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(54) **METHODS AND COMPOSITIONS RELATING TO PBK1**

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C12N 9/12	(2006.01)
A61P 3/10	(2006.01)

(52) **U.S. Cl.** **800/3**; 435/7.21; 435/325; 435/7.2; 435/194

(57) **ABSTRACT**

The invention relates to compositions comprising, and methods utilizing PBK1 protein and DNA, including a method of detecting type 1 diabetes; a mammalian pancreas-derived cell comprising a recombinant nucleic acid encoding a PBK1 protein; a method of identifying a PBK1 modulator; a pharmaceutical composition for treatment of type 2 diabetes in a subject; a method of screening for an agent that treats a metabolic disease; delivery of PBK-1 DNA to a subject to stimulate pancreatic beta cell differentiation and/or regeneration; a method for stimulating cell differentiation and/or regeneration in a pancreatic beta cell; usage of transgenic mice with targeted deletion or overexpression of the PBK-1 gene to test efficacy and specificity of PBK-1 modulator compounds.

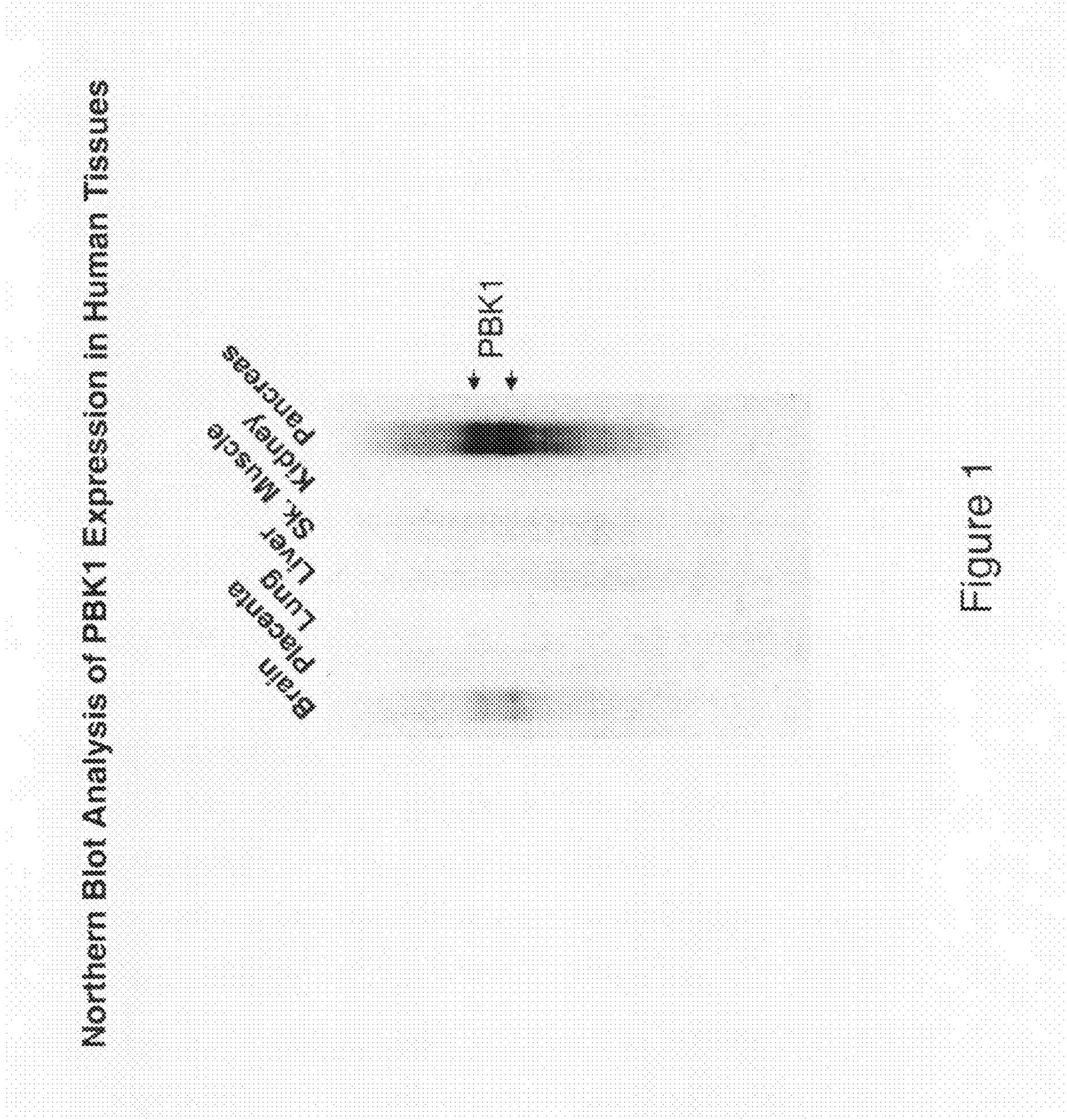


Figure 1

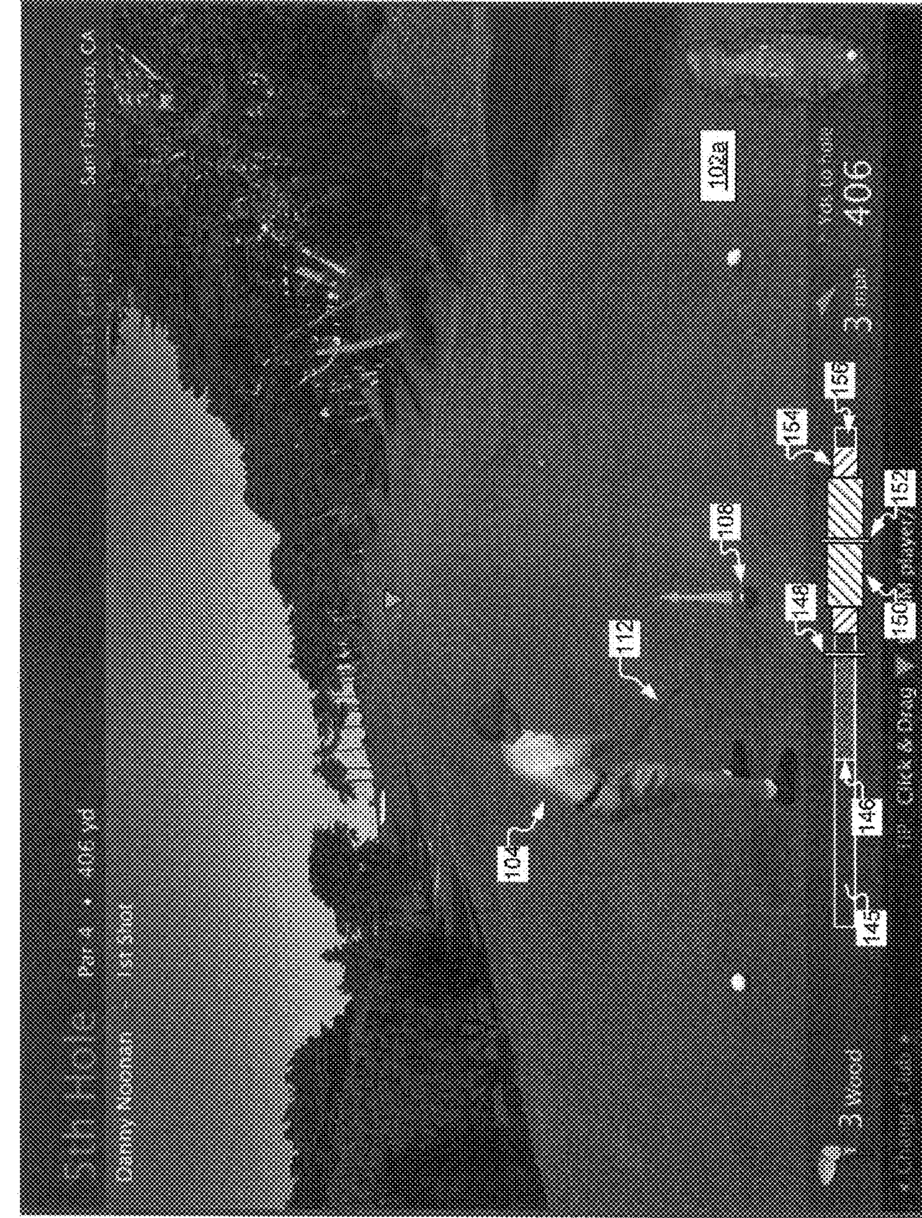


FIG. 1A

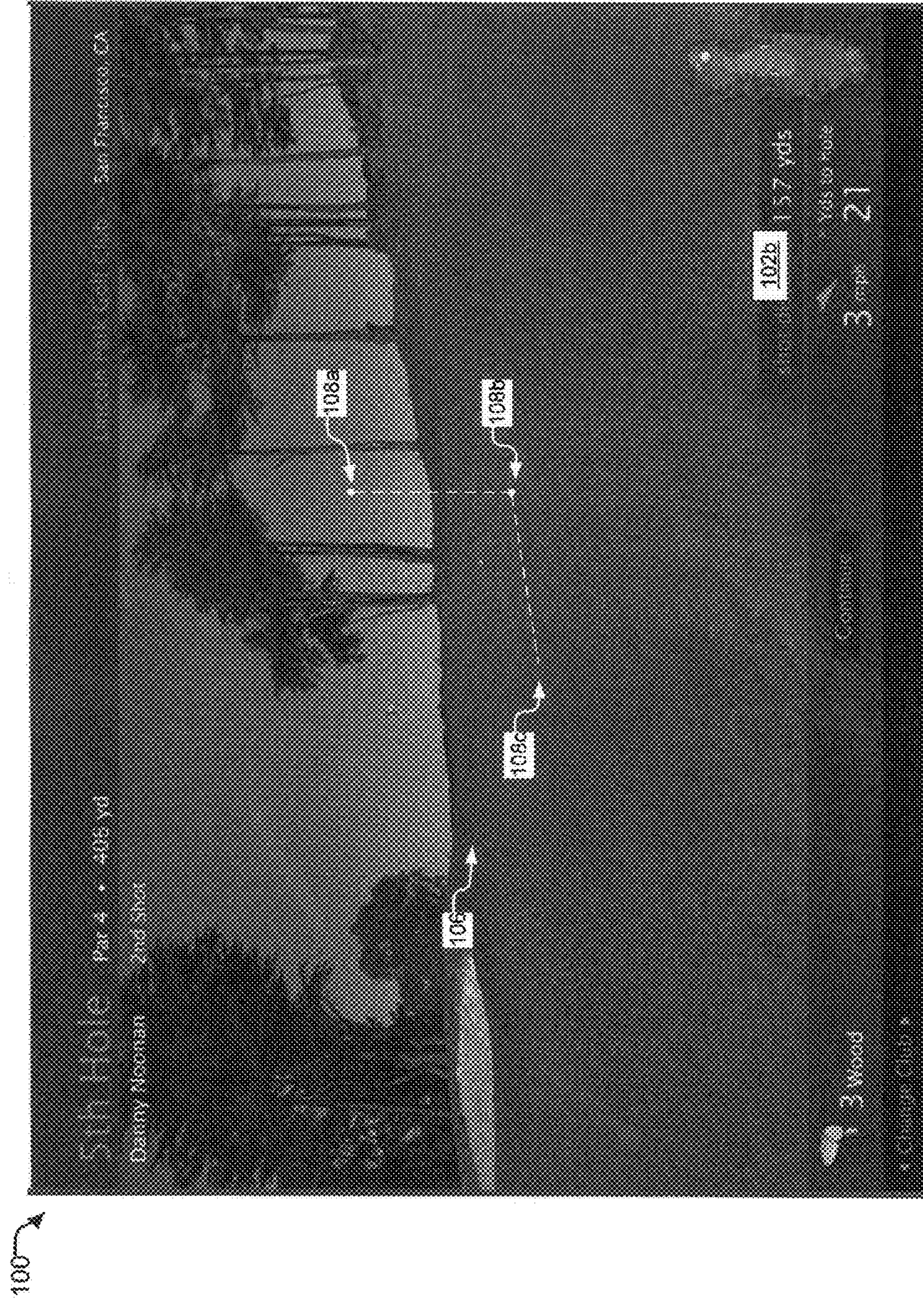


FIG. 1B

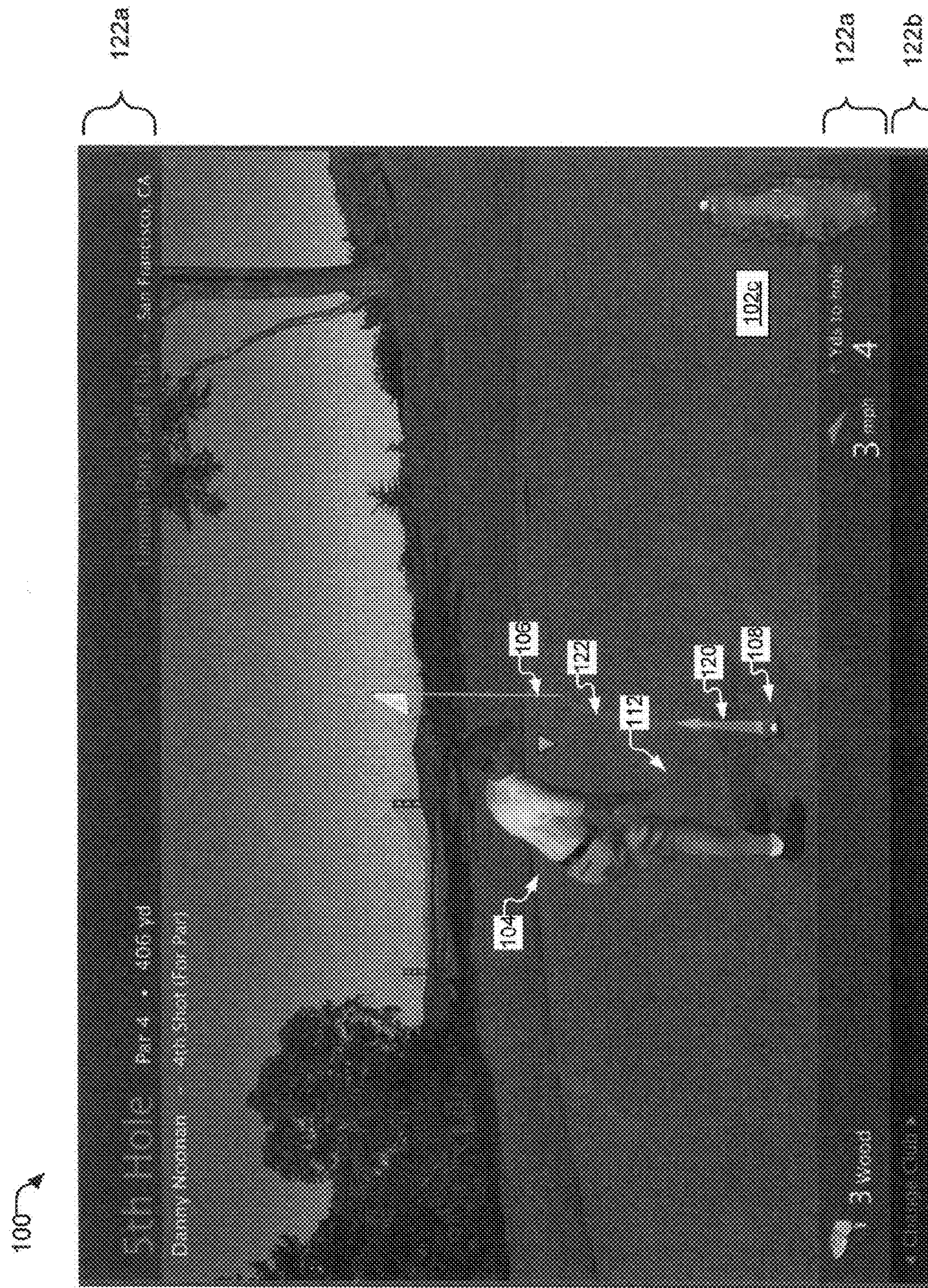


FIG. 1C

Western Blot Analysis of PBK1 Expression in Mouse Pancreas

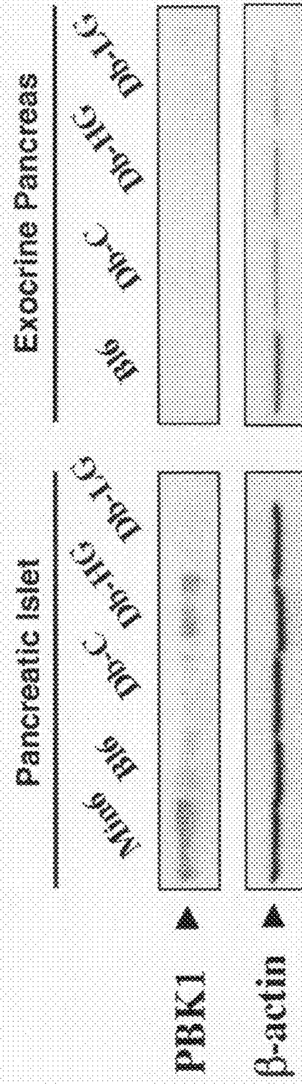
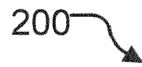


