(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 January 2002 (03.01.2002)

PCT

(10) International Publication Number
WO 02/00854 A2

(51) International Patent Classification[7]: C12N 9/00

(21) International Application Number: PCT/EP01/07289

(22) International Filing Date: 26 June 2001 (26.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/213,998 26 June 2000 (26.06.2000) US
60/293,221 25 May 2001 (25.05.2001) US

(71) Applicant *(for all designated States except US)*: **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **RAMAKRISHNAN, Shyam** [IN/US]; 76 Euston Road Apt. #10, Brighton, MA 02135 (US).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, 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, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, 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, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/00854 A2

(54) Title: REGULATION OF HUMAN PHOSPHODIESTERASE-LIKE ENZYME

(57) Abstract: Reagents which regulate human phosphodiesterase-like enzyme (PDE-like enzyme) activity and reagents which bind to human PDE-like enzyme gene products can be used to regulate degradation of cGMP. Such regulation is particularly useful for treating urinary incontinence, benign prostate hyperplasia, erectile dysfunction, diabetes, cancer, and cardiovascular disorders.

REGULATION OF HUMAN PHOSPHODIESTERASE-LIKE ENZYME

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of regulation of human phosphodiesterase-like enzyme (PDE-like enzyme) activity.

BACKGROUND OF THE INVENTION

3',5' cyclic nucleotide phosphodiesterases (PDEs) catalyze the hydrolysis of 3',5'-cyclic nucleotides to their respective nucleoside 5'-monophosphates. Human PDEs are classified into several families. There is a need in the art for the identification of human PDE-like enzymes, which can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human phosphodiesterase-like enzyme. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a PDE-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 5; and

the amino acid sequence shown in SEQ ID NO: 5.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a PDE-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2.

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 5; and

the amino acid sequence shown in SEQ ID NO: 5.

Binding between the test compound and the PDE-like enzyme polypeptide is detected. A test compound which binds to the PDE-like enzyme polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the PDE-like enzyme.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a PDE-like enzyme polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 4; and

the nucleotide sequence shown in SEQ ID NO: 4.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the PDE-like enzyme through interacting with the PDE-like enzyme mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a PDE-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2;

the amino acid sequence shown in SEQ ID NO: 2;

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 5; and

the amino acid sequence shown in SEQ ID NO: 5.

A PDE-like enzyme activity of the polypeptide is detected. A test compound which increases PDE-like enzyme activity of the polypeptide relative to PDE-like enzyme activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases

PDE-like enzyme activity of the polypeptide relative to PDE-like enzyme activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a PDE-like enzyme product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 4; and

the nucleotide sequence shown in SEQ ID NO: 4.

Binding of the test compound to the PDE-like enzyme product is detected. A test compound which binds to the PDE-like enzyme product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a PDE-like enzyme polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 1;

the nucleotide sequence shown in SEQ ID NO: 1;

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO: 4; and

the nucleotide sequence shown in SEQ ID NO: 4.

PDE-like enzyme activity in the cell is thereby decreased.

The invention thus provides a human phosphodiesterase-like enzyme that can be used to identify test compounds that may act, for example, as activators or inhibitors at the enzyme's active site. Human phosphodiesterase-like enzyme and fragments thereof also are useful in raising specific antibodies that can block the enzyme and effectively reduce its activity.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows the DNA-sequence encoding a PDE-like enzyme polypeptide (SEQ ID NO:1).

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of Fig.1 (SEQ ID NO:2).

Fig. 3 shows the amino acid sequence of PDE-like enzyme polypeptide with SwissProt Accession No: O54735 (SEQ ID NO:3).

Fig. 4 shows the DNA-sequence encoding a PDE-like enzyme polypeptide (SEQ ID NO:4).

Fig. 5 shows the amino acid sequence deduced from the DNA-sequence of Fig. 4 (SEQ ID NO:5).

Fig. 6 shows the BLASTX alignment of PDE-like enzyme polypeptide (SEQ ID NO:2) with SwissProt Accession No. O54735 (SEQ ID NO:3).

Fig. 7 shows the BLASTP alignment of PDE-like enzyme polypeptide (SEQ ID NO: 5) against swiss/O54735/CN5A_RAT (SEQ ID NO:3).

Fig. 8 shows the expression of PDE-like enzyme in various tissues.

Fig. 9 shows the expression of PDE-like enzyme in various tissues. The expression in bladder tissue and is high and in prostate tissue very high with a significant difference between normal and diseased prostate tissue.

Fig. 10 shows the expression of PDE-like enzyme in CNS tissues.

Fig. 11 shows the expression of PDE-like enzyme in a human organ panel.

Fig. 12 shows the expression of PDE-like enzyme in cardiovascular tissues.

Fig. 13 shows the expression of PDE-like enzyme in phage libraries.

Fig. 14 shows the alignment of PDE nucleotide sequences.

Fig. 15 shows the alignment of PDE amino acid sequences.

Fig. 16 shows the alignment of PDE-like enzyme polypeptide with PDE11a4.

Fig. 17 shows the difference in PDE-(like) polynucleotides graphically.

Fig. 18 shows PDE 11A splice variants. Fragments of each transcript corresponding to individual exons are shown as boxes. Numbers above the fragments designate the number of the exon from which the fragment was derived. Dashed lines at the ends of fragments indicate that the transcript sequence is incomplete.

Fig. 19 shows PDE11A genomic structure.

Fig. 20 showsPDE activity of crude lysate from Sf21 cells expressing His-tagged and non-tagged PDE11A4. Assays were performed in 384-well white plates in 20 µl containing the indicated volume of cell lysate and 1 uM [^{3}H]-cGMP (0.025 uCi/well) in 50 mM Tris-HCl, pH 7.5, 8.3 mM $MgCl_2$, 1.7 mM EGTA, 0.5 mg/ml BSA. Reaction mixtures were incubated at RT for 1 h, then quenched with 40 µl of 18 mM $ZnSO_4$ containing 0.25 mg Yttrium SPA PDE beads. The assay plates were sealed, allowed to settle for 30 min at RT, and counted on a TopCount NTX microscintillation counter (Packard). Reactions were run in triplicate.

Fig. 21 shows SDS-Page and western blotting analysis of recombinant human PDE11A4. A, SDS-Page visualized with Coomassie stain. B, western blot with anti-His antibody.

Fig. 22 shows Lineweaver-Burk analysis to determine Km values for cGMP. Reaction mixtures (20 ul/well) containing 1 ng PDE11A4 and various concentrations of cGMP (1.25 mCi/µmol) were incubated at RT. At various time points between 0 and 90 min the reactions were stopped with 30 µl of 18 mM $ZnSO_4$ containing 0.15 mg Yttrium SPA PDE beads. The assay plates were sealed, allowed to settle for 30 min at RT, and counted on a TopCount NTX microscintillation counter (Packard). Reactions were run in triplicate. *A*, Time courses of PDE11A4 reaction with various

concentration of cGMP. *B*, Lineweaver-Burk analysis. The Km value for cGMP was determined as 0.4 μM.

Fig. 23 shows the effect of DMSO in PDE11A4 SPA and stability of PDE11A4. *A*, assays were performed in 384-well white plates in 20 μl containing various concentration of DMSO, 0.4 uM [^{3}H]-cGMP (0.025 uCi/well) and 1 ng PDE11A4 in 50 mM Tris-HCl, pH 7.5, 8.3 mM $MgCl_2$, 1.7 mM EGTA, 0.5 mg/ml BSA. *B*, PDE11A4 solution was preincubated at RT for various duration in 384-well plate. Reactions were then initiated by the addition of [^{3}H]-cGMP.

Reaction mixtures were incubated at RT for 1 h, then quenched with 30 μl of 18 mM $ZnSO_4$ containing 0.15 mg Yttrium SPA PDE beads. The assay plates were sealed, allowed to settle for 30 min at RT, and counted on a TopCount NTX microscintillation counter (Packard). Reactions were run in triplicate.

Fig. 24 shows the inhibition of PDE11A4 activity by IBMX and dipyridamole. Effect of IBMX and dipyridamole was tested. Assays were performed in 384-well white plates in 20 μl containing the indicated concentration of inhibitor, 0.4 uM [^{3}H]-cGMP (0.025 uCi/well) and 1 ng PDE11A4 in 50 mM Tris-HCl, pH 7.5, 8.3 mM $MgCl_2$, 1.7 mM EGTA, 0.5 mg/ml BSA. Reaction mixtures were incubated at RT for 1 h, then quenched with 30 μl of 18 mM $ZnSO_4$ containing 0.15 mg Yttrium SPA PDE beads. The assay plates were sealed, allowed to settle for 30 min at RT, and counted on a TopCount NTX microscintillation counter (Packard). Reactions were run in triplicate. IC_{50} values of IBMX and dipyridamole were calculated to be 41.3 uM and 0.53 uM, respectively.

Fig. 25 shows the expression of PDE-like enzyme in a human cardiovascular (CV) panel. The expression in prostate tissue is very high.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a PDE-like enzyme polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a PDE-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 45% identical to
the amino acid sequence shown in SEQ ID NO: 2;
the amino acid sequence shown in SEQ ID NO: 2;
amino acid sequences which are at least about 45% identical to
the amino acid sequence shown in SEQ ID NO: 5; and
the amino acid sequence shown in SEQ ID NO:5.

b) a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 4;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that activity of a phosphodiesterase-like enzyme (PDE-like enzyme), particularly a human PDE-like enzyme, can be modified to regulate the intracellular level of cGMP in many tissues,

including the smooth muscle of the corpus cavernosum.

Human PDE-like enzyme comprises the amino acid sequence shown in SEQ ID NO:2 or 5. The complement of a coding sequence for SEQ ID NO: 2 is shown in SEQ ID NO:1; the complement of a coding sequence for SEQ ID NO:5 is shown in SEQ ID NO:4. If the complement of SEQ ID NO:1 is translated in the -1 reading frame, the resulting amino acid sequence is 42% identical over 35 amino acids to the rat protein identified with SwissProt Accession No. O54735 and annotated as a cGMP-binding cGMP-specific phosphodiesterase (PDE5A or PDE5) (FIG. 1). If the complement of SEQ ID NO:1 is translated in the -3 reading frame, the resulting amino acid sequence is 29% identical over 65 amino acids to O54735 (FIG. 1). SEQ ID NO:4 is 44% identical over 141 amino acids to swiss|O54735|CN5A_RAT (SEQ ID NO:3) (FIG. 2).

Comparison of the sequence shown in SEQ ID NO:4 with human genomic sequence NT_005232 shows that it contains three complete exons from the PDE11a gene. The first (bases 68-209) and last (bases 297-384) exons are shared with PDE11a variants PDE11a2, PDE11a3, and PDE11a4. The middle exon (bases 210-296) is unique to this PDE variant. The ends of the EST (bases 1-67 and 385-401) do not appear to match with the genomic sequence and therefore may be sequencing misreads or vector-derived. PCR performed with one primer designed within the middle exon sequence and the other designed within either the first or last exon sequence amplifies products of appropriate length as predicted from the EST sequence, confirming that the EST is indeed expressed and that the sequence, at least within the exonic regions, is correct.

Expression profiling using primers that would only amplify this particular splice variant show that it is expressed most highly in skeletal muscle. The expression pattern appears quite different from those of the other four known splice variants.

The middle exon is 86 bp in length, meaning that its inclusion should cause a frame shift. However, the sequence also contains a stop codon (as shown in SEQ ID NO:2) that will prematurely terminate the translation. The location of the stop codon is between the two GAF domains of PDE11a, so the protein will only contain the first GAF domain. Notably, the start codon for the PDE11a1 variant is located slightly 3' of the PDE-like enzyme stop codon, so it contains only the second GAF domain. This may be significant, because both PDE11a1 and PDE-like enzyme are expressed highly in skeletal muscle, making it possible that the PDE11a protein in this tissue (and possible also other tissues) is made of two parts which can be regulated separately. The transcribed portion of the PDE11a gene spans nearly 600,000 bp of the genome and contains at least 24 exons.

Human PDE-like enzyme can be used to develop treatments for various diseases, including diabetes, to develop diagnostic assays for these diseases, and to provide new tools for basic research especially in the fields of medicine and biology. Specifically, the present invention can be used to develop new drugs to inhibit degradation of cGMP in, for example, the smooth muscle tissue of the corpus cavernosum. Human PDE-like enzyme and regulators of human PDE-like enzyme thus can provide treatments for erectile dysfunction. Human PDE-like enzyme also can be regulated to treat urinary incontinence, benign prostate hyperplasia, diabetes, cancer, and cardiovascular disorders.

PDE-like enzyme Polypeptides

Human PDE-like enzyme polypeptides according to the invention comprise at least 5, 10, 15, 25, 50, 75, 100, or 125 contiguous amino acids selected from SEQ ID NO:2 or 5 or from a biologically active variant thereof, as defined below. Thus, a PDE-like enzyme polypeptide can be a portion of a PDE-like enzyme molecule, a full-length PDE-like enzyme molecule, or a fusion protein comprising all or a portion of a PDE-like enzyme molecule. Most preferably, a PDE-like enzyme polypeptide

has a phosphodiesterase activity. Phosphodiesterase activity can be measured, *inter alia*, as described in Example 4.

Biologically Active Variants

PDE-like enzyme variants which are biologically active, *i.e.*, retain a phosphodiesterase activity, also are PDE-like enzyme polypeptides. Preferably, naturally or non-naturally occurring PDE-like enzyme variants have amino acid sequences which are at least about 45, 50, 55, 60, 65, 70, preferably about 75, 80, 85, 90, 96, or 98% identical to an amino acid sequence shown in SEQ ID NO:2 or 5. Percent identity between a putative PDE-like enzyme variant and an amino acid sequence of SEQ ID NO:2 or 5 is determined using the Blast2 alignment program (Blosum62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a PDE-like enzyme polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active PDE-like enzyme polypeptide can readily be determined by assaying for PDE-like enzyme activity, as described, for example, in Example 4.

Fusion Proteins

Fusion proteins are useful for generating antibodies against PDE-like enzyme amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a PDE-like enzyme polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A PDE-like enzyme fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment comprises at least 5, 10, 15, 25, 50, 75, 100, or 125 contiguous amino acids selected from SEQ ID NO:2 or 5 or a biologically active variant, such as those described above. The first protein segment also can comprise full-length PDE-like enzyme.

The second protein segment can be a full-length protein or a protein fragment or polypeptide. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the PDE-like enzyme polypeptide-encoding sequence and the heterologous protein sequence, so that the PDE-like enzyme polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises the complement of SEQ ID NO:1 or 4 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human PDE-like enzyme can be obtained using PDE-like enzyme polynucleotides (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of PDE-like enzyme, and expressing the cDNAs as is known in the art.

PDE-like enzyme Polynucleotides

A PDE-like enzyme polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a PDE-like enzyme polypeptide. The complements of partial nucleotide sequences for a PDE-like enzyme polypeptide are shown in SEQ ID NO:1 and 4.

Degenerate nucleotide sequences encoding human PDE-like enzyme polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 80, 85, 90, 96, or 98% identical to the complements of the nucleotide sequences shown in SEQ ID NO:1 and 4 also are PDE-like enzyme

polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of PDE-like enzyme polynucleotides which encode biologically active PDE-like enzyme polypeptides also are PDE-like enzyme polynucleotides.

Identification of Variants and Homologs of PDE-like enzyme Polynucleotides

Variants and homologs of the PDE-like enzyme polynucleotides described above also are PDE-like enzyme polynucleotides. Typically, homologous PDE-like enzyme polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known PDE-like enzyme polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the PDE-like enzyme polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of PDE-like enzyme polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human PDE-like enzyme polynucleotides or PDE-like enzyme polynucleotides of other species can therefore be identified by hybridizing a putative homologous PDE-like enzyme polynucleotide with a

polynucleotide having a nucleotide sequence of SEQ ID NO:1 or 4 or the complements thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising PDE-like enzyme polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to PDE-like enzyme polynucleotides or their complements following stringent hybridization and/or wash conditions also are PDE-like enzyme polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a PDE-like enzyme polynucleotide having a nucleotide sequence shown in SEQ ID NO:1 or 4 or the complements thereof and a polynucleotide sequence which is at least about 50, 55, 60, 65, 70, preferably about 75, 80, 85, 90, 96, or 98% identical to one of those nucleotide sequences can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$$T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l),$$

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of PDE-like enzyme Polynucleotides

A naturally occurring PDE-like enzyme polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated PDE-like enzyme polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise PDE-like enzyme nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

PDE-like enzyme cDNA molecules can be made with standard molecular biology techniques, using PDE-like enzyme mRNA as a template. PDE-like enzyme cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of PDE-like enzyme polynucleotides using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize PDE-like enzyme polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a PDE-like enzyme polypeptide having, for example, an amino acid sequence shown in SEQ ID NO:2 or 5 or a biologically active variant sequence.

Obtaining Full-Length PDE-like enzyme Polynucleotides

The partial sequence of SEQ ID NO:1 or 4 or their complements can be used to identify the corresponding full length gene(s) from which they were derived. The

partial sequences can be nick-translated or end-labeled with ^{32}P using polynucleotide kinase using labeling methods known to those with skill in the art (BASIC METHODS IN MOLECULAR BIOLOGY, Davis *et al.*, eds., Elsevier Press, N.Y., 1986). For example, a lambda library prepared from human tissue can be screened directly with the labeled sequences of interest or the library can be converted en masse to pBluescript (Stratagene Cloning Systems, La Jolla, Calif. 92037) to facilitate bacterial colony screening (see Sambrook *et al.*, 1989, pg. 1.20).

Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured, and the DNA is fixed to the filters. The filters are hybridized with the labeled probe using hybridization conditions described by Davis *et al.*, 1986. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting autoradiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are selected and expanded, and the DNA is isolated from the colonies for further analysis and sequencing.

Positive cDNA clones are analyzed to determine the amount of additional sequence they contain using PCR with one primer from the partial sequence and the other primer from the vector. Clones with a larger vector-insert PCR product than the original partial sequence are analyzed by restriction digestion and DNA sequencing to determine whether they contain an insert of the same size or similar as the mRNA size determined from Northern blot Analysis.

Once one or more overlapping cDNA clones are identified, the complete sequence of the clones can be determined, for example after exonuclease III digestion (McCombie *et al.*, *Methods 3*, 33-40, 1991). A series of deletion clones are generated, each of which is sequenced. The resulting overlapping sequences are

assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a highly accurate final sequence.

Various PCR-based methods can be used to extend the nucleic acid sequences encoding the disclosed portions of human PDE-like enzyme to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al., Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al., PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining PDE-like enzyme Polypeptides

PDE-like enzyme polypeptides can be obtained, for example, by purification from human smooth muscle cells, by expression of PDE-like enzyme polynucleotides, or by direct chemical synthesis.

Protein Purification

PDE-like enzyme polypeptides can be purified, for example, from human smooth muscle in testis cells or from a cell which has been transfected with a PDE-like enzyme polynucleotide. A purified PDE-like enzyme polypeptide is separated from other compounds which normally associate with the PDE-like enzyme polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. Purification of recombinant PDE5A, a similar enzyme, is taught in Loughney *et al.*, *Gene 330*, 139-47 (1998). A preparation of purified PDE-like enzyme polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis. Enzymatic activity of the purified preparations can be assayed, for example, as described in Example 3.

Expression of PDE-like enzyme Polynucleotides

To express a PDE-like enzyme polypeptide, a PDE-like enzyme polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding PDE-like enzyme polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y, 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a PDE-like enzyme polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a PDE-like enzyme polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for a PDE-like enzyme polypeptide. For example, when a large quantity of a PDE-like enzyme polypeptide is needed for the induction of antibodies,

vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding a PDE-like enzyme polypeptide can be ligated in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989 or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding PDE-like enzyme polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such

techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a PDE-like enzyme polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding PDE-like enzyme polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of PDE-like enzyme polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which PDE-like enzyme polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express PDE-like enzyme polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding PDE-like enzyme polypeptides can be ligated into an adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a PDE-like enzyme polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers such as the Rous sarcoma virus (RSV) enhancer can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are

constructed and delivered to cells via conventional delivery methods (*e.g.,* liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding PDE-like enzyme polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a PDE-like enzyme polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al., Results Probl. Cell Differ. 20,* 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed PDE-like enzyme polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding, and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express PDE-like enzyme polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced PDE-like enzyme sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980) *npt* confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB*, allows cells to utilize indole in place of tryptophan; *hisD*, allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression of PDE-like enzyme Polypeptides

Although the presence of marker gene expression suggests that a PDE-like enzyme polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a PDE-like enzyme polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode the PDE-like enzyme polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a PDE-like enzyme polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of a PDE-like enzyme polynucleotide.

Alternatively, host cells which contain a PDE-like enzyme polynucleotide and which express a PDE-like enzyme polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a PDE-like enzyme polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding the PDE-like enzyme polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding the PDE-like enzyme polypeptide to detect transformants which contain a PDE-like enzyme polynucleotide.

A variety of protocols for detecting and measuring the expression of a PDE-like enzyme polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a PDE-like enzyme

polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding PDE-like enzyme polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a PDE-like enzyme polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of PDE-like enzyme Polypeptides

Host cells transformed with nucleotide sequences encoding a PDE-like enzyme polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode PDE-like enzyme polypeptides can be designed to contain signal sequences which direct secretion of PDE-like enzyme polypeptides through a prokaryotic or eukaryotic cell membrane.

As discussed above, other constructions can be used to join a sequence encoding an PDE-like enzyme polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the PDE-like enzyme polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a PDE-like enzyme polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the PDE-like enzyme polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

<u>*Chemical Synthesis*</u>

Sequences encoding a PDE-like enzyme polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a PDE-like enzyme polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of PDE-like enzyme polypeptides can be

separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic PDE-like enzyme polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the PDE-like enzyme polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered PDE-like enzyme Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce PDE-like enzyme polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter PDE-like enzyme polypeptide-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter

glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a PDE-like enzyme polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a PDE-like enzyme polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a PDE-like enzyme polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a PDE-like enzyme polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to PDE-like enzyme polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a PDE-like enzyme polypeptide from solution.

PDE-like enzyme polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a PDE-like enzyme polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a PDE-like enzyme polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively,

humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a PDE-like enzyme polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to PDE-like enzyme polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to PDE-like enzyme polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as

disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which an PDE-like enzyme polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of PDE-like enzyme gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates,

alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al., Chem. Rev. 90*, 543-583, 1990.

Modifications of PDE-like enzyme gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of a PDE-like enzyme gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a PDE-like enzyme polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a PDE-like enzyme polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent PDE-like enzyme nucleotides, can provide sufficient targeting specificity for PDE-like enzyme mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular PDE-like enzyme polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a PDE-like enzyme polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a PDE-like enzyme polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the PDE-like enzyme polynucleotide. Methods of designing and constructing ribozymes which can cleave RNA molecules in trans in a highly sequence specific manner have been

developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a PDE-like enzyme RNA target can be identified by scanning the PDE-like enzyme target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate PDE-like enzyme RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequences shown in SEQ ID NO:1 or 4 and their complements provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease PDE-like enzyme expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements,

such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Differentially Expressed Genes

Described herein are methods for the identification of genes whose products interact with human PDE-like enzyme. Such genes may represent genes that are differentially expressed in disorders including, but not limited to, urinary incontinence, benign prostate hyperplasia, erectile dysfunction, diabetes, cancer, and cardiovascular disorders. Further, such genes may represent genes that are differentially regulated in response to manipulations relevant to the progression or treatment of such diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human PDE-like enzyme gene or gene product may itself be tested for differential expression.

The degree to which expression differs in a normal versus a diseased state need only be large enough to be visualized via standard characterization techniques such as differential display techniques. Other such standard characterization techniques by which expression differences may be visualized include but are not limited to, quantitative RT (reverse transcriptase), PCR, and Northern analysis.

Identification of Differentially Expressed Genes

To identify differentially expressed genes total RNA or, preferably, mRNA is isolated from tissues of interest. For example, RNA samples are obtained from tissues of experimental subjects and from corresponding tissues of control subjects. Any RNA isolation technique that does not select against the isolation of mRNA may be utilized for the purification of such RNA samples. See, for example, Ausubel *et al.*, ed., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc. New York, 1987-1993. Large numbers of tissue samples may readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, U.S. Patent 4,843,155.

Transcripts within the collected RNA samples that represent RNA produced by differentially expressed genes are identified by methods well known to those of skill in the art. They include, for example, differential screening (Tedder *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 208-12, 1988), subtractive hybridization (Hedrick *et al.*, *Nature 308*, 149-53; Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 2825, 1984), and, preferably, differential display (Liang & Pardee, *Science 257*, 967-71, 1992; U.S. Patent 5,262,311).

The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human PDE-like enzyme. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human PDE-like enzyme. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human PDE-like enzyme gene or gene product are up-regulated or down-regulated.

Screening Methods

The invention provides methods for identifying modulators, *i.e.*, candidate or test compounds which bind to PDE-like enzyme polypeptides or polynucleotides and/or have a stimulatory or inhibitory effect on, for example, expression or activity of the PDE-like enzyme polypeptide or polynucleotide, so as to regulate cGMP specific phosphodiesterase activity. Decreased cGMP specific phosphodiesterase activity is useful, for example, for treating or preventing urinary incontinence, benign prostate hyperplasia, erectile dysfunction. Increased cGMP specific phosphodiesterase activity may be desired, for example, in disorders characterized by inappropriately low levels of cGMP specific phosphodiesterase activity.

