glu Ala Leu Gly Leu His
 125 130 135

Leu Gln Ala Ala Leu Thr Ala Ser Lys Val Gln Val Ser Leu Tyr
 140 145 150

Gly Lys Ser Trp Asp Leu Asn Lys Ile Cys Tyr Lys Ser Gly Val
 155 160 165

35 Pro Leu Ile Glu Asn Gly Met Ile Glu Trp Met Ile Glu Lys Leu
 170 175 180

	Phe Pro Cys Val Ile Leu Thr Pro Leu Asp Cys Phe Trp Glu Gly		
	185	190	195
	Ala Lys Leu Gln Gly Gly Ser Ala Tyr Leu Pro Gly Arg Pro Asp		
	200	205	210
5	Ile Gln Trp Thr Asn Leu Asp Pro Glu Gln Leu Leu Glu Glu Leu		
	215	220	225
	Gly Pro Phe Ala Ser Leu Glu Gly Phe Arg Glu Leu Leu Asp Lys		
	230	235	240
10	Ala Gln Val Gly Gln Ala Tyr Val Gly Arg Pro Cys Leu His Pro		
	245	250	255
	Asp Asp Leu His Cys Pro Pro Ser Ala Pro Asn His His Ser Arg		
	260	265	270
	Gln Ala Pro Asn Val Ala His Glu Leu Ser Gly Gly Cys His Gly		
	275	280	285
15	Phe Ser His Lys Phe Met His Trp Gln Glu Glu Leu Leu Leu Gly		
	290	295	300
	Gly Met Ala Arg Asp Pro Gln Gly Glu Leu Leu Arg Ala Glu Ala		
	305	310	315
20	Leu Gln Ser Thr Phe Leu Ile Met Ser Pro Arg Gln Leu Tyr Glu		
	320	325	330
	His Phe Arg Gly Asp Tyr Gln Thr His Asp Ile Gly Trp Ser Glu		
	335	340	345
	Glu Gln Ala Ser Thr Val Leu Gln Ala Trp Gln Arg Arg Phe Val		
	350	355	360
25	Gln Leu Ala Gln Glu Ala Leu Pro Glu Asn Ala Ser Gln Gln Ile		
	365	370	375
	His Ala Phe Ser Ser Thr Thr Leu Asp Asp Ile Leu His Ala Phe		
	380	385	390
30	Ser Glu Val Ser Ala Ala Arg Val Val Gly Gly Tyr Leu Leu Met		
	395	400	405
	Leu Ala Tyr Ala Cys Val Thr Met Leu Arg Trp Asp Cys Ala Gln		
	410	415	420
	Ser Gln Gly Ser Val Gly Leu Ala Gly Val Leu Leu Val Ala Leu		
	425	430	435
35	Ala Val Ala Ser Gly Leu Gly Leu Cys Ala Leu Leu Gly Ile Thr		
	440	445	450
	Phe Asn Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly		
	455	460	465

	Ile Gly Val Asp Asp Val Phe Leu Leu Ala His Ala Phe Thr Glu		
	470	475	480
	Ala Leu Pro Gly Thr Pro Leu Gln Glu Arg Met Gly Glu Cys Leu		
	485	490	495
5	Gln Arg Thr Gly Thr Ser Val Val Leu Thr Ser Ile Asn Asn Met		
	500	505	510
	Ala Ala Phe Leu Met Ala Ala Leu Val Pro Ile Pro Ala Leu Arg		
	515	520	525
10	Ala Phe Ser Leu Gln Ala Ala Ile Val Val Gly Cys Thr Phe Val		
	530	535	540
	Ala Val Met Leu Val Phe Pro Ala Ile Leu Ser Leu Asp Leu Arg		
	545	550	555
	Arg Arg His Cys Gln Arg Leu Asp Val Leu Cys Cys Phe Ser Ser		
	560	565	570
15	Pro Cys Ser Ala Gln Val Ile Gln Ile Leu Pro Gln Glu Leu Gly		
	575	580	585
	Asp Gly Thr Val Pro Val Gly Ile Ala His Leu Thr Ala Thr Val		
	590	595	600
20	Gln Ala Phe Thr His Cys Glu Ala Ser Ser Gln His Val Val Thr		
	605	610	615
	Ile Leu Pro Pro Gln Ala His Leu Val Pro Pro Pro Ser Asp Pro		
	620	625	630
	Leu Gly Ser Glu Leu Phe Ser Pro Gly Gly Ser Thr Arg Asp Leu		
	635	640	645
25	Leu Gly Gln Glu Glu Glu Thr Arg Gln Lys Ala Ala Cys Lys Ser		
	650	655	660
	Leu Pro Cys Ala Arg Trp Asn Leu Ala His Phe Ala Arg Tyr Gln		
	665	670	675
30	Phe Ala Pro Leu Leu Leu Gln Ser His Ala Lys Ala Ile Val Leu		
	680	685	690
	Val Leu Phe Gly Ala Leu Leu Gly Leu Ser Leu Tyr Gly Ala Thr		
	695	700	705
	Leu Val Gln Asp Gly Leu Ala Leu Thr Asp Val Val Pro Arg Gly		
	710	715	720
35	Thr Lys Glu His Ala Phe Leu Ser Ala Gln Leu Arg Tyr Phe Ser		
	725	730	735
	Leu Tyr Glu Val Ala Leu Val Thr Gln Gly Gly Phe Asp Tyr Ala		
	740	745	750

	His Ser Gln Arg Ala Leu Phe Asp Leu His Gln Arg Phe Ser Ser		
	755	760	765
	Leu Lys Ala Val Leu Pro Pro Pro Ala Thr Gln Ala Pro Arg Thr		
	770	775	780
5	Trp Leu His Tyr Tyr Arg Asn Trp Leu Gln Gly Ile Gln Ala Ala		
	785	790	795
	Phe Asp Gln Asp Trp Ala Ser Gly Arg Ile Thr Arg His Ser Tyr		
	800	805	810
10	Arg Asn Gly Ser Glu Asp Gly Ala Leu Ala Tyr Lys Leu Leu Ile		
	815	820	825
	Gln Thr Gly Asp Ala Gln Glu Pro Leu Asp Phe Ser Gln Leu Thr		
	830	835	840
	Thr Arg Lys Leu Val Asp Arg Glu Gly Leu Ile Pro Pro Glu Leu		
	845	850	855
15	Phe Tyr Met Gly Leu Thr Val Trp Val Ser Ser Asp Pro Leu Gly		
	860	865	870
	Leu Ala Ala Ser Gln Ala Asn Phe Tyr Pro Pro Pro Glu Trp		
	875	880	885
20	Leu His Asp Lys Tyr Asp Thr Thr Gly Glu Asn Leu Arg Ile Pro		
	890	895	900
	Pro Ala Gln Pro Leu Glu Phe Ala Gln Phe Pro Phe Leu Leu Arg		
	905	910	915
	Gly Leu Gln Lys Thr Ala Asp Phe Val Glu Ala Ile Glu Gly Ala		
	920	925	930
25	Arg Ala Ala Cys Ala Glu Ala Gly Gln Ala Gly Val His Ala Tyr		
	935	940	945
	Pro Ser Gly Ser Pro Phe Leu Phe Trp Glu Gln Tyr Leu Gly Leu		
	950	955	960
30	Arg Arg Cys Phe Leu Leu Ala Val Cys Ile Leu Leu Val Cys Thr		
	965	970	975
	Phe Leu Val Cys Ala Leu Leu Leu Asn Pro Trp Thr Ala Gly		
	980	985	990
	Leu Ile Val Leu Val Leu Ala Met Met Thr Val Glu Leu Phe Gly		
	995	1000	1005
35	Ile Met Gly Phe Leu Gly Ile Lys Leu Ser Ala Ile Pro Val Val		
	1010	1015	1020
	Ile Leu Val Ala Ser Val Gly Ile Gly Val Glu Phe Thr Val His		
	1025	1030	1035

Val Ala Leu Gly Phe Leu Thr Thr Gln Gly Ser Arg Asn Leu Arg
 1040 1045 1050

Ala Ala His Ala Leu Glu His Thr Phe Ala Pro Val Thr Asp Gly
 1055 1060 1065

5 Ala Ile Ser Thr Leu Leu Gly Leu Leu Met Leu Ala Gly Ser His
 1070 1075 1080

Phe Asp Phe Ile Val Arg Tyr Phe Phe Ala Ala Leu Thr Val Leu
 1085 1090 1095

10 Thr Leu Leu Gly Leu Leu His Gly Leu Val Leu Leu Pro Val Leu
 1100 1105 1110

Leu Ser Ile Leu Gly Pro Pro Pro Glu Val Ile Gln Met Tyr Lys
 1115 1120 1125

Glu Ser Pro Glu Ile Leu Ser Pro Pro Ala Pro Gln Gly Gly
 1130 1135 1140

15 Leu Arg Trp Gly Ala Ser Ser Ser Leu Pro Gln Ser Phe Ala Arg
 1145 1150 1155

Val Thr Thr Ser Met Thr Val Ala Ile His Pro Pro Pro Leu Pro
 1160 1165 1170

20 Gly Ala Tyr Ile His Pro Ala Pro Asp Glu Pro Pro Trp Ser Pro
 1175 1180 1185

Ala Ala Thr Ser Ser Gly Asn Leu Ser Ser Arg Gly Pro Gly Pro
 1190 1195 1200

Ala Thr Gly
 1203

25 <210> 3
 <211> 228
 <212> DNA
 <213> Homo sapiens

30 <220>
 <221> unknown
 <222> 20, 27, 135, 156, 210
 <223> unknown base

<400> 3
 gctggggatgc acgcctaccc cagcggttcc cccttcctct tctggaaaca 50

35 gtatctgggc ctgcggcgct gcttcctgct ggccgtctgc atcctgctgg 100
 tgtgcacttt cctcgatgt gctctgctgc tcctnaaccc ctggacggct 150
 ggcctnatag tgctggatcc ggcgatgatg acagtggaaac tctttggat 200
 catgggtttn ctgggcatca agctgagt 228

<210> 4
<211> 76
<212> PRT
<213> Homo sapiens

5 <400> 4
Leu Gly Leu Ser Ser Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp
1 5 10 15

Glu Gln Tyr Ile Gly Leu Arg His Trp Leu Leu Leu Phe Ile Ser
20 25 30

10 Val Val Leu Ala Cys Thr Phe Leu Val Cys Ala Val Phe Leu Leu
35 40 45

Asn Pro Trp Thr Ala Gly Ile Ile Val Met Val Leu Ala Leu Met
50 55 60

15 Thr Val Glu Leu Phe Gly Met Met Gly Leu Ile Gly Ile Lys Leu
65 70 75

Ser
76

<210> 5
<211> 125
20 <212> DNA
<213> Homo sapiens

<220>
<221> unknown
<222> 115
25 <223> unknown base

<400> 5
gctgggggtgc acgcctaccc cagcggctcc cccttcctct tctgggaaca 50
gtatctgggc ctgcggcgct gtttcctgct ggccgtctgc atcctgctgg 100
tgtgcacttt cctcntctgt gctct 125

30 <210> 6
<211> 50
<212> DNA
<213> Homo sapiens

<220>
35 <221> unknown
<222> 13-14
<223> unknown base

<400> 6
ccggggcggca tgnngcgaag cggaccacgc tgggggggtgg ctcaggggag 50

40 <210> 7
<211> 1182

<212> PRT

<213> Mus musculus

<400> 7

5	Met Val Arg Pro Leu Ser Leu Gly Glu Leu Pro Pro Ser Tyr Thr	10	15
	1	5	
	Pro Pro Ala Arg Ser Ser Ala Pro His Ile Leu Ala Gly Ser Leu	25	30
	20		
	Gln Ala Pro Leu Trp Leu Arg Ala Tyr Phe Gln Gly Leu Leu Phe	40	45
	35		
10	Ser Leu Gly Cys Arg Ile Gln Lys His Cys Gly Lys Val Leu Phe	55	60
	50		
	Leu Gly Leu Val Ala Phe Gly Ala Leu Ala Leu Gly Leu Arg Val	70	75
	65		
15	Ala Val Ile Glu Thr Asp Leu Glu Gln Leu Trp Val Glu Val Gly	85	90
	80		
	Ser Arg Val Ser Gln Glu Leu His Tyr Thr Lys Glu Lys Leu Gly	100	105
	95		
	Glu Glu Ala Ala Tyr Thr Ser Gln Met Leu Ile Gln Thr Ala His	115	120
	110		
20	Gln Glu Gly Gly Asn Val Leu Thr Pro Glu Ala Leu Asp Leu His	130	135
	125		
	Leu Gln Ala Ala Leu Thr Ala Ser Lys Val Gln Val Ser Leu Tyr	145	150
	140		
25	Gly Lys Ser Trp Asp Leu Asn Lys Ile Cys Tyr Lys Ser Gly Val	160	165
	155		
	Pro Leu Ile Glu Asn Gly Met Ile Glu Arg Met Ile Glu Lys Leu	175	180
	170		
	Phe Pro Cys Val Ile Leu Thr Pro Leu Asp Cys Phe Trp Glu Gly	190	195
	185		
30	Ala Lys Leu Gln Gly Gly Ser Ala Tyr Leu Pro Gly Arg Pro Asp	205	210
	200		
	Ile Gln Trp Thr Asn Leu Asp Pro Gln Gln Leu Leu Glu Leu	220	225
	215		
35	Gly Pro Phe Ala Ser Leu Glu Gly Phe Arg Glu Leu Leu Asp Lys	235	240
	230		
	Ala Gln Val Gly Gln Ala Tyr Val Gly Arg Pro Cys Leu Asp Pro	250	255
	245		
	Asp Asp Pro His Cys Pro Pro Ser Ala Pro Asn Arg His Ser Arg		

	260	265	270
	Gln Ala Pro Asn Val Ala Gln Glu Leu Ser Gly Gly Cys His Gly		
	275	280	285
5	Phe Ser His Lys Phe Met His Trp Gln Glu Glu Leu Leu Leu Gly		
	290	295	300
	Gly Thr Ala Arg Asp Leu Gln Gly Gln Leu Leu Arg Ala Glu Ala		
	305	310	315
	Leu Gln Ser Thr Phe Leu Leu Met Ser Pro Arg Gln Leu Tyr Glu		
	320	325	330
10	His Phe Arg Gly Asp Tyr Gln Thr His Asp Ile Gly Trp Ser Glu		
	335	340	345
	Glu Gln Ala Ser Met Val Leu Gln Ala Trp Gln Arg Arg Phe Val		
	350	355	360
15	Gln Leu Ala Gln Glu Ala Leu Pro Ala Asn Ala Ser Gln Gln Ile		
	365	370	375
	His Ala Phe Ser Ser Thr Thr Leu Asp Asp Ile Leu Arg Ala Phe		
	380	385	390
	Ser Glu Val Ser Thr Thr Arg Val Val Gly Gly Tyr Leu Leu Met		
	395	400	405
20	Leu Ala Tyr Ala Cys Val Thr Met Leu Arg Trp Asp Cys Ala Gln		
	410	415	420
	Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala Leu		
	425	430	435
25	Ala Val Ala Ser Gly Leu Gly Leu Cys Ala Leu Leu Gly Ile Thr		
	440	445	450
	Phe Asn Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly		
	455	460	465
	Ile Gly Val Asp Asp Ile Phe Leu Leu Ala His Ala Phe Thr Lys		
	470	475	480
30	Ala Pro Pro Asp Thr Pro Leu Pro Glu Arg Met Gly Glu Cys Leu		
	485	490	495
	Arg Ser Thr Gly Thr Ser Val Ala Leu Thr Ser Val Asn Asn Met		
	500	505	510
35	Val Ala Phe Phe Met Ala Ala Leu Val Pro Ile Pro Ala Leu Arg		
	515	520	525
	Ala Phe Ser Leu Gln Ala Ala Ile Val Val Gly Cys Asn Phe Ala		
	530	535	540

	Ala Val Met Leu Val Phe Pro Ala Ile Leu Ser Leu Asp Leu Arg		
	545	550	555
	Arg Arg His Arg Gln Arg Leu Asp Val Leu Cys Cys Phe Ser Ser		
	560	565	570
5	Pro Cys Ser Ala Gln Val Ile Gln Met Leu Pro Gln Glu Leu Gly		
	575	580	585
	Asp Arg Ala Val Pro Val Gly Ile Ala His Leu Thr Ala Thr Val		
	590	595	600
10	Gln Ala Phe Thr His Cys Glu Ala Ser Ser Gln His Val Val Thr		
	605	610	615
	Ile Leu Pro Pro Gln Ala His Leu Leu Ser Pro Ala Ser Asp Pro		
	620	625	630
	Leu Gly Ser Glu Leu Tyr Ser Pro Gly Gly Ser Thr Arg Asp Leu		
	635	640	645
15	Leu Ser Gln Glu Glu Gly Thr Gly Pro Gln Ala Ala Cys Arg Pro		
	650	655	660
	Leu Leu Cys Ala His Trp Thr Leu Ala His Phe Ala Arg Tyr Gln		
	665	670	675
20	Phe Ala Pro Leu Leu Gln Thr Arg Ala Lys Ala Leu Val Leu		
	680	685	690
	Leu Phe Phe Gly Ala Leu Leu Gly Leu Ser Leu Tyr Gly Ala Thr		
	695	700	705
	Leu Val Gln Asp Gly Leu Ala Leu Thr Asp Val Val Pro Arg Gly		
	710	715	720
25	Thr Lys Glu His Ala Phe Leu Ser Ala Gln Leu Arg Tyr Phe Ser		
	725	730	735
	Leu Tyr Glu Val Ala Leu Val Thr Gln Gly Gly Phe Asp Tyr Ala		
	740	745	750
30	His Ser Gln Arg Ala Leu Phe Asp Leu His Gln Arg Phe Ser Ser		
	755	760	765
	Leu Lys Ala Val Leu Pro Pro Ala Thr Gln Ala Pro Arg Thr		
	770	775	780
	Trp Leu His Tyr Tyr Arg Ser Trp Leu Gln Gly Ile Gln Ala Ala		
	785	790	795
35	Phe Asp Gln Asp Trp Ala Ser Gly Arg Ile Thr Cys His Ser Tyr		
	800	805	810
	Arg Asn Gly Ser Glu Asp Gly Ala Leu Ala Tyr Lys Leu Leu Ile		
	815	820	825

	Gln Thr Gly Asn Ala Gln Glu Pro Leu Asp Phe Ser Gln Leu Thr		
	830	835	840
	Thr Arg Lys Leu Val Asp Lys Glu Gly Leu Ile Pro Pro Glu Leu		
	845	850	855
5	Phe Tyr Met Gly Leu Thr Val Trp Val Ser Ser Asp Pro Leu Gly		
	860	865	870
	Leu Ala Ala Ser Gln Ala Asn Phe Tyr Pro Pro Pro Glu Trp		
	875	880	885
10	Leu His Asp Lys Tyr Asp Thr Thr Gly Glu Asn Leu Arg Ile Pro		
	890	895	900
	Ala Ala Gln Pro Leu Glu Phe Ala Gln Phe Pro Phe Leu Leu His		
	905	910	915
	Gly Leu Gln Lys Thr Ala Asp Phe Val Glu Ala Ile Glu Gly Ala		
	920	925	930
15	Arg Ala Ala Cys Thr Glu Ala Gly Gln Ala Gly Val His Ala Tyr		
	935	940	945
	Pro Ser Gly Ser Pro Phe Leu Phe Trp Glu Gln Tyr Leu Gly Leu		
	950	955	960
20	Arg Arg Cys Phe Leu Leu Ala Val Cys Ile Leu Leu Val Cys Thr		
	965	970	975
	Phe Leu Val Cys Ala Leu Leu Leu Leu Ser Pro Trp Thr Ala Gly		
	980	985	990
	Leu Ile Val Leu Val Leu Ala Met Met Thr Val Glu Leu Phe Gly		
	995	1000	1005
25	Ile Met Gly Phe Leu Gly Ile Lys Leu Ser Ala Ile Pro Val Val		
	1010	1015	1020
	Ile Leu Val Ala Ser Ile Gly Ile Gly Val Glu Phe Thr Val His		
	1025	1030	1035
30	Val Ala Leu Gly Phe Leu Thr Ser His Gly Ser Arg Asn Leu Arg		
	1040	1045	1050
	Ala Ala Ser Ala Leu Glu Gln Thr Phe Ala Pro Val Thr Asp Gly		
	1055	1060	1065
	Ala Val Ser Thr Leu Leu Gly Leu Leu Met Leu Ala Gly Ser Asn		
	1070	1075	1080
35	Phe Asp Phe Ile Ile Arg Tyr Phe Phe Val Val Leu Thr Val Leu		
	1085	1090	1095
	Thr Leu Leu Gly Leu Leu His Gly Leu Leu Leu Pro Val Leu		
	1100	1105	1110