Figure 2



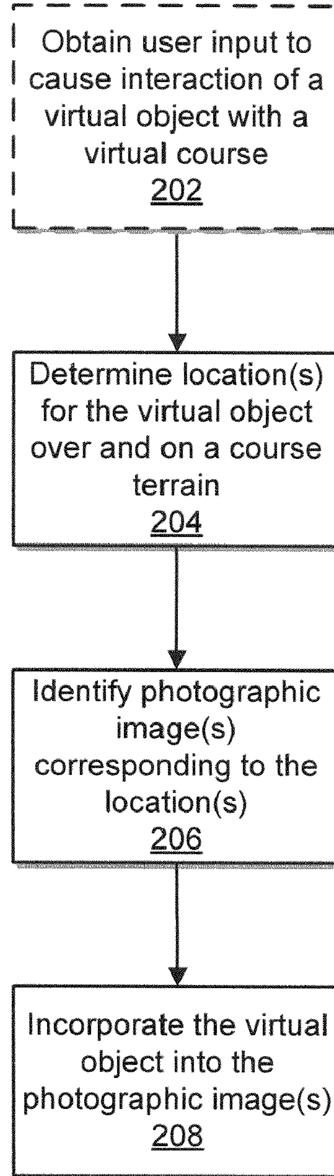
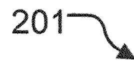