The invention provides assays for screening test compounds which bind to or modulate the activity of a PDE-like enzyme polypeptide or a PDE-like enzyme polynucleotide. A test compound preferably binds to a PDE-like enzyme polypeptide or polynucleotide. More preferably, a test compound decreases a PDE-like enzyme activity of a PDE-like enzyme polypeptide or expression of an PDE-like enzyme polynucleotide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and

synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to PDE-like enzyme polypeptides or polynucleotides or to affect PDE-like enzyme activity or PDE-like enzyme gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 μl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al., Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al., Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the active site of a PDE-like enzyme polypeptide, thereby making the active site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. In binding assays, either the test compound or the PDE-like enzyme polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the PDE-like enzyme polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a PDE-like enzyme polypeptide can be determined without labeling either of the interactants. For example, a micro-physiometer can be used to detect binding of a test compound with a PDE-like enzyme polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a PDE-like enzyme polypeptide. (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a PDE-like enzyme polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a PDE-like enzyme polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300) to identify other proteins which bind to or interact with the PDE-like enzyme polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct a polynucleotide encoding a PDE-like enzyme polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the PDE-like enzyme polypeptide.

It may be desirable to immobilize either a PDE-like enzyme polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the PDE-like enzyme polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are

not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the PDE-like enzyme polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to an PDE-like enzyme polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, a PDE-like enzyme polypeptide is a fusion protein comprising a domain that allows the PDE-like enzyme polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed PDE-like enzyme polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a PDE-like enzyme polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated PDE-like enzyme polypeptides, polynucleotides, or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*,

biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a PDE-like enzyme polypeptide, polynucleotides, or a test compound, but which do not interfere with a desired binding site, such as the active site of the PDE-like enzyme polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to a PDE-like enzyme polypeptide or test compound, enzyme-linked assays which rely on detecting a PDE-like enzyme activity of the PDE-like enzyme polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a PDE-like enzyme polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a PDE-like enzyme polynucleotide or polypeptide can be used in a cell-based assay system. A PDE-like enzyme polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, including smooth muscle cell lines such as the human cell lines HISM, T/G HA-VSMC, Human Aortic Muscle Cells (HASMC) (Cascade Biologics, Inc., Portland OR) and rat cell lines A7r5, A-10, and SV40LT-SMC Clone HEP-5A. An intact cell is contacted with a test compound. Binding of the test compound to a PDE-like enzyme polypeptide or polynucleotide is determined as described above, after lysing the cell to release the PDE-like enzyme polypeptide-or polynucleotide-test compound complex.

PDE-like enzyme Assays

Test compounds can be tested for the ability to increase or decrease a PDE-like enzyme activity of a PDE-like enzyme polypeptide. See Example 3, below. PDE-

like enzyme activity can be measured, for example, by contacting either a purified PDE-like enzyme polypeptide, a cell extract, or an intact cell with a test compound. A test compound which decreases PDE-like enzyme activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing cGMP-specific phosphodiesterase activity. A test compound which increases PDE-like enzyme activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing cGMP phosphodiesterase activity.

PDE-like enzyme Gene Expression

In another embodiment, test compounds which increase or decrease PDE-like enzyme gene expression are identified. A PDE-like enzyme polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the PDE-like enzyme polynucleotide is determined. The level of expression of PDE-like enzyme mRNA or polypeptide in the presence of the test compound is compared to the level of expression of PDE-like enzyme mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of PDE-like enzyme mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of PDE-like enzyme mRNA or polypeptide expression. Alternatively, when expression of PDE-like enzyme mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of PDE-like enzyme mRNA or polypeptide expression.

The level of PDE-like enzyme mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptides. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a PDE-like enzyme polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as

radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a PDE-like enzyme polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a PDE-like enzyme polynucleotide can be used in a cell-based assay system. The PDE-like enzyme polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, including smooth muscle cell lines such as the human cell lines HISM, T/G HA-VSMC, Human Aortic Muscle Cells (HASMC) (Cascade Biologics, Inc., Portland OR) and rat cell lines A7r5, A-10, and SV40LT-SMC Clone HEP-5A.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a PDE-like enzyme polypeptide, PDE-like enzyme polynucleotide, antibodies which specifically bind to PDE-like enzyme, or mimetics, agonists, antagonists, or inhibitors of PDE-like enzyme activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be

administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium

stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

The human PDE-like enzyme gene provides a therapeutic target for decreasing degradation of cGMP, in particular for treating or preventing urinary incontinence, benign prostate hyperplasia, erectile dysfunction, diabetes, cancer, and cardiovascular disorders. PDE-like enzyme can regulate the intracellular level of cGMP in many tissues, including the smooth muscle of the corpus cavernosum. The inhibition of PDE-like enzyme activity can cause cGMP to accumulate in the corpus cavernosum. Increased levels of cGMP causes smooth muscle relaxation of the corpus cavernosum and inflow of blood. Inhibition of PDE-like enzyme activity can therefore be used to treat or prevent urinary incontinence, benign prostate hyperplasia, erectile dysfunction.

Human PDE-like enzyme also can be regulated to treat diabetes. Diabetes mellitus is a common metabolic disorder characterized by an abnormal elevation in blood glucose, alterations in lipids and abnormalities (complications) in the cardiovascular system, eye, kidney and nervous system. Diabetes is divided into two separate diseases: type 1 diabetes (juvenile onset), which results from a loss of cells which make and secrete insulin, and type 2 diabetes (adult onset), which is caused by a defect in insulin secretion and a defect in insulin action.

Type 1 diabetes is initiated by an autoimuune reaction that attacks the insulin secreting cells (beta cells) in the pancreatic islets. Agents that prevent this reaction from occurring or that stop the reaction before destruction of the beta cells has been

accomplished are potential therapies for this disease. Other agents that induce beta cell proliferation and regeneration also are potential therapies.

Type II diabetes is the most common of the two diabetic conditions (6% of the population). The defect in insulin secretion is an important cause of the diabetic condition and results from an inability of the beta cell to properly detect and respond to rises in blood glucose levels with insulin release. Therapies that increase the response by the beta cell to glucose would offer an important new treatment for this disease.

The defect in insulin action in Type II diabetic subjects is another target for therapeutic intervention. Agents that increase the activity of the insulin receptor in muscle, liver, and fat will cause a decrease in blood glucose and a normalization of plasma lipids. The receptor activity can be increased by agents that directly stimulate the receptor or that increase the intracellular signals from the receptor. Other therapies can directly activate the cellular end process, *i.e.* glucose transport or various enzyme systems, to generate an insulin-like effect and therefore a produce beneficial outcome. Because overweight subjects have a greater susceptibility to Type II diabetes, any agent that reduces body weight is a possible therapy.

Both Type I and Type diabetes can be treated with agents that mimic insulin action or that treat diabetic complications by reducing blood glucose levels. Likewise, agents that reduces new blood vessel growth can be used to treat the eye complications that develop in both diseases.

Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation

(angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Activators and/or inhibitors of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

Cardiovascular diseases include the following disorders of the heart and the vascular system: congestive heart failure, myocardial infarction, ischemic diseases of the heart, all kinds of atrial and ventricular arrhythmias, hypertensive vascular diseases, and peripheral vascular diseases.

Heart failure is defined as a pathophysiologic state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirement of the metabolizing tissue. It includes all forms of pumping failure, such as high-output and low-output, acute and chronic, right-sided or left-sided, systolic or diastolic, independent of the underlying cause.

Myocardial infarction (MI) is generally caused by an abrupt decrease in coronary blood flow that follows a thrombotic occlusion of a coronary artery previously narrowed by arteriosclerosis. MI prophylaxis (primary and secondary prevention) is included, as well as the acute treatment of MI and the prevention of complications.

Ischemic diseases are conditions in which the coronary flow is restricted resulting in a perfusion which is inadequate to meet the myocardial requirement for oxygen. This group of diseases includes stable angina, unstable angina, and asymptomatic ischemia.

Arrhythmias include all forms of atrial and ventricular tachyarrhythmias (atrial tachycardia, atrial flutter, atrial fibrillation, atrio-ventricular reentrant tachycardia, preexcitation syndrome, ventricular tachycardia, ventricular flutter, and ventricular fibrillation), as well as bradycardic forms of arrhythmias.

Vascular diseases include primary as well as all kinds of secondary arterial hypertension (renal, endocrine, neurogenic, others). The disclosed gene and its product may be used as drug targets for the treatment of hypertension as well as for the prevention of all complications. Peripheral vascular diseases are defined as vascular diseases in which arterial and/or venous flow is reduced resulting in an

imbalance between blood supply and tissue oxygen demand. It includes chronic peripheral arterial occlusive disease (PAOD), acute arterial thrombosis and embolism, inflammatory vascular disorders, Raynaud's phenomenon, and venous disorders.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an anti-sense nucleic acid molecule, a specific antibody, ribozyme, or a polypeptide-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects PDE-like enzyme activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce PDE-like enzyme activity. The reagent preferably binds to an expression product of an PDE-like enzyme gene. If the expression product is a polypeptide, for example, the reagent can be an antibody or a small chemical compound. For treatment of human cells *ex vivo*, a reagent can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal,

such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung or liver.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques

are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

If the reagent is a single-chain antibody, a polynucleotide encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a PDE-like enzyme gene or the activity of a PDE-like enzyme polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a PDE-

like enzyme gene or the activity of a PDE-like enzyme polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to PDE-like enzyme-specific mRNA, quantitative RT-PCR, immunologic detection of a PDE-like enzyme polypeptide, or measurement of PDE-like enzyme activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Determination of a Therapeutically Effective Dose

Determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases PDE-like enzyme activity relative to PDE-like enzyme activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental

animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts of any particular reagent can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for polypeptides or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

The above disclosure generally describes the present invention, and all patents and patent applications cited in this disclosure are expressly incorporated herein. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of PDE-like enzyme activity

The polynucleotide of SEQ ID NO: 1 is inserted into the expression vector pCEV4 and the expression vector pCEV4-PDE-like enzyme polypeptide obtained is transfected into human embryonic kidney 293 cells. From these cells extracts are obtained and dissolved in 0,1 µl of SET buffer. A volume of 0,2 µl of PDE mix (Tris-HCl 50 mM, pH 7,5, cAMP 50 µM) is added and incubated at 37°C for 20 minutes. The oil rack is heated at 80°C for 30 minutes. A volume of 0,4 µl of "cleaning" mix is added and incubated at 37 °C for 1 hour. The oil rack is heated at 80°C for 30 minutes. A volume of 1 µl of cAMP reaction mix is added and incubated for 1 hour at room temperature. The resultant NADPH is amplified by $NADP^+$ / NADPH cycling. It is shown that the polypeptide of SEQ ID NO: 2 has PDE-like enzyme activity.

EXAMPLE 2

Expression of recombinant human PDE-like enzyme

The *Pichia pastoris* expression vector pPICZB (Invitrogen, San Diego, CA) is used to produce large quantities of a recombinant human PDE-like enzyme in yeast. The encoding DNA sequence is derived from the nucleotide sequences shown in SEQ ID NO:1 or 4 or their complements. Before insertion into vector pPICZB, the DNA

sequence is modified by well known methods in such a way that it contains at its 5'-end an initiation codon and at its 3'-end an enterokinase cleavage site, a His6 reporter tag, and a termination codon. Moreover, at both termini recognition sequences for restriction endonucleases are added.

After digestion of the multiple cloning site of pPICZ B with the corresponding restriction enzymes, the modified DNA sequence is ligated into pPICZB. This expression vector is designed for inducible expression in *Pichia pastoris*, driven by a yeast promoter. The resulting pPICZ/md-His6 vector is used to transform the yeast.

The yeast is cultivated under usual conditions in 5 liter shake flasks, and the recombinantly produced protein isolated from the culture by affinity chromatography (Ni-NTA-Resin) in the presence of 8 M urea. The bound polypeptide is eluted with buffer, pH 3.5, and neutralized. Separation of the polypeptide from the His6 reporter tag is accomplished by site-specific proteolysis using enterokinase (Invitrogen, San Diego, CA) according to manufacturer's instructions. Purified human PDE-like enzyme is obtained.

EXAMPLE 3

Identification of a test compound which binds to a PDE-like enzyme polypeptide

Purified PDE-like enzyme polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. PDE-like enzyme polypeptides comprise an amino acid sequence shown in SEQ ID NO:2 or 5. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a PDE-like enzyme polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound was not incubated is identified as a compound which binds to a PDE-like enzyme polypeptide.

EXAMPLE 4

Identification of a test compound which decreases PDE-like enzyme activity

Cellular extracts from yeast cells transformed with a plasmid encoding a PDE-like enzyme polynucleotide (see Loughney et al. (1998) Gene 216:139) are contacted with test compounds from a small molecule library and assayed for PDE-like enzyme activity. Control extracts in the absence of a test compound also are assayed. PDE-like enzyme activity can be assayed as taught by Loughney *et al. J. Biol. Chem. 271*:796-806 (1996) and Bolger *et al. Mol. Cell. Biol. 13*:6558-71 (1993). A test compound which decreases PDE-like enzyme activity of the extract relative to the control extract by at least 20% is identified as a PDE-like enzyme inhibitor.

EXAMPLE 5

Identification of a test compound which decreases PDE-like enzyme *gene expression*

A test compound is administered to a culture of the smooth muscle cell culture of cell line HISM and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al., Biochem. 18,* 5294-99, 1979). Northern blots are prepared using 20 to 30 μg total RNA and

hybridized with a ^{32}P-labeled PDE-like enzyme-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from SEQ ID NO:1 or 4. A test compound which decreases the PDE-like enzyme-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of PDE-like enzyme gene expression.

EXAMPLE 6

Treatment of erectile dysfunction (ED) with a reagent which specifically binds to a PDE-like enzyme *gene product*

A purified test compound as identified in Example 3 is administered to mature adult male monkeys either *Cercopithecus aethiops* (green monkey) or *Macaca fasciculata* (cynomologous) weighing between 4 and 8 kg. Animals are anesthetized with diazepam (2.5 mg), ketamine chloride (20 µg/kg i.m. supplemented as appropriate) and given the test compound dissolved in saline intracavernosally (0.3 ml). Animals are placed supine, the penis stretched out and a rubber band placed around the root of the base as a tourniquet for three minutes after the injection. The solution is injected through a 27G needle into one of the corpus cavernosa and 5, 10, 25, 30, 60 and 180 minutes later tumescence (increase in volume) and rigidity of the penis is estimated visually and by palpitation.

EXAMPLE 7

Tissue-specific expression of PDE-like enzyme

The qualitative expression pattern of PDE-like enzyme in various tissues is determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). To demonstrate that PDE-like enzyme is involved in the disease process of diabetes, the following whole body panel is screened to show predominant or relatively high expression: subcutaneous and mesenteric adipose tissue, adrenal gland, bone marrow,

brain, colon, fetal brain, heart, hypothalamus, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spleen, stomach, testis, thymus, thyroid, trachea, and uterus. Human islet cells and an islet cell library also are tested. As a final step, the expression of PDE-like enzyme in cells derived from normal individuals with the expression of cells derived from diabetic individuals is compared.

To demonstrate that PDE-like enzyme is involved in cardiovascular disease, the following whole body panel was screened to show predominant or relatively high expression: fetal brain, adult brain, muscle, heart, lung, kidney, liver, thymus, testis, colon, placenta, trachea, pancreas, gastric mucosa, fetal liver, bladder, adrenal gland, spleen, prostate, hypertrophic prostate, cortex, choroid plexus, hippocampus, hypothalamus, amygdala, thalamus, cerebellum, dorsal root ganglion, and spinal cord. Expression in the following cardiovascular tissues was assayed: prostate, aorta, adult heart, fetal heart, apex, left atrium, right atrium, left auricle, right auricle, left ventricle, right ventricle, septum, and node.

To demonstrate that PDE-like enzyme is involved in cancer, expression is determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate), NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung), NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

To demonstrate that PDE-like enzyme is involved in cancer, expression is determined in the following tissues: adrenal gland, bone marrow, brain, cerebellum, colon, fetal brain, fetal liver, heart, kidney, liver, lung, mammary gland, pancreas,

placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, trachea, uterus, and peripheral blood lymphocytes. Expression in the following cancer cell lines also is determined: DU-145 (prostate), NCI-H125 (lung), HT-29 (colon), COLO-205 (colon), A-549 (lung), NCI-H460 (lung), HT-116 (colon), DLD-1 (colon), MDA-MD-231 (breast), LS174T (colon), ZF-75 (breast), MDA-MN-435 (breast), HT-1080, MCF-7 (breast), and U87. Matched pairs of malignant and normal tissue from the same patient also are tested.

Quantitative expression profiling. Quantitative expression profiling was performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, *BioTechnology 10*, 413-17, 1992, and Higuchi *et al.*, *BioTechnology 11*, 1026-30, 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 88*, 7276-80, 1991). Because the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid *et al.*, *Genome Res. 6*, 986-94, 1996, and Gibson *et al.*, *Genome Res. 6*, 995-1001, 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiment, the control of choice is the 18S ribosomal RNA. Because reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence were made in the ABI Prism 7700.

RNA extraction and cDNA preparation. Total RNA from the tissues listed above are used for expression quantification. RNAs labeled "from autopsy" were extracted from autoptic tissues with the TRIzol reagent (Life Technologies, MD) according to the manufacturer's protocol.

Fifty μg of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix: 0.2 U/μl RNase-free DNase I (Roche Diagnostics, Germany); 0.4 U/μl RNase inhibitor (PE Applied Biosystems, CA); 10 mM Tris-HCl pH 7.9; 10mM $MgCl_2$; 50 mM NaCl; and 1 mM DTT.

After incubation, RNA is extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of 3 M NaAcetate, pH5.2, and 2 volumes of ethanol.

Fifty μg of each RNA from the autoptic tissues are DNase treated with the DNA-free kit purchased from Ambion (Ambion, TX). After resuspension and spectro-photometric quantification, each sample is reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) according to the manufacturer's protocol. The final concentration of RNA in the reaction mix is 200ng/μl. Reverse transcription is carried out with 2.5μM of random hexamer primers.

TaqMan quantitative analysis. Specific primers and probe are designed according to the recommendations of PE Applied Biosystems; the probe can be labeled at the 5' end FAM (6-carboxy-fluorescein) and at the 3' end with TAMRA (6-carboxy-tetramethyl-rhodamine). Quantification experiments are performed on 10 ng of reverse transcribed RNA from each sample. Each determination is done in triplicate.

Total cDNA content is normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA using the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

The assay reaction mix is as follows: 1X final TaqMan Universal PCR Master Mix (from 2X stock) (PE Applied Biosystems, CA); 1X PDAR control – 18S RNA (from 20X stock); 300 nM forward primer; 900 nM reverse primer; 200 nM probe; 10 ng cDNA; and water to 25 µl.

Each of the following steps are carried out once: pre PCR, 2 minutes at 50° C, and 10 minutes at 95°C. The following steps are carried out 40 times: denaturation, 15 seconds at 95°C, annealing/extension, 1 minute at 60°C.

The experiment is performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR are processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

The results of whole body expression panels, CNS-specific panels, and cardio-vascular panels are shown in FIGS. 8-11. Tissues such as prostate, bladder, small intestine, colon, and blood vessels are primarily composed of smooth muscle cells. All smooth muscle cells share a similar transcriptional machinery. It is, therefore, likely that PDE-like enzyme, which is expressed in one or more of these tissues (*e.g.*, bladder and prostate), is also expressed to some extent in smooth muscle cells of different blood vessels. This makes it very likely that PDE-like enzyme plays a role in cardiovascular disorders, such as coronary heart disease, hypertension, and arteriosclerosis.

PCR analysis of phage libraries is shown in FIG. 12.

EXAMPLE 8

Diabetes: In vivo testing of compounds/target validation

1. Glucose Production:

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

2. Insulin Sensitivity:

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma

glucose and insulin levels when compared to the vehicle-treated control group.

3. Insulin Secretion

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, test compounds which regulate PDE-like enzyme are administered by different routes (p.o., i.p., s.c., or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4 g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Test compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load

(1 g/kg), bled again after 15, 30, 60, and 90 minutes and plasma glucose levels determined. Test compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

EXAMPLE 9

Diabetes: In vivo testing of compounds/target validation

4. Glucose Production

Over-production of glucose by the liver, due to an enhanced rate of gluconeogenesis, is the major cause of fasting hyperglycemia in diabetes. Overnight fasted normal rats or mice have elevated rates of gluconeogenesis as do streptozotocin-induced diabetic rats or mice fed ad libitum. Rats are made diabetic with a single intravenous injection of 40 mg/kg of streptozotocin while C57BL/KsJ mice are given 40-60 mg/kg i.p. for 5 consecutive days. Blood glucose is measured from tail-tip blood and then compounds are administered via different routes (p.o., i.p., i.v., s.c.). Blood is collected at various times thereafter and glucose measured. Alternatively, compounds are administered for several days, then the animals are fasted overnight, blood is collected and plasma glucose measured. Compounds that inhibit glucose production will decrease plasma glucose levels compared to the vehicle-treated control group.

5. Insulin Sensitivity:

Both ob/ob and db/db mice as well as diabetic Zucker rats are hyperglycemic, hyperinsulinemic and insulin resistant. The animals are pre-bled, their glucose levels measured, and then they are grouped so that the mean glucose level is the same for each group. Compounds are administered daily either q.d. or

b.i.d. by different routes (p.o., i.p., s.c.) for 7-28 days. Blood is collected at various times and plasma glucose and insulin levels determined. Compounds that improve insulin sensitivity in these models will decrease both plasma glucose and insulin levels when compared to the vehicle-treated control group.

6. Insulin Secretion:

Compounds that enhance insulin secretion from the pancreas will increase plasma insulin levels and improve the disappearance of plasma glucose following the administration of a glucose load. When measuring insulin levels, compounds are administered by different routes (p.o., i.p., s.c. or i.v.) to overnight fasted normal rats or mice. At the appropriate time an intravenous glucose load (0.4g/kg) is given, blood is collected one minute later. Plasma insulin levels are determined. Compounds that enhance insulin secretion will increase plasma insulin levels compared to animals given only glucose. When measuring glucose disappearance, animals are bled at the appropriate time after compound administration, then given either an oral or intraperitoneal glucose load (1g/kg), bled again after 15, 30, 60 and 90 minutes and plasma glucose levels determined. Compounds that increase insulin levels will decrease glucose levels and the area-under-the glucose curve when compared to the vehicle-treated group given only glucose.

EXAMPLE 10

Proliferation inhibition assay: Antisense oligonucleotides suppress the growth of cancer cell lines

The cell line used for testing is the human colon cancer cell line HCT116. Cells are cultured in RPMI-1640 with 10-15% fetal calf serum at a concentration of 10,000

cells per milliliter in a volume of 0.5 ml and kept at 37 °C in a 95% air/5%CO_2 atmosphere.

Phosphorothioate oligoribonucleotides are synthesized on an Applied Biosystems Model 380B DNA synthesizer using phosphoroamidite chemistry. A sequence of 24 bases complementary to the nucleotides at position 1 to 24 of SEQ ID NO:1 is used as the test oligonucleotide. As a control, another (random) sequence is used: 5'-TCA ACT GAC TAG ATG TAC ATG GAC-3'. Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate buffered saline at the desired concentration. Purity of the oligonucleotides is tested by capillary gel electrophoresis and ion exchange HPLC. The purified oligonucleotides are added to the culture medium at a concentration of 10 μM once per day for seven days.

The addition of the test oligonucleotide for seven days results in significantly reduced expression of human PDE-like enzyme as determined by Western blotting. This effect is not observed with the control oligonucleotide. After 3 to 7 days, the number of cells in the cultures is counted using an automatic cell counter. The number of cells in cultures treated with the test oligonucleotide (expressed as 100%) is compared with the number of cells in cultures treated with the control oligonucleotide. The number of cells in cultures treated with the test oligonucleotide is not more than 30% of control, indicating that the inhibition of human PDE-like enzyme has an anti-proliferative effect on cancer cells.

EXAMPLE 11

In vivo testing of compounds/target validation

1. Acute Mechanistic Assays

1.1. Reduction in Mitogenic Plasma Hormone Levels

This non-tumor assay measures the ability of a compound to reduce either the endogenous level of a circulating hormone or the level of hormone produced in response to a biologic stimulus. Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.). At a predetermined time after administration of test compound, blood plasma is collected. Plasma is assayed for levels of the hormone of interest. If the normal circulating levels of the hormone are too low and/or variable to provide consistent results, the level of the hormone may be elevated by a pre-treatment with a biologic stimulus (i.e., LHRH may be injected i.m. into mice at a dosage of 30 ng/mouse to induce a burst of testosterone synthesis). The timing of plasma collection would be adjusted to coincide with the peak of the induced hormone response. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

1.2. Hollow Fiber Mechanism of Action Assay

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol, these may include

assays for gene expression (bDNA, PCR, or Taqman), or a specific biochemical activity (i.e., cAMP levels. Results are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2. Subacute Functional *In Vivo* Assays

2.1. Reduction in Mass of Hormone Dependent Tissues

This is another non-tumor assay that measures the ability of a compound to reduce the mass of a hormone dependent tissue (i.e., seminal vesicles in males and uteri in females). Rodents are administered test compound (p.o., i.p., i.v., i.m., or s.c.) according to a predetermined schedule and for a predetermined duration (i.e., 1 week). At termination of the study, animals are weighed, the target organ is excised, any fluid is expressed, and the weight of the organ is recorded. Blood plasma may also be collected. Plasma may be assayed for levels of a hormone of interest or for levels of test agent. Organ weights may be directly compared or they may be normalized for the body weight of the animal. Compound effects are compared to a vehicle-treated control group. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test. Significance is p value ≤ 0.05 compared to the vehicle control group.