Leu Ser Ile Leu Gly Pro Pro Pro Gln Val Val Gln Val Tyr Lys
 1115 1120 1125

Glu Ser Pro Gln Thr Leu Asn Ser Ala Ala Pro Gln Arg Gly Gly
 1130 1135 1140

5 Leu Arg Trp Asp Arg Pro Pro Thr Leu Pro Gln Ser Phe Ala Arg
 1145 1150 1155

Val Thr Thr Ser Met Thr Val Ala Leu His Pro Pro Pro Leu Pro
 1160 1165 1170

Gly Ala Tyr Val His Pro Ala Ser Glu Glu Pro Thr
 10 1175 1180 1182

<210> 8
 <211> 4004
 <212> DNA
 <213> Homo sapiens

15 <400> 8
 cccacgcgtc cgggagaagc tgggggagga ggctgcatac acctctcaga 50
 tgctgataca gaccgcacgc caggagggag agaacatcct cacacccgaa 100
 gcacttggcc tccaccccca ggcagccctc actqccagta aagtccaagt 150
 atcactctat gggaaagtccct gggatttcaa caaaatctgc tacaagtcag 200

20 gagttccccct tattgaaaat ggaatgattt agcggatgat tgagaagctg 250
 tttccgtgcg tgatccctcac cccccctcgac tgcttctggg agggagccaa 300
 actccaaaggg ggctccgcct acctgccgtt cccaaatgtgg ctcacgagct 350
 gagtgggggc tgccatggct tctccacaa attcatgcac tggcaggagg 400
 aattgctgct gggaggcatg gccagagacc cccaaaggaga gctgctgagg 450

25 gcagaggccc tgcagagcac cttcttgctg atgagtcggcc gccagctgta 500
 cgagcatttc cgggggtgact atcagacaca tgacattggc tggagtgagg 550
 agcaggccag cacagtgcta caagcctggc agcggcgctt tgtgcagggtc 600
 ggtatggaca aggacagggg ggtgcctgaa ggccattccc tcctcctgcc 650
 ccctcctatc caccctgttt ctccagctgg cccaggaggc cctgcctgag 700

30 aacgcttccc agcagatcca tgccttctcc tccaccaccc tggatgacat 750
 cctgcattgcg ttctctgaag tcagtgcgtc ccgtgtggtg ggaggctatc 800
 tgctcatggt gggcttgca cctggcacct tgccccacc ccaccccaa 850
 ccagtgcaca ccctggggag cccctgagac tgcccttcc cccacagct 900

ggcctatgcc tgggtgacca tgctgcggtg ggactgcgcc cagtcccagg 950
gttccgtggg ccttgccggg gtactgctgg tggccctggc ggtggcctca 1000
ggccttggc tctgtgcct gctcgccatc accttcaatg ctgccactac 1050
ccaggtacgc caggactgca gggcagactc agtgcagtc accaggctc 1100
5 acgggtcctc agctgcccgc tcctctgccc ctccaggtgc tgcccttctt 1150
gactctggga atcggcgtgg atgacgtatt cctgctggcg catgccttca 1200
cagaggctct gcctggcacc cctctccagg tggggccttg tcccccaagg 1250
ctcatctgag gcagctcagc ttactggta agagcctctt ggtaaagt 1300
accttggct gctaataaac ac ctcggtgcct cttgtccccca tggtaaaca 1350
10 gggaaataa tagtgctgtg tcctaagggt tattgtttgg atcagtgaag 1400
taactcaagt tgaatgctta gaacagccca tcatacgtac atggtaacca 1450
ataaaatgcta gccactgtgt tatgactgcc ccacctctgc accccaagtt 1500
cctgagcctc cccttcaactc cactttgaca cggccctcc ctgtgaccc 1550
gagggcaggt cccactctg tcctggcagg ggcgcattgg cgagtgtctg 1600
15 cagcgcacgg gcaccagggt tggactcaca tccatcaaca acatggccgc 1650
cttcctcatg gctgcctcg ttccatccc tgcgctgcga gccttctccc 1700
tacagcctgg acctacggcg ggcactgc cagcgccttgc atgtgctctg 1750
ctgcttctcc aggtactgcc tgcgcggcag ccccttccctc ccgtgaccc 1800
cgccagcctg tccctcacc agcattcaa ggcacagacc tgcaccc 1850
20 tctctacctc ttccagcccc tgcctctgc aggtgattca gatcctgccc 1900
caggagctgg gggacgggac agtaccagggt ggcattgcc acctcactgc 1950
cacagttcaa gccttaccc actgtgaagc cagcagccag catgtggta 2000
ccatcctgcc tccccaaagcc cacctggcgc ccccaccttc tgacccactg 2050
ggctctgagc tcttcagccc tggagggtcc acacgggacc ttctaggcca 2100
25 ggaggaggag acaaggcaga aggcagcctg caagtccctg ccctgtgcc 2150
gctggaatct tgcccatatc gcccgcatac agtttgcggc gttgctgctc 2200
cagtcacatg ccaaggccat cgtgctggc ctcttggc ctcttctggg 2250
cctgagcctc tacggagcca cttgggtgca agacggcctg gcccgtacgg 2300

atgtggtgcc tcggggcacc aaggagcatg ctttcctgag cgcccagctc 2350
aggtaacttct ccctgtacga ggtggccctg gtgaccagg gtggcttga 2400
ctacgcccac tcccaacgctg ccctcttga tctgcaccag cgcttcagtt 2450
ccctcaaggc ggtgctgccc ccaccggcca cccaggcacc cgcacactgg 2500
5 ctgcactatt accgcaactg gctacaggga atccaggctg ctttgcacca 2550
ggactgggct tctggcgca tcacccgcca ctcgtaccgc aatggctctg 2600
aggatggggc cctggctac aagctgctca tccagactgg agacgcccag 2650
gagcctctgg atttcagcca ggttgggaga gggctggagg ggtccactag 2700
tacaggggct gcaggcctcc tggcccccagg cttcagccc tctctgcctc 2750
10 tgcaagctgac cacaaggaag ctggtgacca gagagggact gattccaccc 2800
gagctttct acatggggct gaccgtgtgg gtgagcagtg acccccctgg 2850
tctggcagcc tcacaggcca acttctaccc cccacccctt gaatggctgc 2900
acgacaaata cgacaccacg ggggagaacc ttcgcagtga gtcttgggg 2950
gagctcgcca agagcctcag cctcgcc:ac acaagccctg agcctgaggc 3000
15 cttgtccact ctgccccgtg ctacccgccc tggccctctc cctttctcc 3050
cttccccctcc cttccacagt cccgcccagct cagcccttgg agtttgcacca 3100
gttcccccttc ctgctgcgtg gcctccagaa gactgcagac tttgtggagg 3150
ccatcgaggg ggcccgccca gcatgcgcag aggccggcca ggctgggtgg 3200
cacgcctacc ccagcggctc ccccttcctc ttctggaaac agtatctggg 3250
20 cctgcggcgc tgcctctgc tggccgtctg catcctgctg gtgtgcactt 3300
tcctcgtctg tgctctgctg ctccctcaacc cctggacggc tggcctcata 3350
gtgagtgctt gcaggagtgg ggacagagac accccacccct tccctgccc 3400
gcctgtcatc cttccctgcca ggagccctct gtgagccctg tctccctcag 3450
gtgctggtcc tggcgatgat gacagtggaa ctcttggta tcatggttt 3500
25 cctgggcata aagctgagtg ccatccccgt ggtgatcctt gtggcctctg 3550
taggcattgg cggtgagttc acagtccacg tggctctggc gagcacgggc 3600
accccgggga gggaccaatc agctgattca gtattcaaca catattgttc 3650
aagccctac tatgtgctag gtactattta agaatttggg ctgggtggac 3700

gtggtggctc attcctgtaa tcccagcaacttgggaggcc gaggcgggtg 3750
gatcacctga ggtcgaggactcgaaaccag cctggccaac atggtaaac 3800
cctgtcttta ctaaaaatac aaaaaattag ccaggcgtgg tggcacatgc 3850
cagtagtccc agtactttg gaggctgagg cagaattgct tgaacctggg 3900
5 aggcgaaggt tgcagtgagc tgagatcgtg ccattgcaactcagcctggg 3950
caacaagagt gcaactctcc gtctcaaaaa aaaaaaaaaa aagggcggcc 4000
gcga 4004

<210> 9
<211> 2082
10 <212> DNA
<213> Homo sapiens

<400> 9
ttccggcatg actcgatcgc cgccccctcag agagctgccc ccgagttaca 50
caccccccagc tcgaaccgca gcaccccaaga tcctagctgg gaggctgaag 100
15 gctccactct ggcttcgtgc ttacttccag ggcctgctct tctctctggg 150
atgcgggatc cagagacatt gtggcaaaagt gctcttctg ggactgttgg 200
cctttggggc cctggcatta ggtctccgca tggccattat tgagacaaac 250
ttggaacagc tctgggtaga agtgggcagc cgggtgagcc aggagctgca 300
ttacaccaag gagaagctgg gggaggaggc tgcatacacc tctcagatgc 350
20 tgatacagac cgcacgcccag gagggagaga acatcctcac acccgaagca 400
cttggcctcc acctccaggc agccctcaact gcccaggtaaag tccaaagtatc 450
actctatggg aagtccctggg atttgaacaa aatctgctac aagtcaaggag 500
ttcccccttat tgaaaatgga atgattgagt ggatgattga gaagctgttt 550
ccgtgcgtga tcctcacccccc cctcgactgc ttctgggagg gagccaaact 600
25 ccaaggggggc tccgcctacc tgcccggccg cccggatatc cagtggacca 650
acctggatcc agagcagctg ctggaggaggc tgggtccctt tgcctccctt 700
gagggcttcc gggagctgct agacaaggca caggtgggcc aggccctacgt 750
ggggcggccc tgtctgcacc ctgatgaccc ccaactgccc cctagtgccc 800
ccaaccatca cagcaggcag gctcccaatg tggctcacga gctgagtggg 850
30 ggctgccatg gcttctccca caaattcatg cactggcagg aggaattgct 900

gctgggaggc atggccagag accccaagg agagctgctg agggcagagg 950
 ccctgcagag cacttcttg ctgatgagtc cccgcccagct gtacgagcat 1000
 ttccgggtg actatcagac acatgacatt ggctggagtg aggagcaggc 1050
 cagcacagtg ctacaagcct ggcaagcggcg ctttgcagc ctggcccagg 1100
 5 aggcctgcc tgagaacgct tccacgaca tccatgcctt ctccctccacc 1150
 accctggata acatcctgca tgcgttctct gaagtcagtg ctgcccgtgt 1200
 ggtgggaggc tatctgctca tgctggccta tgccctgtgt accatgctgc 1250
 ggtgggactg cgcccagtcc cagggttccg tgggccttgc cggggtaactg 1300
 ctggcggccc tggcggtggc ctcaggcctt gggctctgtg ccctgctcgg 1350
 10 catcacccatc aatgctgcca ctacccaggt gctgcccttc ttggctctgg 1400
 gaatcggcgt ggatgacgta ttctgtctgg cgcatgcctt cacagaggct 1450
 ctgcctggca cccctctcca ggagcgcattt ggcgagtgcc tgcagcgcac 1500
 gggaccagt gtcgtactca catccatcaa caacatggcc gcccttcctca 1550
 tggctgcctt cgttccatc cctgcgtgc gaggccttctc cttacagcca 1600
 15 tcctcagcct ggacctacgg cggcgccact gccagcgcct tgaatgtgctc 1650
 tgctgcttctt ccagtcctg ctctgctcag gtgattcaga tcctgccccca 1700
 ggagctgggg gacgggacag taccagtggg cattgcccac ctcactgcca 1750
 cagttcaagc ctttacccac tgtgaagcca gcagccagca tgtggtcacc 1800
 atcctgcctc cccaagccca cctgggtggcc ccaccccttg acccactggg 1850
 20 ctctgagctc ttcagccctg gagggtccac acgggacctt cttaggcagg 1900
 aggaggagac aaggcagaag gcagcctgca agtccctgcc ctgtgcccgc 1950
 tggaaatctt cccatttcgc cccggaattt ctgcagcccg ggggatccac 2000
 tagttctaga gcggccgcca ccgcgggtggc gctccagctt ttgttccctt 2050
 tagtgagggt taattgcgcg cttgggtatc tt 2082

25 <210> 10
 <211> 1315
 <212> PRT
 <213> Homo sapiens

30 <400> 10
 Met Glu Lys Tyr His Val Leu Glu Met Ile Gly Glu Gly Ser Phe
 1 5 10 15

Gly Arg Val Tyr Lys Gly Arg Arg Lys Tyr Ser Ala Gln Val Val
 20 25 30

Ala Leu Lys Phe Ile Pro Lys Leu Gly Arg Ser Glu Lys Glu Leu
 35 40 45

5 Arg Asn Leu Gln Arg Glu Ile Glu Ile Met Arg Gly Leu Arg His
 50 55 60

Pro Asn Ile Val His Met Leu Asp Ser Phe Glu Thr Asp Lys Glu
 65 70 75

Val Val Val Val Thr Asp Tyr Ala Glu Gly Glu Leu Phe Gln Ile
 10 80 85 90

Leu Glu Asp Asp Gly Lys Leu Pro Glu Asp Gln Val Gln Ala Ile
 95 100 105

Ala Ala Gln Leu Val Ser Ala Leu Tyr Tyr Leu His Ser His Arg
 110 115 120

15 Ile Leu His Arg Asp Met Lys Pro Gln Asn Ile Leu Leu Ala Lys
 125 130 135

Gly Gly Gly Ile Lys Leu Cys Asp Phe Gly Phe Ala Arg Ala Met
 140 145 150

Ser Thr Asn Thr Met Val Leu Thr Ser Ile Lys Gly Thr Pro Leu
 20 155 160 165

Tyr Met Ser Pro Glu Leu Val Glu Glu Arg Pro Tyr Asp His Thr
 170 175 180

Ala Asp Leu Trp Ser Val Gly Cys Ile Leu Tyr Glu Leu Ala Val
 185 190 195

25 Gly Thr Pro Pro Phe Tyr Ala Thr Ser Ile Phe Gln Leu Val Ser
 200 205 210

Leu Ile Leu Lys Asp Pro Val Arg Trp Pro Ser Thr Ile Ser Pro
 215 220 225

30 Cys Phe Lys Asn Phe Leu Gln Gly Leu Leu Thr Lys Asp Pro Arg
 230 235 240

Gln Arg Leu Ser Trp Pro Asp Leu Leu Tyr His Pro Phe Ile Ala
 245 250 255

Gly His Val Thr Ile Ile Thr Glu Pro Ala Gly Pro Asp Leu Gly
 260 265 270

35 Thr Pro Phe Thr Ser Arg Leu Pro Pro Glu Leu Gln Val Leu Lys
 275 280 285

Asp Glu Gln Ala His Arg Leu Ala Pro Lys Gly Asn Gln Ser Arg
 290 295 300

	Ile Leu Thr Gln Ala Tyr Lys Arg Met Ala Glu Glu Ala Met Gln		
	305	310	315
	Lys Lys His Gln Asn Thr Gly Pro Ala Leu Glu Gln Glu Asp Lys		
	320	325	330
5	Thr Ser Lys Val Ala Pro Gly Thr Ala Pro Leu Pro Arg Leu Gly		
	335	340	345
	Ala Thr Pro Gln Glu Ser Ser Leu Leu Ala Gly Ile Leu Ala Ser		
	350	355	360
10	Glu Leu Lys Ser Ser Trp Ala Lys Ser Gly Thr Gly Glu Val Pro		
	365	370	375
	Ser Ala Pro Arg Glu Asn Arg Thr Thr Pro Asp Cys Glu Arg Ala		
	380	385	390
	Phe Pro Glu Glu Arg Pro Glu Val Leu Gly Gln Arg Ser Thr Asp		
	395	400	405
15	Val Val Asp Leu Glu Asn Glu Glu Pro Asp Ser Asp Asn Glu Trp		
	410	415	420
	Gln His Leu Leu Glu Thr Thr Glu Pro Val Pro Ile Gln Leu Lys		
	425	430	435
20	Ala Pro Leu Thr Leu Leu Cys Asn Pro Asp Phe Cys Gln Arg Ile		
	440	445	450
	Gln Ser Gln Leu His Glu Ala Gly Gly Gln Ile Leu Lys Gly Ile		
	455	460	465
	Leu Glu Gly Ala Ser His Ile Leu Pro Ala Phe Arg Val Leu Ser		
	470	475	480
25	Ser Leu Leu Ser Ser Cys Ser Asp Ser Val Ala Leu Tyr Ser Phe		
	485	490	495
	Cys Arg Glu Ala Gly Leu Pro Gly Leu Leu Leu Ser Leu Leu Arg		
	500	505	510
30	His Ser Gln Glu Ser Asn Ser Leu Gln Gln Ser Trp Tyr Gly		
	515	520	525
	Thr Phe Leu Gln Asp Leu Met Ala Val Ile Gln Ala Tyr Phe Ala		
	530	535	540
	Cys Thr Phe Asn Leu Glu Arg Ser Gln Thr Ser Asp Ser Leu Gln		
	545	550	555
35	Val Phe Gln Glu Ala Ala Asn Leu Phe Leu Asp Leu Leu Gly Lys		
	560	565	570
	Leu Leu Ala Gln Pro Asp Asp Ser Glu Gln Thr Leu Arg Arg Asp		
	575	580	585

Ser Leu Met Cys Phe Thr Val Leu Cys Glu Ala Met Asp Gly Asn
 590 595 600
 Ser Arg Ala Ile Ser Lys Ala Phe Tyr Ser Ser Leu Leu Thr Thr
 605 610 615
 5 Gln Gln Val Val Leu Asp Gly Leu Leu His Gly Leu Thr Val Pro
 620 625 630
 Gln Leu Pro Val His Thr Pro Gln Gly Ala Pro Gln Val Ser Gln
 635 640 645
 10 Pro Leu Arg Glu Gln Ser Glu Asp Ile Pro Gly Ala Ile Ser Ser
 650 655 660
 Ala Leu Ala Ala Ile Cys Thr Ala Pro Val Gly Leu Pro Asp Cys
 665 670 675
 Trp Asp Ala Lys Glu Gln Val Cys Trp His Leu Ala Asn Gln Leu
 680 685 690
 15 Thr Glu Asp Ser Ser Gln Leu Arg Pro Ser Leu Ile Ser Gly Leu
 695 700 705
 Gln His Pro Ile Leu Cys Leu His Leu Leu Lys Val Leu Tyr Ser
 710 715 720
 20 Cys Cys Leu Val Ser Glu Gly Leu Cys Arg Leu Leu Gly Gln Glu
 725 730 735
 Pro Leu Ala Leu Glu Ser Leu Phe Met Leu Ile Gln Gly Lys Val
 740 745 750
 Lys Val Val Asp Trp Glu Glu Ser Thr Glu Val Thr Leu Tyr Phe
 755 760 765
 25 Leu Ser Leu Leu Val Phe Arg Leu Gln Asn Leu Pro Cys Gly Met
 770 775 780
 Glu Lys Leu Gly Ser Asp Val Ala Thr Leu Phe Thr His Ser His
 785 790 795
 30 Val Val Ser Leu Val Ser Ala Ala Cys Leu Leu Gly Gln Leu
 800 805 810
 Gly Gln Gln Gly Val Thr Phe Asp Leu Gln Pro Met Glu Trp Met
 815 820 825
 Ala Ala Ala Thr His Ala Leu Ser Ala Pro Ala Glu Val Arg Leu
 830 835 840
 35 Thr Pro Pro Gly Ser Cys Gly Phe Tyr Asp Gly Leu Leu Ile Leu
 845 850 855
 Leu Leu Gln Leu Leu Thr Glu Gln Gly Lys Ala Ser Leu Ile Arg
 860 865 870