FIG. 2A



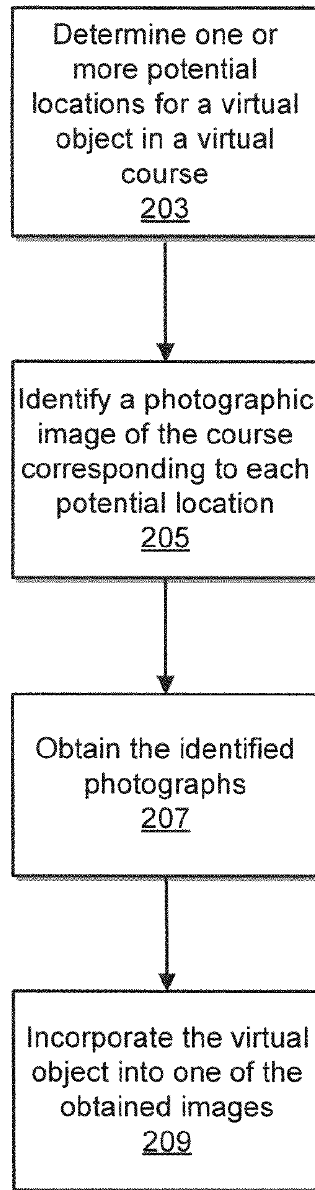


FIG. 2B

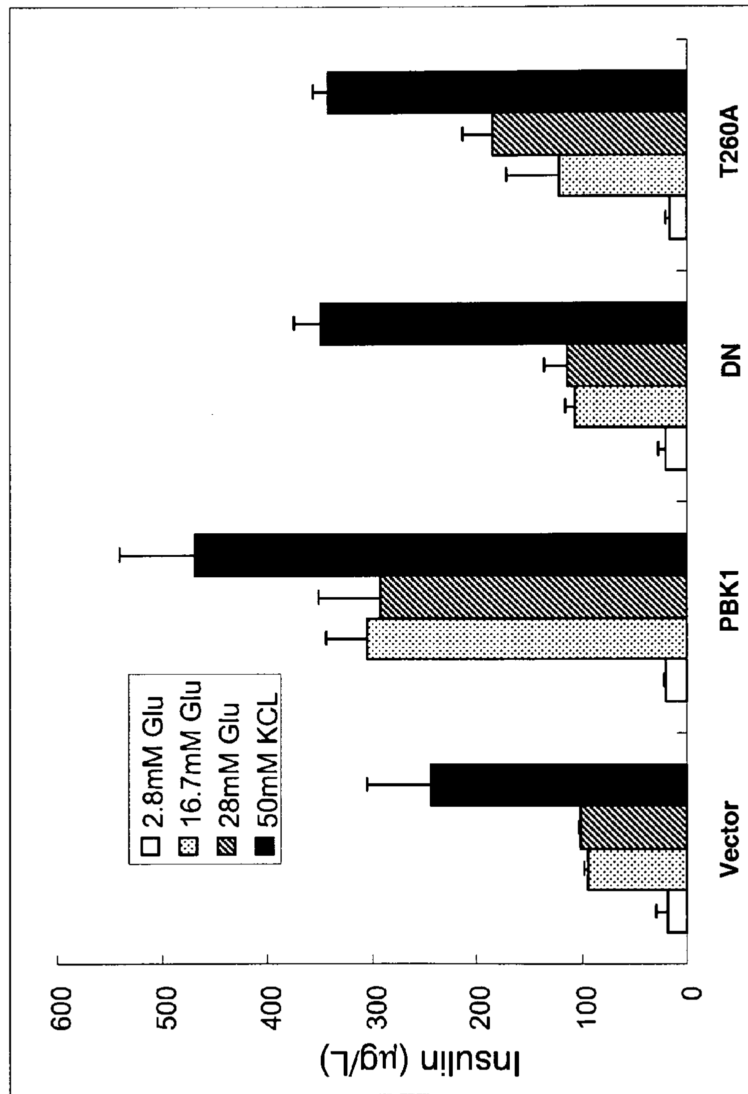


Figure 3

300

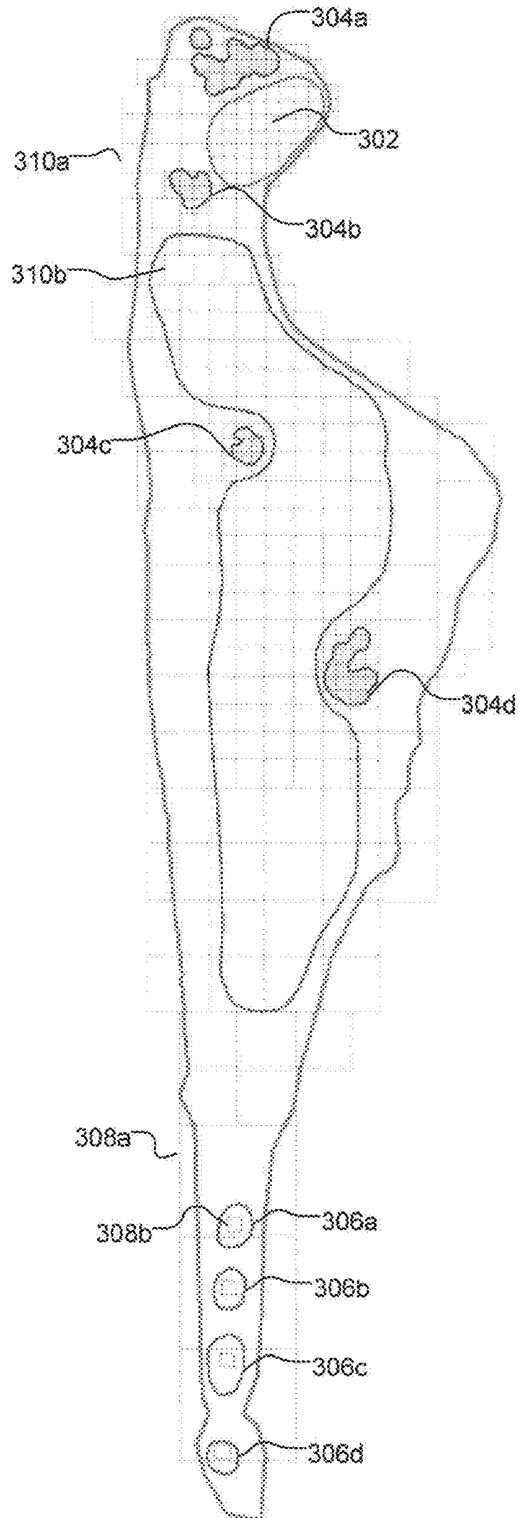


FIG. 3A

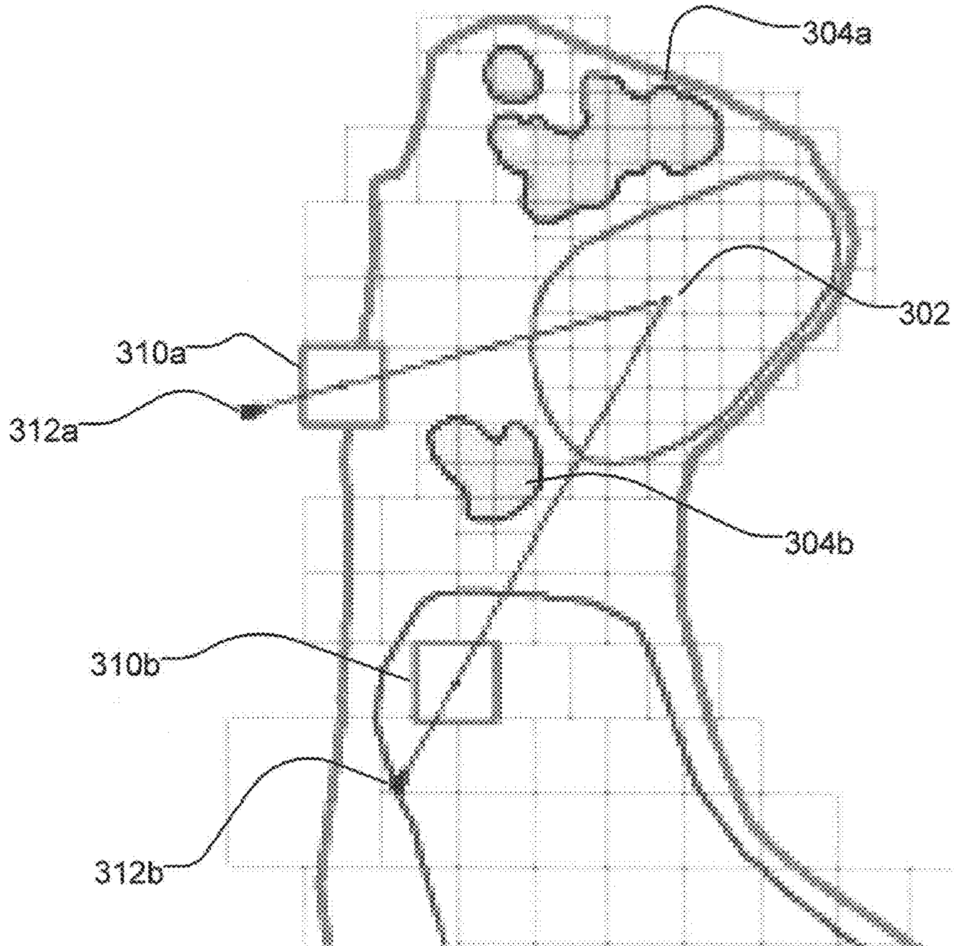


FIG. 3B

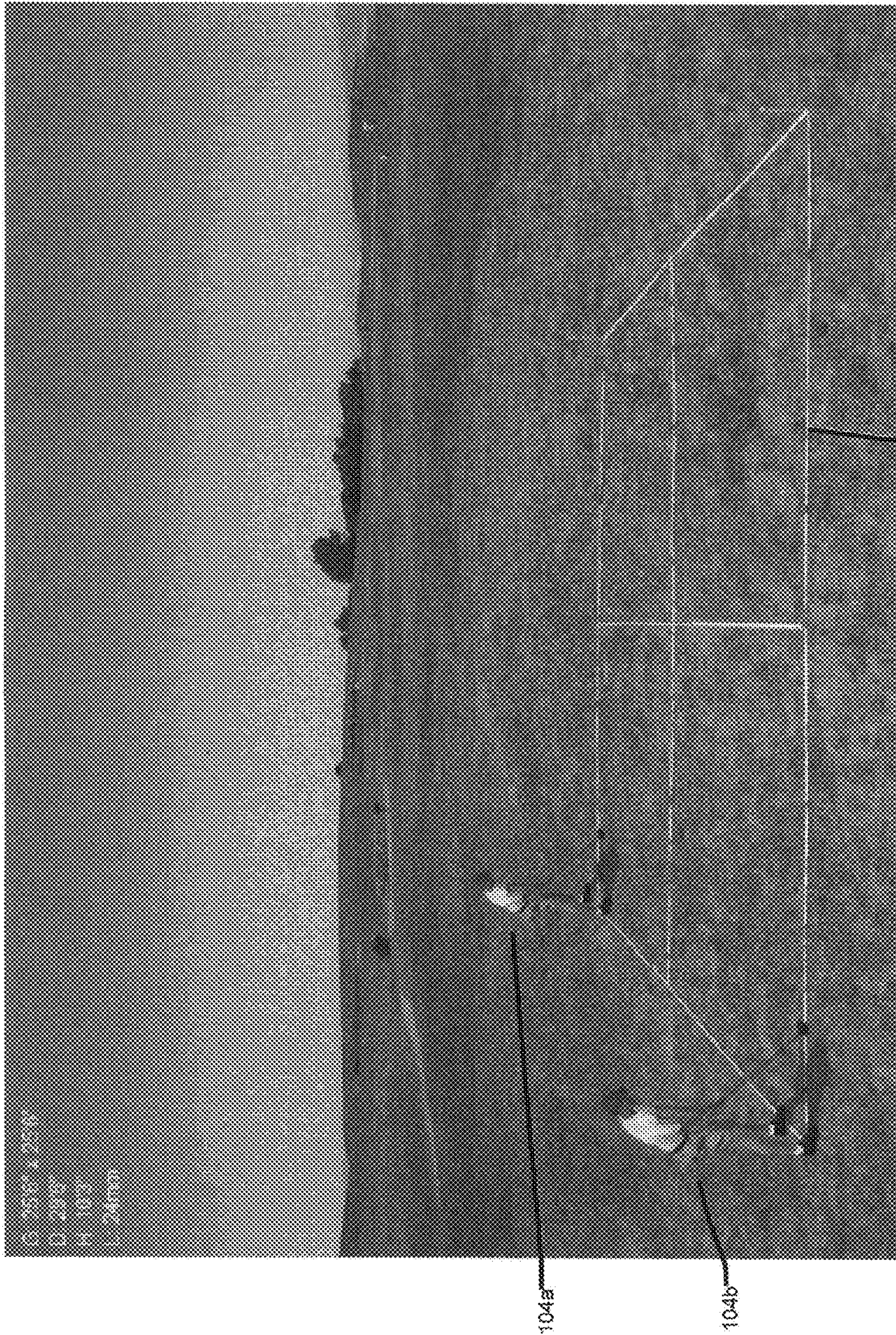


FIG. 3C

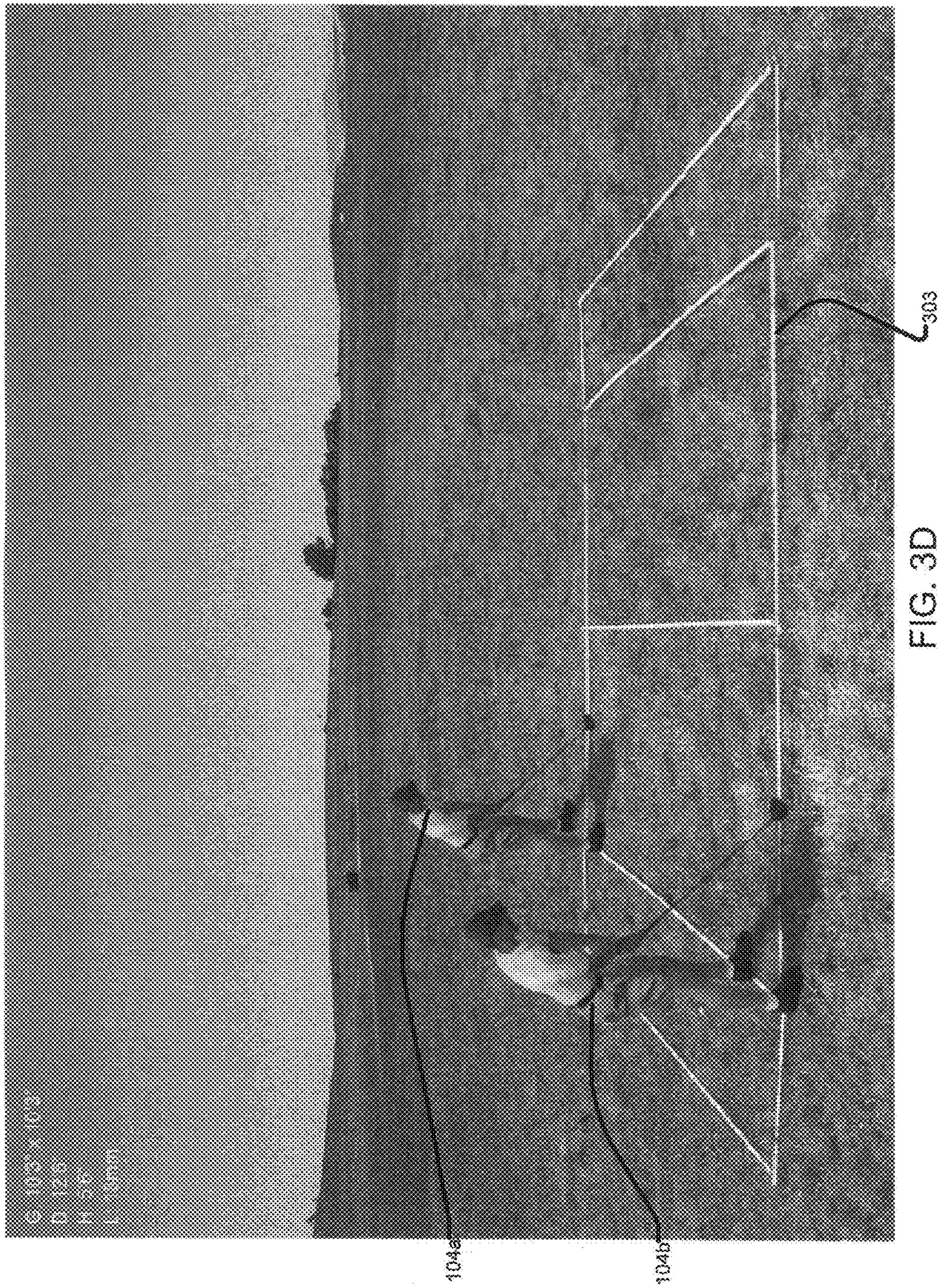


FIG. 3D

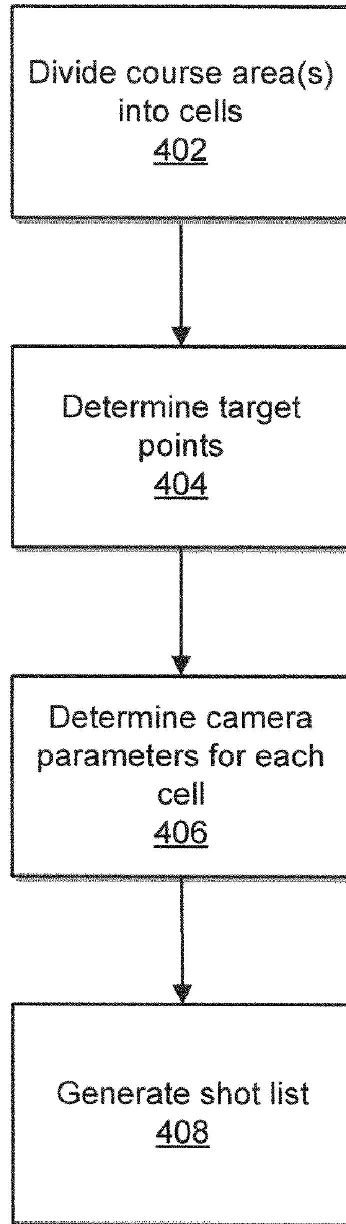


FIG. 4

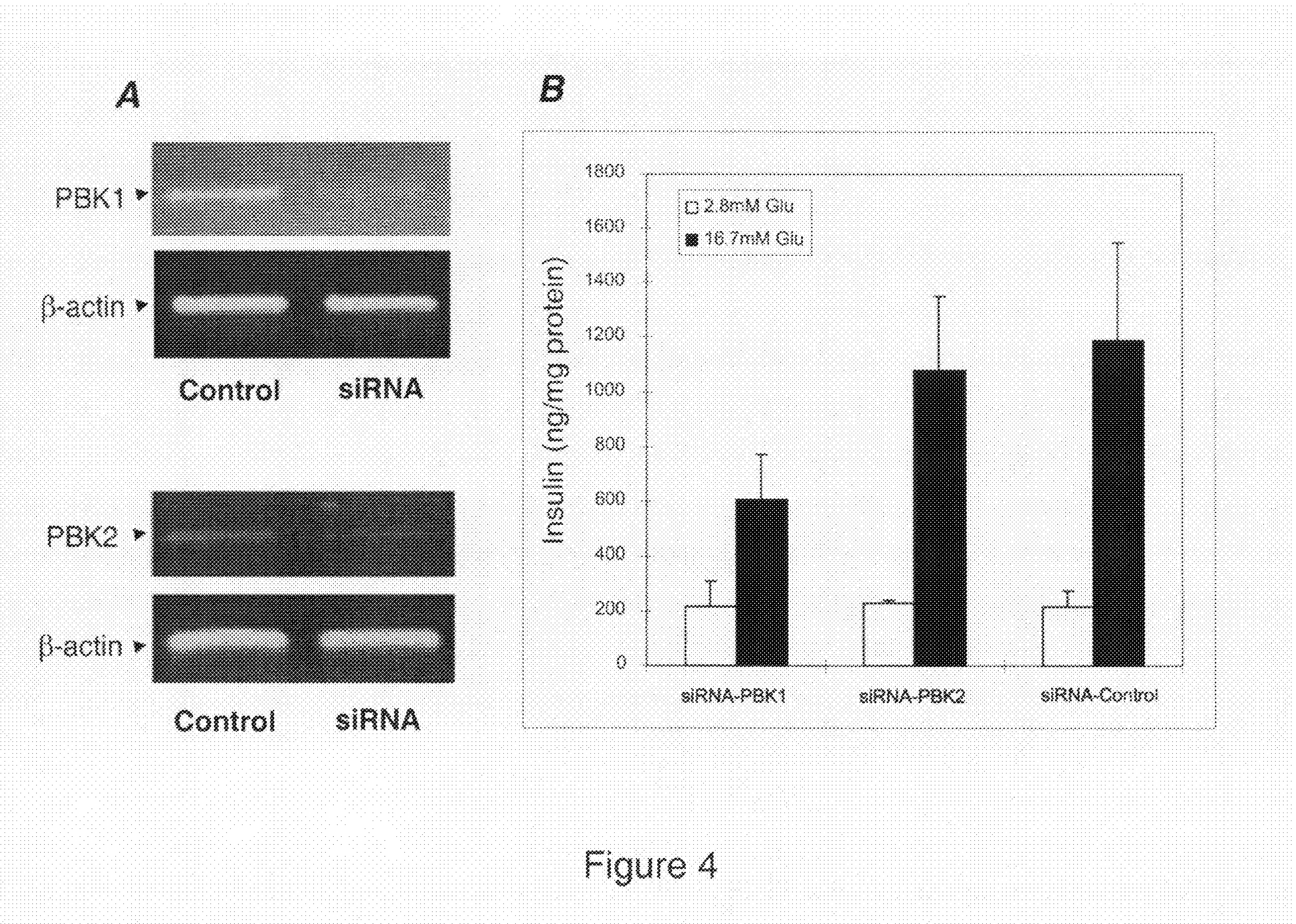


Figure 4

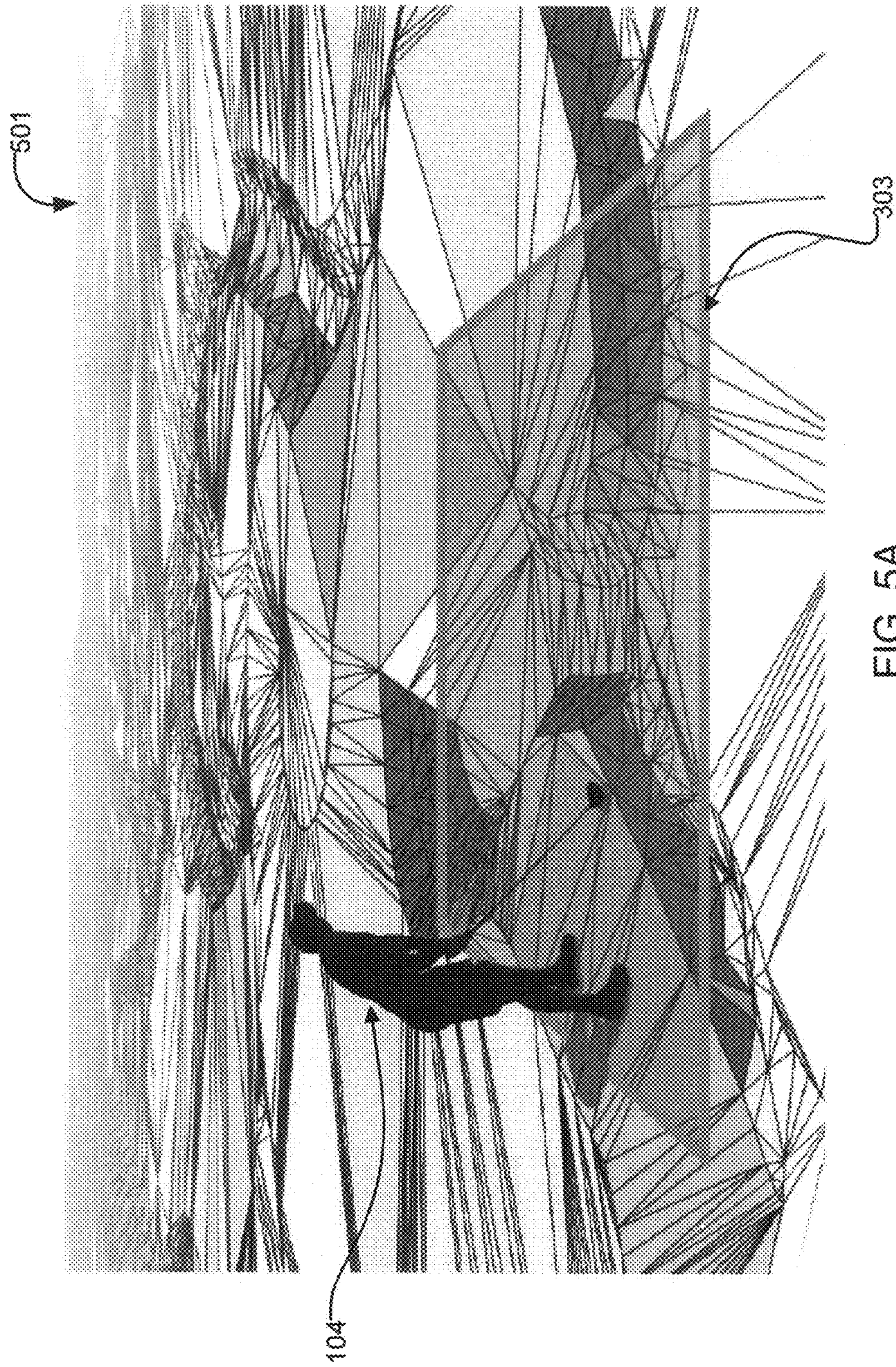


FIG. 5A

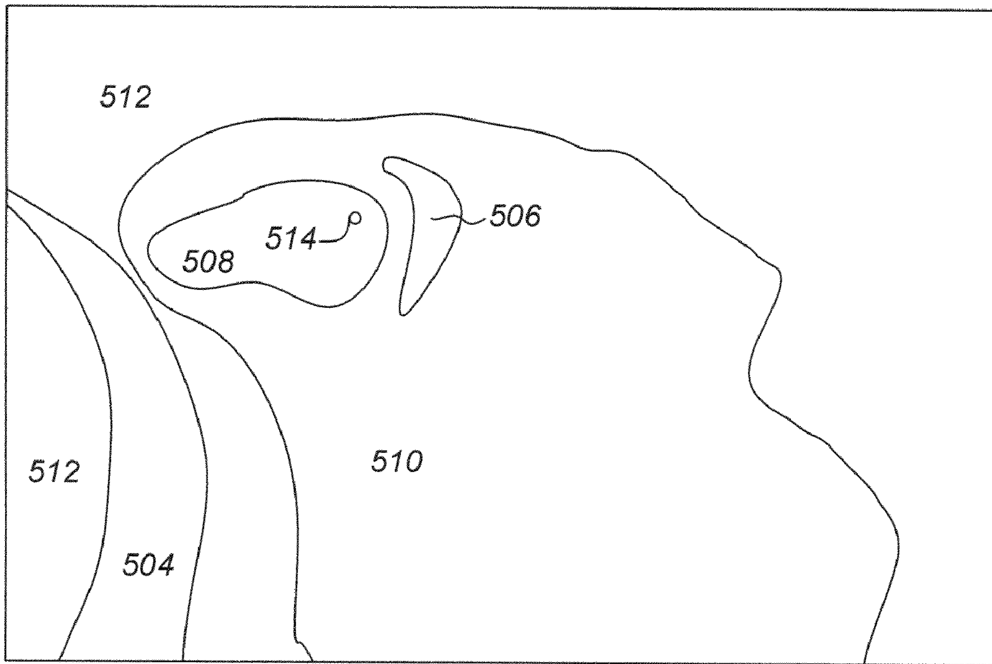


FIG. 5B1

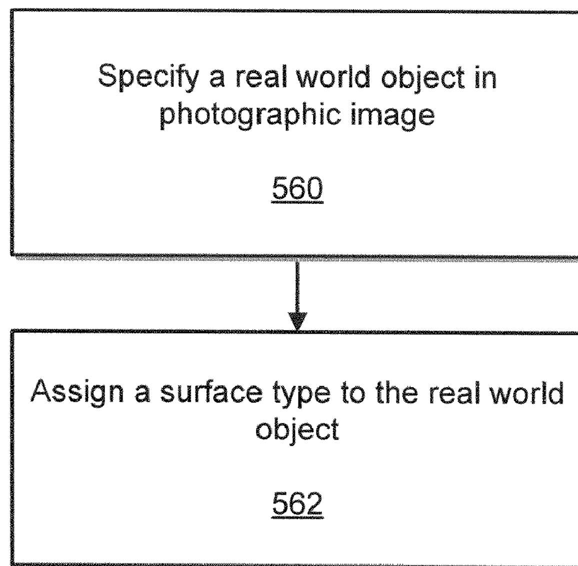


FIG. 5B2

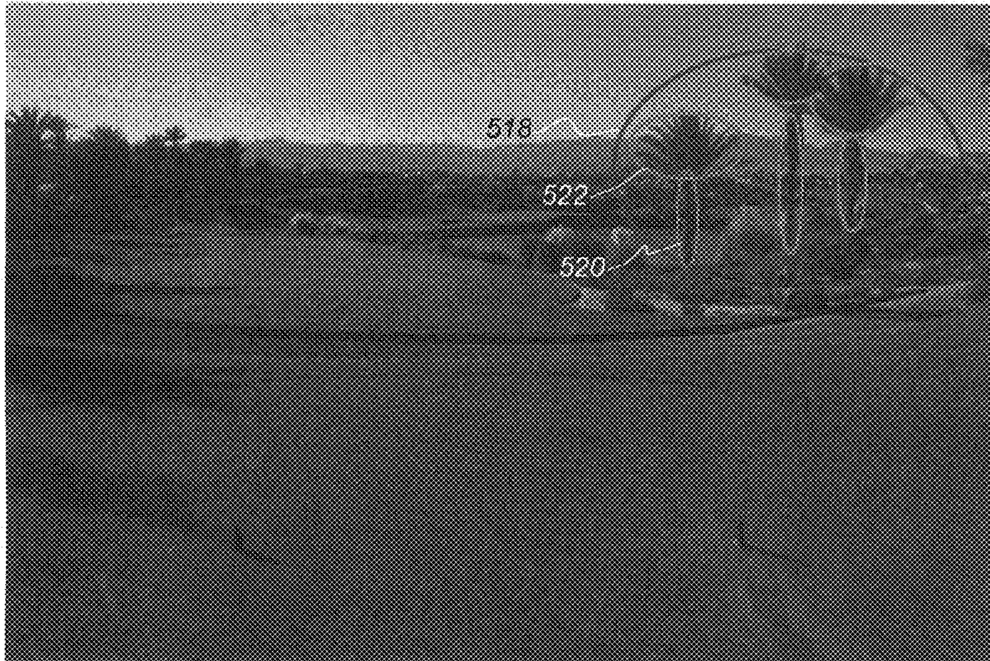


FIG. 5C1

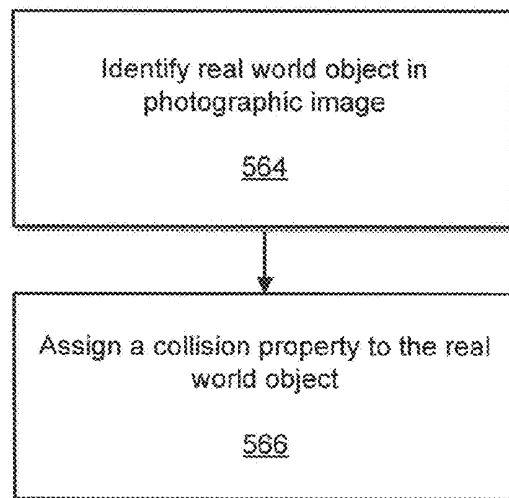