2.2. Hollow Fiber Proliferation Assay

Hollow fibers are prepared with desired cell line(s) and implanted intraperitoneally and/or subcutaneously in rodents. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Fibers are harvested in accordance with specific readout assay protocol. Cell proliferation is

determined by measuring a marker of cell number (i.e., MTT or LDH). The cell number and change in cell number from the starting inoculum are analyzed by Student's t-test or Rank Sum test after the variance between groups is compared by an F-test, with significance at $p \leq 0.05$ as compared to the vehicle control group.

2.3. *Anti-angiogenesis Models*

2.3.1. *Corneal Angiogenesis*

Hydron pellets with or without growth factors or cells are implanted into a micropocket surgically created in the rodent cornea. Compound administration may be systemic or local (compound mixed with growth factors in the hydron pellet). Corneas are harvested at 7 days post implantation immediately following intracardiac infusion of colloidal carbon and are fixed in 10% formalin. Readout is qualitative scoring and/or image analysis. Qualitative scores are compared by Rank Sum test. Image analysis data is evaluated by measuring the area of neovascularization (in pixels) and group averages are compared by Student's t-test (2 tail). Significance is $p \leq 0.05$ as compared to the growth factor or cells only group.

2.3.2. *Matrigel Angiogenesis*

Matrigel, containing cells or growth factors, is injected subcutaneously. Compounds are administered p.o., i.p., i.v., i.m., or s.c. Matrigel plugs are harvested at predetermined time point(s) and prepared for readout. Readout is an ELISA-based assay for hemoglobin concentration and/or histological examination (i.e. vessel count, special staining for endothelial

surface markers: CD31, factor-8). Readouts are analyzed by Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$ as compared to the vehicle control group.

3. Primary Antitumor Efficacy

3.1. Early Therapy Models

3.1.1. Subcutaneous Tumor

Tumor cells or fragments are implanted subcutaneously on Day 0. Vehicle and/or compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting at a time, usually on Day 1, prior to the ability to measure the tumor burden. Body weights and tumor measurements are recorded 2-3 times weekly. Mean net body and tumor weights are calculated for each data collection day. Anti-tumor efficacy may be initially determined by comparing the size of treated (T) and control (C) tumors on a given day by a Student's t-test, after the variance between groups is compared by an F-test, with significance determined at $p \leq 0.05$. The experiment may also be continued past the end of dosing in which case tumor measurements would continue to be recorded to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is $p \leq 0.05$.

3.1.2. Intraperitoneal/Intracranial Tumor Models

Tumor cells are injected intraperitoneally or intracranially on Day 0. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule starting on Day 1. Observations of morbidity and/or mortality are recorded twice daily. Body weights are measured and recorded twice weekly. Morbidity/mortality data is expressed in terms of the median time of survival and the number of long-term survivors is indicated separately. Survival times are used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment.

3.2. Established Disease Model

Tumor cells or fragments are implanted subcutaneously and grown to the desired size for treatment to begin. Once at the predetermined size range, mice are randomized into treatment groups. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by

generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is p value$\leq$ 0.05 compared to the vehicle control group.

3.3. *Orthotopic Disease Models*

3.3.1. *Mammary Fat Pad Assay*

Tumor cells or fragments, of mammary adenocarcinoma origin, are implanted directly into a surgically exposed and reflected mammary fat pad in rodents. The fat pad is placed back in its original position and the surgical site is closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Tumor and body weights are measured and recorded 2-3 times weekly. Mean tumor weights of all groups over days post inoculation are graphed for comparison. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group.

Tumor measurements may be recorded after dosing has stopped to monitor tumor growth delay. Tumor growth delays are expressed as the difference in the median time for the treated and control groups to attain a predetermined size divided by the median time for the control group to attain that size. Growth delays are compared by generating Kaplan-Meier curves from the times for individual tumors to attain the evaluation size. Significance is p value$\leq$ 0.05 compared to the vehicle control group. In addition, this model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor.

Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ, or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.2. Intraprostatic Assay

Tumor cells or fragments, of prostatic adenocarcinoma origin, are implanted directly into a surgically exposed dorsal lobe of the prostate in rodents. The prostate is externalized through an abdominal incision so that the tumor can be implanted specifically in the dorsal lobe while verifying that the implant does not enter the seminal vesicles. The successfully inoculated prostate is replaced in the abdomen and the incisions through the abdomen and skin are closed. Hormones may also be administered to the rodents to support the growth of the tumors. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the lungs), or measuring the target organ weight (i.e., the regional lymph nodes). The means of these endpoints are compared by Student's t-test after conducting an F-test,

with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.3. Intrabronchial Assay

Tumor cells of pulmonary origin may be implanted intrabronchially by making an incision through the skin and exposing the trachea. The trachea is pierced with the beveled end of a 25 gauge needle and the tumor cells are inoculated into the main bronchus using a flat-ended 27 gauge needle with a 90° bend. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the contralateral lung), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

3.3.4. Intracecal Assay

Tumor cells of gastrointestinal origin may be implanted intracecally by making an abdominal incision through the skin and externalizing

the intestine. Tumor cells are inoculated into the cecal wall without penetrating the lumen of the intestine using a 27 or 30 gauge needle. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Body weights are measured and recorded 2-3 times weekly. At a predetermined time, the experiment is terminated and the animal is dissected. The size of the primary tumor is measured in three dimensions using either a caliper or an ocular micrometer attached to a dissecting scope. An F-test is preformed to determine if the variance is equal or unequal followed by a Student's t-test to compare tumor sizes in the treated and control groups at the end of treatment. Significance is $p \leq 0.05$ as compared to the control group. This model provides an opportunity to increase the rate of spontaneous metastasis of this type of tumor. Metastasis can be assessed at termination of the study by counting the number of visible foci per target organ (i.e., the liver), or measuring the target organ weight. The means of these endpoints are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment.

4. Secondary (Metastatic) Antitumor Efficacy

4.1. Spontaneous Metastasis

Tumor cells are inoculated s.c. and the tumors allowed to grow to a predetermined range for spontaneous metastasis studies to the lung or liver. These primary tumors are then excised. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule which may include the period leading up to the excision of the primary tumor to evaluate therapies directed at inhibiting the early stages of tumor metastasis. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded

twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance determined at $p \leq 0.05$ compared to the control group in the experiment for both of these endpoints.

4.2. Forced Metastasis

Tumor cells are injected into the tail vein, portal vein, or the left ventricle of the heart in experimental (forced) lung, liver, and bone metastasis studies, respectively. Compounds are administered p.o., i.p., i.v., i.m., or s.c. according to a predetermined schedule. Observations of morbidity and/or mortality are recorded daily. Body weights are measured and recorded twice weekly. Potential endpoints include survival time, numbers of visible foci per target organ, or target organ weight. When survival time is used as the endpoint the other values are not determined. Survival data is used to generate Kaplan-Meier curves. Significance is $p \leq 0.05$ by a log-rank test compared to the control group in the experiment. The mean number of visible tumor foci, as determined under a dissecting microscope, and the mean target organ weights are compared by Student's t-test after conducting an F-test, with significance at $p \leq 0.05$ compared to the vehicle control group in the experiment for both endpoints.

EXAMPLE 12

Quantitative Expression Profiling PDE-like enzyme polypeptide

Expression profiling is based on a quantitative polymerase chain reaction (PCR) analysis, also called kinetic analysis, first described in Higuchi et al., 1992 and Higuchi et al., 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies. Using this technique, the expression levels of particular genes, which are transcribed from the chromosomes as messenger RNA (mRNA), are measured by first making a DNA copy (cDNA) of the mRNA, and then performing quantitative PCR on the cDNA, a method called quantitative reverse transcription-polymerase chain reaction (quantitative RT-PCR).

Quantitative RT-PCR analysis of RNA from different human tissues was performed to investigate the tissue distribution of PDE-like enzyme polypeptide, cGMP-specific 3',5'-cyclic phosphodiesterase-like mRNA. 25 .mu.g of total RNA from various tissues (Human Total RNA Panel I-V, Clontech Laboratories, Palo Alto, CA, USA) was used as a template to synthsize first-strand cDNA using the SUPERSCRIPT™ First-Strand Synthesis System for RT-PCR (Life Technologies, Rockville , MD, USA). First-strand cDNA synthesis was carried out according to the manufacturer's protocol using oligo (dT) to hybridize to the 3' poly A tails of mRNA and prime the synthesis reaction. 10 ng of the first-strand cDNA was then used as template in a polymerase chain reaction. The polymerase chain reaction was performed in a LightCycler (Roche Molecular Biochemicals, Indianapolis, IN, USA), in the presence of the DNA-binding fluorescent dye SYBR Green I which binds to the minor groove of the DNA double helix, produced only when double-stranded DNA is successfully synthesized in the reaction (Morrison et al., 1998). Upon binding to double-stranded DNA, SYBR Green I emits light that can be quantitatively measured by the LightCycler machine. The polymerase chain reaction was carried out using oligonucleotide primers LBRI54.1-L4 (CAGAGTTTGGGCCCGATGCATTAT) and

LBRI54.1-L4 (AAGCTGCTTTGCTTTGCTAGAGGTGGT) and measurements of the intensity of emitted light were taken following each cycle of the reaction when the reaction had reached a temperature of 82 degrees C. Intensities of emitted light were converted into copy numbers of the gene transcript per nanogram of template cDNA by comparison with simultaneously reacted standards of known concentration.

To correct for differences in mRNA transcription levels per cell in the various tissue types, a normalization procedure was performed using similarly calculated expression levels in the various tissues of five different housekeeping genes: glyceraldehyde-3-phosphatase (G3PDH), hypoxanthine guanine phophoribosyl transferase (HPRT), beta-actin, porphobilinogen deaminase (PBGD), and beta-2-microglobulin. The level of housekeeping gene expression is considered to be relatively constant for all tissues (Adams et al., 1993, Adams et al., 1995, Liew et al., 1994) and therefore can be used as a gauge to approximate relative numbers of cells per .mu.g of total RNA used in the cDNA synthesis step. Except for the use of a slightly different set of housekeeping genes and the use of the LightCycler system to measure expression levels, the normalization procedure was essentially the same as that described in the RNA Master Blot User Manual, Apendix C (1997, Clontech Laboratories, Palo Alto, CA, USA). In brief, expression levels of the five housekeeping genes in all tissue samples were measured in three independent reactions per gene using the LightCycler and a constant amount (25 .mu.g) of starting RNA. The calculated copy numbers for each gene, derived from comparison with simultaneously reacted standards of known concentrations, were recorded and converted into a percentage of the sum of the copy numbers of the gene in all tissue samples. Then for each tissue sample, the sum of the percentage values for each gene was calculated, and a normalization factor was calculated by dividing the sum percentage value for each tissue by the sum percentage value of one of the tissues arbitrarily selected as a standard. To normalize an experimentally obtained value for the expression of a particular gene in a tissue sample, the obtained value was multiplied by the normalization factor for the tissue tested.

Results are given below, showing the experimentally obtained copy numbers of mRNA per 10 ng of first-strand cDNA on the left and the normalized values on the right. RNAs used for the cDNA synthesis, along with their supplier and catalog numbers are shown in table 1.

Exression profiling of PDE-like enzyme polypeptide, cGMP-specific 3',5'-cyclic phosphodiesterase-like mRNA

Sample	Cell Name	Abs. #	Norm. #
Sample 1	Brain-1	8.223	8
Sample 2	Heart-3	18.17	52
Sample 3	Kidney	12.25	20
Sample 4	Liver	36.05	148
Sample 5	Lung	4.261	3
Sample 6	Trachea	33.01	66
Sample 7	Bone Marrow-1	3.04	1
Sample 8	Colon	14.66	13
Sample 9	Small intestine	14.79	22
Sample 10	Spleen-1	.475	0
Sample 11	Stomach	24.63	34
Sample 12	Thymus-1	8.749	9
Sample 13	Mammary gland	13.99	24
Sample 14	Prostate-1	2.122	2
Sample 15	Skeletal muscle-1	918.4	648
Sample 16	Testis	686.8	240
Sample 17	Uterus	17.29	22
Sample 18	Cerebellum	3.54	4
Sample 19	Fetal Brain	31.57	22
Sample 20	Fetal Liver-1	2.577	1
Sample 21	Spinal cord	5.246	6
Sample 22	Placenta-1	1.773	2
Sample 23	Adrenal gland	4.19	5
Sample 24	Pancreas-1	16.27	160
Sample 25	Salivary gland	110	203
Sample 26	Thyroid	27.23	64

Table 1: Whole-body-screen tissues

Tissue	Supplier	Panel name and catalog number
1. brain	Clontech	Human Total RNA Panel I, K4000-1
2. heart	Clontech	Human Total RNA Panel I, K4000-1
3. kidney	Clontech	Human Total RNA Panel I, K4000-1
4. liver	Clontech	Human Total RNA Panel I, K4000-1
5. lung	Clontech	Human Total RNA Panel I, K4000-1
6. trachea	Clontech	Human Total RNA Panel I, K4000-1
7. bone marrow	Clontech	Human Total RNA Panel II, K4001-1
8. colon	Clontech	Human Total RNA Panel II, K4001-1
9. small intestine	Clontech	Human Total RNA Panel II, K4001-1
10. spleen	Clontech	Human Total RNA Panel II, K4001-1
11. stomach	Clontech	Human Total RNA Panel II, K4001-1
12. thymus	Clontech	Human Total RNA Panel II, K4001-1
13. mammary gland	Clontech	Human Total RNA Panel III, K4002-1
14. skeletal muscle	Clontech	Human Total RNA Panel III, K4002-1
15. prostate	Clontech	Human Total RNA Panel III, K4002-1
16. testis	Clontech	Human Total RNA Panel III, K4002-1
17. uterus	Clontech	Human Total RNA Panel III, K4002-1
18. cerebellum	Clontech	Human Total RNA Panel IV, K4003-1
19. fetal brain	Clontech	Human Total RNA Panel IV, K4003-1
20. fetal liver	Clontech	Human Total RNA Panel IV, K4003-1
21. spinal cord	Clontech	Human Total RNA Panel IV, K4003-1
22. placenta	Clontech	Human Total RNA Panel IV, K4003-1
23. adrenal gland	Clontech	Human Total RNA Panel V, K4004-1
24. pancreas	Clontech	Human Total RNA Panel V, K4004-1
25. salivary gland	Clontech	Human Total RNA Panel V, K4004-1
26. thyroid	Clontech	Human Total RNA Panel V, K4004-1

EXAMPLE 13

Activity and inhibition of PDE11A4

Expression of recombinant PDE11A4 was examined by baculovirus expression system in Sf21 cells with or without His-tag at the N-terminus. Since both expression level and enzyme activity of the His-tagged PDE11A4 (His-PDE11A4) was much higher than those of non-tagged enzyme (Fig. 20), His-PDE11A4 was selected for further use. The maximum expression level and activity were obtained from the cells cultured 24 to 48 h after transfection. The His-PDE11A4 was successfully purified by Ni^{2+}-chelating column chromatography (Fig. 21). The assay was carried out in scintillation proximity assay (SPA) using cGMP as substrate. Km value for cGMP was determined to be 0.4 uM (Fig. 22). DMSO did not affect the assay system up to final concentration of 2 % (v/v) (Fig. 23A). The enzyme was stable and any significant reduction of the activity was not observed after 2 h pre-incubation at RT (Fig. 23B). A couple of known inhibitors were assessed by this assay system and IC_{50} values of dipyridamole and IBMX were found to be 0.53 uM and 41 uM, respectively (Fig. 24).

REFERENCES

Yuasa *et al.*, *J. Biol. Chem. 275*, 31469-79, 2000

Fawcett *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 97*, 3702-07, 2000 EP 0 967 284 A1

Higuchi, R., Dollinger, G., Walsh, P.S. and Griffith, R. (1992) Simultaneous amplification and detection of specific DNA sequences. *BioTechnology* 10:413-417.

Higuchi, R., Fockler, C., Dollinger, G. and Watson, R. (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *BioTechnology* 11:1026-1030.

T.B. Morrison, J.J. Weis & C.T. Wittwer .(1998) Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques* 24:954–962.

Adams, M. D., Kerlavage, A. R., Fields, C. & Venter, C. (1993) 3,400 new expressed sequence tags identify diversity of transcripts in human brain. *Nature Genet.* 4:256–265.

Adams, M. D., et al. (1995) Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. *Nature* 377 supp:3–174.

Liew, C. C., Hwang, D. M., Fung, Y. W., Laurenson, C., Cukerman, E., Tsui, S. & Lee, C. Y. (1994) A catalog of genes in the cardiovascular system as identified by expressed sequence tags. *Proc. Natl. Acad. Sci. USA* 91:10145–10649.

CLAIMS

1. An isolated polynucleotide encoding a PDE-like enzyme polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a PDE-like enzyme polypeptide comprising an amino acid sequence selected form the group consisting of:

amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 2;
the amino acid sequence shown in SEQ ID NO: 2;
amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO: 5; and
the amino acid sequence shown in SEQ ID NO: 5.

b) a polynucleotide comprising the sequence of SEQ ID NOS: 1 or 4;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified PDE-like enzyme polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a PDE-like enzyme polypeptide, wherein the method comprises the following steps:

   a) culturing the host cell of claim 3 under conditions suitable for the expression of the PDE-like enzyme polypeptide; and

   b) recovering the PDE-like enzyme polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a PDE-like enzyme polypeptide in a biological sample comprising the following steps:

   a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

   b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a PDE-like enzyme polypeptide of claim 4 comprising the steps of:

   contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the PDE-like enzyme polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a PDE-like enzyme, comprising the steps of:

contacting a test compound with any PDE-like enzyme polypeptide encoded by any polynucleotide of claim1;

detecting binding of the test compound to the PDE-like enzyme polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a PDE-like enzyme.

11. A method of screening for agents which regulate the activity of a PDE-like enzyme, comprising the steps of:

contacting a test compound with a PDE-like enzyme polypeptide encoded by any polynucleotide of claim 1; and

detecting a PDE-like enzyme activity of the polypeptide, wherein a test compound which increases the PDE-like enzyme activity is identified as a potential therapeutic agent for increasing the activity of the PDE-like enzyme, and wherein a test compound which decreases the PDE-like enzyme activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the PDE-like enzyme.

12. A method of screening for agents which decrease the activity of a PDE-like enzyme, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of PDE-like enzyme.

13. A method of reducing the activity of PDE-like enzyme, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any PDE-like enzyme polypeptide of claim 4, whereby the activity of PDE-like enzyme is reduced.

14. A reagent that modulates the activity of a PDE-like enzyme polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claim 10 to 12.

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a PDE-like enzyme in a disease.

17. Use of claim 16 wherein the disease is urinary incontinence, benign prostate hyperplasia, erectile dysfunction, diabetes, cancer or cardiovascular disorder.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5.

19. The cDNA of claim 18 which comprises SEQ ID NO:1 and 4.

20. The cDNA of claim 18 which consists of SEQ ID NO:1 and 4.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO:1 and 4.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO:1 and 4.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO:2 and 5.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO:2 and 5.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5, comprising the steps of:

culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and

isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO:1 and 4.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5, comprising the steps of:

hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 and 4 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and

detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO:1 and 4; and

instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

36. A method of screening for agents which can modulate the activity of a human PDE-like enzyme, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO:2 and 5 and (2) the amino acid sequence shown in SEQ ID NO:2 and 5; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the human PDE-like enzyme.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which modulate an activity of a human PDE-like enzyme, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 45% identical to the amino acid sequence shown in SEQ ID NO:2 and 5 and (2) the amino acid sequence shown in SEQ ID NO:2 and 5; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human PDE-like enzyme, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human PDE-like enzyme.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. A method of screening for agents which modulate an activity of a human PDE-like enzyme, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO:1 and 4; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human PDE-like enzyme.

50. The method of claim 49 wherein the product is a polypeptide.

51. The method of claim 49 wherein the product is RNA.

52. A method of reducing activity of a human PDE-like enzyme, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1 and 4, whereby the activity of a human PDE-like enzyme is reduced.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 53 wherein the reagent is an antibody.

55. The method of claim 52 wherein the product is RNA.

56. The method of claim 55 wherein the reagent is an antisense oligonucleotide.

57. The method of claim 56 wherein the reagent is a ribozyme.

58. The method of claim 52 wherein the cell is *in vitro*.

59. The method of claim 52 wherein the cell is *in vivo*.

60. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5; and

a pharmaceutically acceptable carrier.

61. The pharmaceutical composition of claim 60 wherein the reagent is an antibody.

62. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:1 and 4; and

a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 62 wherein the reagent is a ribozyme.

64. The pharmaceutical composition of claim 62 wherein the reagent is an antisense oligonucleotide.

65. The pharmaceutical composition of claim 62 wherein the reagent is an antibody.

66. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2 and 5; and

a pharmaceutically acceptable carrier.

67. The pharmaceutical composition of claim 66 wherein the expression vector comprises SEQ ID NO:1 and 4.

68. A method of treating a PDE-like enzyme dysfunction related disease, wherein the disease is selected from urinary incontinence, benign prostate hyperplasia, erectile dysfunction, diabetes, cancer and cardiovascular disorder, comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that modulates a function of a human PDE-like enzyme, whereby symptoms of the PDE-like enzyme dysfunction related disease are ameliorated.

69. The method of claim 68 wherein the reagent is identified by the method of claim 36.

70. The method of claim 68 wherein the reagent is identified by the method of claim 45.

71. The method of claim 68 wherein the reagent is identified by the method of claim 49.

Fig. 1

```
ttttataaat aaactcaact ttattccatt cacacagtct tcaactttaa aagataaaag ataacttact
ggtgattcga tgtcctctag gagtaaaaca gaacagcgtt cacatttcag cagagtttgg gcccgatgca
ttattttctt gacaattttc tccaggtcag tctgttcttc aaagaggtca ttaaccacct ctagcaaagc
aaagcagctt ttcctcacta tcaggaagaa acaggttctt ggtcaaactt gagacatttt cactaataca
catcccttac ataacttctg cttctttcat attctttcct tgaggcagca aagagctgag cgttagatat
ggcgattcca caaaatggaa gatacatctg cataaccttg ttatctgcat c
```

Fig. 2

DADNKVMQMY LPFCGIAISN AQLFAASRKE YERSRSYVRD VY//LVKMSQVX PRTCFFLIVR KSCFALLEVV NDLFEEQTDL
EKIVKKIMHR AQTLLKCERCSVLLLEDIES PVSYLLSFKV EDCVNGIKLS LFIK

Fig. 3

```
MLPFGDKTRD MVNAWFSERV HNIPVCKEGI RAHTESCSCS LPQSPHADNT TPGAPARKIS ASEFDRPLRP
IVVKDSEGTV SFLSDSGKKE QMPLTSPRFD SDEGDQCSRL LELVKDISSH LDVTALCHKI FLHIHGLISA
DRYSLFLVCE DSSKDKFLVS RLFDVAEGST LEEASNNCIR LEWNKGIVGH VAAFGEPLNI KDAYEDPRFN
AEVDQITGYK TQSILCMPIK NHREEVVGVA QAINKKSGNG GTFTEKDEKD FAAYLAFCGI VLHNAQLYET
SLLENKRNQV LLDLASLIFE EQQSLEVILK KIAATIISFM QVQKCTIFIV DEDCPDSFSR VFQMEWEEVG
KSSEPLTREH DANKINYMYA QYVKNTMEPL NIPDVTKDNR FPWTNENMGH INTHCIRSLL CTPIKNGKKN
KVIGVCQLVN KMEEKTGKIK AFNQNDEQFL EAFVIFCGLG IQNTQMYEAV ERAMAKQMVT LEVLSYHASA
AEEETRELQA LAAAVVPSAQ TLKITDFSFS DFELSDLETA LCTIRMFTDL NLVQNFQMKH EVLCRWILSV
KKNYRKNVAY HNWRHAFNTA QCMFAALKAG KIQNKLTDLE TLALLIAALS HDLDHRGVNN SYIQRSEHPL
AQLYCHSTME HHHFDQCLMV LNSPGNQILS GLSIEEYKTT LKIIKQAILA TDLALYIKRR GEFFELIRKN
EFSFEDPLQK ELFLAMLMTA CDLSAITKPW PIQQRIAELV AAEFFDQGDR ERKELNMEPA DLMNREKKNK
IPSMQVGFID AICLQLYEAL THVSEDCLPL LDGCRKNRQK WQALADQQEK TLLNGESGQA KRD
```

Fig. 4

```
gatcgacgat tcaatgatga aatcgacaag ctaactggat acaagacaaa atcattattg tgcatgccta
tccgaagcag tgatggtgag attattggtg tggcccaagc gataaataag attcctgaag gagctccatt
tactgaagat gatgaaaaag ttatgcagat gtatcttcca ttttgtggaa tcgccatatc taacgctcag
ctctttgctg cctcaaggaa agaatatgaa agaagcagag ctttgctaga ggtggttaat gacctctttg
aagaacagac tgacctggag aaaattgtca agaaaataat gcatcgggcc caaactctgc tgaaatgtga
acgctgttct gttttactcc tagaggacat cgaatcacca gtaagttatc ttttatcttt taaagttgaa gactgt
```

Fig. 5

```
DRRFNDEIDK LTGYKTKSLL CMPIRSSDGE IIGVAQAINK IPEGAPFTED DEKVMQMYLP FCGIAISNAQ
LFAASRKEYE RSRALLEVVN DLFEEQTDLE KIVKKIMHRA QTLLKCERCS VLLLEDIESP VSYLLSFKVE DC
```