	Asp Met Ser Ser Ser Glu Met Trp Thr Val Leu Trp His Arg Phe		
	875	880	885
	Ser Met Val Leu Arg Leu Pro Glu Glu Ala Ser Ala Gln Glu Gly		
	890	895	900
5	Glu Leu Ser Leu Ser Ser Pro Pro Ser Pro Glu Pro Asp Trp Thr		
	905	910	915
	Leu Ile Ser Pro Gln Gly Met Ala Ala Leu Leu Ser Leu Ala Met		
	920	925	930
10	Ala Thr Phe Thr Gln Glu Pro Gln Leu Cys Leu Ser Cys Leu Ser		
	935	940	945
	Gln His Gly Ser Ile Leu Met Ser Ile Leu Lys His Leu Leu Cys		
	950	955	960
	Pro Ser Phe Leu Asn Gln Leu Arg Gln Ala Pro His Gly Ser Glu		
	965	970	975
15	Phe Leu Pro Val Val Val Leu Ser Val Cys Gln Leu Leu Cys Phe		
	980	985	990
	Pro Phe Ala Leu Asp Met Asp Ala Asp Leu Leu Ile Val Val Leu		
	995	1000	1005
	Ala Asp Leu Arg Asp Ser Glu Val Ala Ala His Leu Leu Gln Val		
20	1010	1015	1020
	Cys Cys Tyr His Leu Pro Leu Met Gln Val Glu Leu Pro Ile Ser		
	1025	1030	1035
	Leu Leu Thr Arg Leu Ala Leu Met Asp Pro Thr Ser Leu Asn Gln		
	1040	1045	1050
25	Phe Val Asn Thr Val Ser Ala Ser Pro Arg Thr Ile Val Ser Phe		
	1055	1060	1065
	Leu Ser Val Ala Leu Leu Ser Asp Gln Pro Leu Leu Thr Ser Asp		
	1070	1075	1080
	Leu Leu Ser Leu Leu Ala His Thr Ala Arg Val Leu Ser Pro Ser		
30	1085	1090	1095
	His Leu Ser Phe Ile Gln Glu Leu Leu Ala Gly Ser Asp Glu Ser		
	1100	1105	1110
	Tyr Arg Pro Leu Arg Ser Leu Leu Gly His Pro Glu Asn Ser Val		
	1115	1120	1125
35	Arg Ala His Thr Tyr Arg Leu Leu Gly His Leu Leu Gln His Ser		
	1130	1135	1140
	Met Ala Leu Arg Gly Ala Leu Gln Ser Gln Ser Gly Leu Leu Ser		
	1145	1150	1155

Leu Leu Leu Leu Gly Leu Gly Asp Lys Asp Pro Val Val Arg Cys
 1160 1165 1170
 Ser Ala Ser Phe Ala Val Gly Asn Ala Ala Tyr Gln Ala Gly Pro
 1175 1180 1185
 5 Leu Gly Pro Ala Leu Ala Ala Val Pro Ser Met Thr Gln Leu
 1190 1195 1200
 Leu Gly Asp Pro Gln Ala Gly Ile Arg Arg Asn Val Ala Ser Ala
 1205 1210 1215
 10 Leu Gly Asn Leu Gly Pro Glu Gly Leu Gly Glu Glu Leu Leu Gln
 1220 1225 1230
 Cys Glu Val Pro Gln Arg Leu Leu Glu Met Ala Cys Gly Asp Pro
 1235 1240 1245
 Gln Pro Asn Val Lys Glu Ala Ala Leu Ile Ala Leu Arg Ser Leu
 1250 1255 1260
 15 Gln Gln Glu Pro Gly Ile His Gln Val Leu Val Ser Leu Gly Ala
 1265 1270 1275
 Ser Glu Lys Leu Ser Leu Leu Ser Leu Gly Asn Gln Ser Leu Pro
 1280 1285 1290
 20 His Ser Ser Pro Arg Pro Ala Ser Ala Lys His Cys Arg Lys Leu
 1295 1300 1305
 Ile His Leu Leu Arg Pro Ala His Ser Met
 1310 1315
 <210> 11
 <211> 48
 25 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Artificial sequence 1-48
 <400> 11
 30 ctatgaaatt aaccctcaact aaaggagct cccgtgagtc cctatgtg 48
 <210> 12
 <211> 48
 <212> DNA
 <213> Artificial sequence
 35 <220>
 <223> Artificial sequence 1-48
 <400> 12
 ggattctaat acgactcaact atagggcccc taaactccgc tgctccac 48
 <210> 13

<211> 396

<212> PRT

<213> Mus musculus

<400> 13

5	Met Ala Leu Pro Ala Ser Leu Leu Pro Leu Cys Cys Leu Ala Leu	1	5	10	15
	Leu Ala Leu Ser Ala Gln Ser Cys Gly Pro Gly Arg Gly Pro Val				
	20		25		30
10	Gly Arg Arg Arg Tyr Val Arg Lys Gln Leu Val Pro Leu Leu Tyr	35	40	45	
	Lys Gln Phe Val Pro Ser Met Pro Glu Arg Thr Leu Gly Ala Ser	50	55	60	
	Gly Pro Ala Glu Gly Arg Val Thr Arg Gly Ser Glu Arg Phe Arg	65	70	75	
15	Asp Leu Val Pro Asn Tyr Asn Pro Asp Ile Ile Phe Lys Asp Glu	80	85	90	
	Glu Asn Ser Gly Ala Asp Arg Leu Met Thr Glu Arg Cys Lys Glu	95	100	105	
20	Arg Val Asn Ala Leu Ala Ile Ala Val Met Asn Met Trp Pro Gly	110	115	120	
	Val Arg Leu Arg Val Thr Glu Gly Trp Asp Glu Asp Gly His His	125	130	135	
	Ala Gln Asp Ser Leu His Tyr Glu Gly Arg Ala Leu Asp Ile Thr	140	145	150	
25	Thr Ser Asp Arg Asp Arg Asn Lys Tyr Gly Leu Leu Ala Arg Leu	155	160	165	
	Ala Val Glu Ala Gly Phe Asp Trp Val Tyr Tyr Glu Ser Arg Asn	170	175	180	
	His Ile His Val Ser Val Lys Ala Asp Asn Ser Leu Ala Val Arg	185	190	195	
30	Ala Gly Gly Cys Phe Pro Gly Asn Ala Thr Val Arg Leu Arg Ser	200	205	210	
	Gly Glu Arg Lys Gly Leu Arg Glu Leu His Arg Gly Asp Trp Val	215	220	225	
35	Leu Ala Ala Asp Ala Ala Gly Arg Val Val Pro Thr Pro Val Leu	230	235	240	
	Leu Phe Leu Asp Arg Asp Leu Gln Arg Arg Ala Ser Phe Val Ala	245	250	255	

	Val Glu Thr Glu Arg Pro Pro Arg Lys Leu Leu Leu Thr Pro Trp		
	260	265	270
	His Leu Val Phe Ala Ala Arg Gly Pro Ala Pro Ala Pro Gly Asp		
	275	280	285
5	Phe Ala Pro Val Phe Ala Arg Arg Leu Arg Ala Gly Asp Ser Val		
	290	295	300
	Leu Ala Pro Gly Gly Asp Ala Leu Gln Pro Ala Arg Val Ala Arg		
	305	310	315
10	Val Ala Arg Glu Glu Ala Val Gly Val Phe Ala Pro Leu Thr Ala		
	320	325	330
	His Gly Thr Leu Leu Val Asn Asp Val Leu Ala Ser Cys Tyr Ala		
	335	340	345
	Val Leu Glu Ser His Gln Trp Ala His Arg Ala Phe Ala Pro Leu		
	350	355	360
15	Arg Leu Leu His Ala Leu Gly Ala Leu Leu Pro Gly Gly Ala Val		
	365	370	375
	Gln Pro Thr Gly Met His Trp Tyr Ser Arg Leu Leu Tyr Arg Leu		
	380	385	390
	Ala Glu Glu Leu Met Gly		
20	395 396		
	<210> 14		
	<211> 437		
	<212> PRT		
	<213> Mus musculus		
25	<400> 14		
	Met Leu Leu Leu Leu Ala Arg Cys Phe Leu Val Ile Leu Ala Ser		
	1	5	10
	15		
	Ser Leu Leu Val Cys Pro Gly Leu Ala Cys Gly Pro Gly Arg Gly		
	20	25	30
30	Phe Gly Lys Arg Arg His Pro Lys Lys Leu Thr Pro Leu Ala Tyr		
	35	40	45
	Lys Gln Phe Ile Pro Asn Val Ala Glu Lys Thr Leu Gly Ala Ser		
	50	55	60
	Gly Arg Tyr Glu Gly Lys Ile Thr Arg Asn Ser Glu Arg Phe Lys		
35	65	70	75
	Glu Leu Thr Pro Asn Tyr Asn Pro Asp Ile Ile Phe Lys Asp Glu		
	80	85	90
	Glu Asn Thr Gly Ala Asp Arg Leu Met Thr Gln Arg Cys Lys Asp		
	95	100	105

Lys Leu Asn Ala Leu Ala Ile Ser Val Met Asn Gln Trp Pro Gly
 110 115 120
 Val Arg Leu Arg Val Thr Glu Gly Trp Asp Glu Asp Gly His His
 125 130 135
 5 Ser Glu Glu Ser Leu His Tyr Glu Gly Arg Ala Val Asp Ile Thr
 140 145 150
 Thr Ser Asp Arg Asp Arg Ser Lys Tyr Gly Met Leu Ala Arg Leu
 155 160 165
 10 Ala Val Glu Ala Gly Phe Asp Trp Val Tyr Tyr Glu Ser Lys Ala
 170 175 180
 His Ile His Cys Ser Val Lys Ala Glu Asn Ser Val Ala Ala Lys
 185 190 195
 Ser Gly Gly Cys Phe Pro Gly Ser Ala Thr Val His Leu Glu Gln
 200 205 210
 15 Gly Gly Thr Lys Leu Val Lys Asp Leu Arg Pro Gly Asp Arg Val
 215 220 225
 Leu Ala Ala Asp Asp Gln Gly Arg Leu Leu Tyr Ser Asp Phe Leu
 230 235 240
 Thr Phe Leu Asp Arg Asp Glu Gly Ala Lys Lys Val Phe Tyr Val
 20 245 250 255
 Ile Glu Thr Leu Glu Pro Arg Glu Arg Leu Leu Leu Thr Ala Ala
 260 265 270
 His Leu Leu Phe Val Ala Pro His Asn Asp Ser Gly Pro Thr Pro
 275 280 285
 25 Gly Pro Ser Ala Leu Phe Ala Ser Arg Val Arg Pro Gly Gln Arg
 290 295 300
 Val Tyr Val Val Ala Glu Arg Gly Gly Asp Arg Arg Leu Leu Pro
 305 310 315
 Ala Ala Val His Ser Val Thr Leu Arg Glu Glu Ala Gly Ala
 320 325 330
 Tyr Ala Pro Leu Thr Ala His Gly Thr Ile Leu Ile Asn Arg Val
 335 340 345
 Leu Ala Ser Cys Tyr Ala Val Ile Glu Glu His Ser Trp Ala His
 350 355 360
 35 Arg Ala Phe Ala Pro Phe Arg Leu Ala His Ala Leu Leu Ala Ala
 365 370 375
 Leu Ala Pro Ala Arg Thr Asp Gly Gly Gly Gly Ser Ile Pro
 380 385 390

Ala Ala Gln Ser Ala Thr Glu Ala Arg Gly Ala Glu Pro Thr Ala
 395 400 405
 Gly Ile His Trp Tyr Ser Gln Leu Leu Tyr His Ile Gly Thr Trp
 410 415 420
 5 Leu Leu Asp Ser Glu Thr Met His Pro Leu Gly Met Ala Val Lys
 425 430 435
 Ala Ser
 437
 <210> 15
 10 <211> 803
 <212> PRT
 <213> Artificial sequence
 <220>
 <223> Artificial sequence 1-803
 15 <400> 15
 Met Ala Ala Gly Arg Pro Val Arg Gly Pro Glu Leu Ala Pro Arg
 1 5 10 15
 Arg Leu Leu Gln Leu Leu Leu Val Leu Leu Gly Gly Arg Gly
 20 25 30
 20 Arg Gly Ala Ala Leu Ser Gly Asn Val Thr Gly Pro Gly Pro Arg
 35 40 45
 Ser Ala Gly Gly Ser Ala Arg Arg Asn Ala Pro Val Thr Ser Pro
 50 55 60
 25 Pro Pro Pro Leu Leu Ser His Cys Gly Arg Ala Ala His Cys Glu
 65 70 75
 Pro Leu Arg Tyr Asn Val Cys Leu Gly Ser Ala Leu Pro Tyr Gly
 80 85 90
 Ala Thr Thr Thr Leu Leu Ala Gly Asp Ser Asp Ser Gln Glu Glu
 95 100 105
 30 Ala His Ser Lys Leu Val Leu Trp Ser Gly Leu Arg Asn Ala Pro
 110 115 120
 Arg Cys Trp Ala Val Ile Gln Pro Leu Leu Cys Ala Val Tyr Met
 125 130 135
 35 Pro Lys Cys Glu Asn Asp Arg Val Glu Leu Pro Ser Arg Thr Leu
 140 145 150
 Cys Gln Ala Thr Arg Gly Pro Cys Ala Ile Val Glu Arg Glu Arg
 155 160 165
 Gly Trp Pro Asp Phe Leu Arg Cys Thr Pro Asp His Phe Pro Glu
 170 175 180

	Gly Cys Pro Asn Glu Val Gln Asn Ile Lys Phe Asn Ser Ser Gly		
	185	190	195
	Gln Cys Glu Ala Pro Leu Val Arg Thr Asp Asn Pro Lys Ser Trp		
	200	205	210
5	Tyr Glu Asp Val Glu Gly Cys Gly Ile Gln Cys Gln Asn Pro Leu		
	215	220	225
	Phe Thr Glu Ala Glu His Gln Asp Met His Ser Tyr Ile Ala Ala		
	230	235	240
10	Phe Gly Ala Val Thr Gly Leu Cys Thr Leu Phe Thr Leu Ala Thr		
	245	250	255
	Phe Val Ala Asp Trp Arg Asn Ser Asn Arg Tyr Pro Ala Val Ile		
	260	265	270
	Leu Phe Tyr Val Asn Ala Cys Phe Phe Val Gly Ser Ile Gly Trp		
	275	280	285
15	Leu Ala Gln Phe Met Asp Gly Ala Arg Arg Glu Ile Val Cys Arg		
	290	295	300
	Ala Asp Gly Thr Met Arg Phe Gly Glu Pro Thr Ser Ser Glu Thr		
	305	310	315
20	Leu Ser Cys Val Ile Ile Phe Val Ile Val Tyr Tyr Ala Leu Met		
	320	325	330
	Ala Gly Val Val Trp Phe Val Val Leu Thr Tyr Ala Trp His Thr		
	335	340	345
	Ser Phe Lys Ala Leu Gly Thr Thr Tyr Gln Pro Leu Ser Gly Lys		
	350	355	360
25	Thr Ser Tyr Phe His Leu Leu Thr Trp Ser Leu Pro Phe Val Leu		
	365	370	375
	Thr Val Ala Ile Leu Ala Val Ala Gln Val Asp Gly Asp Ser Val		
	380	385	390
30	Ser Gly Ile Cys Phe Val Gly Tyr Lys Asn Tyr Arg Tyr Arg Ala		
	395	400	405
	Gly Phe Val Leu Ala Pro Ile Gly Leu Val Leu Ile Val Gly Gly		
	410	415	420
	Tyr Phe Leu Ile Arg Gly Val Met Thr Leu Phe Ser Ile Lys Ser		
	425	430	435
35	Asn His Pro Gly Leu Leu Ser Glu Lys Ala Ala Ser Lys Ile Asn		
	440	445	450
	Glu Thr Met Leu Arg Leu Gly Ile Phe Gly Phe Leu Ala Phe Gly		
	455	460	465

Phe	Val	Leu	Ile	Thr	Phe	Ser	Cys	His	Phe	Tyr	Asp	Phe	Phe	Asn	
					470				475					480	
Gln	Ala	Glu	Trp	Glu	Arg	Ser	Phe	Arg	Asp	Tyr	Val	Leu	Cys	Gln	
				485				490						495	
5	Ala	Asn	Val	Thr	Ile	Gly	Leu	Pro	Thr	Lys	Lys	Pro	Ile	Pro	Asp
					500				505						510
Cys	Glu	Ile	Lys	Asn	Arg	Pro	Ser	Leu	Leu	Val	Glu	Lys	Ile	Asn	
				515				520							525
10	Leu	Phe	Ala	Met	Phe	Gly	Thr	Gly	Ile	Ala	Met	Ser	Thr	Trp	Val
				530				535							540
Trp	Thr	Lys	Ala	Thr	Leu	Leu	Ile	Trp	Arg	Arg	Thr	Trp	Cys	Arg	
				545				550							555
Leu	Thr	Gly	His	Ser	Asp	Asp	Glu	Pro	Lys	Arg	Ile	Lys	Lys	Ser	
				560				565							570
15	Lys	Met	Ile	Ala	Lys	Ala	Phe	Ser	Lys	Arg	Arg	Glu	Leu	Leu	Gln
				575				580							585
Asn	Pro	Gly	Gln	Glu	Leu	Ser	Phe	Ser	Met	His	Thr	Val	Ser	His	
				590				595							600
20	Asp	Gly	Pro	Val	Ala	Gly	Ile	Ala	Phe	Glu	Leu	Asn	Glu	Pro	Ser
				605				610							615
Ala	Asp	Val	Ser	Ser	Ala	Trp	Ala	Gln	His	Val	Thr	Lys	Met	Val	
				620				625							630
Ala	Arg	Arg	Gly	Ala	Ile	Leu	Pro	Gln	Asp	Val	Ser	Val	Thr	Pro	
				635				640							645
25	Val	Ala	Thr	Pro	Val	Pro	Pro	Glu	Glu	Gln	Ala	Asn	Leu	Trp	Leu
				650				655							660
Val	Glu	Ala	Glu	Ile	Ser	Pro	Glu	Leu	Glu	Lys	Arg	Leu	Gly	Arg	
				665				670							675
30	Lys	Lys	Lys	Arg	Arg	Lys	Arg	Lys	Glu	Val	Cys	Pro	Leu	Gly	
				680				685							690
Pro	Ala	Pro	Glu	Leu	His	His	Ser	Ala	Pro	Val	Pro	Ala	Thr	Ser	
				695				700							705
Ala	Val	Pro	Arg	Leu	Pro	Gln	Leu	Pro	Arg	Gln	Lys	Cys	Leu	Val	
				710				715							720
35	Ala	Ala	Asn	Ala	Trp	Gly	Thr	Gly	Glu	Pro	Cys	Arg	Gln	Gly	Ala
				725				730							735
Trp	Thr	Val	Val	Ser	Asn	Pro	Phe	Cys	Pro	Glu	Pro	Ser	Pro	His	
				740				745							750

Gln Asp Pro Phe Leu Pro Gly Ala Ser Ala Pro Arg Val Trp Ala
 755 760 765
 Gln Gly Arg Leu Gln Gly Leu Gly Ser Ile His Ser Arg Thr Asn
 770 775 780
 5 Leu Met Glu Ala Glu Leu Leu Asp Ala Asp Ser Asp Phe Glu Gln
 785 790 795
 Lys Leu Ile Ser Glu Glu Asp Leu
 800 803
 <210> 16
 10 <211> 793
 <212> PRT
 <213> Artificial sequence
 <220>
 <223> Artificial sequence 1-793
 15 <400> 16
 Met Ala Ala Gly Arg Pro Val Arg Gly Pro Glu Leu Ala Pro Arg
 1 5 10 15
 Arg Leu Leu Gln Leu Leu Leu Val Leu Leu Gly Gly Arg Gly
 20 25 30
 20 Arg Gly Ala Ala Leu Ser Gly Asn Val Thr Gly Pro Gly Pro Arg
 35 40 45
 Ser Ala Gly Gly Ser Ala Arg Arg Asn Ala Pro Val Thr Ser Pro
 50 55 60
 25 Pro Pro Pro Leu Leu Ser His Cys Gly Arg Ala Ala His Cys Glu
 65 70 75
 Pro Leu Arg Tyr Asn Val Cys Leu Gly Ser Ala Leu Pro Tyr Gly
 80 85 90
 Ala Thr Thr Thr Leu Leu Ala Gly Asp Ser Asp Ser Gln Glu Glu
 95 100 105
 30 Ala His Ser Lys Leu Val Leu Trp Ser Gly Leu Arg Asn Ala Pro
 110 115 120
 Arg Cys Trp Ala Val Ile Gln Pro Leu Leu Cys Ala Val Tyr Met
 125 130 135
 Pro Lys Cys Glu Asn Asp Arg Val Glu Leu Pro Ser Arg Thr Leu
 140 145 150
 35 Cys Gln Ala Thr Arg Gly Pro Cys Ala Ile Val Glu Arg Glu Arg
 155 160 165
 Gly Trp Pro Asp Phe Leu Arg Cys Thr Pro Asp His Phe Pro Glu
 170 175 180