FIG. 5C2

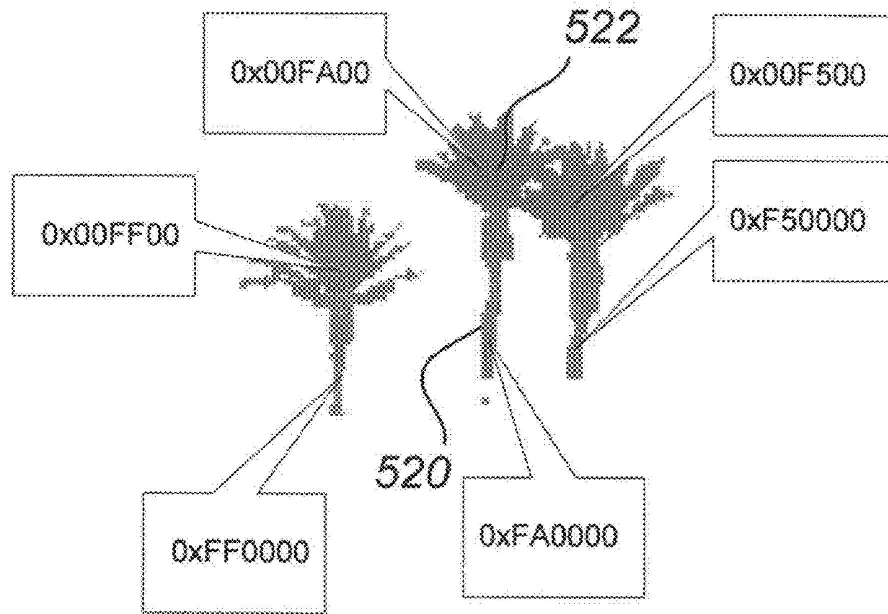


FIG. 5D

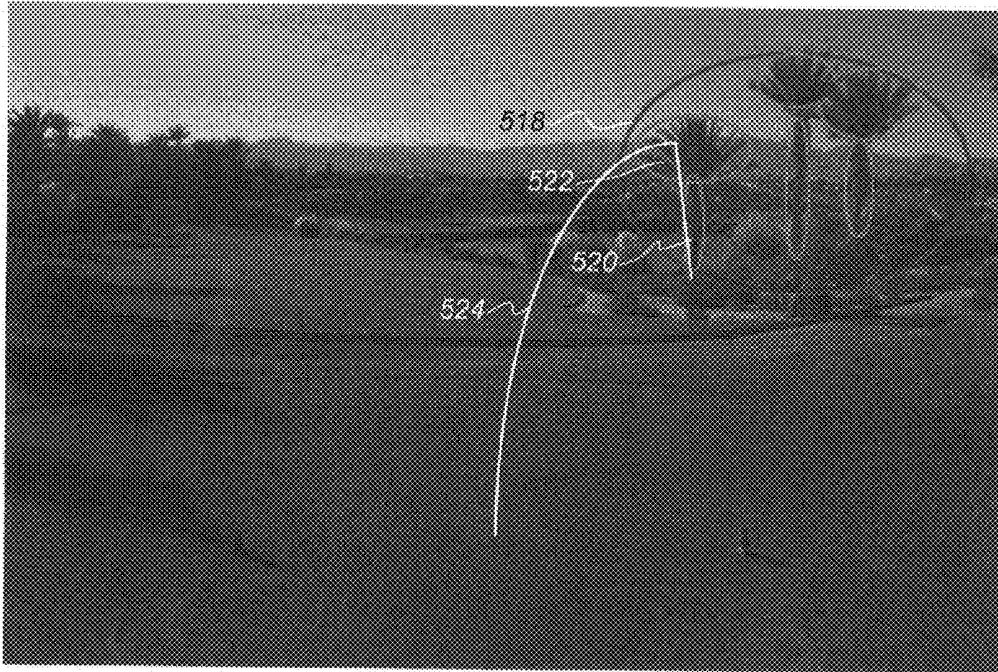


FIG. 5E

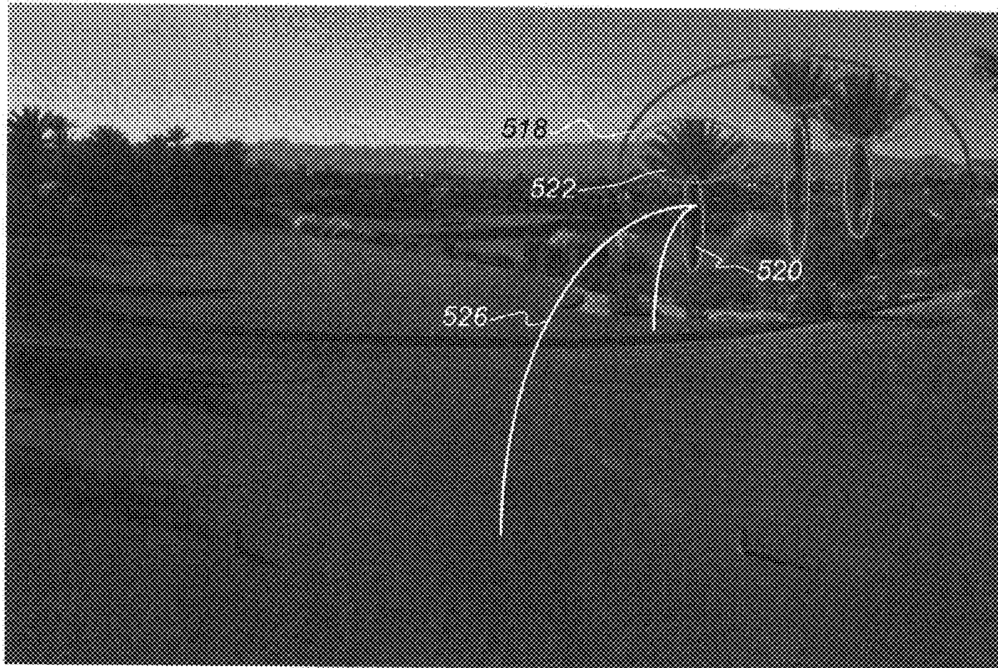


FIG. 5F

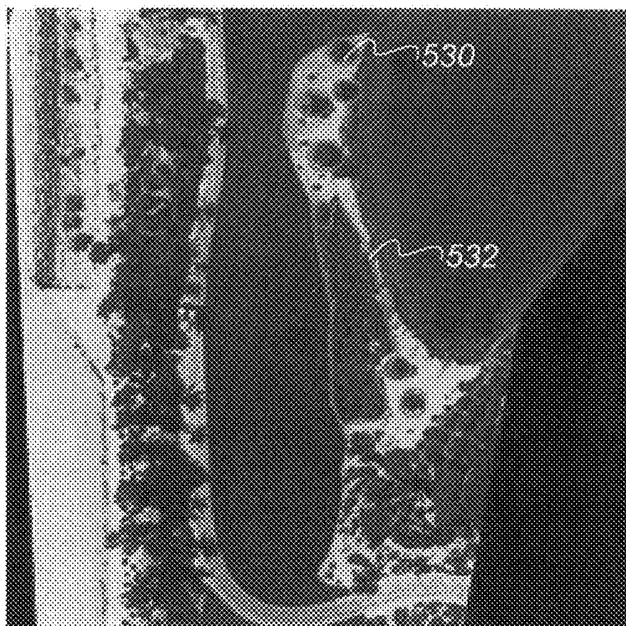


FIG. 5G

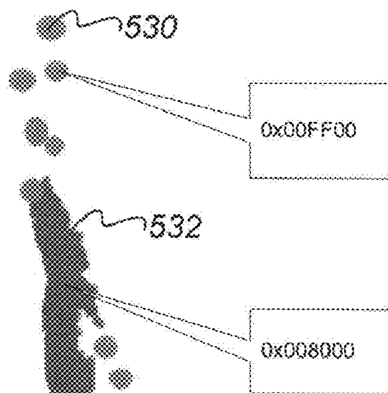


FIG. 5H

503a

503b

FIG. 5I

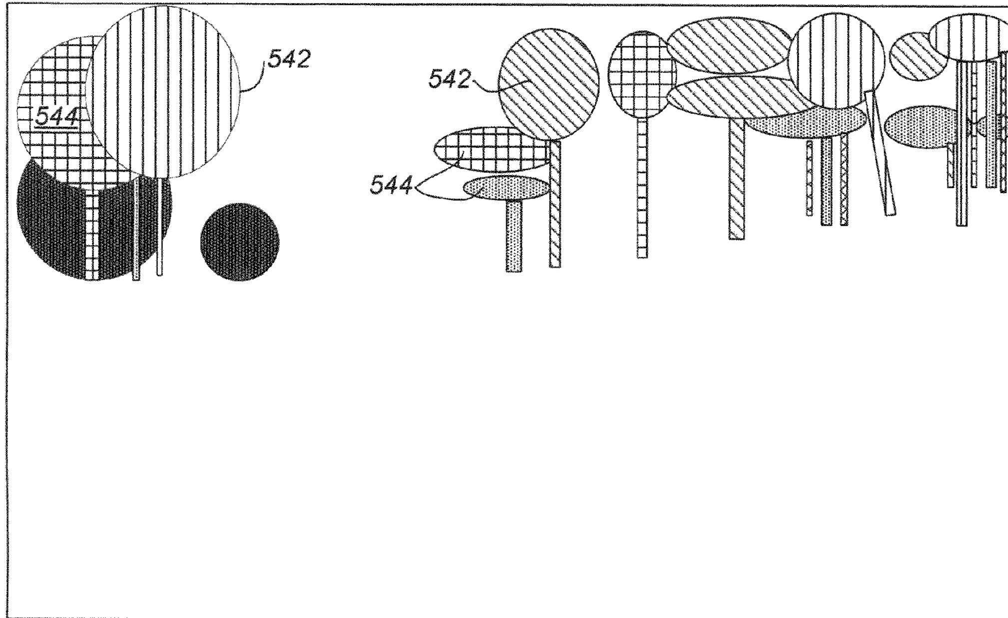


FIG. 5J

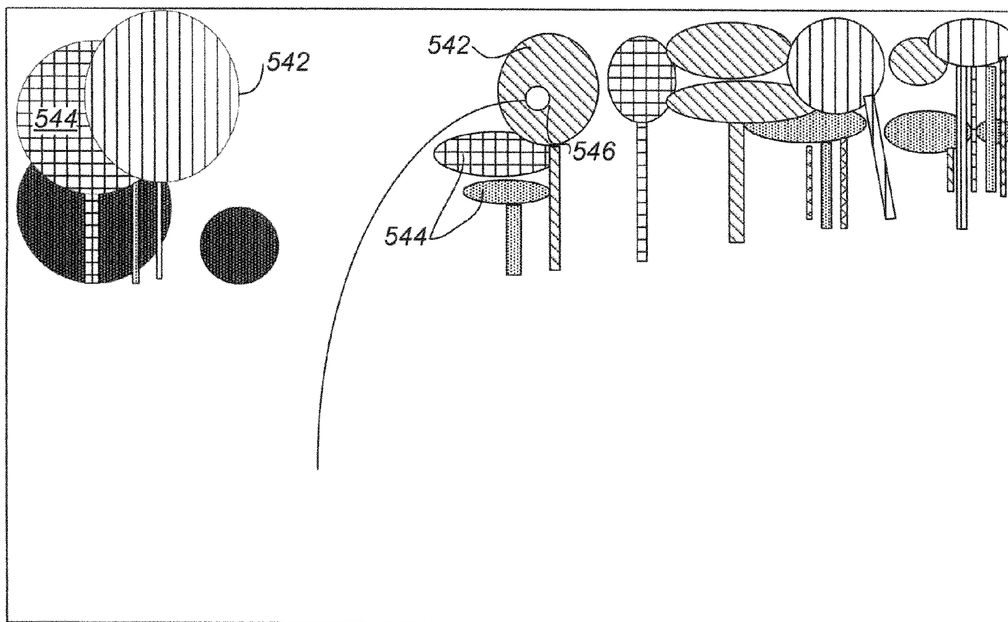


FIG. 5K

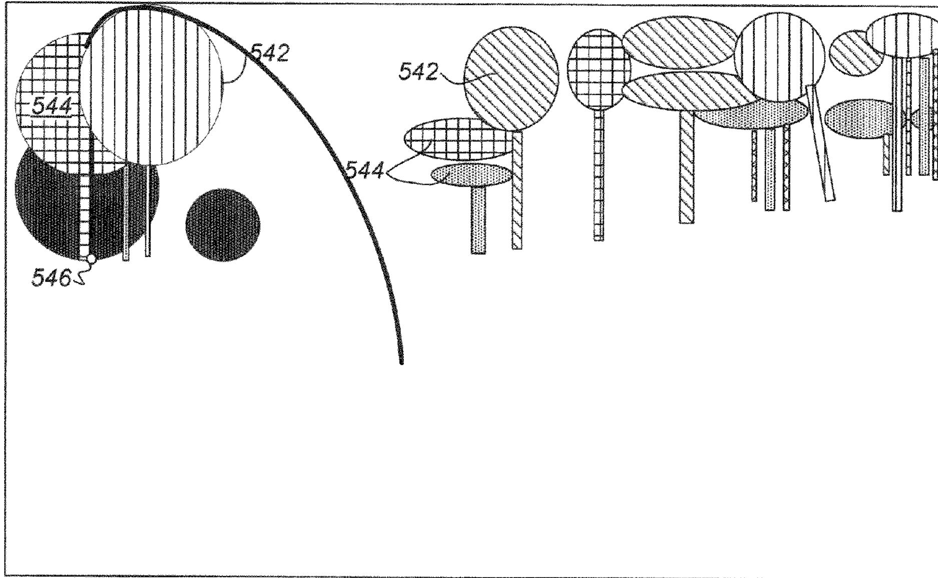


FIG. 5L

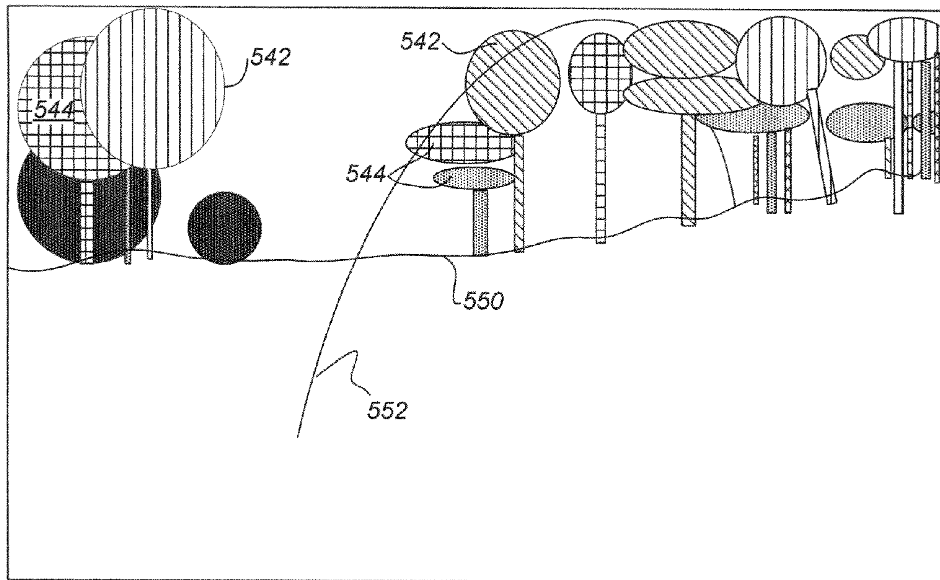


FIG. 5M

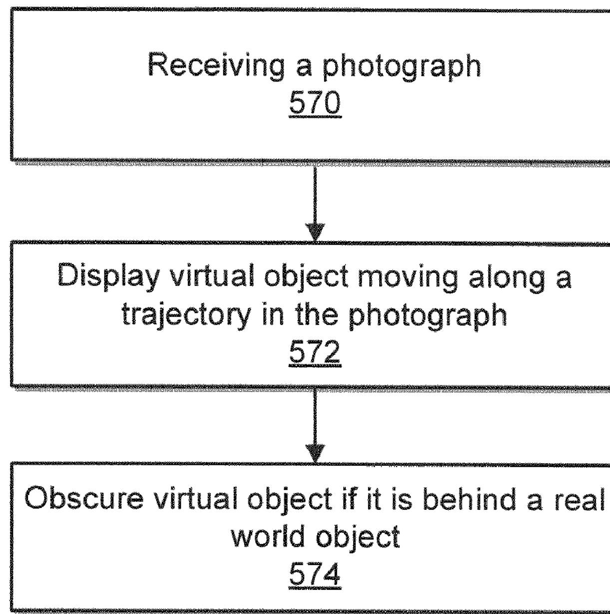


FIG. 5N

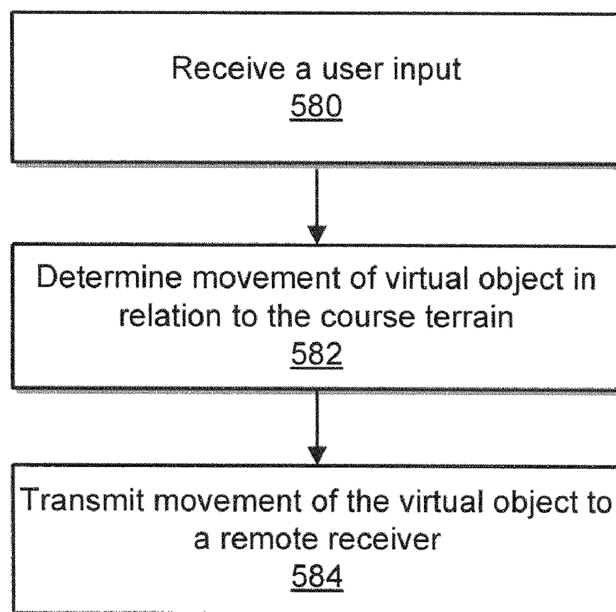


FIG. 5O

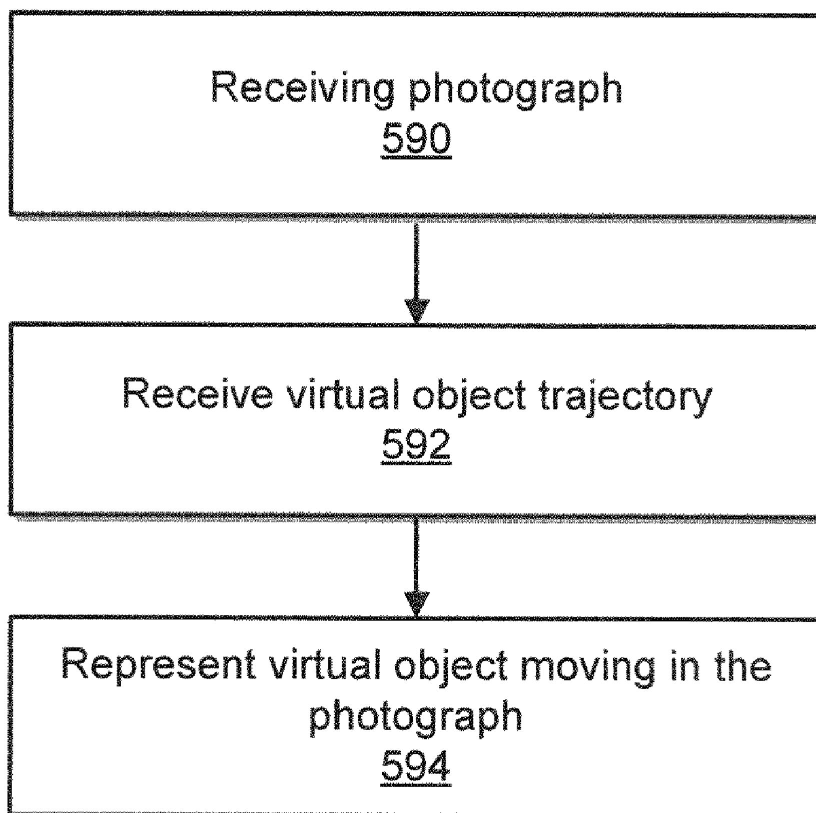


FIG. 5P

Subcellular Localization of PBK-1 Overexpressed in Min6 Cells