Fig. 6

```
BLASTX - alignment of 54 against swiss|O54735|CN5A_RAT
This hit is scoring at : 4e-06 (expectation value)
     Alignment length (overlap) : 35
     Identities : 42 %
     Scoring matrix : BLOSUM62 (used to infer consensus pattern)
     Query reading frame : -1
     Database searched : nrdb
Q:     401 DADNKVMQMYLPFCGIAISNAQLFAASRKEYERSR     297
           :.D.K ...YL.FCGI.:.NAQL:..S  E :R::
 H:    255 EKDEKDFAAYLAFCGIVLHNAQLYETSLLENKRNQ     289
This hit is scoring at : 4e-06 (expectation value)
     Alignment length (overlap) : 65
     Identities : 29 %
     Scoring matrix : BLOSUM62 (used to infer consensus pattern)
     Query reading frame : -3
     Database searched : nrdb
 Q:    234 LIVRKSCFALLEVVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVSYLL
           L: .K.  .LL::.: :FEEQ..LE I:KKI. .. :.::.::C::.::: :.P S:
 H:    282 LLENKRNQVLLDLASLIFEEQQSLEVILKKIAATIISFMQVQKCTIFIVDE-DCPDSFSR
SFKVE      40
            F::E
           VFQME     345
```

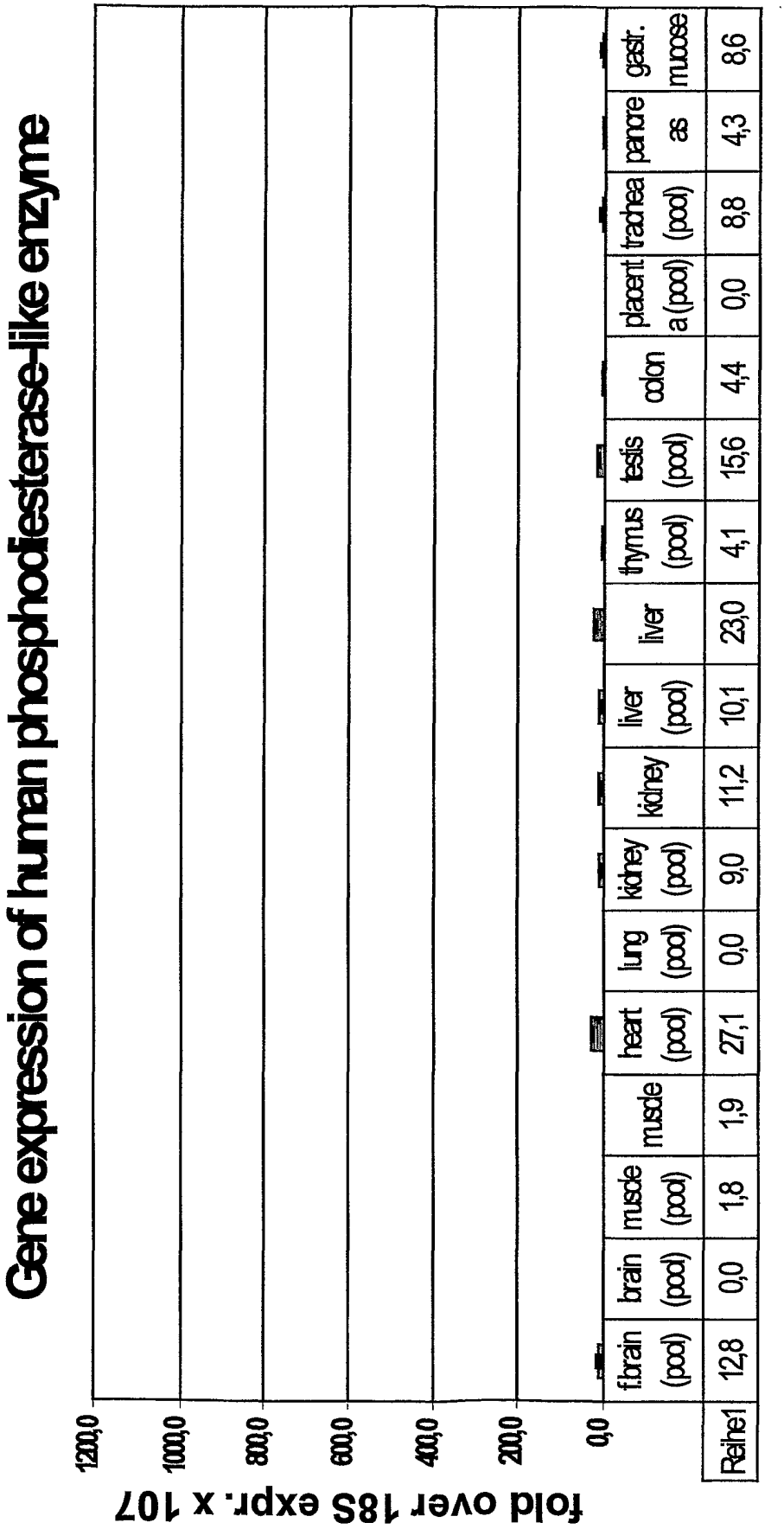
Fig. 7

```
BLASTP alignment of PDE-like enzyme (SEQ ID NO:2) against swiss|O54735|CN5A_RAT
This hit is scoring at : 1e-28 (expectation value)
    Alignment length (overlap) : 141
    Identities : 44 %
    Scoring matrix : BLOSUM62 (used to infer consensus pattern)
    Database searched : nrdb
Q:      1 DRRFNDEIDKLTGYKTKSLLCMPIRSSDGEIIGVAQAINKIP-EGAPFTEDDEKVMQMYL
          D RFN E:D::TGYKT:S:LCMPI::.  E::GVAQAINK . .G..FTE.DEK ...YL
H:    206 DPRFNAEVDQITGYKTQSILCMPIKNHREEVVGVAQAINKKSGNGGTFTEKDEKDFAAYL

          PFCGIAISNAQLFAASRKEYERSRALLEVVNDLFEEQTDLEKIVKKIMHRAQTLLKCERC
          .FCGI.:.NAQL:..S  E :R::.LL::.: :FEEQ..LE I:KKI. .. :.::.::C
          AFCGIVLHNAQLYETSLLENKRNQVLLDLASLIFEEQQSLEVILKKIAATIISFMQVQKC

          SVLLLEDIESPVSYLLSFKVE     140
          ::.:::: :.P S:   F::E
          TIFIVDE-DCPDSFSRVFQME     345
```

Fig. 8


Gene expression of human phosphodiesterase-like enzyme
fold over 18S expr. x 10^7
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
f.brain (pool)
brain (pool)
muscle (pool)
muscle
heart (pool)
lung (pool)
kidney (pool)
kidney
liver (pool)
liver
thymus (pool)
testis (pool)
colon
placenta (pool)
trachea (pool)
pancreas
gastr. mucose
Reihe1
12,8
0,0
1,8
1,9
27,1
0,0
9,0
11,2
10,1
23,0
4,1
15,6
4,4
0,0
8,8
4,3
8,6

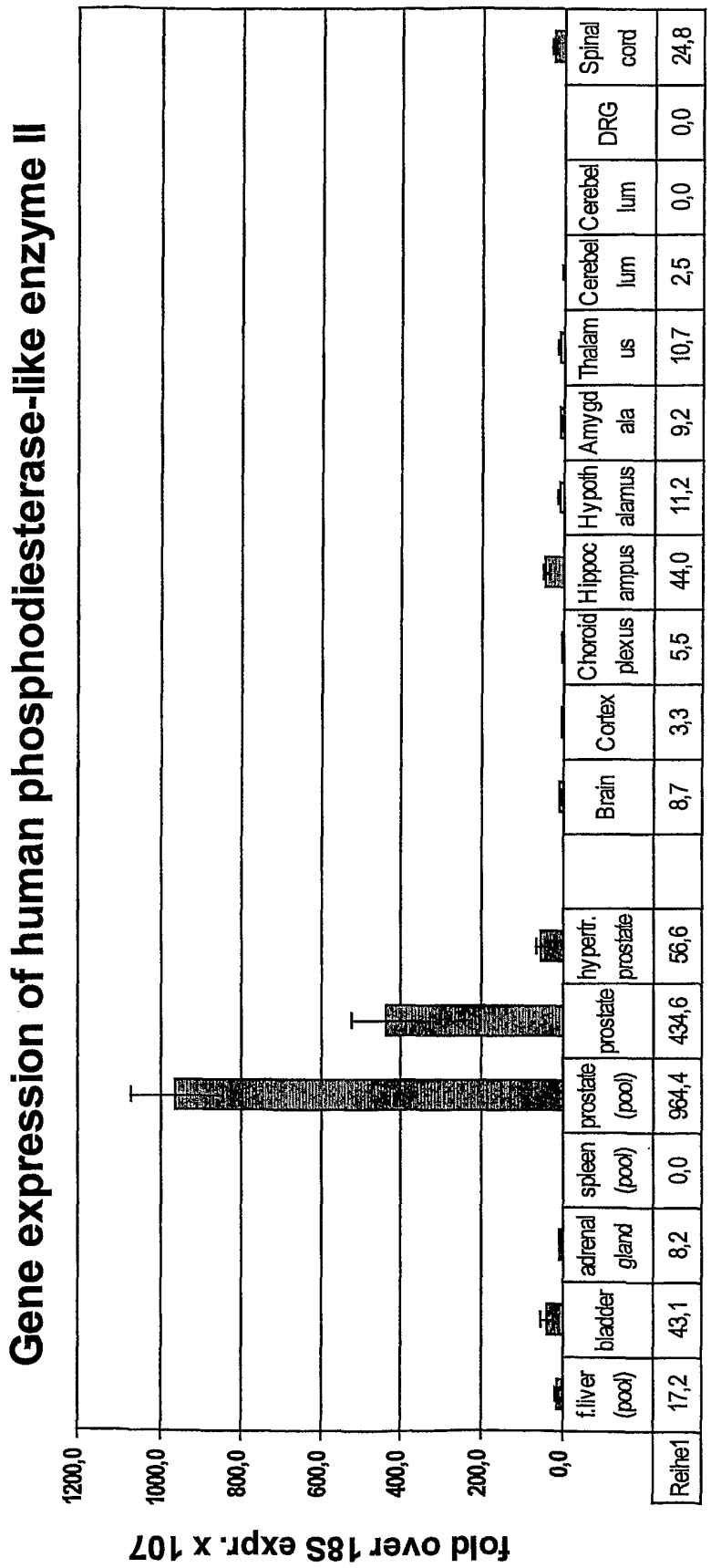
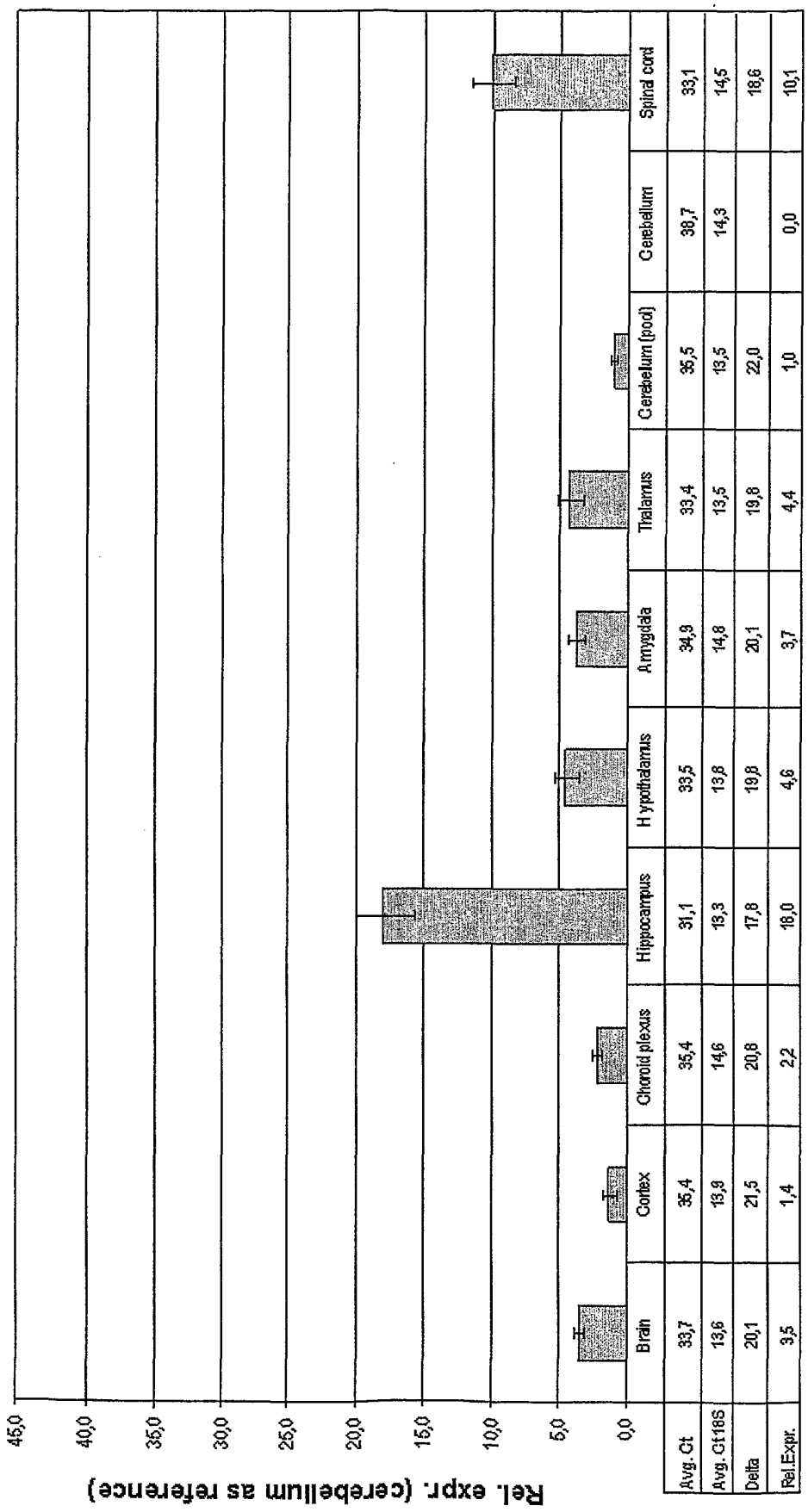

Gene expression of human phosphodiesterase-like enzyme II
fold over 18S expr. x 107
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
f.liver (pool)
bladder
adrenal gland
spleen (pool)
prostate (pool)
prostate
hyperfr. prostate
Brain
Cortex
Choroid plexus
Hippocampus
Hypothalamus
Amygdala
Thalamus
Cerebellum
Cerebellum
DRG
Spinal cord
Reihe1
17,2
43,1
8,2
0,0
964,4
434,6
56,6
8,7
3,3
5,5
44,0
11,2
9,2
10,7
2,5
0,0
0,0
24,8

Fig. 9

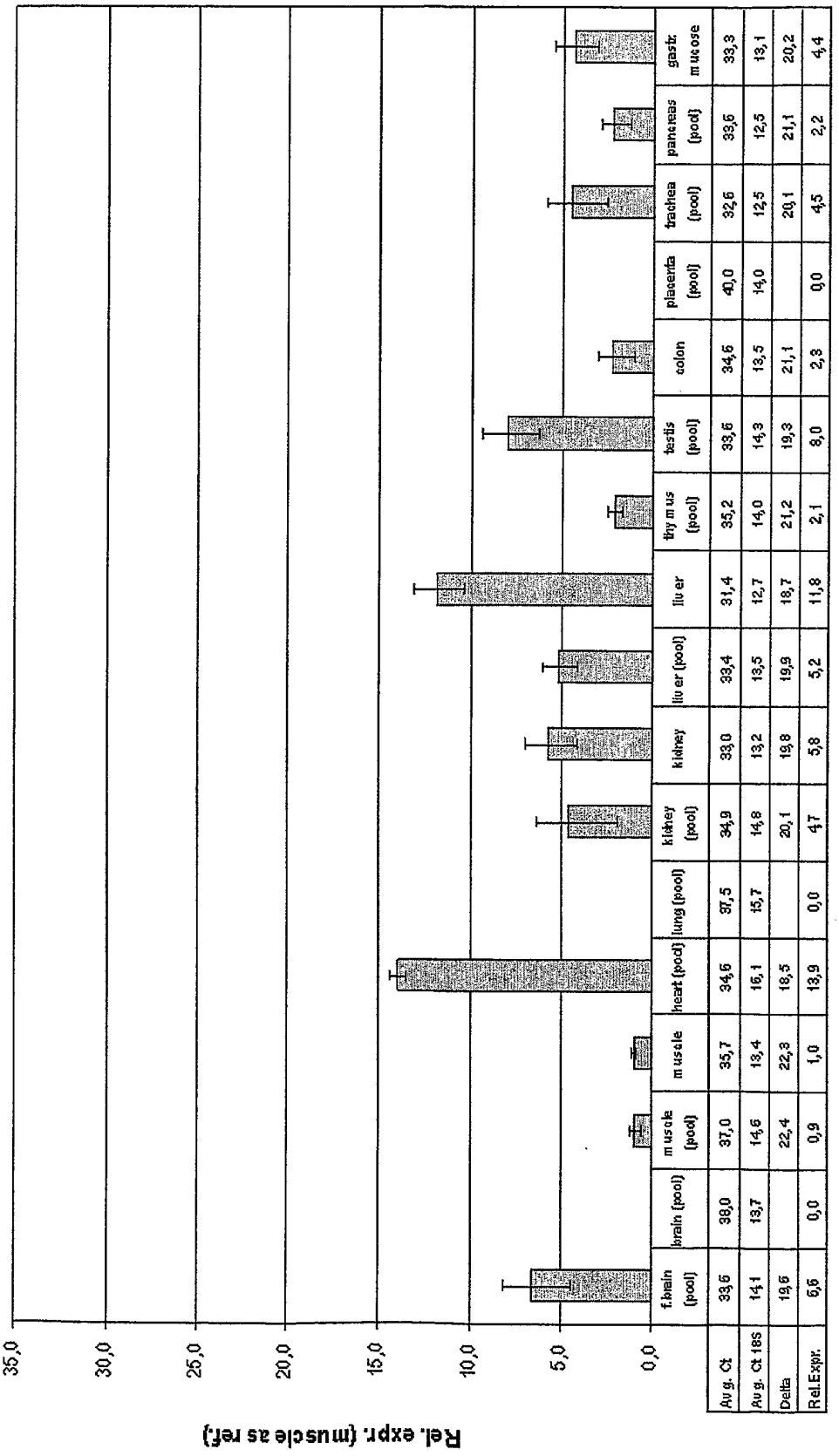
Fig. 10



	Brain	Cortex	Choroid plexus	Hippocampus	Hypothalamus	Amygdala	Thalamus	Cerebellum (pool)	Cerebellum	Spinal cord
Avg.Ct	33,7	36,4	35,4	31,1	33,5	34,9	33,4	35,5	38,7	33,1
Avg.Ct18S	13,6	13,9	14,6	13,3	13,8	14,8	13,5	13,5	14,3	14,5
Delta	20,1	21,5	20,8	17,8	19,8	20,1	19,8	22,0		18,6
Rel.Expr.	3,5	1,4	2,2	18,0	4,6	3,7	4,4	1,0	0,0	10,1

Fig. 11

Gene expression of human phosphodiesterase-like enzyme IV - Human Organ Panel (endog. ref. 18S)



	f.brain (pool)	brain (pool)	muscle (pool)	muscle	heart (pool)	lung (pool)	kidney (pool)	kidney	liver (pool)	liver	thymus (pool)	testis (pool)	colon	placenta (pool)	trachea (pool)	pancreas (pool)	gastr. mucose
Avg. Ct	33,6	38,0	37,0	35,7	34,6	37,5	34,9	33,0	33,4	31,4	35,2	33,6	34,6	40,0	32,6	33,6	33,3
Avg. Ct 18S	14,1	13,7	14,6	13,4	16,1	15,7	14,8	13,2	13,5	12,7	14,0	14,3	13,5	14,0	12,5	12,5	13,1
Delta	19,6		22,4	22,3	18,5		20,1	19,8	19,9	18,7	21,2	19,3	21,1		20,1	21,1	20,2
Rel.Expr.	6,6	0,0	0,9	1,0	13,9	0,0	4,7	5,8	5,2	11,8	2,1	8,0	2,3	0,0	4,5	2,2	4,4

Fig. 12

Gene expression of human phosphodiesterase-like enzyme V

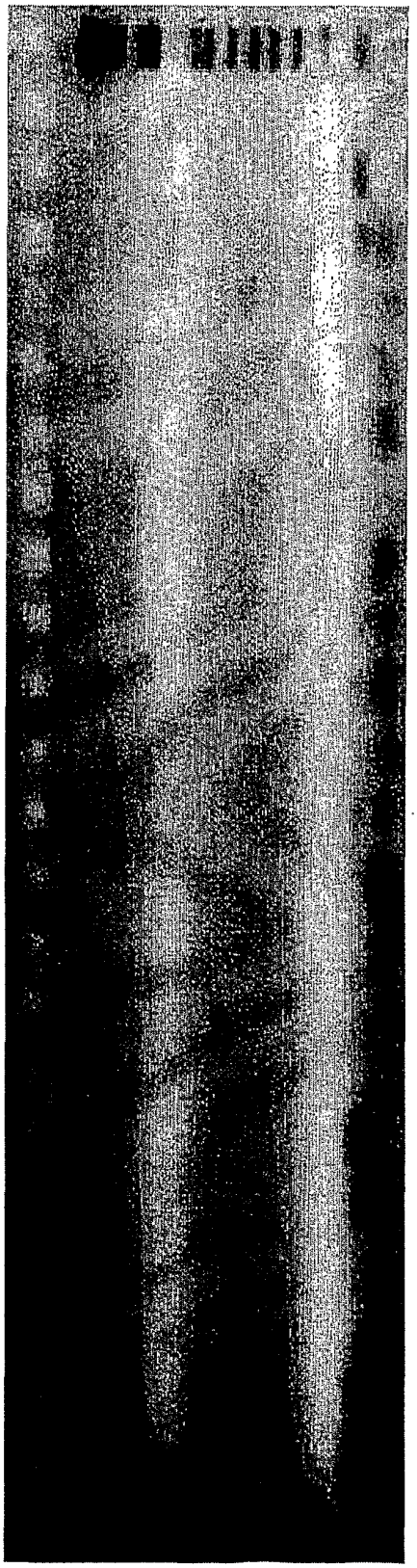
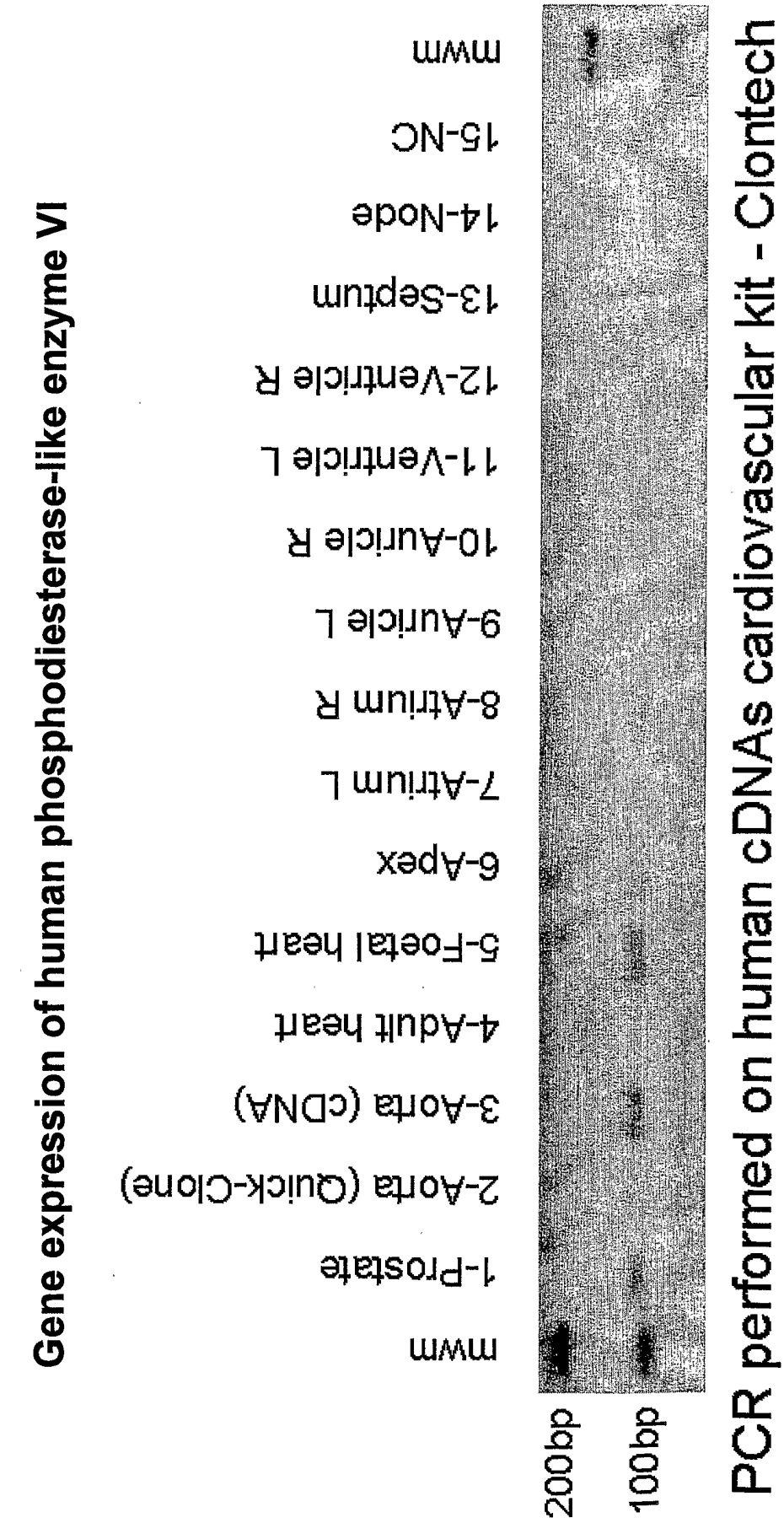