Gly Cys Pro Asn Glu Val Gln Asn Ile Lys Phe Asn Ser Ser Gly
 185 190 195
 Gln Cys Glu Ala Pro Leu Val Arg Thr Asp Asn Pro Lys Ser Trp
 200 205 210
 5 Tyr Glu Asp Val Glu Gly Cys Gly Ile Gln Cys Gln Asn Pro Leu
 215 220 225
 Phe Thr Glu Ala Glu His Gln Asp Met His Ser Tyr Ile Ala Ala
 230 235 240
 Phe Gly Ala Val Thr Gly Leu Cys Thr Leu Phe Thr Leu Ala Thr
 10 245 250 255
 Phe Val Ala Asp Trp Arg Asn Ser Asn Arg Tyr Pro Ala Val Ile
 260 265 270
 Leu Phe Tyr Val Asn Ala Cys Phe Phe Val Gly Ser Ile Gly Trp
 275 280 285
 15 Leu Ala Gln Phe Met Asp Gly Ala Arg Arg Glu Ile Val Cys Arg
 290 295 300
 Ala Asp Gly Thr Met Arg Phe Gly Glu Pro Thr Ser Ser Glu Thr
 305 310 315
 Leu Ser Cys Val Ile Ile Phe Val Ile Val Tyr Tyr Ala Leu Met
 20 320 325 330
 Ala Gly Val Val Trp Phe Val Val Leu Thr Tyr Ala Trp His Thr
 335 340 345
 Ser Phe Lys Ala Leu Gly Thr Thr Tyr Gln Pro Leu Ser Gly Lys
 350 355 360
 25 Thr Ser Tyr Phe His Leu Leu Thr Trp Ser Leu Pro Phe Val Leu
 365 370 375
 Thr Val Ala Ile Leu Ala Val Ala Gln Val Asp Gly Asp Ser Val
 380 385 390
 Ser Gly Ile Cys Phe Val Gly Tyr Lys Asn Tyr Arg Tyr Arg Ala
 30 395 400 405
 Gly Phe Val Leu Ala Pro Ile Gly Leu Val Leu Ile Val Gly Gly
 410 415 420
 Tyr Phe Leu Ile Arg Gly Val Met Thr Leu Phe Ser Ile Lys Ser
 425 430 435
 35 Asn His Pro Gly Leu Leu Ser Glu Lys Ala Ala Ser Lys Ile Asn
 440 445 450
 Glu Thr Met Leu Arg Leu Gly Ile Phe Gly Phe Leu Ala Phe Gly
 455 460 465

	Phe Val Leu Ile Thr Phe Ser Cys His Phe Tyr Asp Phe Phe Asn		
	470	475	480
	Gln Ala Glu Trp Glu Arg Ser Phe Arg Asp Tyr Val Leu Cys Gln		
	485	490	495
5	Ala Asn Val Thr Ile Gly Leu Pro Thr Lys Lys Pro Ile Pro Asp		
	500	505	510
	Cys Glu Ile Lys Asn Arg Pro Ser Leu Leu Val Glu Lys Ile Asn		
	515	520	525
10	Leu Phe Ala Met Phe Gly Thr Gly Ile Ala Met Ser Thr Leu Val		
	530	535	540
	Trp Thr Lys Ala Thr Leu Leu Ile Trp Arg Arg Thr Trp Cys Arg		
	545	550	555
	Leu Thr Gly His Ser Asp Asp Glu Pro Lys Arg Ile Lys Lys Ser		
	560	565	570
15	Lys Met Ile Ala Lys Ala Phe Ser Lys Arg Arg Glu Leu Leu Gln		
	575	580	585
	Asn Pro Gly Gln Glu Leu Ser Phe Ser Met His Thr Val Ser His		
	590	595	600
20	Asp Gly Pro Val Ala Gly Leu Ala Phe Glu Leu Asn Glu Pro Ser		
	605	610	615
	Ala Asp Val Ser Ser Ala Trp Ala Gln His Val Thr Lys Met Val		
	620	625	630
	Ala Arg Arg Gly Ala Ile Leu Pro Gln Asp Val Ser Val Thr Pro		
	635	640	645
25	Val Ala Thr Pro Val Pro Pro Glu Glu Gln Ala Asn Leu Trp Leu		
	650	655	660
	Val Glu Ala Glu Ile Ser Pro Glu Leu Glu Lys Arg Leu Gly Arg		
	665	670	675
30	Lys Lys Lys Arg Arg Lys Arg Lys Lys Glu Val Cys Pro Leu Gly		
	680	685	690
	Pro Ala Pro Glu Leu His His Ser Ala Pro Val Pro Ala Thr Ser		
	695	700	705
	Ala Val Pro Arg Leu Pro Gln Leu Pro Arg Gln Lys Cys Leu Val		
	710	715	720
35	Ala Ala Asn Ala Trp Gly Thr Gly Glu Pro Cys Arg Gln Gly Ala		
	725	730	735
	Trp Thr Val Val Ser Asn Pro Phe Cys Pro Glu Pro Ser Pro His		
	740	745	750

Gln Asp Pro Phe Leu Pro Gly Ala Ser Ala Pro Arg Val Trp Ala
 755 760 765
 Gln Gly Arg Leu Gln Gly Leu Gly Ser Ile His Ser Arg Thr Asn
 770 775 780
 5 Leu Met Glu Ala Glu Leu Leu Asp Ala Asp Ser Asp Phe
 785 790 793
 <210> 17
 <211> 793
 <212> PRT
 10 <213> Homo sapiens
 <400> 17
 Met Ala Ala Gly Arg Pro Val Arg Gly Pro Glu Leu Ala Pro Arg
 1 5 10 15
 Arg Leu Leu Gln Leu Leu Leu Val Leu Leu Gly Gly Arg Gly
 15 20 25 30
 Arg Gly Ala Ala Leu Ser Gly Asn Val Thr Gly Pro Gly Pro Arg
 35 40 45
 Ser Ala Gly Gly Ser Ala Arg Arg Asn Ala Pro Val Thr Ser Pro
 50 55 60
 10 Pro Pro Pro Leu Leu Ser His Cys Gly Arg Ala Ala His Cys Glu
 65 70 75
 Pro Leu Arg Tyr Asn Val Cys Leu Gly Ser Ala Leu Pro Tyr Gly
 80 85 90
 Ala Thr Thr Thr Leu Leu Ala Gly Asp Ser Asp Ser Gln Glu Glu
 25 95 100 105
 Ala His Ser Lys Leu Val Leu Trp Ser Gly Leu Arg Asn Ala Pro
 110 115 120
 Arg Cys Trp Ala Val Ile Gln Pro Leu Leu Cys Ala Val Tyr Met
 125 130 135
 30 Pro Lys Cys Glu Asn Asp Arg Val Glu Leu Pro Ser Arg Thr Leu
 140 145 150
 Cys Gln Ala Thr Arg Gly Pro Cys Ala Ile Val Glu Arg Glu Arg
 155 160 165
 35 Gly Trp Pro Asp Phe Leu Arg Cys Thr Pro Asp His Phe Pro Glu
 170 175 180
 Gly Cys Pro Asn Glu Val Gln Asn Ile Lys Phe Asn Ser Ser Gly
 185 190 195
 Gln Cys Glu Ala Pro Leu Val Arg Thr Asp Asn Pro Lys Ser Trp
 200 205 210

	Tyr Glu Asp Val Glu Gly Cys Gly Ile Gln Cys Gln Asn Pro Leu		
	215	220	225
	Phe Thr Glu Ala Glu His Gln Asp Met His Ser Tyr Ile Ala Ala		
	230	235	240
5	Phe Gly Ala Val Thr Gly Leu Cys Thr Leu Phe Thr Leu Ala Thr		
	245	250	255
	Phe Val Ala Asp Trp Arg Asn Ser Asn Arg Tyr Pro Ala Val Ile		
	260	265	270
10	Leu Phe Tyr Val Asn Ala Cys Phe Phe Val Gly Ser Ile Gly Trp		
	275	280	285
	Leu Ala Gln Phe Met Asp Gly Ala Arg Arg Glu Ile Val Cys Arg		
	290	295	300
	Ala Asp Gly Thr Met Arg Phe Gly Glu Pro Thr Ser Ser Glu Thr		
	305	310	315
15	Leu Ser Cys Val Ile Ile Phe Val Ile Val Tyr Tyr Ala Leu Met		
	320	325	330
	Ala Gly Val Val Trp Phe Val Val Leu Thr Tyr Ala Trp His Thr		
	335	340	345
20	Ser Phe Lys Ala Leu Gly Thr Thr Tyr Gln Pro Le Ser Gly Lys		
	350	355	360
	Thr Ser Tyr Phe His Leu Leu Thr Trp Ser Leu Pro Phe Val Leu		
	365	370	375
	Thr Val Ala Ile Leu Ala Val Ala Gln Val Asp Gly Asp Ser Val		
	380	385	390
25	Ser Gly Ile Cys Phe Val Gly Tyr Lys Asn Tyr Arg Tyr Arg Ala		
	395	400	405
	Gly Phe Val Leu Ala Pro Ile Gly Leu Val Leu Ile Val Gly Gly		
	410	415	420
30	Tyr Phe Leu Ile Arg Gly Val Met Thr Leu Phe Ser Ile Lys Ser		
	425	430	435
	Asn His Pro Gly Leu Leu Ser Glu Lys Ala Ala Ser Lys Ile Asn		
	440	445	450
	Glu Thr Met Leu Arg Leu Gly Ile Phe Gly Phe Leu Ala Phe Gly		
	455	460	465
35	Phe Val Leu Ile Thr Phe Ser Cys His Phe Tyr Asp Phe Phe Asn		
	470	475	480
	Gln Ala Glu Trp Glu Arg Ser Phe Arg Asp Tyr Val Leu Cys Gln		
	485	490	495

	Ala Asn Val Thr Ile Gly Leu Pro Thr Lys Lys Pro Ile Pro Asp		
	500	505	510
	Cys Glu Ile Lys Asn Arg Pro Ser Leu Leu Val Glu Lys Ile Asn		
	515	520	525
5	Leu Phe Ala Met Phe Gly Thr Gly Ile Ala Met Ser Thr Trp Val		
	530	535	540
	Trp Thr Lys Ala Thr Leu Leu Ile Trp Arg Arg Thr Trp Cys Arg		
	545	550	555
10	Leu Thr Gly His Ser Asp Asp Glu Pro Lys Arg Ile Lys Lys Ser		
	560	565	570
	Lys Met Ile Ala Lys Ala Phe Ser Lys Arg Arg Glu Leu Leu Gln		
	575	580	585
	Asn Pro Gly Gln Glu Leu Ser Phe Ser Met His Thr Val Ser His		
	590	595	600
15	Asp Gly Pro Val Ala Gly Leu Ala Phe Glu Leu Asn Glu Pro Ser		
	605	610	615
	Ala Asp Val Ser Ser Ala Trp Ala Gln His Val Thr Lys Met Val		
	620	625	630
20	Ala Arg Arg Gly Ala Ile Leu Pro Gln Asp Val Ser Val Thr Pro		
	635	640	645
	Val Ala Thr Pro Val Pro Pro Glu Glu Gln Ala Asn Leu Trp Leu		
	650	655	660
	Val Glu Ala Glu Ile Ser Pro Glu Leu Glu Lys Arg Leu Gly Arg		
	665	670	675
25	Lys Lys Lys Arg Arg Lys Arg Lys Lys Glu Val Cys Pro Leu Gly		
	680	685	690
	Pro Ala Pro Glu Leu His His Ser Ala Pro Val Pro Ala Thr Ser		
	695	700	705
30	Ala Val Pro Arg Leu Pro Gln Leu Pro Arg Gln Lys Cys Leu Val		
	710	715	720
	Ala Ala Asn Ala Trp Gly Thr Gly Glu Pro Cys Arg Gln Gly Ala		
	725	730	735
	Trp Thr Val Val Ser Asn Pro Phe Cys Pro Glu Pro Ser Pro His		
	740	745	750
35	Gln Asp Pro Phe Leu Pro Gly Ala Ser Ala Pro Arg Val Trp Ala		
	755	760	765
	Gln Gly Arg Leu Gln Gly Leu Gly Ser Ile His Ser Arg Thr Asn		
	770	775	780

Leu Met Glu Ala Glu Leu Leu Asp Ala Asp Ser Asp Asp Phe
785 790 793

5 <210> 18
<211> 228
<212> DNA
<213> Homo sapiens

<400> 18
ctggggctgt ccagttaccc caacggctac cccttcctct tctggagca 50
gtacatcgcc ctccgccact ggctgctgct gttcatcagc gtgggtttgg 100
10 cctgcacatt cctcgtgtgc gctgtcttcc ttctgaaccc ctggacggcc 150
gggatcattg tcatggtcct ggccgtatg acggtcgagc tgttcggcat 200
gatgggcctc atcggaaatca agtcagt 228

15 <210> 19
<211> 18
<212> DNA
<213> Homo sapiens

<400> 19
aggcggggaa tcacagca 18

20 <210> 20
<211> 18
<212> DNA
<213> Homo sapiens

<400> 20
ataccaaaga gttccact 18

25 <210> 21
<211> 45
<212> DNA
<213> Artificial sequence

<220>
30 <223> Artificial sequence 1-45

<400> 21
ctgcggcgct gtttcctgct ggccgtctgc atcctgctgg tgtgc 45

<210> 22
<211> 45
35 <212> DNA
<213> Artificial sequence

<220>
<223> Artificial sequence 1-45

<400> 22
40 agagcacaga cgaggaaagt gcacaccaggc aggtatgcaga cggcc 45

<210> 23
<211> 21
<212> DNA
<213> Artificial sequence

5 <220>
<223> Artificial sequence 1-21

<400> 23
actcctgact ttagcagat t 21

<210> 24
10 <211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Artificial sequence 1-21

15 <400> 24
aggctgcata cacctctcag a 21

<210> 25
<211> 18
<212> DNA
20 <213> Artificial sequence

<220>
<223> Artificial sequence 1-18

<400> 25
gcttaggccc gaggagat 18

25 <210> 26
<211> 20
<212> DNA
<213> Artificial sequence

<220>
30 <223> Artificial sequence 1-20

<400> 26
aactcacaac tttctctcca 20

<210> 27
<211> 48
35 <212> DNA
<213> Artificial sequence

<220>
<223> Artificial sequence 1-48

<400> 27
40 ggattctaat acgactcact ataggccc a tggcctaaa ccgactgc 48

<210> 28

<211> 46
 <212> DNA
 <213> Artificial sequence

 <220>
 5 <223> Artificial sequence 1-46

 <400> 28
 ctatgaaatt aaccctcact aaagggaccc acggcctctc ctcaca 46

 <210> 29
 <211> 449
 10 <212> PRT
 <213> Mus musculus

 <400> 29
 Met Glu Ser Pro Arg Ala Thr Gln Thr Pro Glu Ser Pro Lys Leu
 1 5 10 15

 15 Ser Gln Pro Arg Ala His Leu Ser Ala His Gln Ala Pro Ser Pro
 20 25 30

 Ala Ala Leu Pro Gly Tyr Pro Ala Met Ser Pro Ala Trp Leu Arg
 35 40 45

 20 Pro Arg Leu Arg Phe Cys Leu Phe Leu Leu Leu Leu Val
 50 55 60

 Pro Ala Ala Arg Gly Cys Gly Pro Gly Arg Val Val Gly Ser Arg
 65 70 75

 Arg Arg Pro Pro Arg Lys Leu Val Pro Leu Ala Tyr Lys Gln Phe
 80 85 90

 25 Ser Pro Asn Val Pro Glu Lys Thr Leu Gly Ala Ser Gly Arg Tyr
 95 100 105

 Glu Gly Lys Ile Ala Arg Ser Ser Glu Arg Phe Lys Glu Leu Thr
 110 115 120

 30 Pro Asn Tyr Asn Pro Asp Ile Ile Phe Lys Asp Glu Glu Asn Thr
 125 130 135

 Gly Ala Asp Arg Leu Met Thr Gln Arg Cys Lys Asp Arg Leu Asn
 140 145 150

 Ser Leu Ala Ile Ser Val Met Asn Gln Trp Pro Gly Val Lys Leu
 155 160 165

 35 Arg Val Thr Glu Gly Trp Asp Glu Asp Gly His His Ser Glu Glu
 170 175 180

 Ser Leu His Tyr Glu Gly Arg Ala Val Asp Ile Thr Thr Ser Asp
 185 190 195

 Arg Asp Arg Asn Lys Tyr Gly Leu Leu Ala Arg Leu Ala Val Glu

	200	205	210
	Ala Gly Phe Asp Trp Val Tyr Tyr Glu Ser Lys Ala His Val His		
	215	220	225
	Cys Ser Val Lys Ser Glu His Ser Ala Ala Ala Lys Thr Gly Gly		
5	230	235	240
	Cys Phe Pro Ala Gly Ala Gln Val Arg Leu Glu Asn Gly Glu Arg		
	245	250	255
	Val Ala Leu Ser Ala Val Lys Pro Gly Asp Arg Val Leu Ala Met		
	260	265	270
10	Gly Glu Asp Gly Thr Pro Thr Phe Ser Asp Val Leu Ile Phe Leu		
	275	280	285
	Asp Arg Glu Pro Asn Arg Leu Arg Ala Phe Gln Val Ile Glu Thr		
	290	295	300
	Gln Asp Pro Pro Arg Arg Leu Ala Leu Thr Pro Ala His Leu Leu		
15	305	310	315
	Phe Ile Ala Asp Asn His Thr Glu Pro Ala Ala His Phe Arg Ala		
	320	325	330
	Thr Phe Ala Ser His Val Gln Pro Gly Gln Tyr Val Leu Val Ser		
	335	340	345
20	Gly Val Pro Gly Leu Gln Pro Ala Arg Val Ala Ala Val Ser Thr		
	350	355	360
	His Val Ala Leu Gly Ser Tyr Ala Pro Leu Thr Arg His Gly Thr		
	365	370	375
	Leu Val Val Glu Asp Val Val Ala Ser Cys Phe Ala Ala Val Ala		
25	380	385	390
	Asp His His Leu Ala Gln Leu Ala Phe Trp Pro Leu Arg Leu Phe		
	395	400	405
	Pro Ser Leu Ala Trp Gly Ser Trp Thr Pro Ser Glu Gly Val His		
	410	415	420
30	Trp Tyr Pro Gln Met Leu Tyr Arg Leu Gly Arg Leu Leu Leu Glu		
	425	430	435
	Glu Ser Thr Phe His Pro Leu Gly Met Ser Gly Ala Gly Ser		
	440	445	449
	<210> 30		
35	<211> 228		
	<212> DNA		
	<213> Homo sapiens		
	<400> 30		

ctggggctgt ccagttaccc caacggctac cccttcctct tctgggagca 50
gtacatcgcc ctccgccact ggctgctgct gttcatcagc gtgggtttgg 100
cctgcacatt cctcgtgtgc gctgtttcc ttctgaaccc ctggacggcc 150
gggatcattg tcatggtcct ggcgctgatg acggtcgagc tgttcggcat 200
5 gatgggcctc atcggaatca agctcagt 228

<210> 31
<211> 32
<212> DNA
<213> Artificial sequence

10 <220>
<223> Artificial sequence 1-32

<400> 31
tcgacaagca ggaaacaccc aagtagaagc tc 32

15 <210> 32
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Artificial sequence 1-32

20 <400> 32
tcgacaagca ggaaagtggg aagtagaagc tc 32

The claims defining the invention are as follows:

1. An isolated nucleic acid, comprising DNA having at least a 95% sequence identity to (a) a DNA molecule encoding a *patched-2* polypeptide comprising the sequence of amino acids 1 to about 1203 of Fig. 1 (SEQ ID NO:2), or (b) the complement of (a); and encoding a polypeptide having *patched-2* biological activity.
2. An isolated nucleic acid, comprising DNA having at least a 95% sequence identity to (a) a DNA molecule encoding the same mature polypeptide as that encoded by the cDNA in ATCC 10 Deposit No. 209778 (designation: pRK7.hptc2.Flag-1405), or (b) the complement of the DNA molecule of (a).
3. The isolated nucleic acid of claim 2, comprising the human *patched-2* encoding sequence of the cDNA in ATCC deposit No. 209778 (designation: pRK7.hptc2.Flag-1405), or a sequence which 15 hybridizes thereto under stringent conditions.
4. A vector comprising the nucleic acid of claim 1.
5. The vector of claim 4, operably linked to control sequences recognized by a host cell 20 transformed with the vector.
6. A host cell transformed with the vector of claim 4.
7. The host cell of claim 6, which is mammalian.
8. The host cell of claim 7, wherein said cell is a CHO cell.
9. The host cell of claim 6, which is prokaryotic.
10. The host cell of claim 9, wherein said cell is an E. coli.
11. The host cell of claim 7, wherein said cell is a yeast cell.
12. The host cell of claim 11, which is *Saccharomyces cerevisiae*.
13. A process for producing *patched-2* polypeptides, comprising culturing the host cell of claim 9 under conditions suitable for expression of vertebrate *patched-2* and recovering *patched-2* from the cell culture.