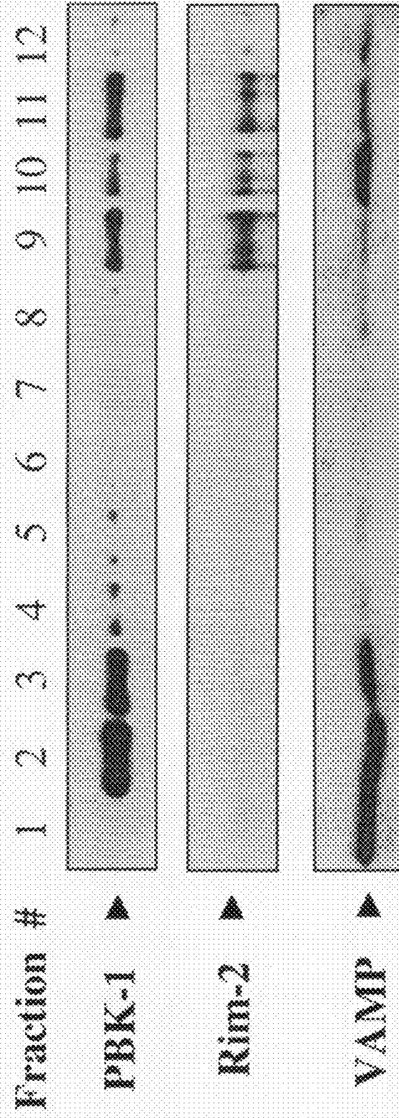


Figure 6

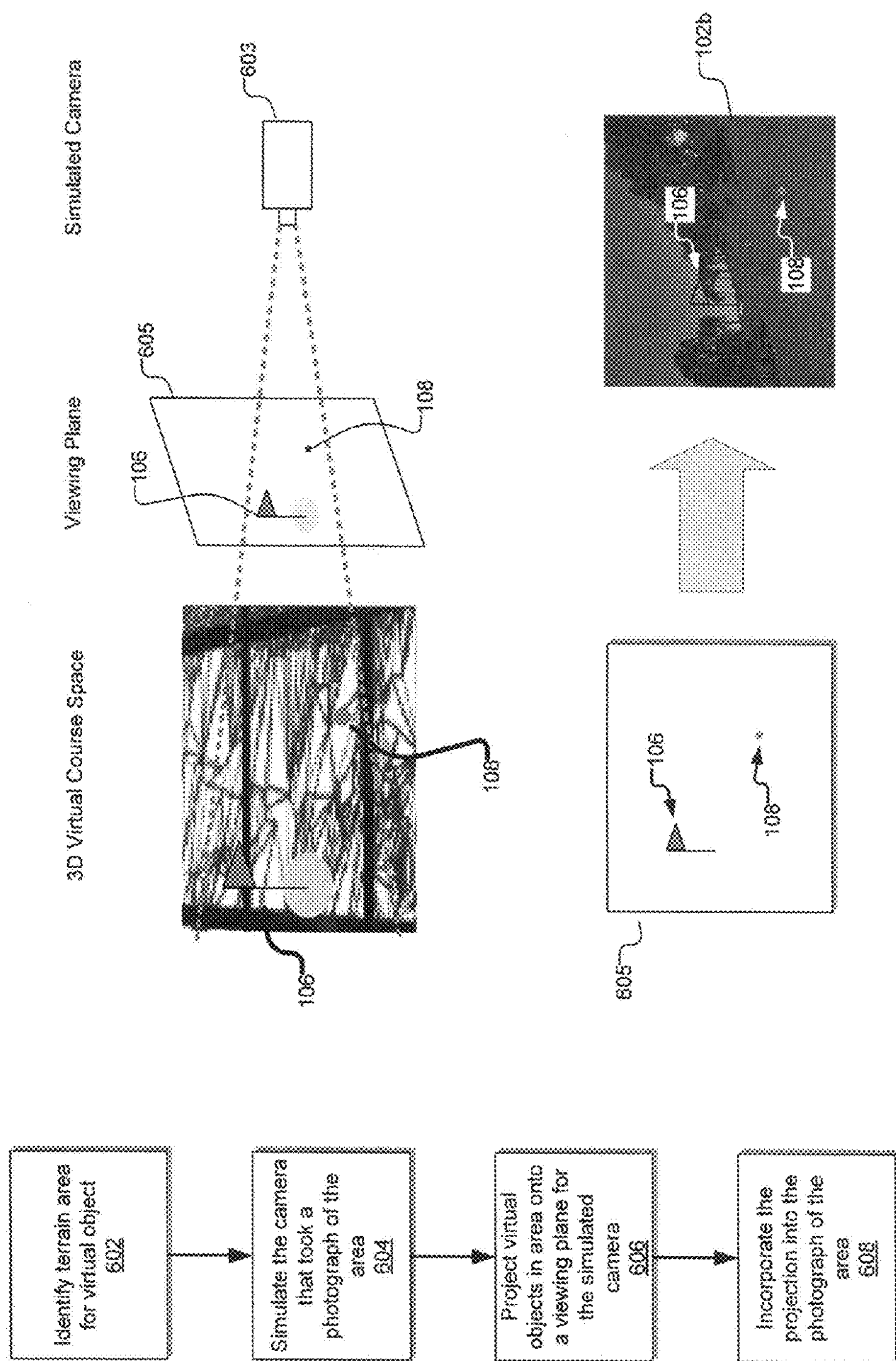


FIG. 6B

FIG. 6A

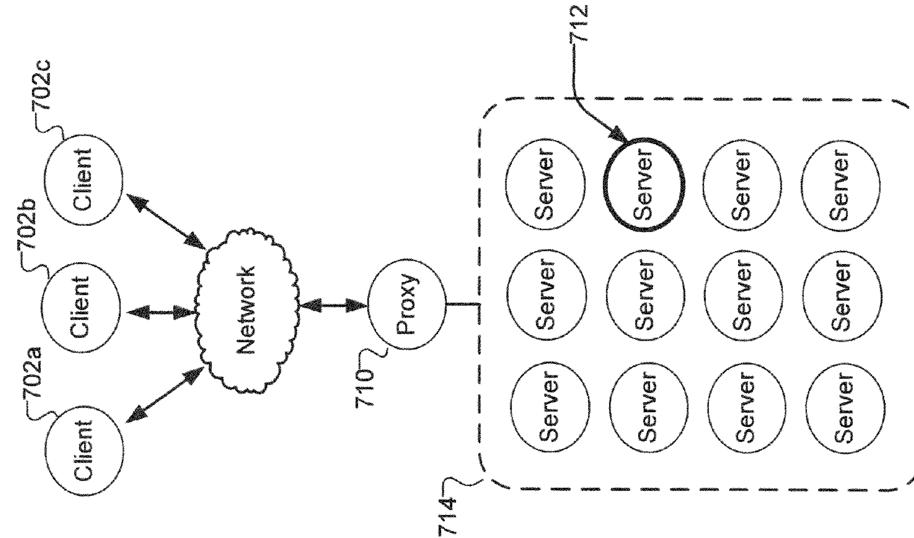


FIG. 7C

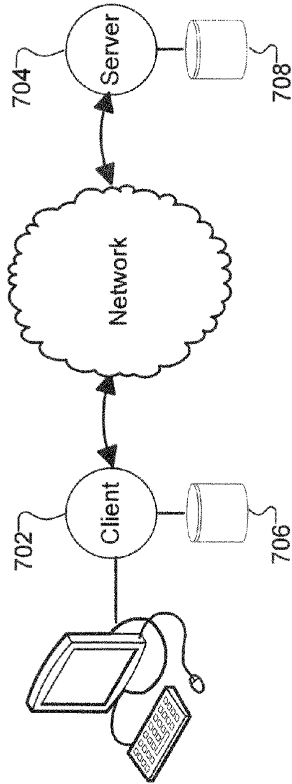


FIG. 7A

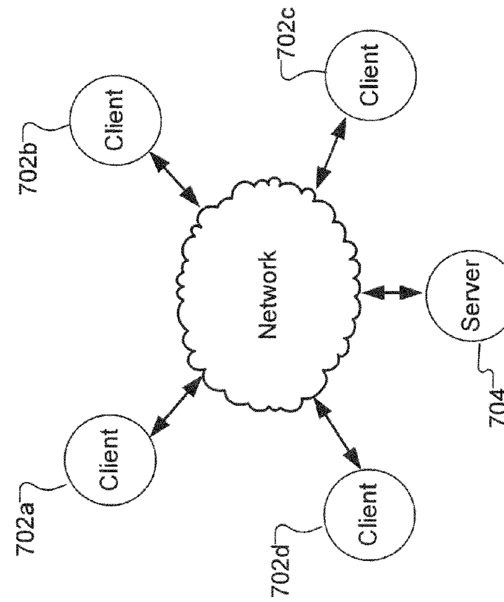


FIG. 7B

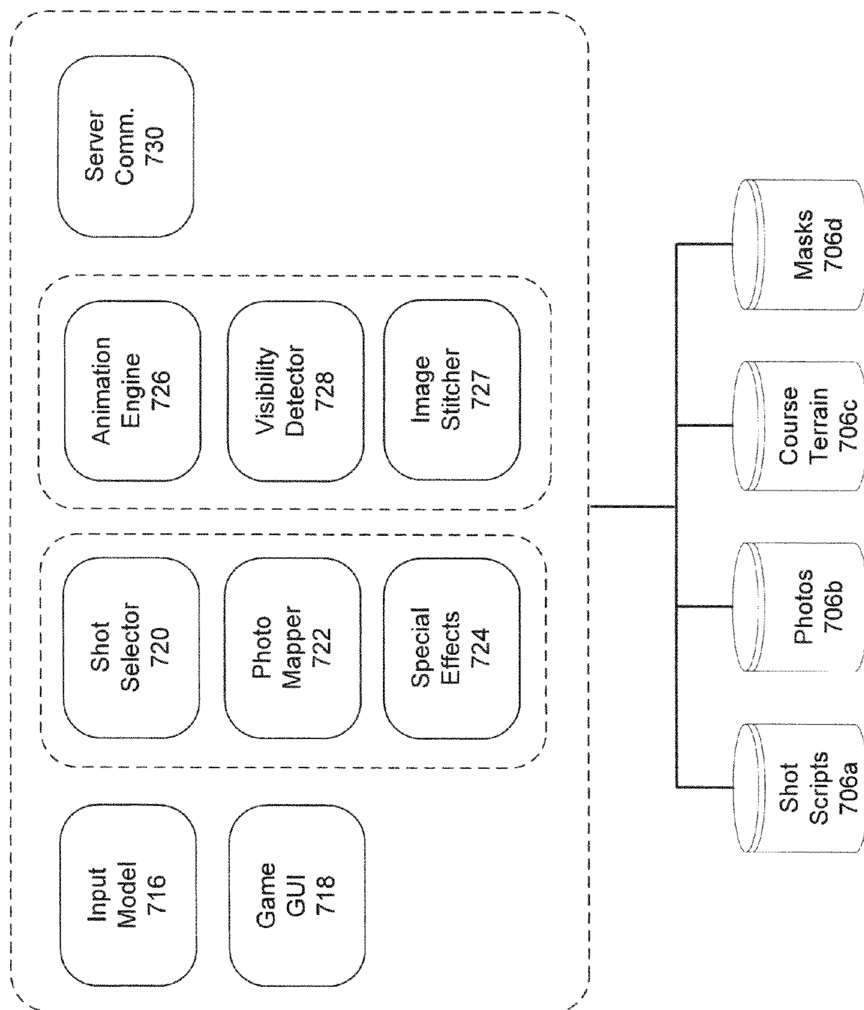


FIG. 7D

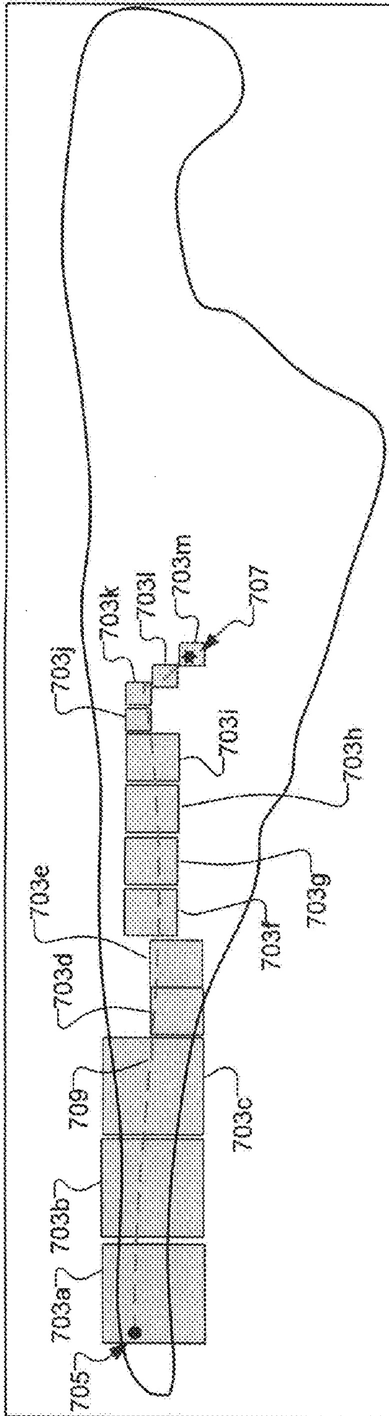


FIG. 7E

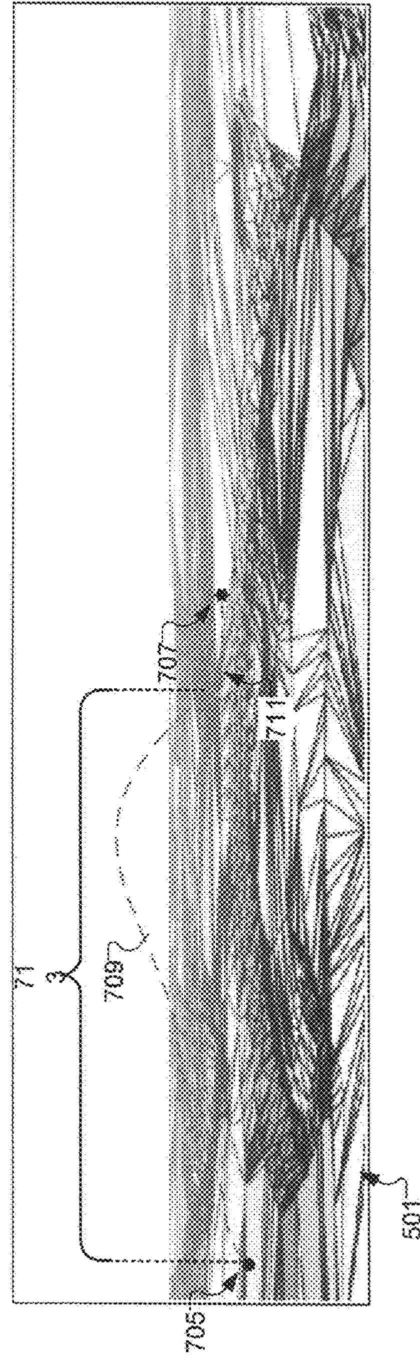
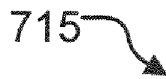


FIG. 7F



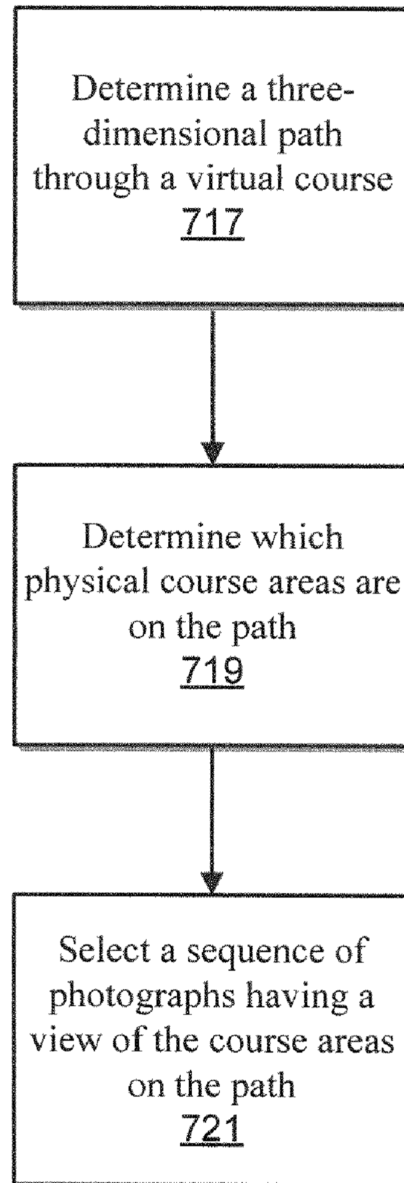


FIG. 7G

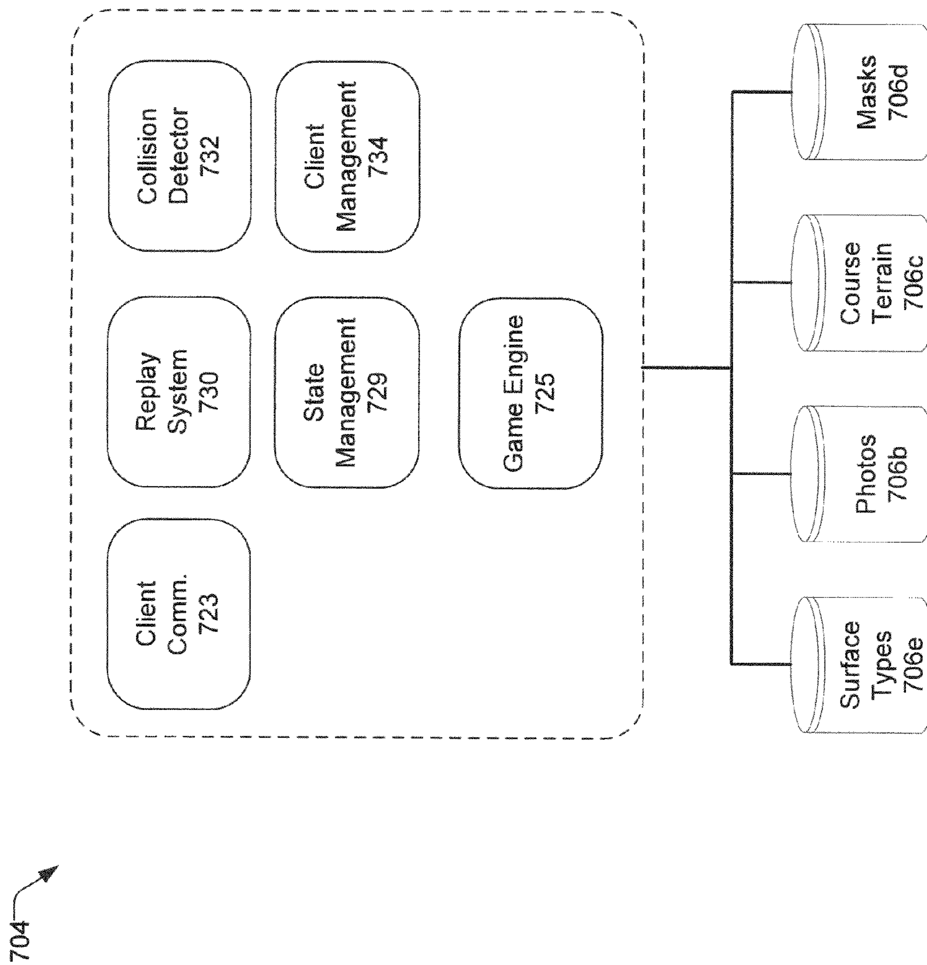


FIG. 7H

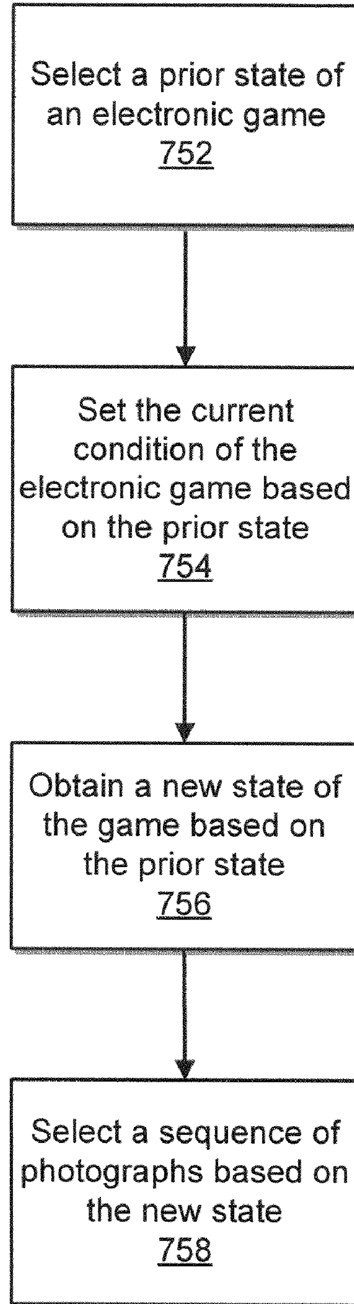


FIG. 7I

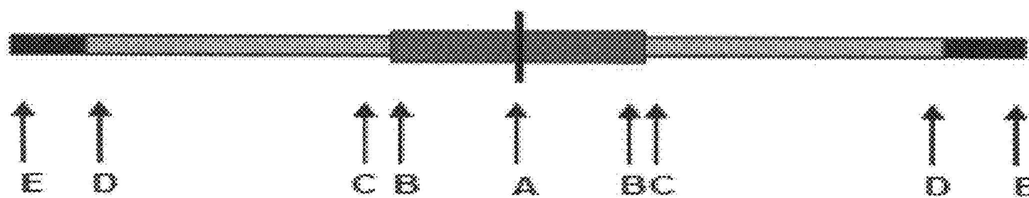


FIG. 7J

METHODS AND COMPOSITIONS RELATING TO PBK1

[0001] This application claims the benefit of priority from U.S. provisional application 60/939,462, filed May 22, 2007, the entire contents of which is hereby incorporated herein by reference.

REFERENCE TO SEQUENCE LISTING APPENDIX

[0002] Amino acid and nucleic acid sequences are shown in appended pages marked “Appendix” and are also being submitted on the accompanying compact disc; said amino acid and nucleic acid sequences are considered part of this application and are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0003] The invention relates to compositions comprising, and methods utilizing PBK1 protein and DNA.