Fig. 13



PCR performed on human cDNAs cardiovascular kit - Clontech

Fig. 14

```
                                   10        20        30        40        50        60
                           ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)         GCAGCGGCGGCAGCCAGAACAGGAGCAGCGATAGCTCGGGTTTCCGGAACAGGAGCCGGG
PDE 11A2 (AF281865)        ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                   ------------------------------------------------------------
PDE-like A  (AI025081)     ------------------------------------------------------------
PDE-like B                 ------------------------------------------------------------

                                   70        80        90       100       110       120
                           ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)         GCAGCGGCGGCAGCTCAGTGCTGGGCACCTGTGCGGAGCAGGAGTAGCAGGACCACGGGG
PDE 11A2 (AF281865)        ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                   ------------------------------------------------------------
PDE-like A  (AI025081)     ------------------------------------------------------------
PDE-like B                 ------------------------------------------------------------

                                  130       140       150       160       170       180
                           ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)         TGGGGTCGGCGCCAGCCACTCTGAGCCAGAGAAGGAAGGGGCATCTCCCAGATTCCACTG
PDE 11A2 (AF281865)        ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                   ------------------------------------------------------------
PDE-like A  (AI025081)     ------------------------------------------------------------
PDE-like B                 ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                    190       200       210       220       230       240
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CTGGGAATAATCTCCAGGGGAGGTGGCGCTGAACTGGGAATACTGGTGGGGGTGAACATG
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    250       260       270       280       290       300
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              TGCAGGAACAGCTAGAGGCCTCGGGGCAGGAAAACATTTGGTTCACGTGTAAACAGGCAA
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------GCAA
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    310       320       330       340       350       360
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GGAAAGCTGTCTGGGACCATGGCAGCCTCCCGCCTGGACTTTGGGGAGGTGGAAACTTTC
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        GGAAAGCTGTCTGGGACCATGGCAGCCTCCCGCCTGGACTTTGGGGAGGTGGAAACTTTC
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                    370       380       390       400       410       420
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CTGGACAGGCACCCAGAGTTGTTTGAAGATTACTTGATGCGGAAGGGGAAGCAGGAGATG
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        CTGGACAGGCACCCAGAGTTGTTTGAAGATTACTTGATGCGGAAGGGGAAGCAGGAGATG
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    430       440       450       460       470       480
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GTTGAAAAGTGGCTGCAGAGGCACAGTCAGGGTCAGGGGGCTTTAGGTCCAAGGCCCTCT
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        GTTGAAAAGTGGCTGCAGAGGCACAGTCAGGGTCAGGGGGCTTTAGGTCCAAGGCCCTCT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    490       500       510       520       530       540
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              TTGGCTGGTACCAGCAGCTTGGCTCACAGCACCTGCAGAGGTGGCAGCAGCGTTGGTGGT
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        TTGGCTGGTACCAGCAGCTTGGCTCACAGCACCTGCAGAGGTGGCAGCAGCGTTGGTGGT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  550       560       570       580       590       600
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GGCACTGGACCAAATGGCTCTGCCCACAGCCAGCCCCTTCCCGGTGGCGGGGACTGTGGT
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  GGCACTGGACCAAATGGCTCTGCCCACAGCCAGCCCCTTCCCGGTGGCGGGGACTGTGGT
PDE-like A (AI025081)     ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  610       620       630       640       650       660
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GGGGTTCCCTTGAGTCCCAGCTGGGCCGGTGGCAGCAGGGGCGATGGGAACCTGCAGCGG
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  GGGGTTCCCTTGAGTCCCAGCTGGGCCGGTGGCAGCAGGGGCGATGGGAACCTGCAGCGG
PDE-like A (AI025081)     ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  670       680       690       700       710       720
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        AGAGCTTCTCAGAAAGAGCTAAGGAAGAGTTTTGCCCGCTCCAAGGCCATCCACGTGAAC
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  AGAGCTTCTCAGAAAGAGCTAAGGAAGAGTTTTGCCCGCTCCAAGGCCATCCACGTGAAC
PDE-like A (AI025081)     ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  730       740       750       760       770       780
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        AGGACCTACGATGAACAGGTGACCTCCCGGGCTCAGGAACCCCTGAGTAGTGTACGACGG
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  AGGACCTACGATGAACAGGTGACCTCCCGGGCTCAGGAACCCCTGAGTAGTGTACGACGG
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  790       800       810       820       830       840
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        AGGGCACTTCTCCGGAAGGCAAGCTCCCTGCCCCCCACCACAGCCCATATTCTCAGTGCG
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  AGGGCACTTCTCCGGAAGGCAAGCTCCCTGCCCCCCACCACAGCCCATATTCTCAGTGCG
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  850       860       870       880       890       900
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CTGCTGGAATCGAGAGTGAATCTGCCTCAGTATCCCCCTACAGCCATCGACTACAAGTGC
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  CTGCTGGAATCGAGAGTGAATCTGCCTCAGTATCCCCCTACAGCCATCGACTACAAGTGC
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                        910       920       930       940       950       960
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CATCTGAAAAAGCATAATGAGCGTCAGTTCTTTCTGGAATTGGTCAAAGATATCTCCAAT
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   --------------------------GTCGACCCACGCGTCCGCGGACGCGTGGGCGCGC
PDE 11A4                        CATCTGAAAAAGCATAATGAGCGTCAGTTCTTTCTGGAATTGGTCAAAGATATCTCCAAT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        970       980       990      1000      1010      1020
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GACCTTGACCTCACCAGCCTGAGCTACAAGATTCTCATCTTTGTCTGCCTTATGGTGGAT
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   GTCCGGCTGCGCTTGCAGCCCAGGGCGTGAGGTGCTCTTTCTGGATCGCCAGCCTCGAAG
PDE 11A4                        GACCTTGACCTCACCAGCCTGAGCTACAAGATTCTCATCTTTGTCTGCCTTATGGTGGAT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                       1030      1040      1050      1060      1070      1080
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GCTGACCGCTGCTCTCTTTTCCTGGTGGAAGGGGCAGCTGCTGGCAAGAAGACCTTGGTC
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   TTGAGGCGTGGACTCTGGCCAGGTGGCTGTTCCAGAGCAACATGGGAGATGCTGAAGCAG
PDE 11A4                        GCTGACCGCTGCTCTCTTTTCCTGGTGGAAGGGGCAGCTGCTGGCAAGAAGACCTTGGTC
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  1090      1100      1110      1120      1130      1140
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                TCCAAATTCTTTGATGTGCATGCAGGAACCCCTCTGCTGCCTTGCAGCAGCACAGAGAAC
PDE 11A2 (AF281865)               ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)     GCAAGAAGACCTTTATTCAGAAATGTGCTCAGTGCCACACAGTGGAAAAAGGTGAAAATC
PDE 11A4                          TCCAAATTCTTTGATGTGCATGCAGGAACCCCTCTGCTGCCTTGCAGCAGCACAGAGAAC
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  1150      1160      1170      1180      1190      1200
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                TCAAATGAGGTGCAGGTCCCCTGGGGCAAAGGTATCATTGGCTATGTCGGGGAGCATGGA
PDE 11A2 (AF281865)               ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)     ACAAGACTGGTCCAAATCTCTGGGGCCTCTTTGGCTGAAAAACAGGAAAAGCACCAGGAT
PDE 11A4                          TCAAATGAGGTGCAGGTCCCCTGGGGCAAAGGTATCATTGGCTATGTCGGGGAGCATGGA
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  1210      1220      1230      1240      1250      1260
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                GAAACGGTCAACATTCCTGATGCCTACCAGGATCGACGATTCAATGATGAAATCGACAAG
PDE 11A2 (AF281865)               ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)     TTTCTTATACAGAGGCAAACAAAAACAAAGGATCGACGATTCAATGATGAAATCGACAAG
PDE 11A4                          GAAACGGTCAACATTCCTGATGCCTACCAGGATCGACGATTCAATGATGAAATCGACAAG
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------GATCGACGATTCAATGATGAAATCGACAAG
```

Fig. 14 (continued)

```
                                        1270      1280      1290      1300      1310      1320
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CTAACTGGATACAAGACAAAATCATTATTGTGCATGCCTATCCGAAGCAGTG--ATGGTG
PDE 11A2 (AF281865)             ----------------------------CGCCCGGGCAGGTGAAAAGTCCAC--TGTGTG
PDE 11A3 (AJ278682,HSA278682)   CTGACTGGATACAAGACAAAATCATTATTGTGCATGCCTATCCGAAGCAGTG--ATGGTG
PDE 11A4                        CTAACTGGATACAAGACAAAATCATTATTGTGCATGCCTATCCGAAGCAGTG--ATGGTG
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      CTAACTGGATACAAGACAAAATCATTATTGTGCATGCCTATCCGAAGCAGTG--ATGGTG

                                        1330      1340      1350      1360      1370      1380
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              AGATTATTGGTGTGGCCCAAGCGATAAATAAGATTCCTG-AAGGAGCTCCATTTACTGAA
PDE 11A2 (AF281865)             TTGGGAATAGATTATGAAGGGCTTTGAGTGTAGGCCCTGTAAGCAATAGGAAGCCATGGA
PDE 11A3 (AJ278682,HSA278682)   AGATTATTGGTGTGGCCCAAGCGATAAATAAGATTCCTG-AAGGAGCTCCATTTACTGAA
PDE 11A4                        AGATTATTGGTGTGGCCCAAGCGATAAATAAGATTCCTG-AAGGAGCTCCATTTACTGAA
PDE-like A  (AI025081)          ------------------------------------------------------------GAT
PDE-like B                      AGATTATTGGTGTGGCCCAAGCGATAAATAAGATTCCTG-AAGGAGCTCCATTTACTGAA

                                        1390      1400      1410      1420      1430      1440
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GATGATGAAAAAGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCATATCTAACGCT
PDE 11A2 (AF281865)             ACAGCCAGAAAGGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCATATCTAACGCT
PDE 11A3 (AJ278682,HSA278682)   GATGATGAAAAAGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCATATCTAACGCT
PDE 11A4                        GATGATGAAAAAGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCATATCTAACGCT
PDE-like A  (AI025081)          GCAGATAACAAGGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCATATCTAACGCT
PDE-like B                      GATGATGAAAAAGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCATATCTAACGCT
```

Fig. 14 (continued)

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                                        1450      1460      1470      1480
                                ....|....|....|....|....|....|....|....|..
PDE 11A (AB036704)              CAGCTCTTTGCTGCCTCAAGGAAAGAATATGAAAGAAGCAGA
PDE 11A2 (AF281865)             CAGCTCTTTGCTGCCTCAAGGAAAGAATATGAAAGAAGCAGA
PDE 11A3 (AJ278682,HSA278682)   CAGCTCTTTGCTGCCTCAAGGAAAGAATATGAAAGAAGCAGA
PDE 11A4                        CAGCTCTTTGCTGCCTCAAGGAAAGAATATGAAAGAAGCAGA
PDE-like A  (AI025081)          CAGCTCTTTGCTGCCTCAAGGAAAGAATATGAAAGAAGCAGAAGTTATGTAAGGGATGTG
PDE-like B                      CAGCTCTTTGCTGCCTCAAGGAAAGAATATGAAAGAAGCAGA

PDE-like A  (AI025081)          TATTAGTGAAAATGTCTCAAGTTTGACCAAGAACCTGTTTCTTCCTGATAGTGAGGAAAA
                                stop codon

                                                1490      1500
                                          ..|....|....|....|
PDE 11A (AB036704)                        GCTTTGCTAGAGGTGGTT
PDE 11A2 (AF281865)                       GCTTTGCTAGAGGTGGTT
PDE 11A3 (AJ278682,HSA278682)             GCTTTGCTAGAGGTGGTT
PDE 11A4                                  GCTTTGCTAGAGGTGGTT
PDE-like A  (AI025081)           GCTGCTTTGCTTTGCTAGAGGTGGTT
PDE-like B                                GCTTTGCTAGAGGTGGTT

                                        1510      1520      1530      1540      1550      1560
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATTGTCAAGAAAATAATGCATCGG
PDE 11A2 (AF281865)             AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATTGTCAAGAAAATAATGCATCGG
PDE 11A3 (AJ278682,HSA278682)   AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATTGTCAAGAAAATAATGCATCGG
PDE 11A4                        AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATTGTCAAGAAAATAATGCATCGG
PDE-like A  (AI025081)          AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATTGTCAAGAAAATAATGCATCGG
PDE-like B                      AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATTGTCAAGAAAATAATGCATCGG
```

Fig. 14 (continued)

```
                                  1570      1580      1590      1600      1610      1620
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GCCCAAACTCTGCTGAAATGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAATCA
PDE 11A2 (AF281865)       GCCCAAACTCTGCTGAAATGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAATCA
PDE 11A3 (AJ278682,HSA278682) GCCCAAACTCTGCTGAAATGTGAGCGCTGTTCTGTTTTACTCCTAGAGGACATCGAATCA
PDE 11A4                  GCCCAAACTCTGCTGAAATGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAATCA
PDE-like A (AI025081)     GCCCAAACTCTGCTGAAATGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAATCA
PDE-like B                GCCCAAACTCTGCTGAAATGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAATCA

                                  1630      1640      1650      1660      1670      1680
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CCAGTGGTGAAATTTACCAAATCCTTTGAATTGATGTCCCCAAAGTGCAGTGCTGATGCT
PDE 11A2 (AF281865)       CCAGTGGTGAAATTTACCAAATCCTTTGAATTGATGTCCCCAAAGTGCAGTGCTGATGCT
PDE 11A3 (AJ278682,HSA278682) CCAGTGGTGAAATTTACCAAATCCTTTGAATTGATGTCCCCAAAGTGCAGTGCTGATGCT
PDE 11A4                  CCAGTGGTGAAATTTACCAAATCCTTTGAATTGATGTCCCCAAAGTGCAGTGCTGATGCT
PDE-like A (AI025081)     CCAGTAAGTTATCTTTT--ATCTTTTAAAGTTGAAGACTGTGTGAA--TGGAATAAAGTT
PDE-like B                CCAGTAAGTTATCTTTT--ATCTTTTAAAGTTGAAGACTGT-------------------

                                  1690      1700      1710      1720      1730      1740
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GAGAACAGTTTCAAAGAAAGCATGGAGAAATCATCATACTCCGACTGGCTAATAAATAAC
PDE 11A2 (AF281865)       GAGAACAGTTTCAAAGAAAGCATGGAGAAATCATCATACTCCGACTGGCTAATAAATAAC
PDE 11A3 (AJ278682,HSA278682) GAGAACAGTTTCAAAGAAAGCATGGAGAAATCATCATACTCCGACTGGCTAATAAATAAC
PDE 11A4                  GAGAACAGTTTCAAAGAAAGCATGGAGAAATCATCATACTCCGACTGGCTAATAAATAAC
PDE-like A (AI025081)     GAGTTTATTTATAAAA--------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

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                                        1750      1760      1770      1780      1790      1800
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              AGCATTGCTGAGCTGGTTGCTTCAACAGGCCTTCCAGTGAACATCAGTGATGCCTACCAG
PDE 11A2 (AF281865)             AGCATTGCTGAGCTGGTTGCTTCAACAGGCCTTCCAGTGAACATCAGTGATGCCTACCAG
PDE 11A3 (AJ278682,HSA278682)   AGCATTGCTGAGCTGGTTGCTTCAACAGGCCTTCCAGTGAACATCAGTGATGCCTACCAG
PDE 11A4                        AGCATTGCTGAGCTGGTTGCTTCAACAGGCCTTCCAGTGAACATCAGTGATGCCTACCAG
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        1810      1820      1830      1840      1850      1860
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GATCCGCGCTTTGATGCAGAGGCAGACCAGATATCTGGTTTTCACATAAGATCTGTTCTT
PDE 11A2 (AF281865)             GATCCGCGCTTTGATGCAGAGGCAGACCAGATATCTGGTTTTCACATAAGATCTGTTCTT
PDE 11A3 (AJ278682,HSA278682)   GATCCGCGCTTTGATGCAGAGGCAGACCAGATATCTGGTTTTCACATAAGATCTGTTCTT
PDE 11A4                        GATCCGCGCTTTGATGCAGAGGCAGACCAGATATCTGGTTTTCACATAAGATCTGTTCTT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        1870      1880      1890      1900      1910      1920
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              TGTGTCCCTATTTGGAATAGCAACCACCAAATAATTGGAGTGGCTCAAGTGTTAAACAGA
PDE 11A2 (AF281865)             TGTGTCCCTATTTGGAATAGCAACCACCAAATAATTGGAGTGGCTCAAGTGTTAAACAGA
PDE 11A3 (AJ278682,HSA278682)   TGTGTCCCTATTTGGAATAGCAACCACCAAATAATTGGAGTGGCTCAAGTGTTAAACAGA
PDE 11A4                        TGTGTCCCTATTTGGAATAGCAACCACCAAATAATTGGAGTGGCTCAAGTGTTAAACAGA
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