14. Isolated native sequence human *patched*-2 polypeptide, comprising amino acid residues 1 to 1203 of Fig. 1 (SEQ ID NO:2).

15. Isolated native sequence human *patched*-2 polypeptide encoded by the nucleotide deposited
5 under accession number ATCC 209778 (Designation: pRK7.hptc2.Flag-1405), having *patched*-2 biological activity.

16. A chimeric molecule comprising vertebrate *patched*-2 polypeptide, linked to a heterologous amino acid sequence.

10

17. The chimeric molecule of claim 16, wherein said heterologous amino acid sequence is an epitope tag sequence.

15

18. The chimeric molecule of claim 17, wherein said heterologous amino acid sequence is a constant region of an immunoglobulin.

19. An antagonist of *patched*-2 which blocks, prevents, inhibits and/or neutralizes the Dhh function in the Dhh signaling pathway, and which is a *patched*-2 specific antibody or a *patched*-2 specific antisense molecule.

20

20. The antagonist of claim 19, which is an antisense nucleotide.

21. The antagonist of claim 19, which is an antibody.

25

22. A method of screening for antagonists or agonists of *patched*-2 biological activity, comprising:
(a) exposing *patched*-2 expressing target cells in culture to a candidate compound and Dhh; and
(b) analyzing cells for binding of Dhh to *patched*-2; or
(c) scoring phenotypic or functional changes in the treated cells;

and comparing the results to control cells which were not exposed to the candidate compound.

30

23. A method of screening for an antagonist or agonist of *patched*-2 biological activity, comprising:
(a) exposing a *patched*-2 ligand and a compound having *patched*-2 biological activity to a candidate antagonist or agonist; and
(b) analyzing the substrate for binding of the ligand to the compound; and

35

comparing the results to control reactions which were not exposed to the candidate molecule.

24. A method according to claim 22 or claim 23, in which the candidate agonist prevents Smo (SEQ ID NO:17) inactivation of *patched*-2 (SEQ ID NO:2).

25. A method of diagnosing to determine whether a particular disorder is modulated by Dhh signaling, comprising:
- (a) culturing test cells or tissues;
- (b) administering a compound which can inhibit *patched-2* modulated Dhh signaling to the cultured cells or tissues; and
- 5 (c) analyzing the level of Dhh binding to *patched-2* or Dhh mediated phenotypic effects in the test cells or tissues,
- in which the compound is a *patched-2* specific antibody or a *patched-2* specific antisense molecule.
- 10
26. An isolated nucleic acid according to claim 1 of claim 2, substantially as herein described with reference to the examples and drawings.
- 15
27. A polypeptide according to claim 14 or claim 15, substantially as herein described with reference to the examples and drawings.
28. A chimeric molecule according to claim 16, substantially as herein described with reference to the examples and drawings.
- 20
29. An antagonist according to claim 19, substantially as herein described with reference to the examples and drawings.
- 25
30. A method according to any one of claims 22, 23 or 25, substantially as herein described with reference to the examples and drawings.
- Dated this 8th day of August 2003
- GENENTECH, INC.
- By their Patent Attorneys
- GRAFFITH HACK
- 30 Fellows Institute of Patent and
- Trade Mark Attorneys of Australia

1 GTTATTTCAG GCCATGGTGT TCGGCCGAAT TAATTCCCGA TCCAGACATG ATAAGATACA TTGATGAGTT TGGACAAACC ACAACTAGAA TGCAGTGAAA
 CAATAAAGTC CGGTACCACA ACGCGCTTA ATTAAGGGCT AGGTCTGTAC TATTCTATGT AACTACTCAA ACCTGTTGG TGGTGTCTT ACGTCACCTT
 (SEQ ID NO:1)

101 AAAATGCTTT ATTTGTGAAA TTTGTGATGC TATTGCTTTA TTTGTAACCA TTATAAGCTG CAATAAACAA GTTGGGCCAT GGCGGCCAAG CTTCTGCAGG
 TTTTACGAAA TAAACACTTT AAACACTACG ATAACGAAAT AAACATTGGT AATATTGAC GTTATTGTT CAACCCGGTA CCGCCGGTTC GAAGACGTCC

201 TCGACTCTAG AGGATCCCCG GGGAAATTCCG GCATGACTCG ATCGCCGCC CTCAGAGAGC TGCCCCGAG TTACACACCC CCAGCTCGAA CCGCAGCACC
 AGCTGAGATC TCCTAGGGC CCCTTAAGGC CGTACTGAGC TAGCGGCGGG GAGTCTCTCG ACGGGGGCTC AATGTGTGGG GGTGAGCTT GGCGTCGTGG

1 M T R S P P L R E L P P S Y T P P A R T A A P
 ~insert starts here (SEQ ID NO:2)

301 CCAGATCCTA GCTGGGAGCC TGAAGGCTCC ACTCTGGCTT CGTGCTTACT TCCAGGGCCT GCTCTTCTCT CTGGGATGCG GGATCCAGAG ACATTGTGGC
 GGTCTAGGAT CGACCCCTCGG ACTTCCGAGG TGAGACCGAA GCACGAATGA AGGTCCCGGA CGAGAAGAGA GACCCCTACGC CCTAGGTCTC TGTAACACCG

24 Q I L A G S L K A P L W L R A Y F Q G L L F S L G C G I Q R H C G

401 AAAGTGCCTCT TTCTGGGACT GTTGGCCTTT GGGGCCCTGG CATTAGGTCT CCGCATGGCC ATTATTGAGA CAAACTGGA ACAGCTCTGG GTAGAAGTGG
 TTTCACGAGA AAGACCCCTGA CAACCGGAAA CCCCGGGACC GTAATCCAGA GGCGTACCGG TAATAACTCT GTTGTGACCT TGTCGAGACC CATCTTCACC

57 K V L F L G L L A F G A L A L G L R M A I I E T N L E Q L W V E V G

501 GCAGCCGGGT GAGCCAGGAG CTGCATTACA CCAAGGAGAA GCTGGGGAG GAGGCTGCAT ACACCTCTCA GATGCTGATA CAGACCGCAC GCCAGGAGGG
 CGTCGGCCCA CTCGGTCCTC GACGTAATGT GGTCTCTT CGACCCCTC CTCCGACGTA TGTGGAGAGT CTACGACTAT GTCTGGCGTG CGGTCCCTCCC

91 S R V S Q E L H Y T K E K L G E E A A Y T S Q M L I Q T A R Q E G

601 AGAGAACATC CTCACACCCG AAGCACTTGG CCTCCACCTC CAGGCAAGCCC TCACTGCCAG TAAAGTCCAA GTATCACTCT ATGGGAAGTC CTGGGATTTG
 TCTCTTGAG GAGTGTGGC TTCGTGAACC GGAGGTGGAG GTCCGTCGG AGTGACGGTC ATTTCAGGTT CATAGTGAGA TACCCCTTCAG GACCCTAAAC

124 E N I L T P E A L G L H L Q A A L T A S K V Q V S L Y G K S W D L

701 AACAAAATCT GCTACAAGTC AGGAGTTCCC CTTATTGAAA ATGGAATGAT TGAGTGGATG ATTGAGAAGC TGTTCCGTG CGTGATCCTC ACCCCCCCTCG
 TTGTTTTAGA CGATGTTCAAGGG GAATAACTTT TACCTTACTA ACTCACCTAC TAACTCTTCG ACAAAAGGCAC GCACTAGGAG TGGGGGGAGC

157 N K I C Y K S G V P L I E N G M I E W M I E K L F P C V I L T P L D

801 ACTGCTTCTG GGAGGGAGCC AAACCTCAAG GGGGCTCCGC CTACCTGCC GGCGCCCGG ATATCCAGTG GACCAACCTG GATCCAGAGC AGCTGCTGGA
 TGACGAAGAC CCTCCCTCGG TTTGAGGTTC CCCCCGAGGCG GATGGACGGG CGGGCGGGCC TATAAGGTAC CTGGTTGGAC CTAGGTCTCG TCGACGACCT

191 C F W E G A K L Q G G S A Y L P G R P D I Q W T N L D P E Q L L E

FIG. 1A

901 GGAGCTGGGT CCCTTGCCT CCCTTGGGG CTTCCGGGAG CTGCTAGACA AGGCACAGGT GGGCCAGGCC TACGTGGGC GGCCTGTCT GCACCCGTAT
 CCTCGACCCA GGGAAACGGA GGGAACTCCC GAAGGCCCTC GACGATCTGT TCCGTGTCCA CCCGGTCCGG ATGCACCCCG CCGGGACAGA CGTGGACTA
 224 E L G P F A S L E G F R E L L D K A Q V G Q A Y V G R P C L H P D

1001 GACCTCCACT GCCCACCTAG TGCCCCAAC CATCACAGCA GGCAGGCTCC CAATGTGGCT CACGAGCTGA GTGGGGCTG CCATGGCTTC TCCCACAAAT
 CTGGAGGTGA CGGGTGGATC ACGGGGGTTG GTAGTGTCGT CCGTCCGAGG GTTACACCGA GTGCTCGACT CACCCCCGAC GGTACCGAAG AGGGTGTAA
 257 D L H C P P S A P N H H S R Q A P N V A H E L S G G C H G F S H K F

1101 TCATGCAC TG GCAGGAGGAA TTGCTGCTGG GAGGCATGGC CAGAGACCCC CAAGGAGAGC TGCTGAGGGC AGAGGCCCTG CAGAGCACCT TCTTGCTGAT
 AGTACGTGAC CGTCCTCCTT AACGACGACC CTCCGTACCG GTCTCTGGG GTTCCTCTCG ACCACTCCC TCTCCGGAC GTCTCGTGGA AGAACGACTA
 291 M H W Q E E L L L G G M A R D P Q G E L L R A E A L Q S T F L L M

1201 GAGTCCCCGC CAGCTGTACG AGCATTCCG GGGTGA CACTAT CAGACACATG ACATTGGCTG GAGTGAGGAG CAGGCCAGCA CAGTGCTACA AGCCTGGCAG
 CTCAGGGCG GTCGACATGC TCGTAAAGGC CCCACTGATA GTCTGTGTAC TGTAACCGAC CTCACTCCTC GTCCGGTCGT GTCACGATGT TCGGACCGTC
 324 S P R Q L Y E H F R G D Y Q T H D I G W S E E Q A S T V L Q A W Q

1301 CGCGCCTTG TGCGAGCTGGC CCAGGAGGCC CTGCCTGAGA ACGCTTCCCA GCAGATCCAT GCCTTCTCCT CCACCACCCCT GGATGACATC CTGCATGCGT
 2/27
 GCCGCGAAAC ACGTCGACCG GGTCCCTCCG GACGGACTCT TGCGAAGGGT CGTCTAGGTA CGGAAGAGGA GGTGGTGGGA CCTACTGTAG GACGTACGCA
 357 R R F V Q L A Q E A L P E N A S Q Q I H A F S S T T L D D I L H A F

1401 TCTCTGAAGT CAGTGCCTGCC CGTGTGGTGG GAGGCTATCT GCTCATGCTG GCCTATGCCT GTGTGACCAT GCTGCGGTGG GACTGCGCCC AGTCCCAGGG
 AGAGACTTCA GTCACGACGG GCACACCACC CTCCGATAGA CGAGTACGAC CGGATACGGA CACACTGGTA CGACGCCACC CTGACGCGGG TCAGGGTCCC
 391 S E V S A A R V V G G Y L L M L A Y A C V T M L R W D C A Q S Q G

1501 TTCCGTGGGC CTTGCCGGG TACTGCTGGT GGCCCTGGCG GTGGCCTCAG GCCTTGGCT CTGTGCCCTG CTCGGCATCA CCTCAATGC TGCCACTACC
 AAGGCACCCG GAACGGCCCC ATGACCGACCA CCGGGACCGC CACCGGAGTC CGGAACCCGA GACACGGGAC GAGCGTAGT GGAAGTTACCG ACGGTGATGG
 424 S V G L A G V L L V A L A V A S G L G L C A L L G I T F N A A T T

1601 CAGGTGCTGC CTTCTTGGC TCTGGGAATC GGCGTGGATG ACGTATTCCCT GCTGGCGCAT GCCTTCACAG AGGCTCTGCC TGGCACCCCT CTCCAGGAGC
 GTCCACCGACG GAAAGAACCG AGACCCCTAG CCGCACCTAC TGCATAAGGA CGACCGCGTA CGGAAGTGTC TCCGAGACGG ACCGTGGGGA GAGGTCTCG
 457 Q V L P F L A L G I G V D D V F L L A H A F T E A L P G T P L Q E R

1701 GCATGGCGA GTGTCTGCAG CGCACGGCA CCAGTGTGCT ACTCACATCC ATCAACAACA TGGCCGCCTT CCTCATGGCT GCCCTCGTTC CCATCCCTGC
 CGTACCCGCT CACAGACGTC GCGTGCCCGT GGTCACAGCA TGAGTGTAGG TAGTTGTTGT ACCGGCGGAA GGAGTACCGA CGGGAGCAAG GGTAGGGACG
 491 M G E C L Q R T G T S V V L T S I N N M A A F L M A A L V P I P A

FIG. 1B

1801 GCTGCGAGCC TTCTCCCTAC AGGCAGCCAT AGTGGTTGGC TGCACCTTG TAGCCGTGAT GCTTGTCTTC CCAGCCATCC TCAGCCTGGA CCTACGGCGG
 CGACGCTCGG AAGAGGGATG TCCGCCGGTA TCACCAACCG ACGTGGAAAC ATCGGCACTA CGAACAGAAG GGTCGGTAGG AGTCGGACCT GGATGCCGCC
 524 L R A F S L Q A A I V V G C T F V A V M L V F P A I L S L D L R R

1901 CGCCCACTGCC AGCGCCTTGA TGTGCTCTGC TGCTTCTCCA GTCCCTGCTC TGCTCAGGTG ATTCAAGATCC TGCCCCAGGA GCTGGGGGAC GGGACAGTAC
 GCGGTGACGG TCGCGGAAC ACACGAGACG ACGAAGAGGT CAGGGACGAG ACGAGTCCAC TAAGTCTAGG ACGGGGTCCT CGACCCCCCTG CCCTGTCATG
 557 R H C Q R L D V L C C F S S P C S A Q V I Q I L P Q E L G D G T V P

2001 CAGTGGGCAT TGCCCACCTC ACTGCCACAG TTCAAGCCTT TACCCACTGT GAAGCCAGCA GCCAGCATGT GGTCACCATC CTGCCTCCCC AAGCCACCT
 GTCACCCGTA ACGGGTGGAG TGACGGTGTGTC AAGTTGGAA ATGGGTGACA CTTCGGTCTG CGGTGTCACA CCAGTGGTAG GACGGAGGGG TTCGGGTGGA
 591 V G I A H L T A T V Q A F T H C E A S S Q H V V T I L P P Q A H L

2101 GGTGCCCCCA CCTTCTGACC CACTGGCCTC TGAGCTCTTC AGCCCTGGAG GGTCCACACG GGACCTTCTA GGCCAGGAGG AGGAGACAAG GCAGAAGGCA
 CCACGGGGGT GGAAGACTGG GTGACCCGAG ACTCGAGAAG TCGGGACCTC CCAGGTGTGC CCTGGAAGAT CGGTCCCTCC TCCTCTGTTC CGTCTCCGT
 624 V P P P S D P L G S E L F S P G G S T R D L L G Q E E E T R Q K A

2201 GCCTGCAAGT CCCTGCCCTG TGCCCGCTGG AATCTGCCCG ATTTGCCCG CTATCAGTTT GCCCCGTTGC TGCTCCAGTC ACATGCCAAG GCCATCGTGC
 CGGACGTTCA GGGACGGGAC ACGGGCGACC TTAGAACGGG TAAAGCGGGC GATAGTCAA CGGGCAACG ACGAGGTCAAG TGTACGGTTC CGGTAGCAGC
 657 A C K S L P C A R W N L A H F A R Y Q F A P L L L Q S H A K A I V L

2301 TGGTGCTCTT TGGTGCTCTT CTGGGCTGA GCCTCTACGG AGCCACCTG GTGCAAGACG GCCTGGCCCT GACGGATGTG GTGCCTCGGG GCACCAAGGA
 ACCACGAGAA ACCACGAGAA GACCCGGACT CGGAGATGCC TCGGTGGAAC CACGTTCTGC CGGACCGGGG CTGCCTACAC CACGGAGCCC CGTGGTTCC
 691 V L F G A L L G L S L Y G A T L V Q D G L A L T D V V P R G T K E

2401 GCATGCCCTC CTGAGCGCCC AGCTCAGGTA CTTCTCCCTG TACGAGGTGG CCCTGGTGAC CCAGGGTGGC TTTGACTACG CCCATTCCCA ACGGCCCTC
 CGTACGGAAG GACTCGCGGG TCGAGTCCAT GAAGAGGGAC ATGCTCCACC GGGACCACG GGTCCCACCG AACTGATGC GGGTAAGGGT TGCGCGGGAG
 724 H A F L S A Q L R Y F S L Y E V A L V T Q G G F D Y A H S Q R A L

2501 TTTGATCTGC ACCAGCGCTT CAGTTCCCTC AAGGCGGTGC TGCCCCCACC GGCCACCCAG GCACCCCGCA CCTGGCTGCA CTATTACCGC AACTGGCTAC
 AACTAGACG TGGTCGCGAA GTCAAGGGAG TTCCGCCACG ACGGGGGTGG CCGGTGGTGC CGTGGGGCGT GGACCGACGT GATAATGGCG TTGACCGATG
 757 F D L H Q R F S S L K A V L P P P A T Q A P R T W L H Y Y R N W L Q

2601 AGGGAATCCA GGCTGCCTTT GACCAGGACT GGGCTTCTGG GCGCATCACC CGCCACTCGT ACCGCAATGG CTCTGAGGAT GGGGCCCTGG CCTACAAGCT
 TCCCTTAGGT CCGACGGAAA CTGGTCTGA CCCGAAGACC CGCGTAGTGG CGGGTGAGCA TGGCGTTACC GAGACTCCTA CCCCCGGGACC GGATGTTCGA
 791 G I Q A A F D Q D W A S G R I T R H S Y R N G S E D G A L A Y K L

FIG. 1C

2701 GCTCATCCAG ACTGGAGACG CCCAGGAGCC TCTGGATTTC AGCCAGCTGA CCACAAGGAA GCTGGTGGAC AGAGAGGGAC TGATTCCACC CGAGCTCTTC
 CGAGTAGGTC TGACCTCTGC GGGTCCCTCGG AGACCTAAAG TCGGTGCACT GGTGTTCCCT CGACCACCTG TCTCTCCCTG ACTAAGGTGG GCTCGAGAAG
 824 L I Q T G D A Q E P L D F S Q L T T R K L V D R E G L I P P E L F

 2801 TACATGGGGC TGACCGTGTG GGTGAGCAGT GACCCCTGG GTCTGGCAGC CTCACAGGCC AACTTCTACC CCCCACCTCC TGAATGGCTG CACGACAAAT
 ATGTACCCCG ACTGGCACAC CCACTCGTCA CTGGGGGACC CAGACCGTGTG GAGTGTCCGG TTGAAGATGG GGGGTGGAGG ACTTACCGAC GTGCTGTTA
 857 Y M G L T V W V S S D P L G L A A S Q A N F Y P P P P E W L H D K Y