BACKGROUND OF THE INVENTION

[0004] It was estimated that approximately 150 million people worldwide had type 2 diabetes (T2D) in the year 2000, with the prediction that this number could double to 300 million by 2025 (1). T2D is characterized by insulin resistance in peripheral tissues and deficient β-cell insulin-secretory response to glucose. Glucose-sensing by pancreatic β-cell plays an important role in regulating glucose homeostasis and onset of T2D. Normal pancreatic β-cells are able to sense minor changes in blood glucose levels, and promptly respond to such changes by adjusting insulin secretion rates to maintain normoglycemia (2). In patients with insulin resistance, the pancreatic β-cells have to secret higher levels of insulin as a compensatory response to insulin resistance in order to maintain normoglycemia, resulting in hyperinsulinemia. Consequently, T2D develops only in subjects that are unable to sustain this β-cell compensatory response (3, 4). This is supported by results from longitudinal studies of subjects that develop T2D. These patients show a rise in insulin levels in the normoglycemic and prediabetes phases, followed by a decline in insulin secretion when β cells loss their ability to sense glucose, resulting pancreatic β cell failure and onset of diabetes (5). A longitudinal study in Pima Indians also confirmed that β-cell dysfunction was the major determinant of progression from normoglycemia to diabetes (6). Furthermore, the natural history of T2D entails progressive deterioration in β-cell function (7) and loss of β cell mass due to apoptosis (8, 9).

[0005] Current treatment options for type 2 diabetes include insulin, sulfonylureas, glitinides, acarbose, metformin, thiazolidinediones. These drugs lower blood glucose through diverse mechanisms of action. However, many of the drugs cannot prevent β-cell death or re-establish β-cell mass, and most of the oral hypoglycemic agents lose their efficacy over time, resulting in progressive deterioration in β-cell function and loss of glycemic control. Moreover, sulfonylurea therapy has been shown to induce apoptosis in rodent β-cells (10) or cultured human islets (11), thus likely exacerbating β-cell loss in T2D patients. Consequently, there has been intense interest in the development of therapeutic agents that preserve or restore functional β-cell mass (12). Several agents with the potential to inhibit β-cell apoptosis and/or increase β-cell mass have been identified in preclinical studies (12). One of the agents, a GLP-1 analogue, commercially known as Byetta (exenatide), has been shown to lower blood glucose level by improving β-cell function (β-15). Byetta is a peptide derived from the venom of the Gila monster, a poisonous lizard. Treatment of β-cell with Byetta has been shown to improve β-cell glucose sensing concurrent with preservation of β-cell mass and stimulation of β-cell regeneration (16). However, Byetta must be administered by injection twice daily, and long term usage of the drug has been associated with development of anti-exenatide antibodies in diabetic subjects. Additionally, the drug slows gastric emptying, and causes gastrointestinal discomfort.

[0006] Thus, there is a continuing need for compositions and methods for detection and treatment of diabetes in a subject. Further, development, of an oral antidiabetic drug that can improve glucose sensing by pancreatic β-cells is required for treatment of type 2 diabetes.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. **1** depicts a northern blot that indicates that PBK1 mRNA is most abundantly expressed in human pancreas.

[0008] FIG. **2** depicts a western blot that indicates that PBK1 is predominantly expressed in endocrine islets, and that PBK1 protein expression was significantly up-regulated in islets of diabetic db mice.

[0009] FIG. **3** graphically represents insulin levels at indicated glucose concentrations for Min-6 cells comprising vector, PBK1, a dominant negative mutant of PBK1 (DN), and a PKA phosphorylation defective mutant of PBK1 (T260A); demonstrating that PBK1 overexpression improved glucose-sensing by Min-6 cells.

[0010] FIG. **4** depicts RT-PCR and insulin secretion data related to inhibition of PBK1 and PBK2 mRNA expression.

[0011] FIG. **5** graphically represents a time course for oral glucose tolerance in PBK1 heterozygous knockout mice and wild type controls.

[0012] FIG. **6** depicts western blot data related to subcellular localization of recombinant PBK1.

SUMMARY OF THE INVENTION

[0013] The invention relates to compositions comprising-, and methods utilizing PBK1 protein and DNA.

[0014] In one aspect, the invention provides a method of detecting type 1 diabetes in a patient, comprising: reacting a patient sample with a PBK1 protein or a fragment thereof under conditions that promote antibody-antigen binding; and detecting the presence of an autoantibody in said sample.

[0015] In one embodiment of the present invention, the PBK1 protein is immobilized on a solid support. In another embodiment, the patient sample is selected from the group consisting of: blood, serum, plasma, and a biopsy tissue. In another, the detecting of the autoantibody comprises reacting the autoantibody with an antibody having a detectable label.

[0016] In another aspect, the invention provides a mammalian pancreas-derived cell comprising a recombinant nucleic acid encoding a PBK1 protein.

[0017] In one embodiment of the present invention, the cell is a cell of a mammalian pancreas-derived cell line. In another embodiment, the cell is a Min6 cell transfected with a recombinant nucleic acid encoding a PBK1 protein.

[0018] In another aspect, the invention provides a method of detecting a PBK1 modulator, comprising: contacting PBK1 with a compound; and detecting a change in PBK1 activity, the change in PBK1 activity indicative that the compound is a PBK1 modulator.

[0019] In a related aspect, the invention encompasses a pharmaceutical composition for treatment of type 2 diabetes in a subject, the composition comprising a modulator of PBK1.

[0020] In a further related aspect, the invention provides a method of identifying a PBK1 modulator, comprising one or more of: a) contacting a PBK1 protein with a test agent under conditions that promote kinase activity of said PBK1 protein, and detecting an increase in the kinase activity; and b) contacting a cell that expresses a PBK1 protein with a test agent under conditions that promote glucose-stimulated insulin secretion from said cell, and detecting an increase in the glucose-stimulated insulin secretion from the cell; wherein said increase in a) or b) each indicates that the test agent is a PBK1 modulator.

[0021] In one embodiment of the present invention, the method further comprises determining that the increase in the glucose-stimulated insulin secretion comprises potentiation of secretagogue-stimulated insulin secretion from a cell that expresses a PBK1 protein. In another embodiment, the cell is a mammalian pancreas-derived cell. In another, the cell is a MIN6 cell. In another, the cell contains a recombinant nucleic acid encoding a PBK1 protein.

[0022] In another aspect, the invention provides a method of screening for an agent that treats a metabolic disease, said method comprising one or more of:

[0023] a) contacting a PBK1 protein with a test agent under conditions that promote binding of said test agent to said PBK1 protein, and detecting binding of said test agent to said PBK protein;

[0024] b) contacting a PBK1 protein with a test agent under conditions that promote kinase activity of said PBK1 protein, and detecting an increase in the kinase activity; and

[0025] c) contacting a cell that expresses a PBK protein with a test agent under conditions that promote glucose-stimulated insulin secretion from said cell, and detecting an increase in the glucose-stimulated insulin secretion from the cell; wherein said disorder is selected from diabetes, type 2 diabetes, obesity, and diabetic retinopathy.

[0026] In one embodiment of the present invention, the screening method further comprises designating said test agent as an agent that treats said disease. In another embodiment, the screening method further comprises designating said test agent as an agent that treats type 2 diabetes.

[0027] In another embodiment, the PBK1 protein is selected from a protein having a polypeptide sequence that has at least 73%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the protein having a polypeptide sequence set forth in SEQ ID NO: 2.

[0028] In another embodiment, the PBK1 protein is selected from a protein having a polypeptide sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 6.

[0029] In another embodiment, step a) is performed prior to step c). In another, step b) is performed prior to step c). In another, step a) is performed prior to b), and step b) is performed prior to step c).

[0030] In another embodiment, the screening method further comprises determining that the increase in the glucose-stimulated insulin secretion comprises potentiation of secretagogue-stimulated insulin secretion from a cell that expresses a PBK1 protein. In another, the cell is a mammalian pancreas-derived cell. In another, the cell is a MTN6 cell. In another, the cell contains a recombinant nucleic acid encoding a PBK1 protein.

[0031] In another aspect, the invention comprehends delivery of PBK-1 DNA to a subject to stimulate pancreatic beta cell differentiation and/or regeneration.

[0032] In a related aspect, the invention provides a method for stimulating cell differentiation and/or regeneration in a pancreatic beta cell comprising delivering PBK-1 DNA to said cell. In one embodiment of the present invention, the DNA is operably linked to a promoter that drives expression of said DNA in said cell. In one embodiment, the promoter is an adenoviral promoter, retroviral promoter, or any promoter that can direct expression of PBK1 expression in mammalian cells, tissues, or living body.

[0033] In another aspect, the invention comprehends the usage of transgenic mice with targeted deletion or overexpression of the PBK-1 gene to test efficacy and specificity of PBK-1 modulator compounds.

[0034] In a related aspect, the invention provides a method for in vivo screening for an agent that treats diabetes, said method comprising: a) subjecting a mammal comprising a PBK1 knockout genome to a diet that promotes diabetes; b) treating said mammal with a test agent; c) determining blood glucose level of said mammal; and d) determining whether said mammal is glucose tolerant. In one embodiment of the present invention, the animal is heterozygous for the PBK1 knockout. In another, the animal is homozygous for the PBK1 knockout.

DETAILED DESCRIPTION OF THE INVENTION

[0035] PBK1 (pancreas brain kinase), is shown herein to play a key role in regulating glucose-sensing. Overexpression of PBK1 in MIN6 cells greatly improves their glucose-sensing by more than three fold, much greater than that by GLP-1. Moreover, data detailed herein show that PBK1 also enhances insulin secretion stimulated by GLP-1 and other insulin secretagogues by improving the stimulus-secretion coupling function of β-cells. It is further demonstrated that PBK1 is a "master" regulator of the networks that regulate glucose-sensing, since PBK1 is associated with both synaptic-like microvesicles and insulin secretion granules. PBK1 is predominantly expressed in brain and pancreas, thus eliminating or reducing potential problems of functionally related side effects of an oral antidiabetic compound. Various embodiments described herein refer to type 1 diabetes and/or type 2 diabetes. For the purposes of the invention described herein these references to the types of diabetes are made consistently with the 1997 recommendations of the American Diabetes Association expert committee for universal adoption of simplified terminology, with which recommendations the National Institute of Diabetic and Digestive and Kidney Diseases (NIDDK) agreed. Accordingly, for the purposes of the invention described herein, type 1 diabetes encompasses art-recognized references to type I diabetes, juvenile diabetes, insulin-dependent diabetes mellitus, and/or IDDM; and type 2 diabetes encompasses art-recognized references to type II diabetes, adult-onset diabetes, noninsulin-dependent diabetes mellitus, and/or NIDDM.

[0036] In accordance with the present invention, various techniques and terms, including, but not limited to, conventional molecular biology, microbiology, immunology and

recombinant DNA techniques and terms may be used which are known by those of skill in the art. Such techniques and terms are described and/or defined in detail in standard references such as Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; Ausubel, F. et al., Current Protocols in Molecular Biology, John Wiley & Sons, 1994; Wild, D., The Immunoassay Handbook, 3rd Ed., Elsevier Science, 2005; Gosling, J. P., Immunoassays: A Practical Approach, Practical Approach Series, Oxford University Press, 2005; and Harlow, E. and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988; and other references described herein.

[0037] PBK1 protein provided in compositions and methods described herein may be isolated from natural sources, such as brain or pancreas of an organism. Alternatively, PBK1 protein may be generated recombinantly, such as be expression using an expression construct, in vitro or in vivo. Nucleic acid sequences encoding PBK1 have been isolated as exemplified by nucleic acid sequences described herein along with amino acid sequences of PBK1. Methods and compositions are not limited to PBK1 having the amino acid sequence described herein in detail. Homologs, including orthologs, of PBK1 may be used. In addition, as will be appreciated by one of skill in the art, due to the degeneracy of the genetic code, more than one nucleic acid will encode an identical protein. Thus, nucleic acids encoding PBK1 or a homolog thereof are not limited to those nucleic acids described herein in detail.

[0038] "PBK1 protein" means a protein having at least 73%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the protein having a polypeptide sequence set forth in SEQ ID NO: 1, or a protein encoded by a nucleic acid sequence that hybridizes under high stringency hybridization conditions to the nucleic acid set forth in SEQ ID NO: 1 or a complement thereof so long as the protein effects the function described in the particular inventive method comprising use of the protein. For example, in a method of screening of the invention, a protein encoded by a nucleic acid sequence that hybridizes under high stringency hybridization conditions to the nucleic acid set forth in SEQ ID NO: 1 or a complement thereof is operable in the inventive method so long as the protein effects the described binding, kinase activity, or glucose-stimulated insulin secretion.

[0039] High stringency hybridization conditions are known to the ordinarily skilled artisan. For instance, high stringency conditions can be achieved by incubating the blot overnight (e.g., at least 12 hours) with a long polynucleotide probe in a hybridization solution containing 7% SDS, 0.5 M $NaPO_4$, pH 7, 1 mM EDTA at 65° C., followed by one or more washes with a 0.1% SDS, 1×SSC solution at 65° C. Whereas high stringency washes can allow for less than 5% mismatch, reduced or low stringency conditions can permit up to 20% nucleotide mismatch. A fragment of PBK1 protein is any fragment of a PBK1 protein that is operable in the described method utilizing the fragment, as understood by the ordinarily skilled artisan. A fragment of PBK1 protein is operative in any of the inventive methods described herein utilizing a PBK1 protein. For example, in a method of the invention comprising reacting a patient sample with a PBK1 protein or a fragment thereof under conditions that promote antibody-antigen binding, a fragment of the PBK1 will be such understood to be operative, so long as the fragment effects the described antibody-antigen binding. Furthermore, it is understood by the ordinarily skilled artisan that such binding refers to specific binding as determinable by use of appropriate controls to distinguish it from nonspecific binding. Similarly, it is understood by the ordinarily skilled artisan that binding of a test agent or modulator to PBK1 protein refers to specific binding as determinable by use of appropriate controls to distinguish it from nonspecific binding.

[0040] "PBK1 DNA" means an isolated DNA molecule having a sequence that has at least 73%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the DNA sequence set forth in SEQ ID NO: 1, or an isolated DNA molecule having a sequence that hybridizes under high stringency hybridization conditions to the nucleic acid set forth in SEQ ID NO: 1 or a complement thereof, so long as the DNA effects the function described in the particular inventive method comprising use of the DNA. PBK1 DNA includes an isolated DNA molecule that has a sequence set forth in SEQ ID NO: 1, SEQ ID NO: 5, or SEQ ID NO: 18. A fragment of PBK1 DNA is any fragment of a PBK1 protein that is operable in the described method utilizing the fragment, as understood by the ordinarily skilled artisan. A fragment of PBK1 DNA is operative in any of the inventive methods described herein utilizing a PBK1 DNA.

[0041] A "test agent" or "modulator" as described in any of the inventive methods provided herein comprehends a compound; small molecule; biochemical; cytokine; biological including protein, peptide, antibody, or fragments thereof.

[0042] Conditions that promote binding, kinase activity, or glucose-stimulated insulin secretion as described in any of the inventive methods provided herein are well known in the art; including such conditions described in the references provided herein, and otherwise described or illustrated herein.

[0043] Methods of detecting type 1 diabetes are provided according to the present invention which include detecting an autoantibody in a patient sample by reacting the patient sample with a PBK1 protein. Detection of an autoantibody in the sample is indicative of type 1 diabetes in the patient. In particular embodiments, methods of the present invention for detecting type 1 diabetes are advantageously used to screen patients suspected of developing type 1 diabetes or likely to be susceptible to developing type 1 diabetes. For example, an inventive assay is used in a subject having a medical history or genetic background which predisposes the subject to development of type 1 diabetes.

[0044] Any of various techniques may be used to detect an autoantibody to PBK1 in a sample obtained from a subject. Standard immunomethods including, but not limited to, ELISA, radioimmunoassay, immunoblotting, immunoprecipitation assay, or immunodiffusion assay, may be used to detect an autoantibody to PBK1 in a sample. Immunomethods are described in detail in standard references such as Wild, D., The Immunoassay Handbook, 3rd Ed., Elsevier Science, 2005; Gosling, J. P., Immunoassays: A Practical Approach, Practical Approach Series, Oxford University Press, 2005; and Harlow, E. and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988.