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                                  1930      1940      1950      1960      1970      1980
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CTTGATGGGAAACCTTTTGATGATGCAGATCAACGACTTTTTGAGGCTTTTGTCATCTTT
PDE 11A2 (AF281865)       CTTGATGGGAAACCTTTTGATGATGCAGATCAACGACTTTTTGAGGCTTTTGTCATCTTT
PDE 11A3 (AJ278682,HSA278682) CTTGATGGGAAACCTTTTGATGATGCAGATCAACGACTTTTTGAGGCTTTTGTCATCTTT
PDE 11A4                  CTTGATGGGAAACCTTTTGATGATGCAGATCAACGACTTTTTGAGGCTTTTGTCATCTTT
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  1990      2000      2010      2020      2030      2040
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        TGTGGACTTGGCATCAACAACACAATTATGTATGATCAAGTGAAGAAGTCCTGGGCCAAG
PDE 11A2 (AF281865)       TGTGGACTTGGCATCAACAACACAATTATGTATGATCAAGTGAAGAAGTCCTGGGCCAAG
PDE 11A3 (AJ278682,HSA278682) TGTGGACTTGGCATCAACAACACAATTATGTATGATCAAGTGAAGAAGTCCTGGGCCAAG
PDE 11A4                  TGTGGACTTGGCATCAACAACACAATTATGTATGATCAAGTGAAGAAGTCCTGGGCCAAG
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  2050      2060      2070      2080      2090      2100
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CAGTCTGTGGCTCTTGATGTGCTATCATACCATGCAACATGTTCAAAAGCTGAAGTTGAC
PDE 11A2 (AF281865)       CAGTCTGTGGCTCTTGATGTGCTATCATACCATGCAACATGTTCAAAAGCTGAAGTTGAC
PDE 11A3 (AJ278682,HSA278682) CAGTCTGTGGCTCTTGATGTGCTATCATACCATGCAACATGTTCAAAAGCTGAAGTTGAC
PDE 11A4                  CAGTCTGTGGCTCTTGATGTGCTATCATACCATGCAACATGTTCAAAAGCTGAAGTTGAC
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                    2110      2120      2130      2140      2150      2160
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              AAGTTTAAGGCAGCCAACATCCCTCTGGTGTCAGAACTTGCCATCGATGACATTCATTTT
PDE 11A2 (AF281865)             AAGTTTAAGGCAGCCAACATCCCTCTGGTGTCAGAACTTGCCATCGATGACATTCATTTT
PDE 11A3 (AJ278682,HSA278682)   AAGTTTAAGGCAGCCAACATCCCTCTGGTGTCAGAACTTGCCATCGATGACATTCATTTT
PDE 11A4                        AAGTTTAAGGCAGCCAACATCCCTCTGGTGTCAGAACTTGCCATCGATGACATTCATTTT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    2170      2180      2190      2200      2210      2220
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GATGACTTTTCTCTCGACGTTGATGCCATGATCACAGCTGCTCTCCGGATGTTCATGGAG
PDE 11A2 (AF281865)             GATGACTTTTCTCTCGACGTTGATGCCATGATCACAGCTGCTCTCCGGATGTTCATGGAG
PDE 11A3 (AJ278682,HSA278682)   GATGACTTTTCTCTCGACGTTGATGCCATGATCACAGCTGCTCTCCGGATGTTCATGGAG
PDE 11A4                        GATGACTTTTCTCTCGACGTTGATGCCATGATCACAGCTGCTCTCCGGATGTTCATGGAG
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    2230      2240      2250      2260      2270      2280
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CTGGGGATGGTACAGAAATTTAAAATTGACTATGAGACACTGTGTAGGTGGCTTTTGACA
PDE 11A2 (AF281865)             CTGGGGATGGTACAGAAATTTAAAATTGACTATGAGACACTGTGTAGGTGGCTTTTGACA
PDE 11A3 (AJ278682,HSA278682)   CTGGGGATGGTACAGAAATTTAAAATTGACTATGAGACACTGTGTAGGTGGCTTTTGACA
PDE 11A4                        CTGGGGATGGTACAGAAATTTAAAATTGACTATGAGACACTGTGTAGGTGGCTTTTGACA
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                    2290      2300      2310      2320      2330      2340
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GTGAGGAAAAACTATCGGATGGTTCTATACCACAACTGGAGACATGCCTTCAACGTGTGT
PDE 11A2 (AF281865)             GTGAGGAAAAACTATCGGATGGTTCTATACCACAACTGGAGACATGCCTTCAACGTGTGT
PDE 11A3 (AJ278682,HSA278682)   GTGAGGAAAAACTATCGGATGGTTCTATACCACAACTGGAGACATGCCTTCAACGTGTGT
PDE 11A4                        GTGAGGAAAAACTATCGGATGGTTCTATACCACAACTGGAGACATGCCTTCAACGTGTGT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    2350      2360      2370      2380      2390      2400
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CAGCTGATGTTCGCGATGTTAACCACTGCTGGGTTTCAAGACATTCTGACCGAGGTGGAA
PDE 11A2 (AF281865)             CAGCTGATGTTCGCGATGTTAACCACTGCTGGGTTTCAAGACATTCTGACCGAGGTGGAA
PDE 11A3 (AJ278682,HSA278682)   CAGCTGATGTTCGCGATGTTAACCACTGCTGGGTTTCAAGACATTCTGACCGAGGTGGAA
PDE 11A4                        CAGCTGATGTTCGCGATGTTAACCACTGCTGGGTTTCAAGACATTCTGACCGAGGTGGAA
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    2410      2420      2430      2440      2450      2460
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              ATTTTAGCGGTGATTGTGGGATGCCTGTGTCATGACCTCGACCACAGGGGAACCAACAAT
PDE 11A2 (AF281865)             ATTTTAGCGGTGATTGTGGGATGCCTGTGTCATGACCTCGACCACAGGGGAACCAACAAT
PDE 11A3 (AJ278682,HSA278682)   ATTTTAGCGGTGATTGTGGGATGCCTGTGTCATGACCTCGACCACAGGGGAACCAACAAT
PDE 11A4                        ATTTTAGCGGTGATTGTGGGATGCCTGTGTCATGACCTCGACCACAGGGGAACCAACAAT
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  2470      2480      2490      2500      2510      2520
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GCCTTCCAAGCTAAGAGTGGCTCTGCCCTGGCCCAACTCTATGGAACCTCTGCTACCTTG
PDE 11A2 (AF281865)       GCCTTCCAAGCTAAGAGTGGCTCTGCCCTGGCCCAACTCTATGGAACCTCTGCTACCTTG
PDE 11A3 (AJ278682,HSA278682) GCCTTCCAAGCTAAGAGTGGCTCTGCCCTGGCCCAACTCTATGGAACCTCTGCTACCTTG
PDE 11A4                  GCCTTCCAAGCTAAGAGTGGCTCTGCCCTGGCCCAACTCTATGGAACCTCTGCTACCTTG
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  2530      2540      2550      2560      2570      2580
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GAGCATCACCATTTCAACCACGCCGTGATGATCCTTCAAAGTGAGGGTCACAATATCTTT
PDE 11A2 (AF281865)       GAGCATCACCATTTCAACCACGCCGTGATGATCCTTCAAAGTGAGGGTCACAATATCTTT
PDE 11A3 (AJ278682,HSA278682) GAGCATCACCATTTCAACCACGCCGTGATGATCCTTCAAAGTGAGGGTCACAATATCTTT
PDE 11A4                  GAGCATCACCATTTCAACCACGCCGTGATGATCCTTCAAAGTGAGGGTCACAATATCTTT
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  2590      2600      2610      2620      2630      2640
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GCTAACCTGTCCTCCAAGGAATATAGTGACCTTATGCAGCTTTTGAAGCAGTCAATATTG
PDE 11A2 (AF281865)       GCTAACCTGTCCTCCAAGGAATATAGTGACCTTATGCAGCTTTTGAAGCAGTCAATATTG
PDE 11A3 (AJ278682,HSA278682) GCTAACCTGTCCTCCAAGGAATATAGTGACCTTATGCAGCTTTTGAAGCAGTCAATATTG
PDE 11A4                  GCTAACCTGTCCTCCAAGGAATATAGTGACCTTATGCAGCTTTTGAAGCAGTCAATATTG
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  2650      2660      2670      2680      2690      2700
                                  ....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                GCAACAGACCTCACGCTGTACTTTGAGAGGAGAACTGAATTCTTTGAACTTGTCAGTAAA
PDE 11A2 (AF281865)               GCAACAGACCTCACGCTGTACTTTGAGAGGAGAACTGAATTCTTTGAACTTGTCAGTAAA
PDE 11A3 (AJ278682,HSA278682)     GCAACAGACCTCACGCTGTACTTTGAGAGGAGAACTGAATTCTTTGAACTTGTCAGTAAA
PDE 11A4                          GCAACAGACCTCACGCTGTACTTTGAGAGGAGAACTGAATTCTTTGAACTTGTCAGTAAA
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  2710      2720      2730      2740      2750      2760
                                  ....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                GGAGAATACGATTGGAACATCAAAAACCATCGTGATATATTTCGATCAATGTTAATGACA
PDE 11A2 (AF281865)               GGAGAATACGATTGGAACATCAAAAACCATCGTGATATATTTCGATCAATGTTAATGACA
PDE 11A3 (AJ278682,HSA278682)     GGAGAATACGATTGGAACATCAAAAACCATCGTGATATATTTCGATCAATGTTAATGACA
PDE 11A4                          GGAGAATACGATTGGAACATCAAAAACCATCGTGATATATTTCGATCAATGTTAATGACA
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  2770      2780      2790      2800      2810      2820
                                  ....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                GCCTGTGACCTTGGAGCCGTGACCAAACCGTGGGAGATCTCCAGACAGGTGGCAGAACTT
PDE 11A2 (AF281865)               GCCTGTGACCTTGGAGCCGTGACCAAACCGTGGGAGATCTCCAGACAGGTGGCAGAACTT
PDE 11A3 (AJ278682,HSA278682)     GCCTGTGACCTTGGAGCCGTGACCAAACCGTGGGAGATCTCCAGACAGGTGGCAGAACTT
PDE 11A4                          GCCTGTGACCTTGGAGCCGTGACCAAACCGTGGGAGATCTCCAGACAGGTGGCAGAACTT
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  2830      2840      2850      2860      2870      2880
                                  ....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                GTAACCAGTGAGTTCTTCGAACAAGGAGATCGGGAGAGATTAGAGCTCAAACTCACTCCT
PDE 11A2 (AF281865)               GTAACCAGTGAGTTCTTCGAACAAGGAGATCGGGAGAGATTAGAGCTCAAACTCACTCCT
PDE 11A3 (AJ278682,HSA278682)     GTAACCAGTGAGTTCTTCGAACAAGGAGATCGGGAGAGATTAGAGCTCAAACTCACTCCT
PDE 11A4                          GTAACCAGTGAGTTCTTCGAACAAGGAGATCGGGAGAGATTAGAGCTCAAACTCACTCCT
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  2890      2900      2910      2920      2930      2940
                                  ....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                TCAGCAATTTTTGATCGGAACCGGAAGGATGAACTGCCTCGGTTGCAACTGGAGTGGATT
PDE 11A2 (AF281865)               TCAGCAATTTTTGATCGGAACCGGAAGGATGAACTGCCTCGGTTGCAACTGGAGTGGATT
PDE 11A3 (AJ278682,HSA278682)     TCAGCAATTTTTGATCGGAACCGGAAGGATGAACTGCCTCGGTTGCAACTGGAGTGGATT
PDE 11A4                          TCAGCAATTTTTGATCGGAACCGGAAGGATGAACTGCCTCGGTTGCAACTGGAGTGGATT
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  2950      2960      2970      2980      2990      3000
                                  ....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                GATAGCATCTGCATGCCTTTGTATCAGGCACTGGTGAAGGTCAACGTGAAACTGAAGCCG
PDE 11A2 (AF281865)               GATAGCATCTGCATGCCTTTGTATCAGGCACTGGTGAAGGTCAACGTGAAACTGAAGCCG
PDE 11A3 (AJ278682,HSA278682)     GATAGCATCTGCATGCCTTTGTATCAGGCACTGGTGAAGGTCAACGTGAAACTGAAGCCG
PDE 11A4                          GATAGCATCTGCATGCCTTTGTATCAGGCACTGGTGAAGGTCAACGTGAAACTGAAGCCG
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                        3010      3020      3030      3040      3050      3060
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              ATGCTAGATTCAGTAGCTACAAACAGAAGTAAGTGGGAAGAGCTACACCAAAAACGACTG
PDE 11A2 (AF281865)             ATGCTAGATTCAGTAGCTACAAACAGAAGTAAGTGGGAAGAGCTACACCAAAAACGACTG
PDE 11A3 (AJ278682,HSA278682)   ATGCTAGATTCAGTAGCTACAAACAGAAGTAAGTGGGAAGAGCTACACCAAAAACGACTG
PDE 11A4                        ATGCTAGATTCAGTAGCTACAAACAGAAGTAAGTGGGAAGAGCTACACCAAAAACGACTG
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        3070      3080      3090      3100      3110      3120
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              CTGGCCTCAACTGCCTCATCCTCCTCCCCTGCCAGTGTTATGGTAGCCAAGGAAGACAGG
PDE 11A2 (AF281865)             CTGGCCTCAACTGCCTCATCCTCCTCCCCTGCCAGTGTTATGGTAGCCAAGGAAGACAGG
PDE 11A3 (AJ278682,HSA278682)   CTGGCCTCAACTGCCTCATCCTCCTCCCCTGCCAGTGTTATGGTAGCCAAGGAAGACAGG
PDE 11A4                        CTGGCCTCAACTGCCTCATCCTCCTCCCCTGCCAGTGTTATGGTAGCCAAGGAAGACAGG
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        3130      3140      3150      3160      3170      3180
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              AACTAAACCTCCAGGTCAGCTGCAGCTGCAAAATGACTACAGCCTGAAGGGCCATTTTCA
PDE 11A2 (AF281865)             AACTAAACCTCCAGCTCAG-----------------------------------------
PDE 11A3 (AJ278682,HSA278682)   AACTAAACCTCCAGCTCAG-----------------------------------------
PDE 11A4                        AACTAAACCTCCAGGTCAGCTGCAGCTGCAAAATGACTACAGCCTGAAGGGCCATTTTCA
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  3190      3200      3210      3220      3230      3240
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GTCCAGCAATGTCATCCTTTTGTTCTTTTAGCTCAGAAAGACCTAACATCTCAAGGATGC
PDE 11A2 (AF281865)       ------------------------------------AAAGACCTAACATCTCAAGGATGC
PDE 11A3 (AJ278682,HSA278682) ------------------------------------AAAGACCTAACATCTCAAGGATGC
PDE 11A4                  GTCCAGCA------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  3250      3260      3270      3280      3290      3300
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        ACTGGGAACCATGCCTGGGCTTTCACCTTGAAGCATGGTCAGCAGCAGAGAGAGCAACGG
PDE 11A2 (AF281865)       ACTGGGAACCATGCCTGGGCTTTCACCTTGAAGCATGGTCAGCAGCAGAGAGAGCAACGG
PDE 11A3 (AJ278682,HSA278682) NCTGGGAACCATGCCTGGGCTTTCACCTTGAAGCATGGTCAGCAGCAGAGAGAGCAACGG
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  3310      3320      3330      3340      3350      3360
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        GAAGGACAAAGAAAGAGGTGGGGCAGGGAGCACACCCCAGGACCCTCACTTTTCCCTAAT
PDE 11A2 (AF281865)       GAAGGACAAAGAAAGAGGTGGG-CAGGGAGCACACCCCAGGACCCTCACTTTTCCCTAAT
PDE 11A3 (AJ278682,HSA278682) GAAGGACAAAGAAAGAGGTGGG-CAGGGAGCACACCCCAGGACCCTCACTTTTCCCTAAT
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                        3370      3380      3390      3400      3410      3420
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GAACACGCATGGGCTGAAATGAAGGCTCTGGGTAGGGGACTGTTTTGGATCCAAGGACCT
PDE 11A2 (AF281865)             GAACACGCATGGGCTGAAATGAAGGCTCTGGGTAGGGGACTGTTTTGGATCCAAGGACCT
PDE 11A3 (AJ278682,HSA278682)   GAACACGCATGGGCTGAAATGAAGGCTCTGGGTAGGGGACTGTTTTGGATCCAAGGACCT
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        3430      3440      3450      3460      3470      3480
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GTGGACAGTCGGCCTACTTACTCTGAGCTGAGGGAACACTGAACAGTAAAAGCGTCATTA
PDE 11A2 (AF281865)             GTGGACAGTCGGCCTACTTACTCTGAGCTGAGGGAACACTGAACAGTAAAAGCGTCATTA
PDE 11A3 (AJ278682,HSA278682)   GTGGACAGTCGGCCTACTTACTNTGAGCTGAGGGAACNCTGAACAGTAAAAGCGTCATTA
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        3490      3500      3510      3520      3530      3540
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GCGCTGCTTCATTTTGTATAGGGCTTTTCTGTTTGTTACAAGCCAAACATTGCCTGTCTT
PDE 11A2 (AF281865)             GCGCTGAAAAAAAAAAAAAAAAAAAAAAA-------------------------------
PDE 11A3 (AJ278682,HSA278682)   GCGCTGAAAAAAAAAAAAAAAAAAAAAAA-------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  3550      3560      3570      3580      3590      3600
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        TGCTTCCCGTCCCTGAATGCCTTTTTGTGCCAGACTGTCCCAAGAATCCTAATTTGTATT
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  3610      3620      3630      3640      3650      3660
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CCATAGAGGTATTTTATTTTTAATCCTAGAGCTTCTTATTGATGGATCCTTTAGAATTGC
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  3670      3680      3690      3700      3710      3720
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CTACCTAAAAGGTAAACTATACTATCCTTATAAATACTGATCAATCCCAGTTCTCCCCCT
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  3730      3740      3750      3760      3770      3780
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        AAAAATGAATACATAGTAGGACTATAGCAAATGTGTTTGATGGGTAATTCTAGACTGGGA
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  3790      3800      3810      3820      3830      3840
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CTATGGTACCCTTTTCCAGAGTTTTAAAATTCAACCTTCATTACAGACAAAGTTTTCTCC
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------

                                  3850      3860      3870      3880      3890      3900
                          ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)        CAGAAGGAATGGATTGATAGATTTTGATTAAAGTAAGGGTGGAAGGAAATCTGTAGCTGG
PDE 11A2 (AF281865)       ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682) ------------------------------------------------------------
PDE 11A4                  ------------------------------------------------------------
PDE-like A  (AI025081)    ------------------------------------------------------------
PDE-like B                ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                    3910      3920      3930      3940      3950      3960
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              ATTTACCACAAGTGACATCTAGAAACTATAGTTCACAGGACAGAGCAGAGCCATGGAGAA
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    3970      3980      3990      4000      4010      4020
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              TAAGCATTGACTACCTTGAGTTCTCCTAGTGAGGAGTTCTGGTATAAAATTTAAGATTAC
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    4030      4040      4050      4060      4070      4080
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              TACCAGTAACCAACTTAAAGCAAACTATAGGGGTCCCTAATTTTGGATTTTTCCTTAAGT
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                    4090      4100      4110      4120      4130      4140
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              GTAAGAAACAATGCTTCAAATGTTAAGAAATAACAGTCTGGGCAAAGAACGCATATTCTA
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    4150      4160      4170      4180      4190      4200
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              TAGGAAGCCAGGTTTACAATAGGTAAGAATAAACTGTATTAAGTAGATGTAATGACTAGA
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    4210      4220      4230      4240      4250      4260
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)              AAGCTGCTTTGCTCCCTATATTGAGAAATTGTGGACATGGTATGTGTTATCCAAAGAACA
PDE 11A2 (AF281865)             ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ------------------------------------------------------------
PDE 11A4                        ------------------------------------------------------------
PDE-like A  (AI025081)          ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                  4270      4280      4290      4300      4310      4320
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                TTGGGCTAGAAGATAGATTTCTATCCTTAGCTTTGGCATTATTGACTGGATTGACTTGAA
PDE 11A2 (AF281865)               ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)     ------------------------------------------------------------
PDE 11A4                          ------------------------------------------------------------
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  4330      4340      4350      4360      4370      4380
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                CAAGTCGCTTAACTTCTACAAGCTTGTTTCCTTATTTGTCAAATTAGATTACACTAGGAA
PDE 11A2 (AF281865)               ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)     ------------------------------------------------------------
PDE 11A4                          ------------------------------------------------------------
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------

                                  4390      4400      4410      4420      4430      4440
                                  ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704)                ACGATTCTCGAACATGTTTTAACCTTACAACTCTTTGTTCAAATAAATCTTTCAATGAAT
PDE 11A2 (AF281865)               ------------------------------------------------------------
PDE 11A3 (AJ278682,HSA278682)     ------------------------------------------------------------
PDE 11A4                          ------------------------------------------------------------
PDE-like A  (AI025081)            ------------------------------------------------------------
PDE-like B                        ------------------------------------------------------------
```

Fig. 14 (continued)

```
                                        4450      4460      4470
                                ....|....|....|....|....|....|....|....
PDE 11A (AB036704)              CCCCAACATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
PDE 11A2 (AF281865)             ----------------------------------------
PDE 11A3 (AJ278682,HSA278682)   ----------------------------------------
PDE 11A4                        ----------------------------------------
PDE-like A  (AI025081)          ----------------------------------------
PDE-like B                      ----------------------------------------
```

Fig. 15

```
                                        10        20        30        40        50        60
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            MAASRLDFGEVETFLDRHPELFEDYLMRKGKQEMVEKWLQRHSQGQGALGPRPSLAGTSS
PDE 11A2 (AF281865_1)           ------------------------------------------------------------
PDE 11A3 (AJ278682, 11136969)   ------------------------------------------------------------
PDE 11A4 TR                     MAASRLDFGEVETFLDRHPELFEDYLMRKGKQEMVEKWLQRHSQGQGALGPRPSLAGTSS
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        70        80        90       100       110       120
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            LAHSTCRGGSSVGGGTGPNGSAHSQPLPGGGDCGGVPLSPSWAGGSRGDGNLQRRASQKE
PDE 11A2 (AF281865_1)           ------------------------------------------------------------
PDE 11A3 (AJ278682, 11136969)   ------------------------------------------------------------
PDE 11A4 TR                     LAHSTCRGGSSVGGGTGPNGSAHSQPLPGGGDCGGVPLSPSWAGGSRGDGNLQRRASQKE
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                       130       140       150       160       170       180
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            LRKSFARSKAIHVNRTYDEQVTSRAQEPLSSVRRRALLRKASSLPPTTAHILSALLESRV
PDE 11A2 (AF281865_1)           ------------------------------------------------------------
PDE 11A3 (AJ278682, 11136969)   --------------------------MLKQARRPLFRNVLSATQWKKVKITRLVQISGA
PDE 11A4 TR                     LRKSFARSKAIHVNRTYDEQVTSRAQEPLSSVRRRALLRKASSLPPTTAHILSALLESRV
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 15 (continued)

```
                                        190       200       210       220       230       240
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            NLPQYPPTAIDYKCHLKKHNERQFFLELVKDISNDLDLTSLSYKILIFVCLMVDADRCSL
PDE 11A2 (AF281865_1)           ------------------------------------------------------------
PDE 11A3 (AJ278682, 11136969)   SLAEKQEKHQDFLIQRQTKTK--------------------------------------
PDE 11A4 TR                     NLPQYPPTAIDYKCHLKKHNERQFFLELVKDISNDLDLTSLSYKILIFVCLMVDADRCSL
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        250       260       270       280       290       300
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            FLVEGAAAGKKTLVSKFFDVHAGTPLLPCSSTENSNEVQVPWGKGIIGYVGEHGETVNIP
PDE 11A2 (AF281865_1)           ------------------------------------------------------------
PDE 11A3 (AJ278682, 11136969)   ------------------------------------------------------------
PDE 11A4 TR                     FLVEGAAAGKKTLVSKFFDVHAGTPLLPCSSTENSNEVQVPWGKGIIGYVGEHGETVNIP
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                        310       320       330       340       350       360
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            DAYQDRRFNDEIDKLTGYKTKSLLCMPIRSSDGEIIGVAQAINKIPEGAPFTEDDEKVMQ
PDE 11A2 (AF281865_1)           ------------------------------------------------------------MQ
PDE 11A3 (AJ278682, 11136969)   ----DRRFNDEIDKLTGYKTKSLLCMPIRSSDGEIIGVAQAINKIPEGAPFTEDDEKVMQ
PDE 11A4 TR                     DAYQDRRFNDEIDKLTGYKTKSLLCMPIRSSDGEIIGVAQAINKIPEGAPFTEDDEKVMQ
PDE-like A                      ---------------------------------------------------DADNKVMQ
PDE-like B                      ----DRRFNDEIDKLTGYKTKSLLCMPIRSSDGEIIGVAQAINKIPEGAPFTEDDEKVMQ
```

Fig. 15 (continued)

```
                                        370       380       390       400       410       420
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            MYLPFCGIAISNAQLFAASRKEYERSR-----------------------------ALLE
PDE 11A2 (AF281865_1)           MYLPFCGIAISNAQLFAASRKEYERSR-----------------------------ALLE
PDE 11A3 (AJ278682, 11136969)   MYLPFCGIAISNAQLFAASRKEYERSR-----------------------------ALLE
PDE 11A4 TR                     MYLPFCGIAISNAQLFAASRKEYERSR-----------------------------ALLE
PDE-like A                      MYLPFCGIAISNAQLFAASRKEYERSRSYVRDVYLVKMSQVXPRTCFFLIVRKSCFALLE
PDE-like B                      MYLPFCGIAISNAQLFAASRKEYERSR-----------------------------ALLE

                                        430       440       450       460       470       480
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            VVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVVKFTKSFELMSPKCSA
PDE 11A2 (AF281865_1)           VVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVVKFTKSFELMSPKCSA
PDE 11A3 (AJ278682, 11136969)   VVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVVKFTKSFELMSPKCSA
PDE 11A4 TR                     VVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVVKFTKSFELMSPKCSA
PDE-like A                      VVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVS-YLLSFKVEDCVNGI
PDE-like B                      VVNDLFEEQTDLEKIVKKIMHRAQTLLKCERCSVLLLEDIESPVS-YLLSFKVEDC----

                                        490       500       510       520       530       540
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            DAENSFKESMEKSSYSDWLINNSIAELVASTGLPVNISDAYQDPRFDAEADQISGFHIRS
PDE 11A2 (AF281865_1)           DAENSFKESMEKSSYSDWLINNSIAELVASTGLPVNISDAYQDPRFDAEADQISGFHIRS
PDE 11A3 (AJ278682, 11136969)   DAENSFKESMEKSSYSDWLINNSIAELVASTGLPVNISDAYQDPRFDAEADQISGFHIRS
PDE 11A4 TR                     DAENSFKESMEKSSYSDWLINNSIAELVASTGLPVNISDAYQDPRFDAEADQISGFHIRS
PDE-like A                      KLSLFIK-------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 15 (continued)

```
                                    550       560       570       580       590       600
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            VLCVPIWNSNHQIIGVAQVLNRLDGKPFDDADQRLFEAFVIFCGLGINNTIMYDQVKKSW
PDE 11A2 (AF281865_1)           VLCVPIWNSNHQIIGVAQVLNRLDGKPFDDADQRLFEAFVIFCGLGINNTIMYDQVKKSW
PDE 11A3 (AJ278682, 11136969)   VLCVPIWNSNHQIIGVAQVLNRLDGKPFDDADQRLFEAFVIFCGLGINNTIMYDQVKKSW
PDE 11A4 TR                     VLCVPIWNSNHQIIGVAQVLNRLDGKPFDDADQRLFEAFVIFCGLGINNTIMYDQVKKSW
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    610       620       630       640       650       660
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            AKQSVALDVLSYHATCSKAEVDKFKAANIPLVSELAIDDIHFDDFSLDVDAMITAALRMF
PDE 11A2 (AF281865_1)           AKQSVALDVLSYHATCSKAEVDKFKAANIPLVSELAIDDIHFDDFSLDVDAMITAALRMF
PDE 11A3 (AJ278682, 11136969)   AKQSVALDVLSYHATCSKAEVDKFKAANIPLVSELAIDDIHFDDFSLDVDAMITAALRMF
PDE 11A4 TR                     AKQSVALDVLSYHATCSKAEVDKFKAANIPLVSELAIDDIHFDDFSLDVDAMITAALRMF
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    670       680       690       700       710       720
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            MELGMVQKFKIDYETLCRWLLTVRKNYRMVLYHNWRHAFNVCQLMFAMLTTAGFQDILTE
PDE 11A2 (AF281865_1)           MELGMVQKFKIDYETLCRWLLTVRKNYRMVLYHNWRHAFNVCQLMFAMLTTAGFQDILTE
PDE 11A3 (AJ278682, 11136969)   MELGMVQKFKIDYETLCRWLLTVRKNYRMVLYHNWRHAFNVCQLMFAMLTTAGFQDILTE
PDE 11A4 TR                     MELGMVQKFKIDYETLCRWLLTVRKNYRMVLYHNWRHAFNVCQLMFAMLTTAGFQDILTE
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 15 (continued)

```
                                    730       740       750       760       770       780
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            VEILAVIVGCLCHDLDHRGTNNAFQAKSGSALAQLYGTSATLEHHHFNHAVMILQSEGHN
PDE 11A2 (AF281865_1)           VEILAVIVGCLCHDLDHRGTNNAFQAKSGSALAQLYGTSATLEHHHFNHAVMILQSEGHN
PDE 11A3 (AJ278682, 11136969)   VEILAVIVGCLCHDLDHRGTNNAFQAKSGSALAQLYGTSATLEHHHFNHAVMILQSEGHN
PDE 11A4 TR                     VEILAVIVGCLCHDLDHRGTNNAFQAKSGSALAQLYGTSATLEHHHFNHAVMILQSEGHN
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    790       800       810       820       830       840
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            IFANLSSKEYSDLMQLLKQSILATDLTLYFERRTEFFELVSKGEYDWNIKNHRDIFRSML
PDE 11A2 (AF281865_1)           IFANLSSKEYSDLMQLLKQSILATDLTLYFERRTEFFELVSKGEYDWNIKNHRDIFRSML
PDE 11A3 (AJ278682, 11136969)   IFANLSSKEYSDLMQLLKQSILATDLTLYFERRTEFFELVSKGEYDWNIKNHRDIFRSML
PDE 11A4 TR                     IFANLSSKEYSDLMQLLKQSILATDLTLYFERRTEFFELVSKGEYDWNIKNHRDIFRSML
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------

                                    850       860       870       880       890       900
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            MTACDLGAVTKPWEISRQVAELVTSEFFEQGDRERLELKLTPSAIFDRNRKDELPRLQLE
PDE 11A2 (AF281865_1)           MTACDLGAVTKPWEISRQVAELVTSEFFEQGDRERLELKLTPSAIFDRNRKDELPRLQLE
PDE 11A3 (AJ278682, 11136969)   MTACDLGAVTKPWEISRQVAELVTSEFFEQGDRERLELKLTPSAIFDRNRKDELPRLQLE
PDE 11A4 TR                     MTACDLGAVTKPWEISRQVAELVTSEFFEQGDRERLELKLTPSAIFDRNRKDELPRLQLE
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------
```

Fig. 15 (continued)

```
                                        910       920       930       940       950       960
                                ....|....|....|....|....|....|....|....|....|....|....|....|
PDE 11A (AB036704_1)            WIDSICMPLYQALVKVNVKLKPMLDSVATNRSKWEELHQKRLLASTASSSSPASVMVAKE
PDE 11A2 (AF281865_1)           WIDSICMPLYQALVKVNVKLKPMLDSVATNRSKWEELHQKRLLASTASSSSPASVMVAKE
PDE 11A3 (AJ278682, 11136969)   WIDSICMPLYQALVKVNVKLKPMLDSVATNRSKWEELHQKRLLASTASSSSPASVMVAKE
PDE 11A4 TR                     WIDSICMPLYQALVKVNVKLKPMLDSVATNRSKWEELHQKRLLASTASSSSPASVMVAKE
PDE-like A                      ------------------------------------------------------------
PDE-like B                      ------------------------------------------------------------


                                ...
PDE 11A (AB036704_1)            DRN
PDE 11A2 (AF281865_1)           DRN
PDE 11A3 (AJ278682, 11136969)   DRN
PDE 11A4 TR                     DRN
PDE-like A                      ---
PDE-like B                      ---
```

FIG. 16

Alignment of PDE-like enzyme polypeptide with PDE11a4
Two GAF domains are underlined; PDE-like enzyme polypeptide stop codon is bold.