 2901 ACGACACCAC GGGGGAGAAC CTTCGCATCC CGCCAGCTCA GCCCTTGGAG TTTGCCAGT TCCCCCTTCCT GCTGCGTGGC CTCCAGAAGA CTGCAGACTT
 TGCTGTGGTG CCCCCCTCTTG GAAGCGTAGG CGGGTCGAGT CGGGAACCTC AAACGGGTCA AGGGGAAGGA CGACGCACCG GAGGTCTTCT GACGTCTGAA
 891 D T T G E N L R I P P A Q P L E F A Q F P F L L R G L Q K T A D F

 3001 TGTGGAGGCC ATCGAGGGGG CCCGGGCAGC ATGCGCAGAG GCCGGCCAGG CTGGGGTGCA CGCCTACCCC AGCGGCTCCC CCTTCCTCTT CTGGGAACAG
 ACACCTCCGG TAGCTCCCCC GGGCCCGTCG TACCGTCTC CGGCCGGTCC GACCCACGT GC GGATGGGG TCGCCGAGGG GGAAGGAGAA GACCCTGTC
 924 V E A I E G A R A A C A E A G Q A G V H A Y P S G S P F L F W E Q

 3101 TATCTGGGCC TGGGGCGCTG CTTCTGCTG GCCGTCTGCA TCCTGCTGGT GTGCACTTTC CTCGTCTGTG CTCTGCTGCT CCTCAACCCC TGGACGGCTG
 ATAGACCCGG ACGCCGCGAC GAAGGACGAC CGGCAGACGT AGGACGACCA CACGTGAAAG GAGCAGACAC GAGACGACGA GGAGTTGGGG ACCTGCCGAC
 957 Y L G L R R C F L L A V C I L L V C T F L V C A L L L L N P W T A G 27

 3201 GCCTCATAGT GCTGGTCCTG GCGATGATGA CAGTGGAACT CTTGGTATC ATGGGTTCC TGGGCATCAA GCTGAGTGCC ATCCCCGTGG TGATCCTTGT
 CGGAGTATCA CGACCAGGAC CGCTACTACT GTCACCTTGA GAAACCATAG TACCCAAAGG ACCCGTAGTT CGACTCACGG TAGGGGCACC ACTAGGAACA
 991 L I V L V L A M M T V E L F G I M G F L G I K L S A I P V V I L V

 3301 GGCCTCTGTA GGCATTGGCG TTGAGTCAC AGTCCACGTG GCTCTGGCT TCCTGACCAC CCAGGGCAGC CGGAACCTGC GGGCCGCCCA TGCCCTTGAG
 CCGGAGACAT CCGTAACCGC AACTCAAGTG TCAGGTGCAC CGAGACCCGA AGGACTGGTG GGTCCCGTCG GCCTTGACG CCCGGCGGGT ACGGAAACTC
 1024 A S V G I G V E F T V H V A L G F L T T Q G S R N L R A A H A L E

 3401 CACACATTG CCCCCGTGAC CGATGGGCC ATCTCCACAT TGCTGGTCT GCTCATGCTT GCTGGTTCCC ACTTGACTT CATTGTAAGG TACTTCTTTG
 GTGTGTAAAC GGGGGCACTG GCTACCCCG TAGAGGTGTA ACGACCCAGA CGAGTACGAA CGACCAAGGG TGAAACTGAA GTAACATTCC ATGAAGAAAC
 1057 H T F A P V T D G A I S T L L G L L M L A G S H F D F I V R Y F F A

 3501 CGGCGCTGAC AGTGCTCAGC CTCTGGCC TCCTCCATGG ACTCGTGTGCT CTGCCTGTGC TGCTGTCCAT CCTGGGCCCG CGGCCAGAGG TGATACAGAT
 GCCGCGACTG TCACGAGTGC GAGGACCCGG AGGAGGTACC TGAGCACGAC GACGGACACG ACGACAGGTA GGACCCGGC GGC GGCTCCTCC ACTATGTCTA
 1091 A L T V L T L L G L L H G L V L L P V L L S I L G P P P E V I Q M

FIG. 1D

3601 GTACAAGGAA AGCCCAGAGA TCCTGAGTCC ACCAGCTCCA CAGGGAGGCG GGCTTAGGTG GGGGGCATCC TCCTCCCTGC CCCAGAGCTT TGCCAGAGTG
 CATGTTCTT TCGGGTCTCT AGGACTCAGG TGGTCGAGGT GTCCCTCCGC CCGAATCCAC CCCCCGTAGG AGGAGGGACG GGGTCTCGAA ACGGTCTCAC
 1124 Y K E S P E I L S P P A P Q G G G L R W G A S S S L P Q S F A R V

3701 ACTACCTCCA TGACCGTGGC CATCCACCCA CCCCCCTGC CTGGTGCCTA CATCCATCCA GCCCCTGATG AGCCCCCTTG GTCCCTGCT GCCACTAGCT
 TGATGGAGGT ACTGGCACCG GTAGGTGGGT GGGGGGGACG GACCACGGAT GTAGGTAGGT CGGGGACTAC TCGGGGGAAC CAGGGGACGA CGGTGATCGA
 1157 T T S M T V A I H P P P L P G A Y I H P A P D E P P W S P A A T S S

3801 CTGGCAACCT CAGTTCCAGG GGACCAGGTC CAGCCACTGG GTGAAAGAGC AGCTGAAGCA CAGAGACCAT GTGTGGGCG TGTGGGTCA CTGGGAAGCA
 GACCGTTGGA GTCAAGGTCC CCTGGTCCAG GTCGGTGACC CACTTCTCG TCGACTTCGT GTCTCTGGTA CACACCCCGC ACACCCAGT GACCCTTCGT
 1191 G N L S S R G P G P A T G O 1203 5/27

3901 CTGGGCTGG TGTAGACGC AGGACGGACC CCTGGAGGCG CCTGCTGCTG CTGCATCCCC TCTCCGACC CAGCTGTCAT GGGCCTCCCT GATATCGAAT
 GACCCAGACC ACAATCTGCG TCCTGCCTGG GGACCTCCCG GGACGACGAC GACGTAGGGG AGAGGGCTGG GTCGACAGTA CCCGGAGGGA CTATAGCTTA
 ^T to C (silent) pRK follows, this is the Prime end of vector^

4001 TCAATCGATA GAACCGAGGT GCAGTTGGAC
 AGTTAGCTAT CTTGGCTCCA CGTCAACCTG

FIG. 1E

6/27

(SEQ ID NO: 3)

905531	30	40	50	60	70
	GCTGGGGTGCACGCCAACNCAGCGGNTCCCCCTTCCTCTTCTGGGAACA				
	3010	3020	3030	3040	3050
hpatched	CTGGGGCTGTCCAGTTACCCCAACGGCTACCCCTTCCTCTTCTGGGAGCA				

(SEQ ID NO: 4)

905531	80	90	100	110	120
	GTATCTGGGCCTGCGCGCTGCTTCCTGCTGGCCGTCTGCATCCTGCTGG				
	3060	3070	3080	3090	3100
hpatched	GTACATCGGCCTCCGCCACTGGCTGCTGCTGTTCATCAGCGTGGTGGTGG				

905531	130	140	150	160	170
	TGTGCACTTCCTCGTCTGTGCTCTGCTGCTCCTNAACCCCTGGACGGCT				
	3110	3120	3130	3140	3150
hpatched	CCTGCACATTCCCTCGTGTGCGCTGCTTCCTCTGAACCCCTGGACGGCC				

905531	180	190	200	210	220
	GGCCTNATAGTGTGGTCCTGGCGATGATGACAGTGGAACTCTTGTTAT				
	3160	3170	3180	3190	3200
hpatched	GGGATCATTGTGATGGTCCTGGCGCTGATGACGGTCGAGCTGTTGGCAT				

905531	230	240	250		
	CATGGGTTTNTGGGCATCAAGCTGAGT				
	3210	3220	3230		
hpatched	GATGGGCCTCATCGGAATCAAGCTCAGT				

905531	80	90	100	110	120
	TCTGGGCCTGCGCGCTGCTTCCTGCTGGCCGTCTGCATCCTGCTGGTGT				
	3090	3100	3110	3120	
hpatched	GCTGCTGCTGTTCATCAGCGTGGTGGCC---TGCACATTCCCTCGTGT				

905531	130	140	150		
	GCACACTTCCTCGTCTGTGCTCTGCTGCT				
	3130	3140	3150		
hpatched	GCGCTGTCTCCTCTGAACCCCTGGAC				

FIG. 2A

7/27

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

FIG. 3A

PTCH	450	L	A	Y	A	C	L	T	M	L	R	W	D	C	S	K	S	Q	G	A	V	G	L	A	G	V	L	L	V	A	L	S	V	A	A	G	L	G	C	S	L	I	G	I	S	F	N	A	A
PTCH2	406	L	A	Y	A	C	V	T	M	L	R	W	D	C	A	O	S	Q	G	S	V	G	L	A	G	V	L	L	V	A	A	S	G	I	G	C	A	L	L	G	T	F	N	A	A				

TM3

PTCH	500	T	O	V	L	P	F	L	A	L	G	V	G	V	D	D	V	F	L	L	A	H	A	F	S	E	T	G	Q	N	K	R	I	P	F	E	D	R	T	G	E	C	L	K	R	T	G	A	S	V	A
PTCH2	456	T	O	V	L	P	F	L	A	L	G	I	G	V	V	D	D	V	F	L	L	A	H	A	F	T	E	A	L	P	G	- - -	T	P	L	Q	E	R	M	G	E	C	L	Q	R	T	G	T	S	V	V

TM4

PTCH	550	I	T	S	I	S	N	V	T	A	F	F	M	A	A	L	J	P	I	P	A	L	R	A	F	S	L	Q	A	A	V	V	V	F	N	F	A	M	V	V	L	I	F	P	A	I	L	S	M	D		
PTCH2	504	I	T	S	I	S	N	N	M	A	A	F	F	I	M	A	A	L	V	P	I	P	A	L	R	A	F	S	L	Q	A	A	V	V	V	F	N	F	A	M	V	V	L	I	F	P	A	I	L	S	M	D

TM5

PTCH	600	I	Y	R	R	E	D	R	R	L	D	I	F	C	C	F	T	S	P	C	V	S	R	R	V	I	Q	V	E	P	Q	A	Y	T	D	T	H	D	N	T	R	Y	S	P	P	P	P	Y	S	S	H	S
PTCH2	554	I	L	R	R	E	D	R	R	L	D	I	F	C	C	F	T	S	P	C	V	S	R	R	V	I	Q	V	E	P	Q	A	Y	T	D	T	H	D	N	T	R	Y	S	P	P	P	Y	S	S	H	S	

TM6

PTCH	650	F	A	H	E	T	Q	I	T	M	Q	S	T	V	O	L	R	T	E	Y	D	P	H	T	H	V	Y	Y	T	T	A	E	P	R	S	E	I	S	V	Q	P	V	T	V	T	Q	D	T	L	S	C
PTCH2	593	I	A	H	- - -	- - -	- - -	L	T	A	T	V	O	A	F	T	H	C	E	A	S	S	Q	H	V	V	T	I	L	P	P	Q	A	H	L	V	P	P	P	- - -	S	D	P	L	G	S					

TM7

PTCH	748	K	A	K
------	-----	---	---	---

PTCH 847 Y F R D W L Q G L C D A F D S D W E T G K I M P N N Y K N G S D D G V L A Y K L L V O T G S R D K P
 PTCH2 784 Y Y R N W L Q G I Q A A F D Q D W A S G R I T R H S Y R N G S E D G A L A Y K L L I Q T G D A Q E P

PTCH 897 I D I S Q L T K Q R L V D A D G I I N P S A F Y I Y L T A W V S N D P V A Y A A S Q A N I R P H R P
 PTCH2 834 L D F S Q L T T R K L V D R E G L I P P E L F Y M G L T V W V S S D P L G L A A S Q A N F Y P P P P

PTCH 947 E W V H D K A D Y M P E T R L R I P A A E P I E Y A Q F P F Y L N G L R D T S D F V E A I E K V R T
 PTCH2 884 E W L H D K Y D - T T G E N L R I P P A Q P L E F A Q F P F L L R G L Q K T A D F V E A I E G A R A

TM8

PTCH 997 I C S N Y T S L G L S S Y P N G Y P F L F W E Q Y I G L R H W L L F I S V V L A C T F L V C A V F
 PTCH2 933 A C A E A G Q A G V H A Y P S G S P F L F W E Q Y L G L R R C F L I A V C I L L V C T F L V C A L L

TM9

PTCH 1047 L L N P W T A G I I V M V L A L M T V E L F G M M G L Q G I K L S A V P V V I L I A S V G I G V E F
 PTCH2 983 L L N P W T A G L I I V L V L A M M T V E L F G I M G F L Q G I K L S A I P V V I L V A S V G I G V E F

TM10

PTCH 1097 T V H V A L A F L T A I G D K N R R A V L A L E H M F A P V L D G A V S T L L G V L L M L A G S E F D
 PTCH2 1033 T V H V A L G F L T Q G S R N L R A A H A L E H T F A P V T D G A I S T L L G L L M L A G S H F D

TM11

PTCH 1147 F I V R Y F F A V L A I L T I L G V L N G L V L L P V L L S F F G P Y P E V S P A N G L N R L P T P
 PTCH2 1083 F I V R Y F F A A L T V L T L L G L L H G L V L L P V L L S I L G P P P E V I Q M Y K E S P E I L S

PTCH 1197 S P E P P P S V V R F A M P P G H T H S G S D S S D S E Y S S Q T T V S G L S E E L R H Y E A Q Q G
 PTCH2 1133 P P A P Q G G G L R W G A S S L P Q S - F A R V T T S M T V A I H P P P L P G A Y I H P A P D E P

FIG. 3C

PTCH 1247 A G G **P A** H Q V I V E A T E N P V F A H S T V V H P E S R H H P P S N P R Q Q P H L D S G S L P P G
PTCH2 1182 P W S **P A** T S S G N L S S R G P G P A T G