>PDE11a4	#1	GCAAGGAAAGCTGTCTGGGACCATGGCAGCCTCCCGCCT
>PDE11a4	#40	GGACTTTGGGGAGGTGGAAACTTTCCTGGACAGGCACCC
>PDE11a4	#79	AGAGTTGTTTGAAGATTACTTGATGCGGAAGGGGAAGCA
>PDE11a4	#118	GGAGATGGTTGAAAAGTGGCTGCAGAGGCACAGTCAGGG
>PDE11a4	#157	TCAGGGGGCTTTAGGTCCAAGGCCCTCTTTGGCTGGTAC
>PDE11a4	#196	CAGCAGCTTGGCTCACAGCACCTGCAGAGGTGGCAGCAG
>PDE11a4	#235	CGTTGGTGGTGGCACTGGACCAAATGGCTCTGCCCACAG
>PDE11a4	#274	CCAGCCCCTTCCCGGTGGCGGGGACTGTGGTGGGGTTCC
>PDE11a4	#313	CTTGAGTCCCAGCTGGGCCGGTGGCAGCAGGGGCGATGG
>PDE11a4	#352	GAACCTGCAGCGGAGAGCTTCTCAGAAAGAGCTAAGGAA
>PDE11a4	#391	GAGTTTTGCCCGCTCCAAGGCCATCCACGTGAACAGGAC
>PDE11a4	#430	CTACGATGAACAGGTGACCTCCCGGGCTCAGGAACCCCT
>PDE11a4	#469	GAGTAGTGTACGACGGAGGGCACTTCTCCGGAAGGCAAG
>PDE11a4	#508	CTCCCTGCCCCCCACCACAGCCCATATTCTCAGTGCGCT
>PDE11a4	#547	GCTGGAATCGAGAGTGAATCTGCCTCAGTATCCCCCTAC
>PDE11a4	#586	AGCCATCGACTACAAGTGCCATCTGAAAAAGCATAATGA
>PDE11a4	#625	GCGTCAGTTCTTTCTGGAATTGGTCAAAGATATCTCCAA

FIG. 16 (continued)

```
>PDE11a4    #664     TGACCTTGACCTCACCAGCCTGAGCTACAAGATTCTCAT
>PDE11a4    #703     CTTTGTCTGCCTTATGGTGGATGCTGACCGCTGCTCTCT
>PDE11a4    #742     TTTCCTGGTGGAAGGGGCAGCTGCTGGCAAGAAGACCTT
>PDE11a4    #781     GGTCTCCAAATTCTTTGATGTGCATGCAGGAACCCCTCT
>PDE11a4    #820     GCTGCCTTGCAGCAGCACAGAGAACTCAAATGAGGTGCA
>PDE11a4    #859     GGTCCCCTGGGGCAAAGGTATCATTGGCTATGTCGGGGA
>PDE11a4    #898     GCATGGAGAAACGGTCAACATTCCTGATGCCTACCAGGA
>PDE11a4    #937     TCGACGATTCAATGATGAAATCGACAAGCTAACTGGATA
>PDE11a4    #976     CAAGACAAAATCATTATTGTGCATGCCTATCCGAAGCAG
>PDE11a4    #1015    TGATGGTGAGATTATTGGTGTGGCCCAAGCGATAAATAA
>PDE11a4    #1054    GATTCCTGAAGGAGCTCCATTTACTGAAGATGATGAAAA
<PDE-LIKE   #1       GTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCAT
>PDE11a4    #1093    AGTTATGCAGATGTATCTTCCATTTTGTGGAATCGCCAT
<PDE-LIKE   #39      ATCTAACGCTCAGCTCTTTGCTGCCTCAAGGAAAGAATA
>PDE11a4    #1132    ATCTAACGCTCAGCTCTTTGCTGCCTCAAGGAAAGAATA
<PDE-LIKE   #78      TGAAAGAAGCAGAAGTTATGTAAGGGATGTGTATTAGTG
>PDE11a4    #1171    TGAAAGAAGCAGA:::::::::::::::::::::::::
<PDE-LIKE   #117     AAAATGTCTCAAGTTTGACCAAGAACCTGTTTCTTCCTG
>PDE11a4    #1210    ::::::::::::::::::::::::::::::::::::::
<PDE-LIKE   #156     ATAGTGAGGAAAAGCTGCTTTGCTTTGCTAGAGGTGGTT
>PDE11a4    #1249    ::::::::::::::::::::GCTTTGCTAGAGGTGGTT
```

FIG. 16 (continued)

<PDE-LIKE	#195	AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATT
>PDE11a4	#1288	AATGACCTCTTTGAAGAACAGACTGACCTGGAGAAAATT
<PDE-LIKE	#234	GTCAAGAAAATAATGCATCGGGCCCAAACTCTGCTGAAA
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<PDE-LIKE	#273	TGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAA
>PDE11a4	#1366	TGTGAACGCTGTTCTGTTTTACTCCTAGAGGACATCGAA
<PDE-LIKE	#312	TCACCAGT
>PDE11a4	#1405	TCACCAGTGGTGAAATTTACCAAATCCTTTGAATTGATG
>PDE11a4	#1444	TCCCCAAAGTGCAGTGCTGATGCTGAGAACAGTTTCAAA
>PDE11a4	#1483	GAAAGCATGGAGAAATCATCATACTCCGACTGGCTAATA
>PDE11a4	#1522	AATAACAGCATTGCTGAGCTGGTTGCTTCAACAGGCCTT
>PDE11a4	#1561	CCAGTGAACATCAGTGATGCCTACCAGGATCCGCGCTTT
>PDE11a4	#1600	GATGCAGAGGCAGACCAGATATCTGGTTTTCACATAAGA
>PDE11a4	#1639	TCTGTTCTTTGTGTCCCTATTTGGAATAGCAACCACCAA
>PDE11a4	#1678	ATAATTGGAGTGGCTCAAGTGTTAAACAGACTTGATGGG
>PDE11a4	#1717	AAACCTTTTGATGATGCAGATCAACGACTTTTTGAGGCT
>PDE11a4	#1756	TTTGTCATCTTTTGTGGACTTGGCATCAACAACACAATT
>PDE11a4	#1795	ATGTATGATCAAGTGAAGAAGTCCTGGGCCAAGCAGTCT
>PDE11a4	#1834	GTGGCTCTTGATGTGCTATCATACCATGCAACATGTTCA
>PDE11a4	#1873	AAAGCTGAAGTTGACAAGTTTAAGGCAGCCAACATCCCT
>PDE11a4	#1912	CTGGTGTCAGAACTTGCCATCGATGACATTCATTTTGAT

FIG. 16 (continued)

>PDE11a4	#1951	GACTTTTCTCTCGACGTTGATGCCATGATCACAGCTGCT
>PDE11a4	#1990	CTCCGGATGTTCATGGAGCTGGGGATGGTACAGAAATTT
>PDE11a4	#2029	AAAATTGACTATGAGACACTGTGTAGGTGGCTTTTGACA
>PDE11a4	#2068	GTGAGGAAAAACTATCGGATGGTTCTATACCACAACTGG
>PDE11a4	#2107	AGACATGCCTTCAACGTGTGTCAGCTGATGTTCGCGATG
>PDE11a4	#2146	TTAACCACTGCTGGGTTTCAAGACATTCTGACCGAGGTG
>PDE11a4	#2185	GAAATTTTAGCGGTGATTGTGGGATGCCTGTGTCATGAC
>PDE11a4	#2224	CTCGACCACAGGGGAACCAACAATGCCTTCCAAGCTAAG
>PDE11a4	#2263	AGTGGCTCTGCCCTGGCCCAACTCTATGGAACCTCTGCT
>PDE11a4	#2302	ACCTTGGAGCATCACCATTTCAACCACGCCGTGATGATC
>PDE11a4	#2341	CTTCAAAGTGAGGGTCACAATATCTTTGCTAACCTGTCC
>PDE11a4	#2380	TCCAAGGAATATAGTGACCTTATGCAGCTTTTGAAGCAG
>PDE11a4	#2419	TCAATATTGGCAACAGACCTCACGCTGTACTTTGAGAGG
>PDE11a4	#2458	AGAACTGAATTCTTTGAACTTGTCAGTAAAGGAGAATAC
>PDE11a4	#2497	GATTGGAACATCAAAAACCATCGTGATATATTTCGATCA
>PDE11a4	#2536	ATGTTAATGACAGCCTGTGACCTTGGAGCCGTGACCAAA
>PDE11a4	#2575	CCGTGGGAGATCTCCAGACAGGTGGCAGAACTTGTAACC
>PDE11a4	#2614	AGTGAGTTCTTCGAACAAGGAGATCGGGAGAGATTAGAG
>PDE11a4	#2653	CTCAAACTCACTCCTTCAGCAATTTTTGATCGGAACCGG