PTCH 1297 R Q G Q Q P R R D P P R E G L W P P L Y R P R R D A F E I S T E G H S G P S N R A R W G P R G A R S

PTCH 1347 H N P R N P A S T A M G S S V P G Y C Q P I T T V T A S A S V T V A V H P P P V P G P G R N P R G G

PTCH 1397 L C P G Y P E T D H G L F E D P H V P F H V R C E R R D S K V E V I E L Q D V E C E E R P R G S S S

PTCH 1447 N

FIG. 3D

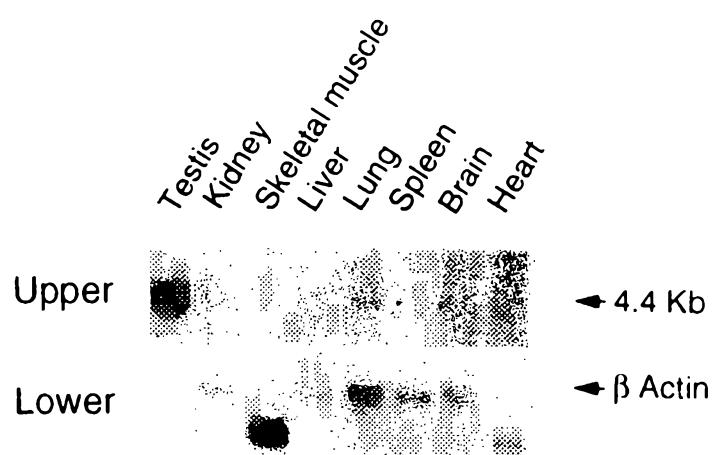


FIG. 4



FIG. 5



FIG. 6C



FIG. 6B

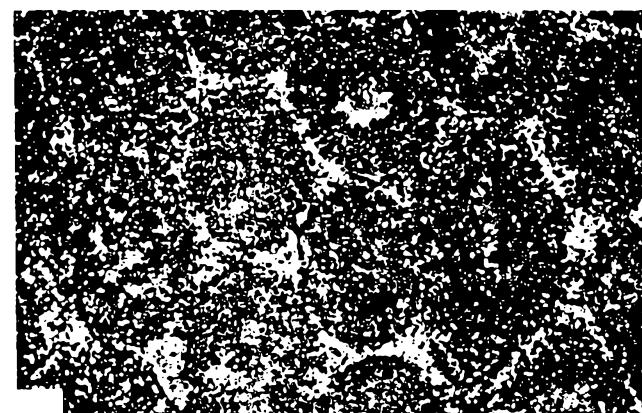


FIG. 6A

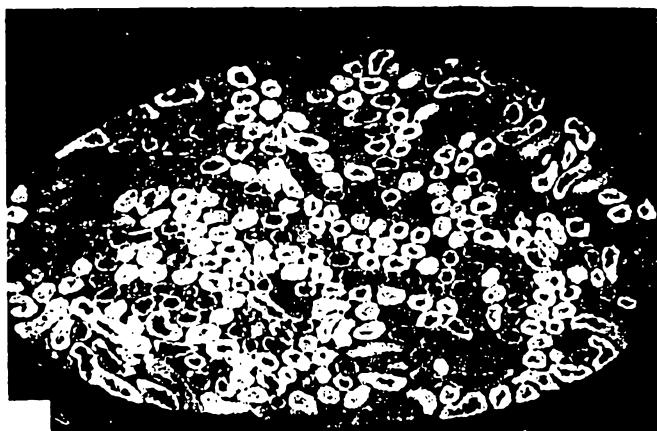


FIG. 6F

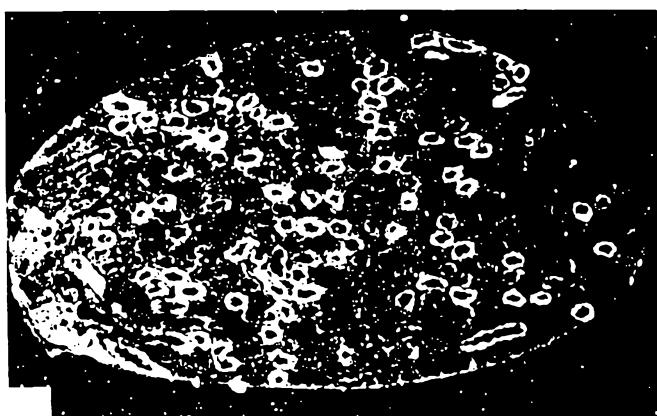


FIG. 6E



FIG. 6D

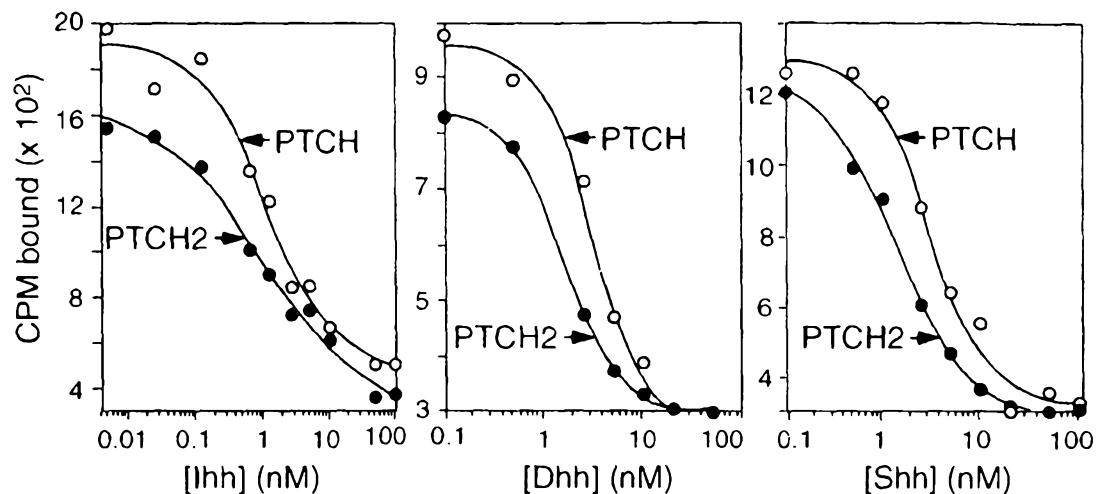


FIG. 7A

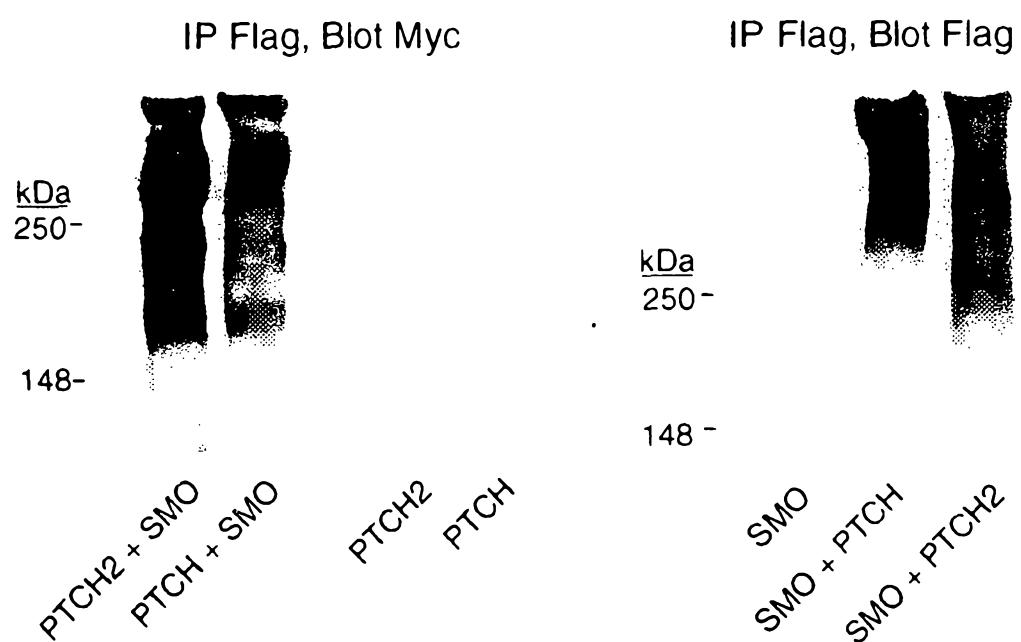


FIG. 7B

17/27

	10	20	30	40	50
hPtch-2	MTRSPPLRELPPSYTPPARTAAPQILAGSLKAPLWLRAYFQGLLFSLGCG	*****.*****.*****.*****.*****.*****.*****.*****.*****			
mPatched2 (SEQ ID NO:7)	MVRPLSLGELPPSYTPPARSSAPHILAGSLQAPLWLRAYFQGLLFSLGCR				
	10	20	30	40	50
hPtch-2	60	70	80	90	100
mPatched2	IQRHCGKVLFLGLLAFGALALGLRMAI IETNLEQLWVEVGSRVSQELHYT	*****.*****.*****.*****.*****.*****.*****.*****.*****			
hPtch-2	60	70	80	90	100
mPatched2	IQKHCAGKVLFLGLVAFGALALGLRVAVIETDLEQLWVEVGSRVSQELHYT				
	110	120	130	140	150
hPtch-2	KEKLGEAAAYTSQMLIQTARQEGENILTPEALGLHLQAALTASKVQVSLY	*****.*****.*****.*****.*****.*****.*****.*****.*****			
mPatched2	KEKLGEAAAYTSQMLIQTAKQEGGNVLTPPEALDLHLQAALTASKVQVSLY				
hPtch-2	110	120	130	140	150
mPatched2	110	120	130	140	150
	160	170	180	190	200
hPtch-2	GKSWDLNKICYKSGVPLIENGMIIEWMIEKLFPCVILTPLDCFWEGAKLQG	*****.*****.*****.*****.*****.*****.*****.*****.*****			
mPatched2	GKSWDLNKICYKSGVPLIENGMIERMIEKLFPCVILTPLDCFWEGAKLQG				
hPtch-2	160	170	180	190	200
mPatched2	160	170	180	190	200
	210	220	230	240	250
hPtch-2	GSAYLPGRPDIQWTNLDPEQLLEELGPFASLEGFRELLDKAQVGQAYVGR	*****.*****.*****.*****.*****.*****.*****.*****.*****			
mPatched2	GSAYLPGRPDIQWTNLDPQLLEELGPFASLEGFRELLDKAQVGQAYVGR				
hPtch-2	210	220	230	240	250
mPatched2	210	220	230	240	250
	260	270	280	290	300
hPtch-2	PCLHPDDLHCPPSAPNHRSQAPNVAHELSGGCHGFSHKFMHWQEELLLG	*****.*****.*****.*****.*****.*****.*****.*****.*****			
mPatched2	PCLDPDDPHCPPSAPNRHSRQAPNVAQELSGGCHGFSHKFMHWQEELLLG				
hPtch-2	260	270	280	290	300
mPatched2	260	270	280	290	300
	310	320	330	340	350
hPtch-2	GMARDPQGELLRAEALQSTFLLMSPRQLYEHFRGDYQTHDIGWSEEQAST	*****.*****.*****.*****.*****.*****.*****.*****.*****			
mPatched2	GTARDLQGQLLRAEALQSTFLLMSPRQLYEHFRGDYQTHDIGWSEEQASM				
hPtch-2	310	320	330	340	350
mPatched2	310	320	330	340	350

FIG. 8A

18 / 27

	360	370	380	390	400
hPtch-2	VLQAWQRRFVQLAQEALPENASQQIHAFSSTLDDILHAFSEVSAARVVG	*****	*****	*****	*****
mPatched2	VLQAWQRRFVQLAQEALPANASQQIHAFSSTLDDILRASFSEVSTTRVVG	*****	*****	*****	*****
	360	370	380	390	400
hPtch-2	410	420	430	440	450
mPatched2	GYLLMLAYACVTMLRWDCAQSQGSVGLAGVLLVALAVASGLGLCALLGIT	*****	*****	*****	*****
	410	420	430	440	450
hPtch-2	460	470	480	490	500
mPatched2	FNAATTQVLPFLALGIGVDDVFLLAHAFTTEALPGTPLQERMGECLQRTGT	*****	*****	*****	*****
	460	470	480	490	500
hPtch-2	510	520	530	540	550
mPatched2	SVVLTSINNMAAFLMAALVPIPALRAFSLQAAIVVGCTFVAVMLVFPAIL	***	***	***	***
	510	520	530	540	550
hPtch-2	560	570	580	590	600
mPatched2	SLDLRRRHQRQLDVLCCFSSPCSAQVIQILPQELGDGTVPVGIAHLTATV	*****	*****	*****	*****
	560	570	580	590	600
hPtch-2	610	620	630	640	650
mPatched2	QAFTHCEASSQHVVTILPPQAHLVPPPSDPLGSELPSPGGSTRDLLQEE	*****	*****	*****	*****
	610	620	630	640	650
hPtch-2	660	670	680	690	700
mPatched2	ETRQKAACKSLPCARWNLAHFARYQFAPLLLQSHAKAIVLVLFGALLGLS	*	*****	*****	*****
	660	670	680	690	700
hPtch-2	GTGPQAACRPLLCAHWTIAHFARYQFAPLLLQTRAKALVLLFFGALLGLS	*****	*****	*****	*****
mPatched2					

FIG. 8B

19/27

	710	720	730	740	750
<i>hPtch-2</i>	LYGATLVQDGLALTDVVPRGTKEHAFLSAQLRYFSLYEVALVTQGGFDYA				

<i>mPatched2</i>	LYGATLVQDGLALTDVVPRGTKEHAFLSAQLRYFSLYEVALVTQGGFDYA				
	710	720	730	740	750
	760	770	780	790	800
<i>hPtch-2</i>	HSQRALFDLHQRFSSLKAVLPPPATQAPRTWLHYYRNWLQGIQAAFDQDW				

<i>mPatched2</i>	HSQRALFDLHQRFSSLKAVLPPPATQAPRTWLHYYRSWLQGIQAAFDQDW				
	760	770	780	790	800
	810	820	830	840	850
<i>hPtch-2</i>	ASGRITRHSYRNGSEDGALAYKLLIQTGDAQEPLDFSQLTTRKLVDREGL				

<i>mPatched2</i>	ASGRITCHSYRNGSEDGALAYKLLIQTGNAQEPLDFSQLTTRKLVDKEGL				
	810	820	830	840	850
	860	870	880	890	900
<i>hPtch-2</i>	IPPELFYMGLTVWVSSDPLGLAASQANFYPPPPEWLHDKYDTTGENLRIP				

<i>mPatched2</i>	IPPELFYMGLTVWVSSDPLGLAASQANFYPPPPEWLHDKYDTTGENLRIP				
	860	870	880	890	900
	910	920	930	940	950
<i>hPtch-2</i>	PAQPLEFAQFPFLRLGLQKTADFVEAIEGARAACAEAGQAGVHAYPSGSP				

<i>mPatched2</i>	AAQPLEFAQFPFLRHGLQKTADFVEAIEGARAACTEAGQAGVHAYPSGSP				
	910	920	930	940	950
	960	970	980	990	1000
<i>hPtch-2</i>	FLFWEQYLGLRRCFLLAVCILLVCTFLVCALLLNWTAGLIVLVLAMMT				

<i>mPatched2</i>	FLFWEQYLGLRRCFLLAVCILLVCTFLVCALLLSPWTAGLIVLVLAMMT				
	960	970	980	990	1000
	1010	1020	1030	1040	1050
<i>hPtch-2</i>	VELFGIMGFLGIKLSAIPVVLVAVSIGIGVEFTVHVALGFLTTQGSRNLR				

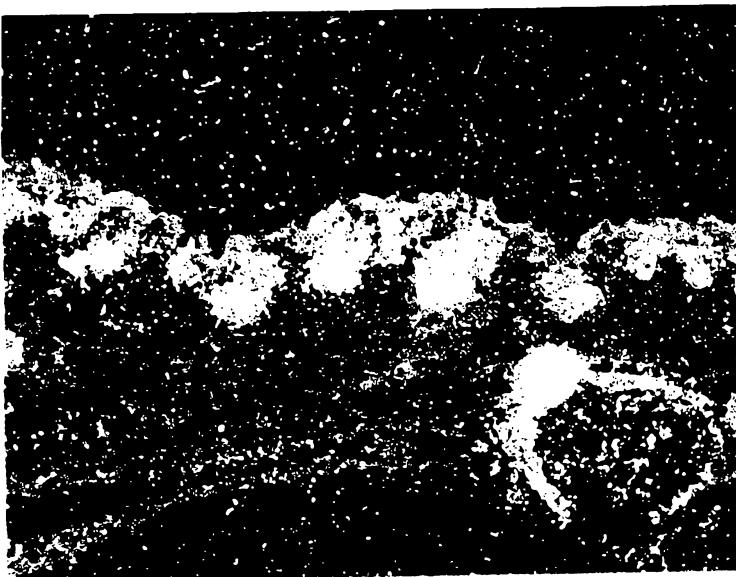
<i>mPatched2</i>	VELFGIMGFLGIKLSAIPVVLVAVSIGIGVEFTVHVALGFLTSQGSRNLR				
	1010	1020	1030	1040	1050

FIG. 8C

	1060	1070	1080	1090	1100
hPtch-2	AAHALEHTFAPVTDGAISTLLGLLMLAGSHFDFIVRYFFAALTVLTLGL	***	***	***	*****
mPatched2	AASALEQTFAPVTDGAVSTLLGLLMLAGSNFDFIIRYFFVVLTVLTLGL	1060	1070	1080	1090
	1110	1120	1130	1140	1150
hPtch-2	LHGLVLLPVLLSILGPPPEVIQMYKESPEILSPPAPQGGGLRWGASSSLP	*****	*****	*****	*****
mPatched2	LHGLLLLPVLLSILGPPPQVVQVYKESPQTLNSAAPQRGGLRWDRPPTLP	1110	1120	1130	1140
	1160	1170	1180	1190	1200
hPtch-2	QSFARVTTSMIAHPPPLPGAYIHPAPDEPPWSPAATSSGNLSSRGPGP	*****	*****	*****	*****
mPatched2	QSFARVTTSMIAHPPPLPGAYVHPASEEPT	1160	1170	1180	
hPtch-2	ATG				

FIG. 8D

PTCH



PTCH2



FIG. 9

> Consensus Sequence of human patched 2 cDNA clone
 > length: 4004 bp

(SEQ ID NO:8)

1 CCCACCGCGTC CGGGAGAAGC TGGGGGAGGA GGCTGCATAC ACCTCTCAGA TGCTGATACA GACCGCACGC CAGGAGGGAG AGAACATCCT CACACCCGAA
 GGGTGCAG GGCCTCTTCG ACCCCCTCCT CCGACGTATG TGGAGAGTCT ACGACTATGT CTGGCGTGC GTCCTCCCTC TCTTGTAGGA GTGTGGCTT
 race 6

101 GCACTTGGCC TCCACCTCCA GGCAGCCCTC ACTGCCAGTA AAGTCCAAGT ATCACTCTAT GGGAAAGTCCT GGGATTGAA CAAAATCTGC TACAAGTCAG
 CGTGAACCGG AGGTGGAGGT CGTCGGGAG TGACGGTCAT TTCAGGTTCA TAGTGAGATA CCCTTCAGGA CCCTAAACTT GTTTTAGACG ATGTTCAAGTC
 race 5'

201 GAGTTCCCCT TATTGAAAAT GGAATGATTG AGCGGATGAT TGAGAAGCTG TTTCGCGTG TGATCCTCAC CCCCCCTCGAC TGCTTCTGGG AGGGAGCCAA
 CTCAAGGGGA ATAACCTTTA CCTTACTAAC TCGCCTACTA ACTCTTCGAC AAAGGCACGC ACTAGGAGTG GGGGGAGCTG ACGAAGACCC TCCCTCGGTT

301 ACTCCAAGGG GGCTCCGCCT ACCTGCCGCT CCCAATGTGG CTCACGAGCT GAGTGGGGC TGCCATGGCT TCTCCCACAA ATTCACTGCAC TGGCAGGAGG
 TGAGGTTCCC CCGAGGCGGA TGGACGGCGA GGGTTACACC GAGTGCTCGA CTCACCCCCG ACGGTACCGA AGAGGGTGT TAAGTACGTG ACCGTCCCTCC

401 AATTGCTGCT GGGAGGCATG GCCAGAGACC CCCAAGGAGA GCTGCTGAGG GCAGAGGCC CTCAGAGCAC CTTCTTGCTG ATGAGTCCCC GCCAGCTGTA
 TTAACGACGA CCCTCCGTAC CGGTCTCTGG GGGTTCTCT CGACGACTCC CGTCTCCGGG ACGTCTCGTG GAAGAACGAC TACTCAGGGG CGGTCGACAT

501 CGAGCATTTC CGGGGTGACT ATCAGACACA TGACATTGGC TGGAGTGAGG AGCAGGCCAG CACAGTGTCA AAAGCCTGGC AGCGGCCCTT TGTGCAGGTC
 GCTCGTAAAG GCCCCACTGA TAGTCTGTGT ACTGTAACCG ACCTCACTCC TCGTCCGGTC GTGTACGAT GTTCGGACCG TCGCCCGAA ACACGTCCAG

601 GGTATGGACA AGGACAGGGG GGTGCCCTGA GGCCATTCCC TCCTCCTGCC CCCTCCTATC CACCCCTGTTT CTCCAGCTGG CCCAGGAGGC CCTGCCTGAG
 CCATACCTGT TCCTGTCCCC CCACGGGACT CCGGTAAGGG AGGAGGACGG GGGAGGATAG GTGGGACAAA GAGGTCGACC GGGTCCTCCG GGACGGACTC

701 AACGCTTCCC AGCAGATCCA TGCTTCTCC TCCACCAACCC TGGATGACAT CCTGCATGCG TTCTCTGAAG TCAGTGCTGC CGGTGTGGTG GGAGGCTATC
 TTGCGAAGGG TCGTCTAGGT ACGGAAGAGG AGGTGGTGGG ACCTACTGTA GGACGTACGC AAGAGACTTC ACTCACGACG GGACACACAC CCTCCGATAG

801 TGCTCATGGT GGGTCTTGCA CCTGGCACCT TGCCCCACC CCACCTCAA CCAGTGCCA CCCTGGGAG CCCCTGAGAC TGCCCTTCC CCCCACAGCT
 ACGAGTACCA CCCAGAACGT GGACCGTGG ACGGGGGTGG GGTGGAGGT GGTACGGGT GGGACCCCTC GGGGACTCTG ACGGGAAAGG GGGGTGTCGA

FIG. 10A

901 GGCCTATGCC TGTGTGACCA TGCTGCGGTG GGACTGCGCC CAGTCCCAGG GTTCCGTGGG CCTTGCCGGG GTACTGCTGG TGGCCCTGGC GGTGGCCTCA
 CCGGATACGG ACACACTGGT ACGACGCCAC CCTGACGCCGG GTCAGGGTCC CAAGGCACCC GGAACGGCCC CATGACGACC ACCGGGACCG CCACCGGAGT

 1001 GGCCTGGGC TCTGTGCCCT GCTCGGCATC ACCTTCAATG CTGCCACTAC CCAGGTACGC CAGGACTGCA GGGCAGACTC AGTGCCAGTC ACCAGGCTTC
 CCGGAACCG AGACACGGGA CGAGCCGTAG TGGAAAGTTAC GACGGTGATG GGTCCATGCG GTCCTGACGT CCCGTCTGAG TCACGGTCAG TGGTCCGAAG

 1101 ACGGGTCCTC AGCTGCCCGC TCCTCTGCCCT CTCCAGGTGC TGCCCTTCTT GACTCTGGGA ATCGGCCTGG ATGACGTATT CCTGCTGGCG CATGCCCTCA
 TGCCCAGGAG TCGACGGCG AGGAGACGGG GAGGTCCACG ACGGGAAGAA CTGAGACCCCT TAGCCGCACC TACTGCATAA GGACGACCGC GTACGGAAGT

 1201 CAGAGGCTCT GCCTGGCACC CCTCTCCAGG TGGGGCCTTG TCCCCCAGGG CTCATCTGAG GCAGCTCAGC TTACTGGTTA AGAGCCTCTT GGTTCAAGTG
 GTCTCCGAGA CGGACCGTGG GGAGAGGTCC ACCCCGGAAC AGGGGGTCCC GAGTAGACTC CGTCGAGTCG AATGACCAAT TCTCGGAGAA CCAAGTTCAC

 1301 ACCTTGGGCT GCTAAATGAAC CTCGGTGCCT CTTGTCCCCA TGTGTAAACA GGGGAAATAA TAGTGTGTG TCCTAAGGGT TATTGTTGG ATCAGTGAAG
 TGGAACCCGA CGATTACTTG GAGCCACGGA GAACAGGGGT ACACATTGT CCCCTTTATT ATCACGACAC AGGATTCCA ATAACAAACC TAGTCACTTC

 1401 TAACTCAAGT TGAATGCTTA GAACAGCCCA TCATACGTAC ATGGTACCCA ATAAATGCTA GCCACTGTGT TATGACTGCC CCACCTCTGC ACCCCAAAGT
 ATTGAGTTCA ACTTACGAAT CTTGTCGGGT AGTATGCATG TACCATGGGT TATTACGAT CGGTGACACA ATACTGACGG GGTGGAGACG TGGGGTTCAA

 1501 CCTGAGCCTC CCCTTCACTC CACTTGACA CGGGCCCTCC CTTGTGACCT GAGGGCAGGT CCCCACTCTG TCCTGGCAGG AGCGCATGGG CGAGTGTCTG
 GGACTCGGAG GGGAAAGTGAG GTGAAACTGT GCCGGGGAGG GAACACTGGA CTCCCGTCCA GGGTGAGAC AGGACCGTCC TCGCGTACCC GCTCACAGAC

 1601 CAGCGCACGG GCACCAGTGT TGTACTCACA TCCATCAACA ACATGGCCGC CTTCCTCATG GCTGCCCTCG TTCCCATCCC TGCGCTGCGA GCCTTCTCCC
 GTCGCGTGCC CGTGGTCACA ACATGAGTGT AGGTAGTTGT TGTACCGGCG GAAGGAGTAC CGACGGGAGC AAGGGTAGGG ACGCGACGCT CGGAAGAGGG

 1701 TACAGCCTGG ACCTACGGCG GCGCCACTGC CAGCGCCTTG ATGTGCTCTG CTGCTTCTCC AGGTACTGCC TGCGCCCCAG CCCCTCCTC CCGTGACCCA
 ATGTCGGACC TGGATGCCGC CGCGGTGACG GTCGCGGAAC TACACGAGAC GACGAAGAGG TCCATGACGG ACGCGGGGTC GGGGAAGGAG GGCACGGGT

 1801 CGCCAGCCTG TCCCCCTCACC AGCATTCAA GGCACAGACC TGTCATCCAC TCTCTACCTC TTCCAGTCCC TGCTCTGCTC AGGTGATTCA GATCCTGCC
 GCGGTCGGAC AGGGGAGTGG TCGTAAAGTT CCGTGTCTGG ACAGTAGGTG AGAGATGGAG AAGGTCAAGG ACGAGACGAG TCCACTAAGT CTAGGACGGG

 1901 CAGGAGCTGG GGGACGGGAC AGTACCAAGTG GGCATTGCC ACCTCACTGC CACAGTTCAA GCCTTACCC ACTGTGAAGC CAGCAGCCAG CATGTGGTCA
 GTCCTCGACC CCCTGCCCTG TCATGGTCAC CCGTAACGGG TGGAGTGACG GTGTCAAGTT CGGAAATGGG TGACACTTCG GTCGTCGGTC GTACACCAGT

 2001 CCATCCTGCC TCCCCAAGCC CACCTGGTGC CCCCACCTTC TGACCCACTG GGCTCTGAGC TCTTCAGCCC TGGAGGGTCC ACACGGGACC TTCTAGGCCA
 GGTAGGACGG AGGGGTTCCGG GTGGACCAAG GGGGTGGAAG ACTGGGTGAC CCGAGACTCG AGAAGTCGGG ACCTCCCAGG TGTGCCCTGG AAGATCCGGT

FIG. 10B

2101 GGAGGAGGAG ACAAGGCAGA AGGCAGCCTG CAAGTCCCTG CCCTGTGCC GCTGGAATCT TGCCCATTTG GCCCGCTATC AGTTTGCCCC GTTGCTGCTC
 CCTCCTCCTC TGTTCCGTCT TCCGTCGGAC GTTCAGGGAC GGGACACGGG CGACCTTAGA ACGGGTAAAG CGGGCGATAG TCAAACGGGG CAACGACGAG

 2201 CAGTCACATG CCAAGGCAT CGTGCTGGTG CTCTTGGTG CTCTTCTGGG CCTGAGCCTC TACGGAGCCA CCTGGTGCAGA AGACGGCCTG CCCCTGACGG
 GTCAGTGTAC GGTTCCGGTA GCACGACAC GAGAAACAC GAGAAGACCC GGACTCGGAG ATGCCTCGGT GGAACCACGT TCTGCCGGAC CGGGACTGCC

 2301 ATGTGGTGCCT CGGGGGCACC AAGGAGCATG CCTTCCTGAG CGCCCAGCTC AGGTACTTCT CCCTGTACGA GGTGGCCCTG GTGACCCAGG GTGGCTTTGA
 TACACCACGG AGCCCCGTGG TTCCTCGTAC GGAAGGACTC GCGGGTCGAG TCCATGAAGA GGGACATGCT CCACCGGGAC CACTGGTCC CACCGAAACT

 2401 CTACGCCAC TCCCAACCGCG CCCTCTTGA TCTGCACCAAG CGCTTCAGTT CCCTCAAGGC GGTGCTGCC CCACCGGCCA CCCAGGCACC CCGCACCTGG
 GATGCGGGTG AGGGTTGCGC GGGAGAAACT AGACGTGGTC GCGAAGTCAA GGGAGTTCCG CCACGACGGG GGTGGCCGGT GGGTCCGTGG GGCCTGGACC

 2501 CTGCACTATT ACCGCAACTG GCTACAGGGAG ATCCAGGCTG CCTTGACCA GGACTGGCT TCTGGCGCA TCACCCGCCA CTCGTACCGC AATGGCTCTG
 GACGTGATAA TGGCGTTGAC CGATGTCCCT TAGGTCCGAC GGAAACTGGT CCTGACCGCA AGACCCGCGT AGTGGGCGGT GAGCATGGCG TTACCGAGAC

 2601 AGGATGGGGC CCTGGCCTAC AAGCTGCTCA TCCAGACTGG AGACGCCAG GAGCCTCTGG ATTTCAGCCA GGTTGGGAGA GGGCTGGAGG GGTCCACTAG
 TCCTACCCCG GGACCGGATG TTCGACGAGT AGGTCTGACC TCTGCGGGTC CTCGGAGACC TAAAGTCGGT CCAACCCCTCT CCCGACCTCC CCAGGTGATC

 2701 TACAGGGGCT GCAGGCCCTCC TGGGCCAGG CCTTCAGCCC TCTCTGCCCTC TGCGACTGAC CACAAGGAAG CTGGTGGACA GAGAGGGACT GATTCCACCC
 ATGTCCCCGA CGTCCGGAGG ACCCGGGTCC GGAAGTCGGG AGAGACGGAG ACGTCGACTG GTGTTCCCTC GACCACCTGT CTCTCCCTGA CTAAGGTGGG

 2801 GAGCTCTTCT ACATGGGGCT GACCGTGTGG GTGAGCAGTG ACCCCCTGGG TCTGGCAGCC TCACAGGCCA ACTTCTACCC CCCACCTCC GAATGGCTGC
 CTCGAGAAGA TGTACCCCGA CTGGCACACC CACTCGTCAC TGGGGGACCC AGACCGTCGG AGTGTCCGGT TGAAGATGGG GGGTGGAGGA CTTACCGACG

 2901 ACGACAAATA CGACACCACG GGGGAGAACCC TTGCGAGTGA GTCTGGGGG GAGCTGGCA AGAGCCTCAG CCTCGCCAC ACAAGCCCTG AGCCTGAGGC
 TGCTGTTTAT GCTGTGGTGC CCCCTCTTGG AAGCGTCACT CAGAACCCCC CTCGAGCCGT TCTCGGAGTC GGAGCGGGTG TGTTCCGGAC TCGGACTCCG

 3001 CCTGCCCACT CTGCCCCGTG CTCACCGCCCC TGTCCTCTC CCTCTTCTCC CTTCCCTCC CCTCCACAGT CCCGCCAGCT CAGCCCTTGG AGTTTGCCCCA
 GGACGGGTGA GACGGGGCAC GAGTGGCGGG ACAGGGAGAG GGAGAAGAGG GAAGGGGAGG GGAGGTGTCA GGGCGGTGA GTCGGGAACC TCAAACGGGGT

FIG. 10C

3101 GTTCCCCTTC CTGCTCGTG GCCTCCAGAA GACTGCAGAC TTTGTGGAGG CCATCGAGGG GGCCCGGGCA GCATGCGCAG AGGCCGGCCA GGCTGGGTG
 CAAGGGGAAG GACGACGCAC CGGAGGTCTT CTGACGTCTG AAACACCTCC GGTAGCTCCC CCGGGCCCGT CGTACGCGTC TCCGGCCGGT CCGACCCAC

 3201 CACGCCTACC CCAGCGGCTC CCCCTTCCTC TTCTGGGAAC AGTATCTGGG CCTGCGGCAG TGCTTCCTGC TGGCCGTCTG CATCCTGCTG GTGTGCACTT
 GTGCGGATGG GGTCGCGAG GGGGAAGGAG AAGACCCCTG TCATAGACCC GGACGCCGCG ACGAAGGACG ACCGGCAGAC GTAGGACGAC CACACGTGAA

 3301 TCCTCGTCTG TGCTCTGCTG CTCCTCAACC CCTGGACGGC TGGCCTCATA GTGAGTGCCT GCAGGAGTGG GGACAGAGAC ACCCCACCCCT TCCCTGCCA
 AGGAGCAGAC ACGAGACGAC GAGGAGTTGG GGACCTGCCG ACCGGAGTAT CACTCACGAA CGTCTCACC CCTGTCTCTG TGGGTGGGA AGGGACGGGT

 3401 GCCTGTCATC CCTCCTGCCA GGAGCCCTCT GTGAGCCCTG TCTCCCTCAG GTGCTGGTCC TGGCGATGAT GACAGTGGAA CTCTTGGTA TCATGGGTTT
 CGGACAGTAG GGAGGACGGT CCTCGGGAGA CACTCGGGAC AGAGGGAGTC CACGACCAGG ACCGCTACTA CTGTCACCTT GAGAAACCAT AGTACCCAAA

 3501 CCTGGGCATC AAGCTGAGTG CCATCCCCGT GGTGATCCTT GTGGCCTCTG TAGGCATTGG CGTTGAGTTC ACAGTCCACG TGGCTCTGGT GAGCACGGGC
 GGACCCGTAG TTCGACTCAC GGTAGGGCA CCACTAGGAA CACCGGAGAC ATCCGTAACC GCAACTCAAG TGTCAGGTGC ACCGAGACCA CTCGTGCCG

 3601 ACCCCGGGGA GGGACCAATC AGCTGATTCA GTATTCAACA CATATTGTT AAGCCCCTAC TATGTGCTAG GTACTATTAA AGAATTGGG CTGGGTGGAC
 TGGGGCCCT CCCTGGTTAG TCGACTAAAGT CATAAGTTGT GTATAACAAG TTCGGGGATG ATACACGATC CATGATAAAT TCTTAAACCC GACCCACCTG

 3701 GTGGTGGCTC ATTCCGTAA TCCCAGCACT TTGGGAGGCC GAGGCGGGTG GATCACCTGA GGTGGGAGT TCGAAACCAG CCTGGCCAAC ATGGTGAAAC
 CACCACCGAG TAAGGACATT AGGGTCGTGA AACCCCTCCGG CTCCGCCAC CTAGTGGACT CCAGCCCTCA AGCTTGGTC GGACCGGTTG TACCACTTTG

 3801 CCTGTCTTTA CTAAAAATAC AAAAATTAG CCAGCGTGG TGGCACATGC CAGTAGTCCC AGCTACTTTG GAGGCTGAGG CAGAATTGCT TGAACCTGGG
 GGACAGAAAT GATTTTATG TTTTTAATC GGTCCGCACC ACCGTGTACG GTCATCAGGG TCGATGAAAC CTCCGACTCC GTCTAACGA ACTTGGACCC

 3901 AGGCGAAGGT TGCAGTGAGC TGAGATCGTG CCATTGCACT CCAGCCTGGG CAACAAGAGT GCAACTCTCC GTCTAAAAA AAAAAAAA AAGGGCGGCC
 TCCGCTTCCA ACGTCACTCG ACTCTAGCAC GGTAACGTGA GGTGGACCC GTTGTCTCA CGTTGAGAGG CAGAGTTTTT TTTTTTTTTT TTCCCGCCGG

 4001 GCGA
 CGCT

FIG. 10D

Clone 16.1 human patched 2
> length: 2082 bp

> (SEQ ID NO:9)

1 TTCCGGCATG ACTCGATCGC CGCCCCTCAG AGAGCTGCC CCGAGTTACA CACCCCCAGC TCGAACCGCA GCACCCCAGA TCCTAGCTGG GAGCCTGAAG AAGGCCGTAC TGAGCTAGCG GCGGGGAGTC TCTCGACGGG GGCTCAATGT GTGGGGGTG AGCTTGGCGT CGTGGGGTCT AGGATCGACC CTCGGACTTC
101 GCTCCACTCT GGCTTCGTGC TTACTCCAG GGCCTGCTCT TCTCTCTGGG ATGCGGGATC CAGAGACATT GTGGCAAAGT GCTCTTCTG GGACTGTTGG CGAGGTGAGA CGAACGCACG AATGAAGGTC CCGGACGAGA AGAGAGACCC TACGCCCTAG GTCTCTGTAA CACCGTTCA CGAGAAAGAC CCTGACAACC
201 CCTTTGGGGC CCTGGCATTG GGTCTCCGCA TGGCCATTAT TGAGACAAAC TTGGAACAGC TCTGGTAGA AGTGGGCAGC CGGGTGAGCC AGGAGCTGCA GGAAACCCCG GGACCGTAAT CCAGAGGCCT ACCGGTAATA ACTCTGTTG AACCTTGTG AGACCCATCT TCACCCGTCG GCCCACTCGG TCCTCGACGT
301 TTACACCAAG GAGAACGCTGG GGGAGGAGGC TGCATACACC TCTCAGATGC TGATACAGAC CGCACGCCAG GAGGGAGAGA ACATCCTCAC ACCCGAACGA AATGTGGTTC CTCTTCGACC CCCTCCTCCG ACGTATGTGG AGAGTCTACG ACTATGTCTG GCGTGCCTGC CTCCCTCTCT TGTAGGAGTG TGGGCTTCGT
401 CTTGGCCTCC ACCTCCAGGC AGCCCTCACT GCCAGTAAG TCCAAGTATC ACTCTATGGG AAGTCCTGGG ATTTGAACAA AATCTGCTAC AAGTCAGGAG GAACCGGAGG TGGAGGTCCG TCGGGAGTGA CGGTCAATTG AGTTCTAG TGAGATACCC TTCAGGACCC TAAACTTGTG TTAGACGATG TTCAGTCCTC
501 TTCCCCTTAT TGAAAATGGA ATGATTGAGT GGATGATGTA GAAGCTGTTT CCGTGCCTGA TCCTCACCCCC CCTCGACTGC TTCTGGGAGG GAGCCAAACT AAGGGGAATA ACTTTTACCT TACTAACTCA CCTACTAACT CTTCGACAAA GGCACGCACT AGGAGTGGGG GGAGCTGACG AAGACCCCTCC CTCGGTTGA
601 CCAAGGGGGC TCCGCCTACC TGCCCAGGCC CCCGGATATC CAGTGGACCA ACCTGGATCC AGAGCAGCTG CTGGAGGAGC TGGGTCCCTT TGCCTCCCTT GGTTCCCCCG AGCGGGATGG ACGGGCCGGC GGGCCTATAG GTCACCTGGT TGGACCTAGG TCTCGTCAC GACCTCCTCG ACCCAGGGAA ACGGAGGGAA
701 GAGGGCTTCC GGGAGCTGCT AGACAAGGCA CAGGTGGGCC AGGCCTACGT GGGGCGGGCC TGTCTGCACC CTGATGACCT CCACTGCCCA CCTAGTGCCCTT CTCCCGAAGG CCCTCGACGA TCTGTTCCGT GTCCACCCGG TCCGGATGCA CCCCCGGGGG ACAGACGTGG GACTACTGGA GGTGACGGGT GGATCACGGC
801 CCAACCATCA CAGCAGGCAG GCTCCAATG TGGCTCACGA GCTGAGTGGG GGCTGCCATG GCTTCTCCCA CAAATTCACTG CACTGGCAGG AGGAATTGCT GGTTGGTAGT GTCGTCCGTC CGAGGGTTAC ACCGAGTGCT CGACTCACCC CCGACGGTAC CGAAGAGGGT GTTTAAGTAC GTGACCGTCC TCCTTAACGA
901 GCTGGGAGGC ATGGCCAGAG ACCCCCAAGG AGAGCTGCTG AGGGCAGAGG CCCTGCAGAG CACCTTCTTG CTGATGAGTC CCCGCCAGCT GTACGAGCAT CGACCCCTCCG TACCGGTCTC TGGGGGTTCC TCTCGACGAC TCCCGTCTCC GGGACGTCTC GTGGAAGAAC GACTACTCAG GGGCGGTGCA CATGCTCGTA

26 / 27

FIG. 11A

1001 TTCCGGGGTG ACTATCAGAC ACATGACATT GGCTGGAGTG AGGAGCAGGC CAGCACAGTG CTACAAGCCT GGCAGCGGCG CTTTGTGCAG CTGGCCCAGG
 AAGGCCAAC TGATAGTCTG TGTACTGTAA CCGACCTCAC TCCTCGTCCG GTCGTGTAC GATGTTCGGA CCGTCGCCGC GAAACACGTC GACCGGGTCC

 1101 AGGCCCTGCC TGAGAACGCT TCCCAGCAGA TCCATGCCTT CTCCTCCACC ACCCTGGATA ACATCCTGCA TGCGTTCTCT GAAGTCAGTG CTGCCCGTGT
 TCCGGGACGG ACTCTTGCAGA AGGGTCGTCT AGGTACGGAA GAGGAGGTGG TGGGACCTAT TGTAGGACGT ACGCAAGAGA CTTCAGTCAC GACGGGCACA

 1201 GGTGGGAGGC TATCTGCTCA TGCTGGCCTA TGCCGTGTG ACCATGCTGC GGTGGGACTG CGCCCAAGTCC CAGGGTTCCG TGGGCTTGC CGGGGTACTG
 CCACCCCTCCG ATAGACGAGT ACGACCGGAT ACGGACACAC TGGTACGACG CCACCCCTGAC GCGGGTCAGG GTCCCAAGGC ACCCGGAACG GCCCCATGAC

 1301 CTGGTGGCCC TGGCGGTGGC CTCAGGCCTT GGGCTCTGTG CCCTGCTCGG CATCACCTTC AATGCTGCCA CTACCCAGGT GCTGCCCTTC TTGGCTCTGG
 GACCACCGGG ACCGCCACCG GAGTCCGGAA CCCGAGACAC GGGACGAGCC GTAGTGGAAAG TTACGACGGT GATGGGTCCA CGACGGGAAG AACCGAGACC

 1401 GAATCGGCGT GGATGACGTA TTCCCTGCTGG CGCATGCCTT CACAGAGGCT CTGCCTGGCA CCCCTCTCCA GGAGCGCATG GGCGAGTGTC TGCAGCGCAC
 CTTAGCCGCA CCTACTGCAT AAGGACGACC GCGTACGGAA GTGTCTCCGA GACGGACCCT GGGGAGAGGT CCTCGCGTAC CCGCTCACAG ACGTCGCGTG

 1501 GGGCACCAGT GTCGTACTCA CATCCATCAA CAACATGGCC GCCTCCTCA TGGCTGCCCT CGTTCCCATC CCTGCGCTGC GAGCCTTCTC CTTACAGCCA
 CCCGTGGTCA CAGCATGAGT GTAGGTAGTT GTTGTACCGG CGGAAGGAGT ACCGACGGGA GCAAGGGTAG GGACCGCAGC CTCGGAAGAG GAATGTCGGT

 1601 TCCTCAGCCT GGACCTACGG CGGCCCACT GCCAGCGCCT TGATGTGCTC TGCTGCTTCT CCAGTCCCTG CTCTGCTCAG GTGATTCAAGA TCCTGCCCTA
 AGGAGTCGGA CCTGGATGCC GCCCGGGTGA CGGTGCGGGA ACTACACGAG ACGACGAAGA GGTCAAGGGAC GAGACGAGTC CACTAAGTCT AGGACGGGGT

 1701 GGAGCTGGGG GACGGGACAG TACCACTGGG CATTGCCAC CTCACTGCCA CAGTTCAAGC CTTTACCCAC TGTGAAGCCA GCAGCCAGCA TGTGGTCACC
 CCTCGACCCC CTGCCCTGTC ATGGTCACCC GTAACGGGTG GAGTGACGGT GTCAAGTTCG GAAATGGGTG ACACTTCGGT CGTCGGTCGT ACACCAGTGG

 1801 ATCCTGCCTC CCCAAGCCCA CCTGGTGCC CCACCTCTG ACCCACTGGG CTCTGAGCTC TTCAGCCCTG GAGGGTCCAC ACGGGACCTT CTAGGCCAGG
 TAGGACGGAG GGGTTGGGT GGACCACGGG GGTGGAAGAC TGGGTGACCC GAGACTCGAG AAGTCGGGAC CTCCCAGGTG TGCCCTGGAA GATCCGGTCC

 1901 AGGAGGAGAC AAGGCAGAAG GCAGCCTGCC AGTCCCTGCC CTGTGCCCGC TGGAAATCTTG CCCATTCGC CCCGGAATTG CTGCAGCCCG GGGGATCCAC
 TCCTCCTCTG TTCCGTCTTC CGTCGGACGT TCAGGGACGG GACACGGGCG ACCTTAGAAC GGGTAAAGCG GGGCCTTAAG GACGTGGGC CCCCTAGGTG

 2001 TAGTTCTAGA CGGGCCGCCA CCGCGGTGGA GCTCCAGCTT TTGTTCCCTT TAGTGAGGGT TAATTGCGCG CTTGGGTATC TT
 ATCAAGATCT CGCCGGCGGT GGCGCCACCT CGAGGTCGAA AACAGGGAA ATCACTCCCA ATTAACGCGC GAACCCATAG AA

FIG. 11B