FIG. 16 (continued)

```
>PDE11a4    #2692    AAGGATGAACTGCCTCGGTTGCAACTGGAGTGGATTGAT
>PDE11a4    #2731    AGCATCTGCATGCCTTTGTATCAGGCACTGGTGAAGGTC
>PDE11a4    #2770    AACGTGAAACTGAAGCCGATGCTAGATTCAGTAGCTACA
>PDE11a4    #2809    AACAGAAGTAAGTGGGAAGAGCTACACCAAAAACGACTG
>PDE11a4    #2848    CTGGCCTCAACTGCCTCATCCTCCTCCCCTGCCAGTGTT
>PDE11a4    #2887    ATGGTAGCCAAGGAAGACAGGAACTAAACCTCCAGGTCA
>PDE11a4    #2926    GCTGCAGCTGCAAAATGACTACAGCCTGAAGGGCCATTT
>PDE11a4    #2965    TCAGTCCAGCA
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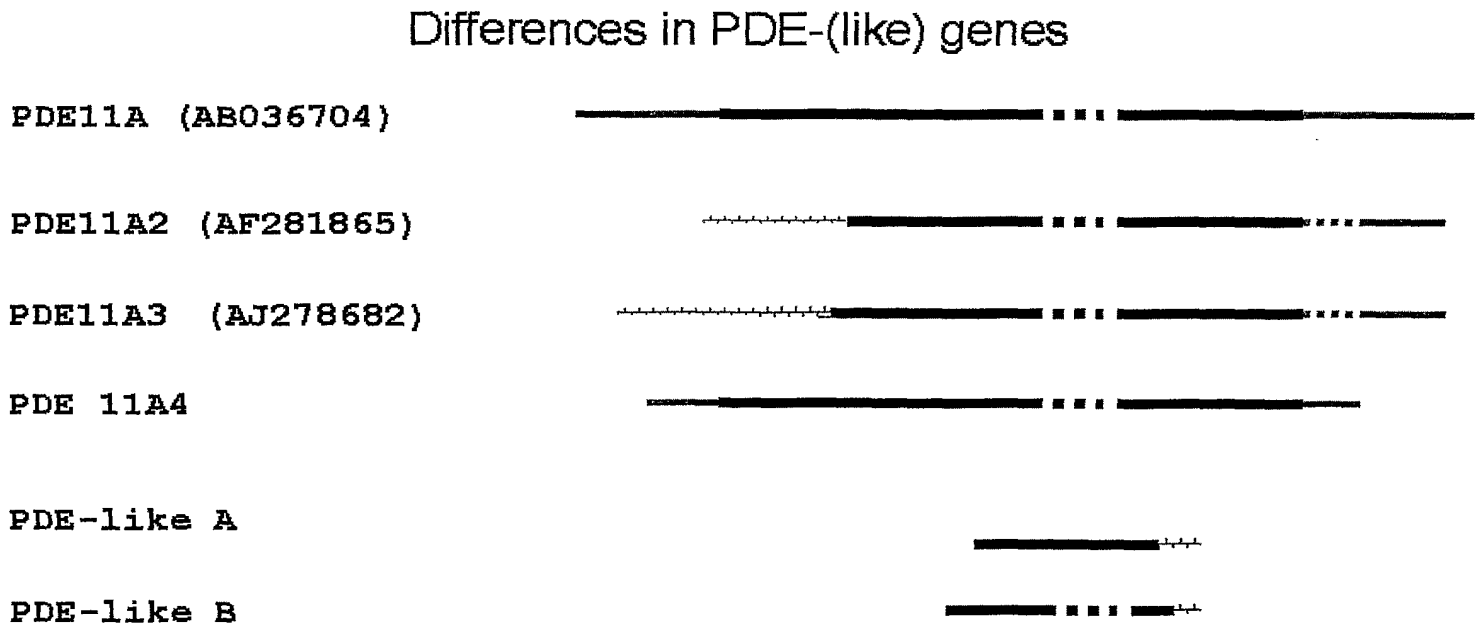
Fig. 17

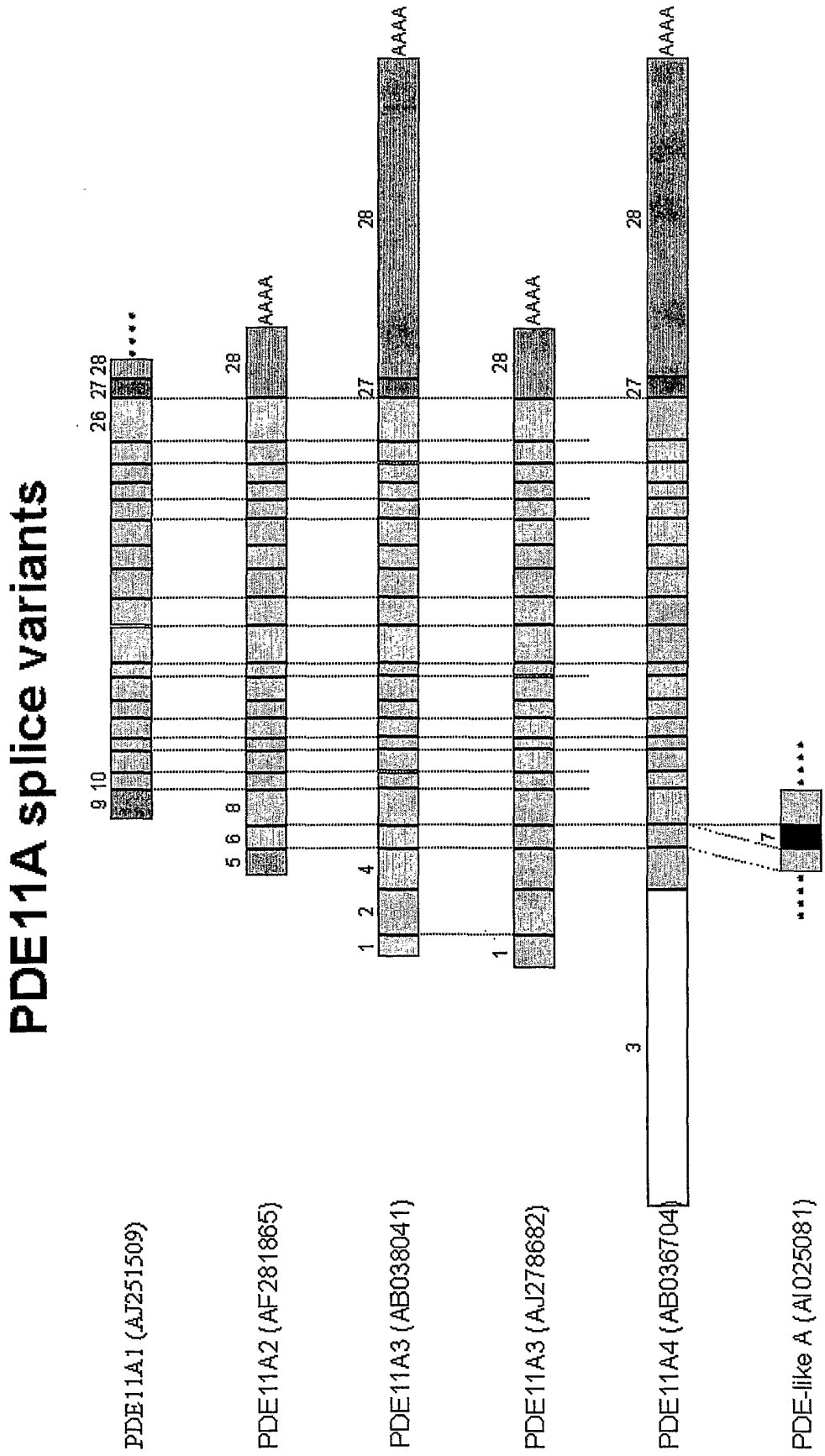


CDS in blue, UTR in green Not to scale

▪▪▪▪ or ···· Indicates missing in some

or Indicates different

Fig. 18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PDE11A2 (AF281865)
PDE11A3 (AB038041)
PDE11A3 (AJ278682)
PDE11A4 (AB036704)
PDE-like A (AI025081)
AAAA
AAAA
AAAA
AAAA
1
2
3
4
5
6
7
8
9
10
26
27
28

Fig. 19

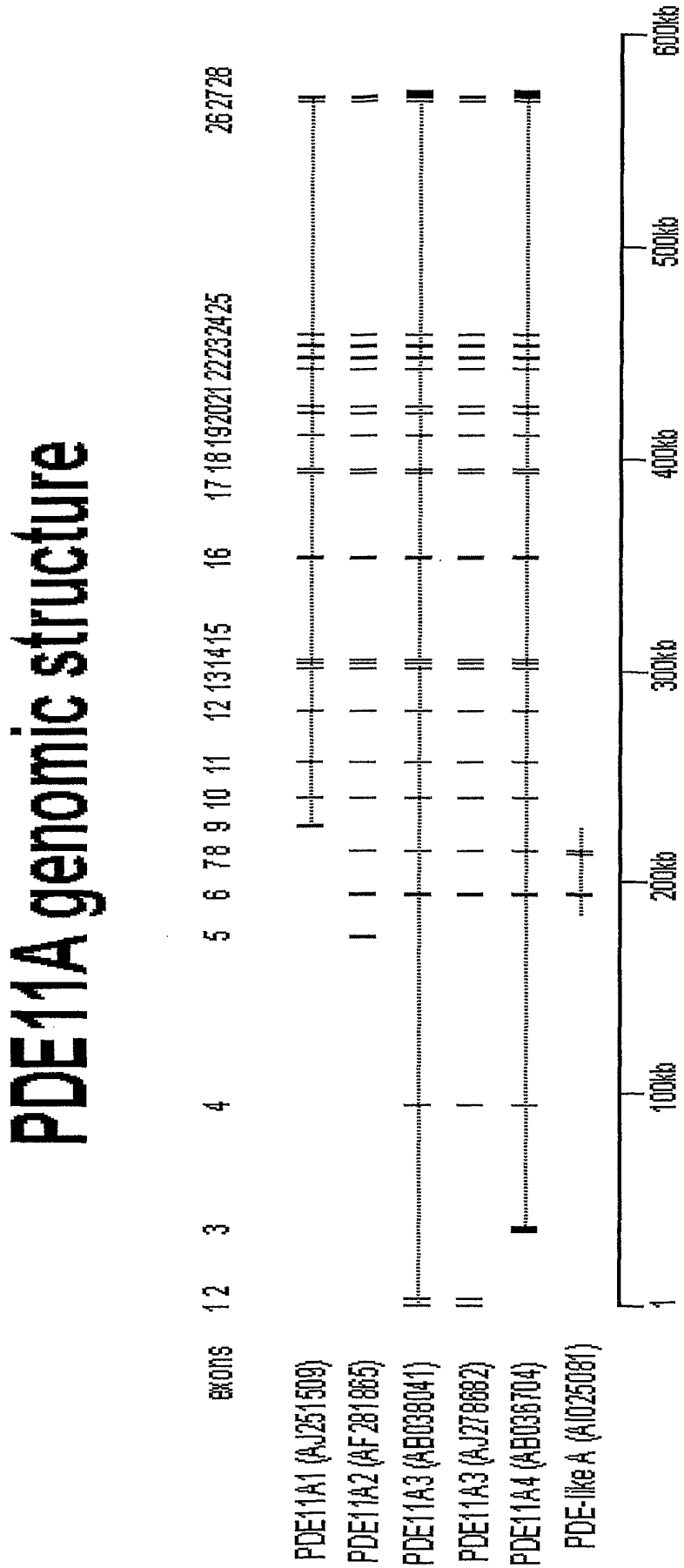

PDE11A genomic structure
exons
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
PDE11A1 (AJ251509)
PDE11A2 (AF281865)
PDE11A3 (AB039041)
PDE11A3 (AJ278682)
PDE11A4 (AB036704)
PDE-like A (AI025081)
1
100kb
200kb
300kb
400kb
500kb
600kb

Fig. 20

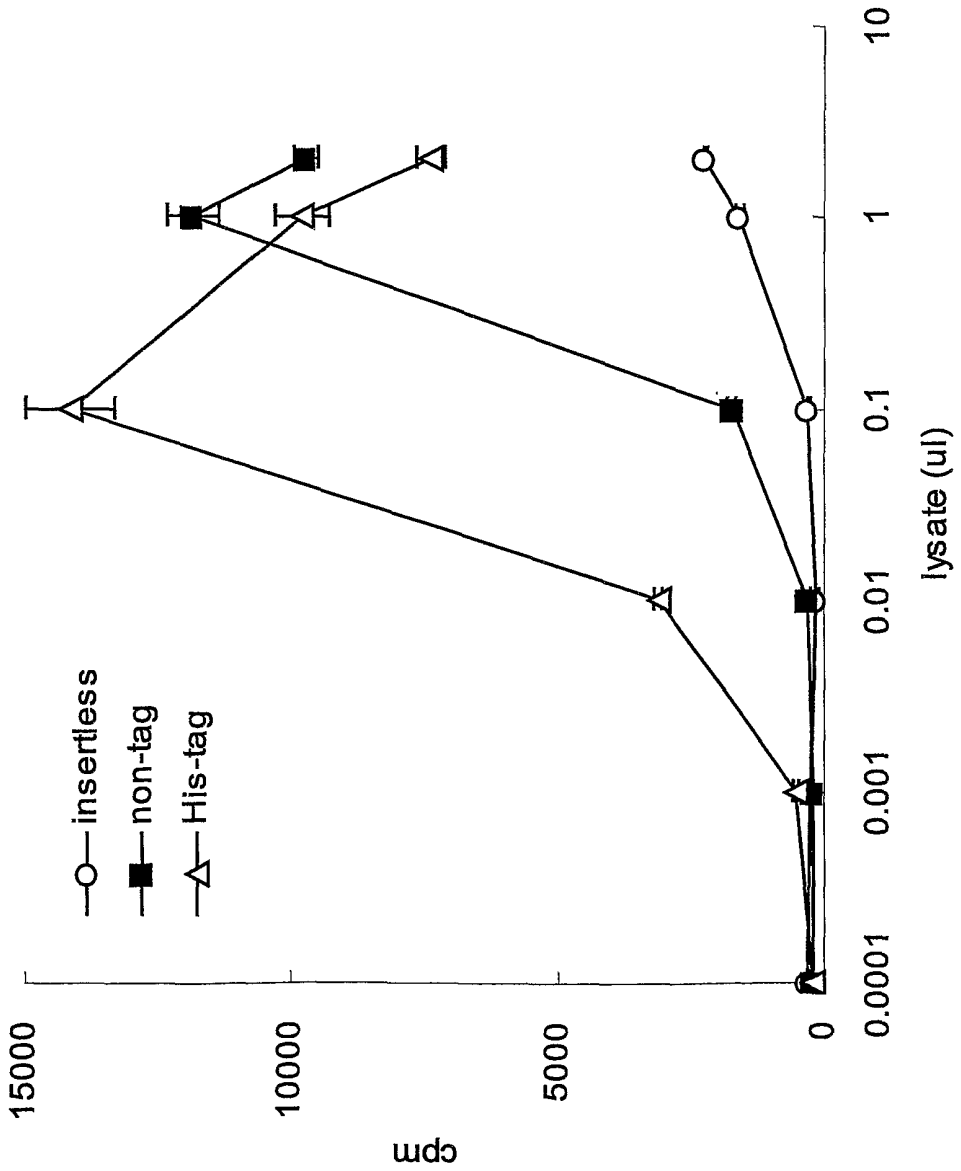

insertless
non-tag
His-tag
15000
10000
5000
0
cpm
0.0001
0.001
0.01
0.1
1
10
lysate (ul)

Fig. 21

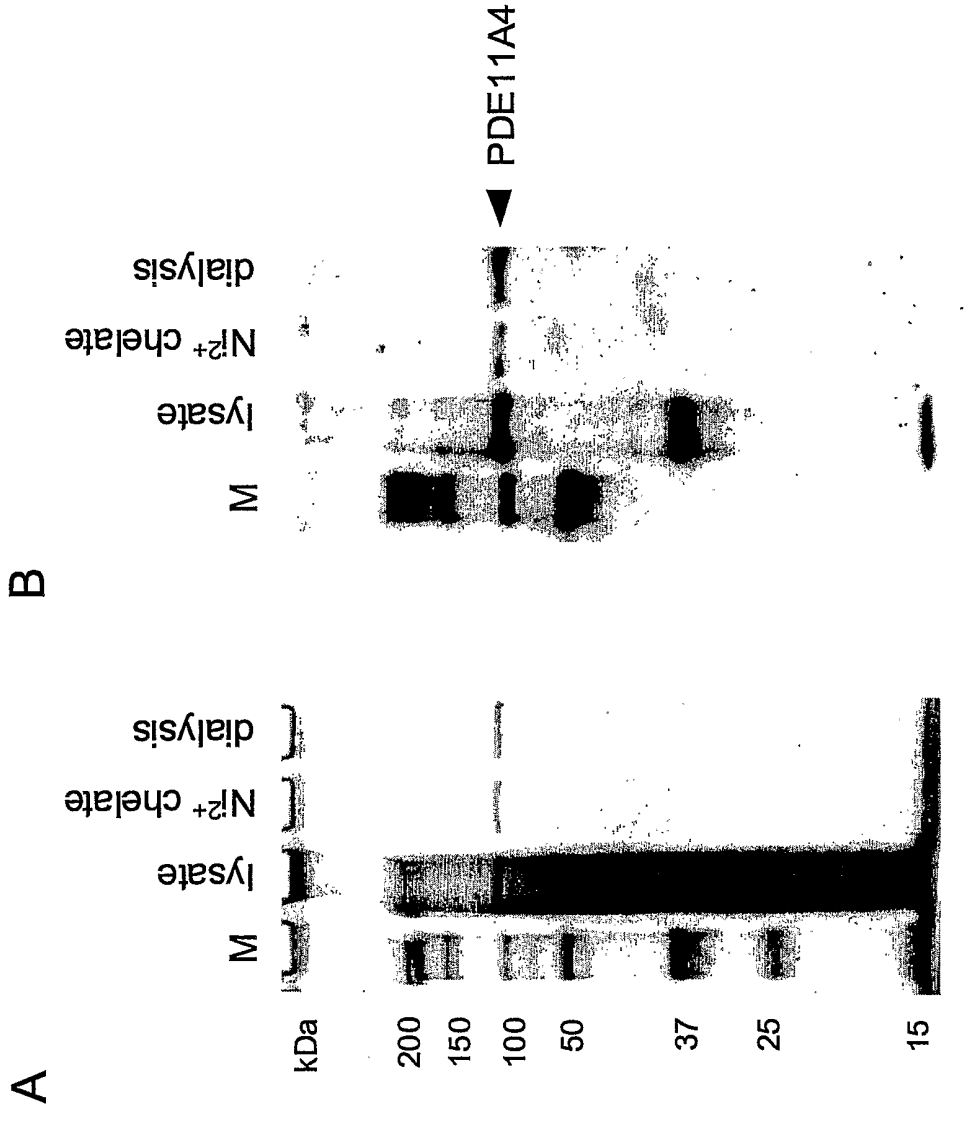

A
kDa
200
150
100
50
37
25
15
M
lysate
Ni2+ chelate
dialysis
B
M
lysate
Ni2+ chelate
dialysis
PDE11A4

Fig. 22

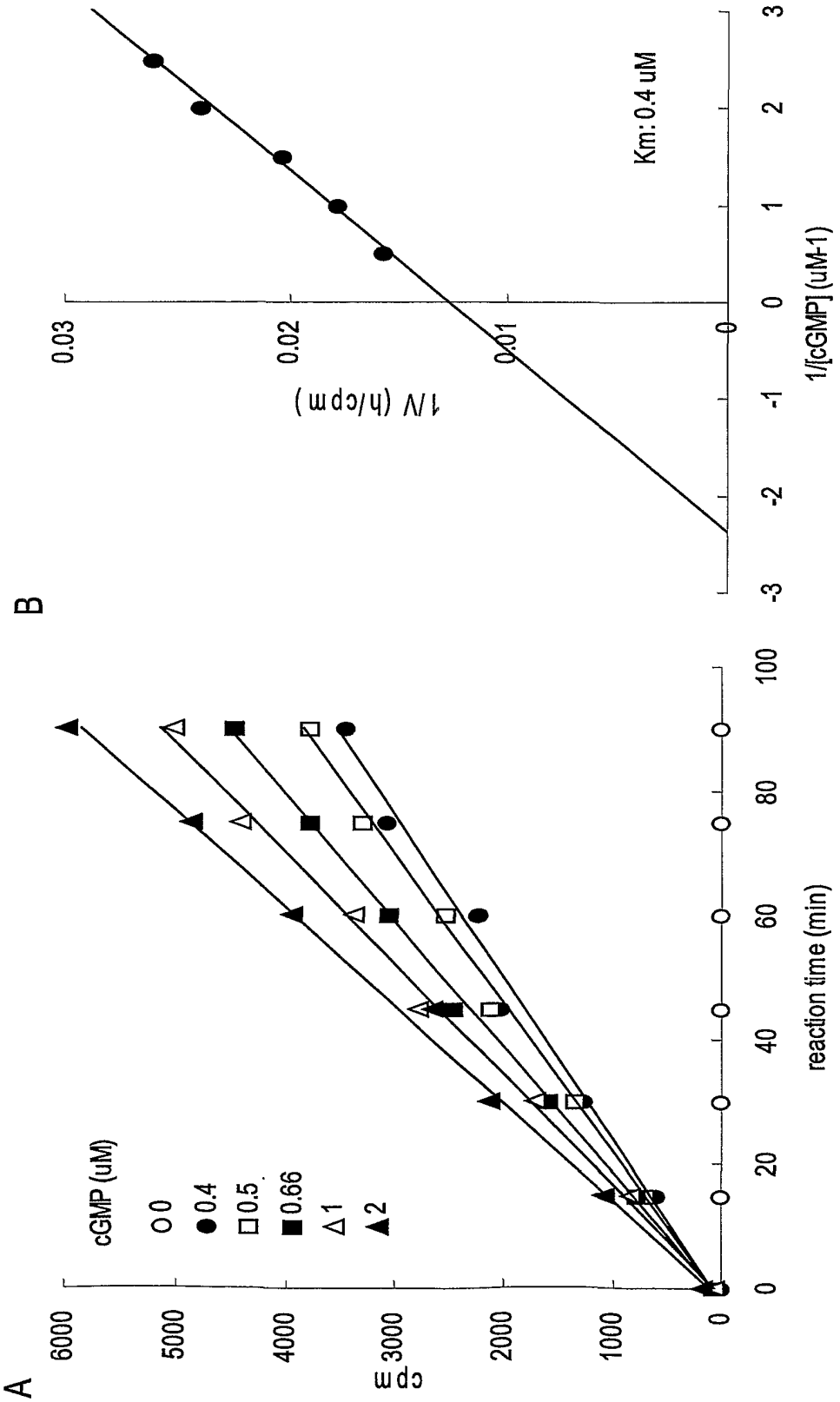

A
cpm
6000
5000
4000
3000
2000
1000
0
cGMP (uM)
0
0.4
0.5
0.66
1
2
0
20
40
60
80
100
reaction time (min)
B
1/V (h/cpm)
0.03
0.02
0.01
0
-3
-2
-1
0
1
2
3
1/[cGMP] (uM-1)
Km: 0.4 uM

Fig. 23

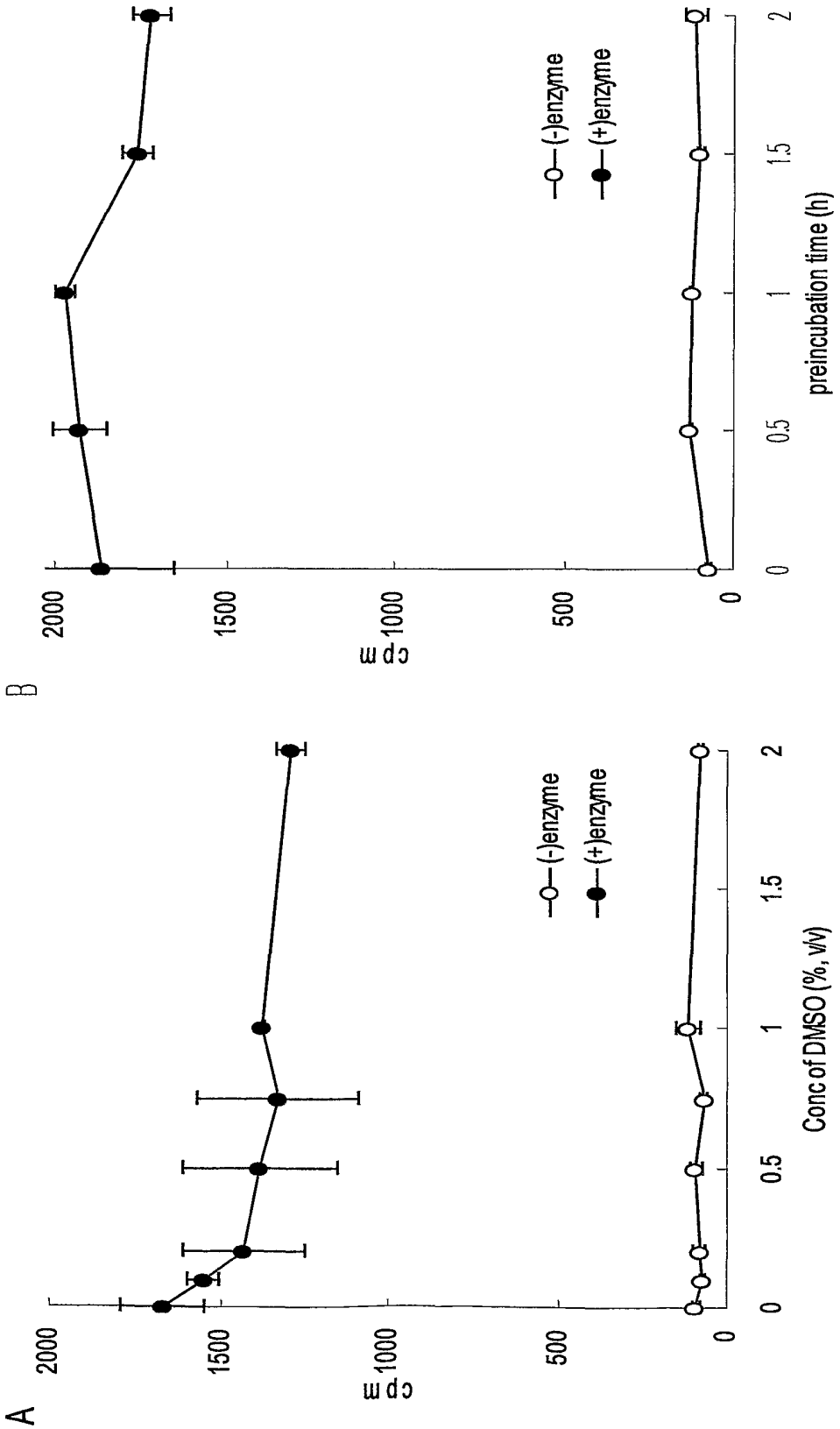

A
B
cpm
2000
1500
1000
500
0
0
0.5
1
1.5
2
Conc of DMSO (%, v/v)
preincubation time (h)
(-)enzyme
(+)enzyme

Fig. 24

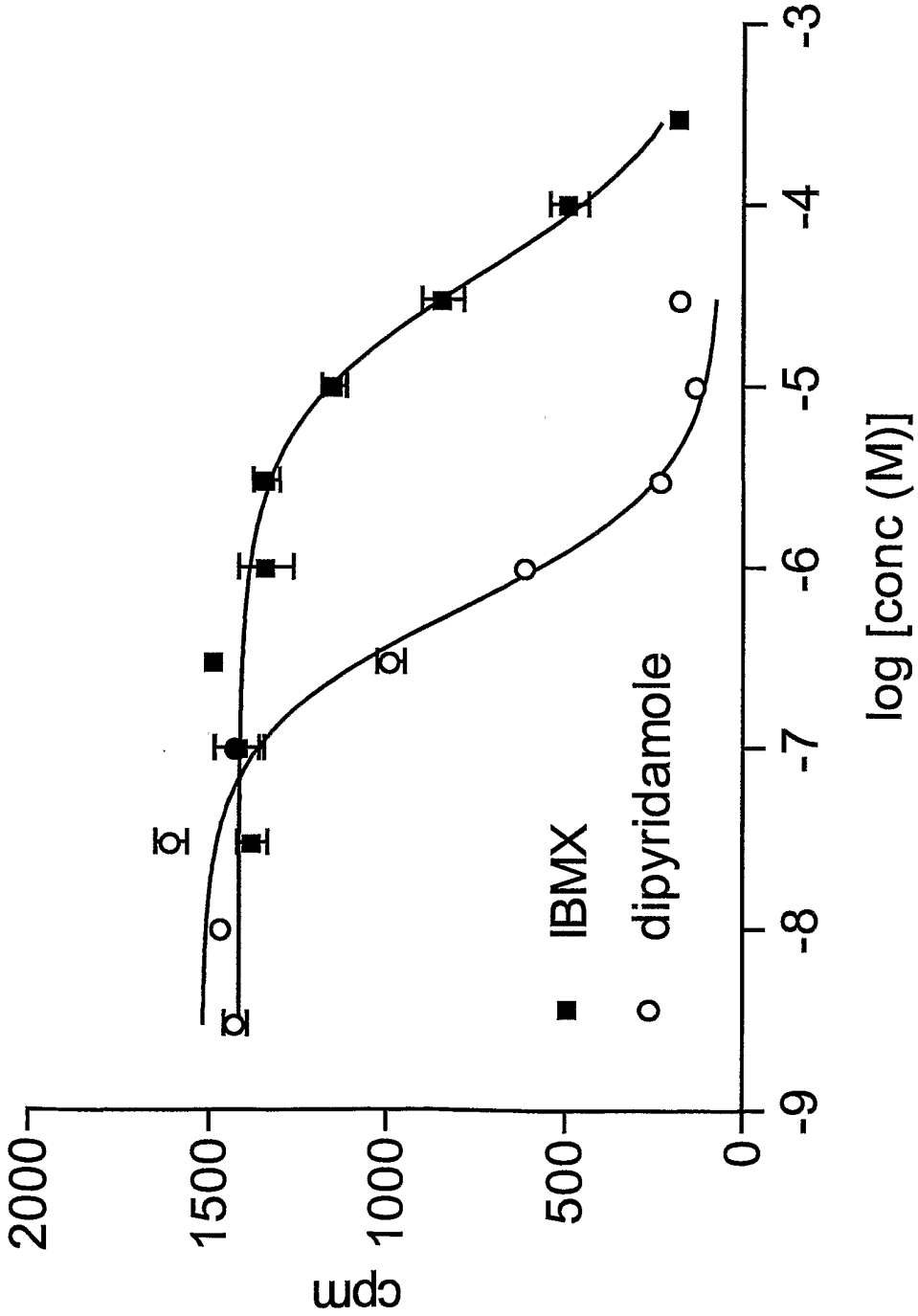
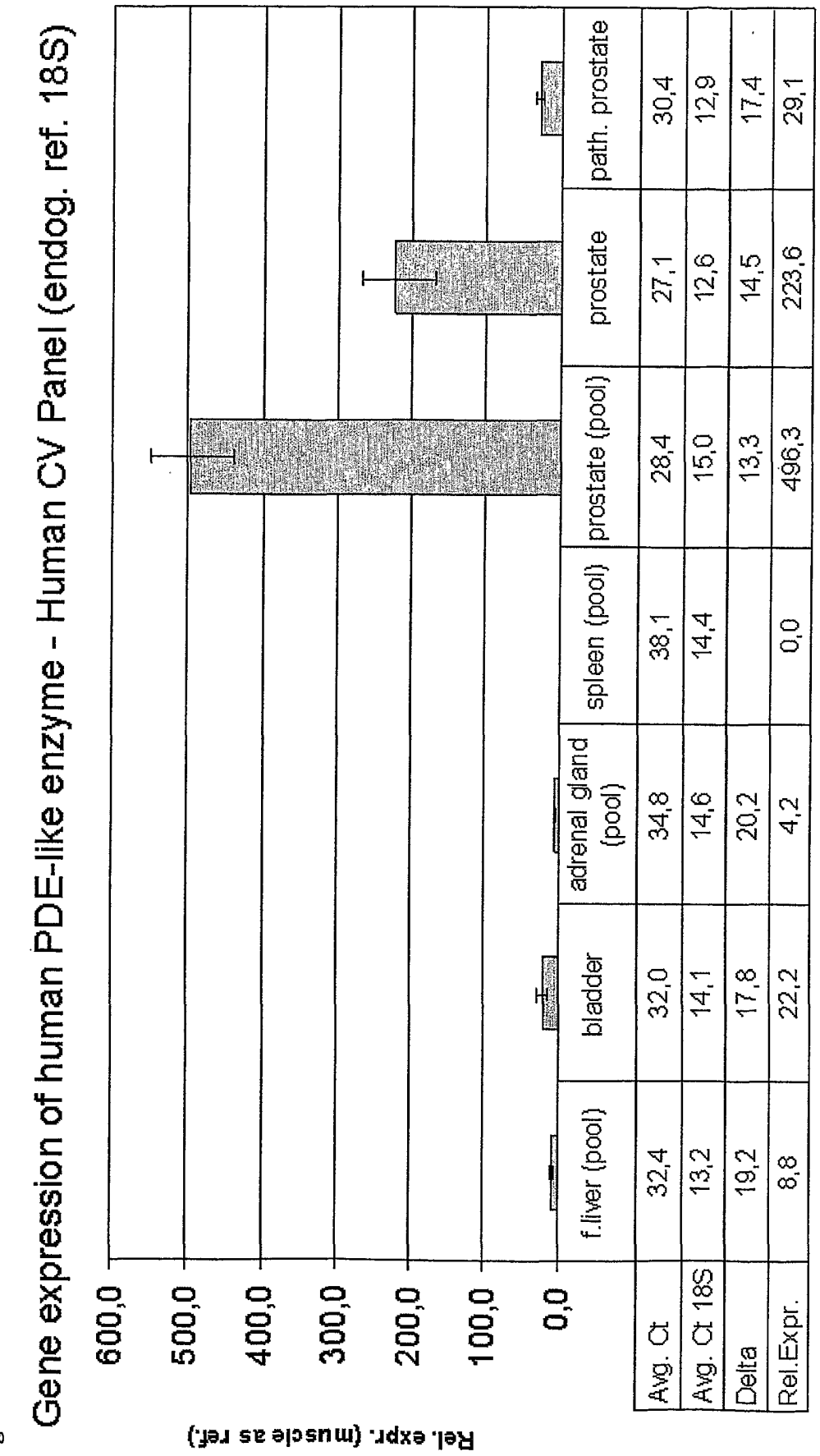

2000
1500
1000
500
0
cpm
-9
-8
-7
-6
-5
-4
-3
log [conc (M)]
IBMX
dipyridamole

Fig. 25



	f.liver (pool)	bladder	adrenal gland (pool)	spleen (pool)	prostate (pool)	prostate	path. prostate
Avg. Ct	32,4	32,0	34,8	38,1	28,4	27,1	30,4
Avg. Ct 18S	13,2	14,1	14,6	14,4	15,0	12,6	12,9
Delta	19,2	17,8	20,2		13,3	14,5	17,4
Rel.Expr.	8,8	22,2	4,2	0,0	496,3	223,6	29,1

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Arg Ser Arg Ser Tyr Val Arg Asp Val Tyr Xaa Leu Val Lys Met Ser
35 40 45

Gln Val Xaa Pro Arg Thr Cys Phe Phe Leu Ile Val Arg Lys Ser Cys
50 55 60

Phe Ala Leu Leu Glu Val Val Asn Asp Leu Phe Glu Glu Gln Thr Asp
65 70 75 80

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

Lys Cys Glu Arg Cys Ser Val Leu Leu Leu Glu Asp Ile Glu Ser Pro
100 105 110

Val Ser Tyr Leu Leu Ser Phe Lys Val Glu Asp Cys Val Asn Gly Ile
115 120 125

Lys Leu Ser Leu Phe Ile Lys
130 135

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His Thr Glu Ser Cys Ser Cys Ser Leu Pro Gln Ser Pro His Ala Asp
35 40 45

Asn Thr Thr Pro Gly Ala Pro Ala Arg Lys Ile Ser Ala Ser Glu Phe
50 55 60

Asp Arg Pro Leu Arg Pro Ile Val Val Lys Asp Ser Glu Gly Thr Val
65 70 75 80

Ser Phe Leu Ser Asp Ser Gly Lys Lys Glu Gln Met Pro Leu Thr Ser
85 90 95

Pro Arg Phe Asp Ser Asp Glu Gly Asp Gln Cys Ser Arg Leu Leu Glu
100 105 110

Leu Val Lys Asp Ile Ser Ser His Leu Asp Val Thr Ala Leu Cys His
115 120 125

Lys Ile Phe Leu His Ile His Gly Leu Ile Ser Ala Asp Arg Tyr Ser
130 135 140

Leu Phe Leu Val Cys Glu Asp Ser Ser Lys Asp Lys Phe Leu Val Ser
145 150 155 160

Arg Leu Phe Asp Val Ala Glu Gly Ser Thr Leu Glu Glu Ala Ser Asn
165 170 175

Asn Cys Ile Arg Leu Glu Trp Asn Lys Gly Ile Val Gly His Val Ala
180 185 190

Ala Phe Gly Glu Pro Leu Asn Ile Lys Asp Ala Tyr Glu Asp Pro Arg
195 200 205

Phe Asn Ala Glu Val Asp Gln Ile Thr Gly Tyr Lys Thr Gln Ser Ile
210 215 220

Leu Cys Met Pro Ile Lys Asn His Arg Glu Glu Val Val Gly Val Ala
225 230 235 240

Gln Ala Ile Asn Lys Lys Ser Gly Asn Gly Gly Thr Phe Thr Glu Lys
245 250 255

Asp Glu Lys Asp Phe Ala Ala Tyr Leu Ala Phe Cys Gly Ile Val Leu
260 265 270

His Asn Ala Gln Leu Tyr Glu Thr Ser Leu Leu Glu Asn Lys Arg Asn
275 280 285

Gln Val Leu Leu Asp Leu Ala Ser Leu Ile Phe Glu Glu Gln Gln Ser
290 295 300

Leu Glu Val Ile Leu Lys Lys Ile Ala Ala Thr Ile Ile Ser Phe Met
305 310 315 320

Gln Val Gln Lys Cys Thr Ile Phe Ile Val Asp Glu Asp Cys Pro Asp
325 330 335

Ser Phe Ser Arg Val Phe Gln Met Glu Trp Glu Glu Val Gly Lys Ser
340 345 350

Ser Glu Pro Leu Thr Arg Glu His Asp Ala Asn Lys Ile Asn Tyr Met
355 360 365

Tyr Ala Gln Tyr Val Lys Asn Thr Met Glu Pro Leu Asn Ile Pro Asp
370 375 380

Val Thr Lys Asp Asn Arg Phe Pro Trp Thr Asn Glu Asn Met Gly His
385 390 395 400

Ile Asn Thr His Cys Ile Arg Ser Leu Leu Cys Thr Pro Ile Lys Asn
405 410 415

Gly Lys Lys Asn Lys Val Ile Gly Val Cys Gln Leu Val Asn Lys Met
420 425 430

Glu Glu Lys Thr Gly Lys Ile Lys Ala Phe Asn Gln Asn Asp Glu Gln
435 440 445

Phe Leu Glu Ala Phe Val Ile Phe Cys Gly Leu Gly Ile Gln Asn Thr
450 455 460

Gln Met Tyr Glu Ala Val Glu Arg Ala Met Ala Lys Gln Met Val Thr
465 470 475 480

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485 490 495

Glu Leu Gln Ala Leu Ala Ala Ala Val Val Pro Ser Ala Gln Thr Leu
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Lys Ile Thr Asp Phe Ser Phe Ser Asp Phe Glu Leu Ser Asp Leu Glu
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Thr Ala Leu Cys Thr Ile Arg Met Phe Thr Asp Leu Asn Leu Val Gln
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Asn Phe Gln Met Lys His Glu Val Leu Cys Arg Trp Ile Leu Ser Val
545 550 555 560

Lys Lys Asn Tyr Arg Lys Asn Val Ala Tyr His Asn Trp Arg His Ala
565 570 575

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Gln Asn Lys Leu Thr Asp Leu Glu Thr Leu Ala Leu Leu Ile Ala Ala
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Leu Ser His Asp Leu Asp His Arg Gly Val Asn Asn Ser Tyr Ile Gln
610 615 620

Arg Ser Glu His Pro Leu Ala Gln Leu Tyr Cys His Ser Thr Met Glu
625 630 635 640

His His His Phe Asp Gln Cys Leu Met Val Leu Asn Ser Pro Gly Asn
645 650 655

Gln Ile Leu Ser Gly Leu Ser Ile Glu Glu Tyr Lys Thr Thr Leu Lys
660 665 670

Ile Ile Lys Gln Ala Ile Leu Ala Thr Asp Leu Ala Leu Tyr Ile Lys
675 680 685

Arg Arg Gly Glu Phe Phe Glu Leu Ile Arg Lys Asn Glu Phe Ser Phe
690 695 700

Glu Asp Pro Leu Gln Lys Glu Leu Phe Leu Ala Met Leu Met Thr Ala
705 710 715 720

Cys Asp Leu Ser Ala Ile Thr Lys Pro Trp Pro Ile Gln Gln Arg Ile
725 730 735

Ala Glu Leu Val Ala Ala Glu Phe Phe Asp Gln Gly Asp Arg Glu Arg
740 745 750

Lys Glu Leu Asn Met Glu Pro Ala Asp Leu Met Asn Arg Glu Lys Lys
755 760 765

Asn Lys Ile Pro Ser Met Gln Val Gly Phe Ile Asp Ala Ile Cys Leu
770 775 780

Gln Leu Tyr Glu Ala Leu Thr His Val Ser Glu Asp Cys Leu Pro Leu
785 790 795 800

Leu Asp Gly Cys Arg Lys Asn Arg Gln Lys Trp Gln Ala Leu Ala Asp
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820 825 830

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attcctgaag gagctccatt tactgaagat gatgaaaaag ttatgcagat gtatcttcca 180
ttttgtggaa tcgccatatc taacgctcag ctctttgctg cctcaaggaa agaatatgaa 240
agaagcagag ctttgctaga ggtggttaat gacctctttg aagaacagac tgacctggag 300
aaaattgtca agaaaataat gcatcgggcc caaactctgc tgaaatgtga acgctgttct 360
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35 40 45

Glu Asp Asp Glu Lys Val Met Gln Met Tyr Leu Pro Phe Cys Gly Ile
50 55 60

Ala Ile Ser Asn Ala Gln Leu Phe Ala Ala Ser Arg Lys Glu Tyr Glu
65 70 75 80

Arg Ser Arg Ala Leu Leu Glu Val Val Asn Asp Leu Phe Glu Glu Gln
85 90 95

Thr Asp Leu Glu Lys Ile Val Lys Lys Ile Met His Arg Ala Gln Thr
100 105 110

Leu Leu Lys Cys Glu Arg Cys Ser Val Leu Leu Leu Glu Asp Ile Glu
115 120 125

Ser Pro Val Ser Tyr Leu Leu Ser Phe Lys Val Glu Asp Cys

130 135 140