[0045] PBK1 protein or its peptide analogues may be immobilized on a solid support for incubation with the patient sample in particular embodiments.

[0046] PBK1 protein used in methods of the present invention for detection of type 1 diabetes is full-length PBK1 in particular embodiments. In further embodiments, an anti-

genic fragment of PBK1 is used. An antigenic fragment of PBK1 is a portion of a PBK1 protein which stimulates an immune response in a subject.

[0047] A patient sample is typically a blood, serum, plasma, and/or biopsy tissue sample. A particular biopsy tissue used in a method of the present invention is pancreas biopsy tissue. In addition to detection of type 1 diabetes, methods and compositions for use in treatment of diabetes are provided according to embodiments of the present invention.

[0048] In a particular embodiment, a mammalian pancreas-derived cell including a recombinant nucleic acid encoding PBK1 is provided. A mammalian pancreas-derived cell including a recombinant nucleic acid encoding PBK1 may be a primary pancreas cell, such as a pancreatic beta cell or a cell of a mammalian pancreas-derived cell line. In particular embodiments a cell of a mammalian pancreas-derived cell line is a Min6 cell. Further embodiments include any human pancreatic beta cell line including a recombinant nucleic acid encoding PBK1.

[0049] A method of treating type 1 diabetes in a subject is provided which includes delivering a mammalian pancreas-derived cell including a recombinant nucleic acid encoding PBK1 to a subject. In preferred embodiments, the cell is a glucose-sensing cell competent to secrete insulin. In particular embodiments, the mammalian pancreas-derived cell including a recombinant nucleic acid encoding PBK1 is delivered to the pancreas. In further embodiments, the microencapsulated mammalian pancreas-derived cell including a recombinant nucleic acid encoding PBK1 is delivered to liver or the kidney capsule of the subject.

[0050] An in vivo animal model for transplantation of exogenous cells expressing a transgene to the pancreas is exemplified by a model of type 1 diabetes generated by treatment of an animal with streptozotocin (STZ). Streptozotocin is well-known as a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas. The STZ-diabetic animals will be transplanted with pancreatic beta cell line stably overexpressing PBK1 gene to test efficacy of the transplantation in the treatment of type 1 diabetes. Animals which may be used in a streptozotocin model of type 1 diabetes include, but are not limited to, rodents, rabbits and dogs.

[0051] In further embodiments, a nucleic acid encoding PBK1 is delivered to a subject to stimulate pancreatic beta cell differentiation and/or regeneration. Pancreatic β-cell differentiation/regeneration is measured by cell number counting and by immunofluorescence analysis of BrdU incorporation in pancreatic β-cell lines overexpressing the PBK-1 kinase.

[0052] Methods of identifying a PBK1 modulator are provided according to embodiments of the present invention which include contacting PBK1 with a putative modulator compound and assaying for a change in PBK1 activity. For example, a detected increase in PBK1 activity is indicative that the putative activator compound is an PBK1 activator. A detected decrease in PBK1 activity is indicative that the putative inhibitor compound is a PBK1 inhibitor. It is understood by the ordinarily skilled artisan that the detected increase or decrease in any of the inventive methods described herein comprehends reference to appropriate controls for the purposes of the particular method.

[0053] An assay used in methods of identifying a PBK1 modulator or in methods of screening described herein may have any of various formats, including, but not limited to, cell-based and array assays. An array assay refers to an ordered array of one or more materials, such as an arrangement of addressable regions including putative activators, for example.

[0054] In particular embodiments, the inventive method includes detection of a change in secretagogue-stimulated insulin secretion, such as glucose-stimulated, GLP-1-stimulated and/or KCl-stimulated insulin secretion from a cell expressing PBK1. A cell expressing PBK1 is a mammalian pancreas-derived cell in particular embodiments of an inventive method. For example, a cell expressing PBK1 for use in an assay for detecting modulated PBK1 activity following contact with a putative modulator is a MIN6 cell containing a recombinant nucleic acid encoding PBK1.

[0055] Assays for modulator activity or for the screening described herein are optionally performed using a transgenic non-human animal, such as a transgenic mouse, having targeted deletion or overexpression of the PBK-1 gene, for example to test efficacy and specificity of PBK-1 modulator compounds. Methods for targeted deletion or overexpression of a gene in a cell and/or in an animal, such as a transgenic mouse, are known in the art as exemplified by description in references including, but not limited to, U.S. Pat. Nos. 5,994,618 and 6,891,082, Nagy, A. et al., Manipulating the Mouse Embryo: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2002 and Pinkert, C. A., Transgenic Animal Technology, A Laboratory Handbook, 2nd ed., Academic Press, 2002. See also Kishi M. et al. (2005) Science 37: 929-931.

[0056] A pharmaceutical composition for treatment of type 2 diabetes in a subject is provided which includes a modulator of PBK1. A PBK1 modulator is an activator or inhibitor of PBK-1 activity in particular embodiments.

[0057] In particular embodiments, a PBK1 modulator or agent described herein is formulated for oral delivery to a subject, in particular a subject having need of treatment for type 2 diabetes. In further embodiments, the pharmaceutical composition is formulated for parenteral, nasal, topical, ocular, buccal, pulmonary, or rectal delivery to the subject.

[0058] Formulation of pharmaceutical compositions for particular routes of administration is known in the art and is described in detail in references such as Allen, L. V. et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, 8th ed., Lippincott, Williams & Wilkins, 2005; and Remington: The Science and Practice of Pharmacy, 21st ed., Lippincott, Williams & Wilkins, 2006.

[0059] The terms "subject" and "patient" are used interchangeably herein and refer to individual animals, particularly mammals, including, but not limited to, humans.

[0060] The invention comprehends PBK1 as a drug target to screen compounds to be used for the treatment of type 2 diabetes. In particular embodiments, activators of AMPK (5'-AMP-activated protein kinase), related compounds and derivatives are PBK1 activators for the treatment of type 2 diabetes which improve glucose-sensing of the pancreatic beta-cells.

[0061] In further embodiments, inhibitors of PBK-1 are identified. PBK-1 inhibitors are useful, for instance, to prevent/treat hyperglycemia-induced apoptosis of pancreatic beta cells, or used as reference compounds in the methods described herein.

[0062] Embodiments of inventive compositions and methods are illustrated in the following examples. These examples

are provided for illustrative purposes and are not considered limitations on the scope of inventive compositions and methods.

EXAMPLES

Example 1

Cloning and Expression

Molecular Cloning of PBK1

[0063] The coding region of the human PBK1 cDNA (SEQ ID NO: 18; NCBI Acc. AF533876) is amplified by PCR from human fetal brain marathon-ready cDNA(BD Bioscience) using primers by 5'-CGATGACATCGACGGGGAAGGAC-3' (SEQ ID NO: 7) and 5'-GATGGCCTCGTGGAGGTGA-CATG-3' (SEQ ID NO: 8) and subcloned into Srf-1 site of pCR-Script-Amp SK(+) vector. A flag-tagged version of PBK1 is generated by PCR amplification by using primer pairs 5'-GCCACCATGGATTACAAGGATGACGAC-GATAAGACATCGACGGGGAAGGACGGCG GC-3' (SEQ ID NO: 9), and 5'-GATGGCCTCGTGGAGGTGA-CATG-3' (SEQ ID NO: 10). The resulting PCR product is cloned into Srf-1 sites of pCR-Script-Amp SK(+) vector, sequenced, and subcloned to the HindIII-Not1 sites of pcDNA3.1.

Retrovirus Expression Vector

[0064] The insert is excised with BamH1-Xho1 from pcDNA3.1-PBK1 and ligated into the BamH1 and Sal1 sites of pBabe-puro to generate pBabe-PBK1 for retroviral expression in MIN6 cells.

Mutants

[0065] Site-directed mutagenesis is performed by using Quickchange multi-site kit (Strategene). Amino acid residue Arg-48(K48M) or Thr-260(T260A) of PBK1 was replaced by Met and Ala, respectively. The primers for these two mutants are 5'-CCAGAAGGTGGCCATCATGATCGTCAAC-CGTGAG-3' (SEQ ID NO: 11) (K48M) and 5'-CGCCG-CACGCCGCCTCGCGCTAGAGCACATTCAG-3' (SEQ ID NO: 12) (T260A). The mutants are confirmed by sequencing analysis.

Cell Culture and Retroviral Infection

[0066] Pancreatic beta-cell line, Min6 cells, described in Miyazaki et al., Endocrinology, 1990 July; 127(1):126-32, are cultured in DMEM with 15% FBS, 25 mM glucose, 100 micromolar beta-mercaptoethanol, 100 units/ml penicillin/streptomycin. For retroviral infection, pBabe-puro constructs are used to transfect 293T-derived phoenix cells by the calcium phosphate method. Two days after transfection, supernatants containing viral particles are harvested and used to infect MIN6 cells. The cells are then selected by 1 microgram/ml puromycin.

Example 2

Human Tissue Distribution of PBK1 mRNA

[0067] Tissue distribution of PBK1 (pancreas brain kinase-1), also known as BRSK2, SAD-A, SAD1, STK29, PEN11B, C11orf7 (18, 19) is examined. Northern blot analysis shows that PBK1 is almost exclusively expressed in human pancreas and brain, and most abundantly in human pancreas (FIG. **1**); implicating an important role for PBK1 in regulating pancreas function. Similar results were obtained when heart tissue was included in Northern blots. Northern blot analysis was carried out using multi-tissue blot from Clontech and radio-labeled human PBK1 as a probe.

Example 3

PBK1 Protein is Predominantly Expressed in Endocrine Islets

[0068] PBK1 protein expression were analyzed by western blot analysis using mouse monoclonal antibody to PBK1 and protein lysate from Min6 cells, pancreatic islets and exocrine pancreas isolated from C57B16 (B16), non-diabetic control db mouse (Db-C), hyperglycemic db mouse (Db-HG), or db mouse under normalglycemia (Db-LG), respectively. The same blot was also used to analyze the expression level of β-actin as an internal control for sample loading. The results showed that PBK1 is predominantly expressed in endocrine islet, but not detectable in exocrine pancreas. The results also showed that PBK1 protein expression was significantly up-regulated in islets of diabetic db mice. See FIG. **2**.

Example 4

PBK1 Overexpression Improved Glucose-Sensing by Min-6 Cells

[0069] Min-6 is a stable cell line derived from mouse pancreatic beta cells (25). In comparison with other pancreatic beta-cell lines, MIN6 cells maintain the integrity of glucose-sensing ability. To achieve high transfection rates for the expression of PBK1 in MIN6 cells, a recombinant retroviral expression system was created for PBK1 as described above, and a flag tagged expression vector for PBK1 is generated to facilitate analysis of its expression in MIN6 cells, a mouse islet beta-cell line. As shown in FIG. **3**, overexpression of PBK1 in MIN6 cells significantly enhances glucose and KCl stimulated insulin secretion. The effect of PBK1 on insulin secretion is fully dependent on its kinase activity.

[0070] The Min-6 cells were cultured in DMEM medium containing 4.5 g/l glucose and L-glutamine, and were infected with recombinant retroviruses overexpressing the human PBK1, a dominant negative mutant of PBK1 (DN), or a PKA phosphorylation defective mutant of PBK1 (T260A). The dominant negative mutant was generated by replacing lysine at aa 48 with methionine. The PKA defective mutant was generated by replacing threonine at aa 260 with an alanine. The infected Min-6 cells were selected for puromycin resistance as a stable pool. For measurement of glucose sensing by Min-6 cells, the stable pool of the infected Min-6 cells were preincubated for 1 hour at 37° C. in Krebs Ringer buffer containing 0 mM glucose, followed by 2 hour incubation with the indicated concentration of 2.8 mM, 16.7 mM, and 28 mM of glucose or 50 mM KCl. The supernatants were then collected and analyzed for insulin levels by radioimmuno assays using a kit from Linco Research, Inc. With respect to DN, mutation of the key amino acids for the kinase activation of PBK completely abolished its effect on insulin secretion. With respect to T260A, the mutant PBK1 partially lost its ability to improve insulin secretion by glucose and KCl (FIG.

3). The results showed that the wild type PBK1, but not the mutants, potentiated glucose- and KCl-stimulated insulin secretion.

Example 5

PBK1 Deficiency Impaired Glucose-Stimulated Insulin Secretion from Min-6 Cells

[0071] Endogenous mouse PBK1 (also known as SAD-A; BRSK2) and PBK2 (also known as SAD-B; BRSK1) mRNA expression was inhibited by transient transfection of Min-6 cells with 200 nM of siRNAs targeting the coding region of the mouse PBK1 (194-214: 5'-AGCGAGAGATTGC-CATCTTGA-3') (SEQ ID NO: 16) and PBK2 (468-488: 5-GCCAGAGAACCTGCTGTTGGA-3') (SEQ ID NO: 17) coding regions or a scrambled-sequence siRNA as a negative control (Dharmacon) using Lipofectamine 2000 (Invitrogen). Efficiency of PBK1 and PBK2 knockdown were assessed by RT-PCR. The results showed more than 70% reduction of the endogenous PBK1 and PBK2 mRNA expression (A). Sixty hours after the transfection, insulin secretion was analyzed from the Min-6 cells as described in FIG. **4**. The results showed that deficiency of PBK1, but not PBK2, resulted in impaired glucose sensing by Min-6 cells (B).

Example 6

PBK1 Deficiency in Mice Caused Diabetes

[0072] Oral glucose tolerance test were carried out in awakening PBK1 heterozygous knockout mice and the wild type controls to analyze the onset of diabetes. The heterozygous PBK1 knockout (n=4) and the wild type control mice (n=4) at three months of age on regular chow were fasted overnight and then analyzed for blood glucose levels using blood samples collected by tail bleeding (0 min). The mice were then given 50% glucose solution by oral gavage at 2.5 g glucose/kg body weight. Blood samples were collected from tail-bleeding at 30, 60, and 120 minutes, respectively, after the glucose overload and analyzed for glucose levels by glucose meter. The results (FIG. **5**) showed that the heterozygous PBK1 knockout mice have higher fasting blood glucose level (panel A). One of the heterozygous mice developed diabetes as judged by the fasting blood glucose level and glucose tolerance test (panel B).

Example 7

Subcellular Fractionation of PBK1

[0073] PBK1 is associated with both synaptic-like microvesicles and insulin secretion granules. Subcellular localization of the recombinant PBK1 in MIN-6 cells was analyzed by sucrose gradient and Western blot analysis using markers for synaptic-like microvesicles and insulin secretion granules. As shown by FIG. **6**, the recombinant PBK1 is co-localized with both Rim2 and VAMP2 in subcellular fractions segregated by sucrose gradient. Rim2 is a putative effector protein for Rab3s, synaptic GTP-binding proteins. RIM2 is localized close to the active zone at the synapse and regulates neurotransmitter releases (20). In insulin-secreting β-cells, Rim2 interacts with cAMP-binding protein cAMP-GEFII (or Epac 2), and mediates cAMP-dependent, PKA-independent insulin secretion (21). VAMP2 is associated with insulin secretory granules and regulates insulin exocytosis from pancreatic β-cells (22). The data support a dynamic role of PBK1 in regulating insulin secretion by interacting with proteins of different networks involved in insulin secretion.

[0074] Sucrose gradient fractionation is performed by the method of Mizuta et all (17). Briefly, a stable MIN6 cell clone overexpressing PBK1 is harvested by homogenization buffer containing 200 mM sucrose, 50 mM NaCl, 2 mM EGTA, 10 mM HEPES at pH7.2, and 1 mM phenylmethylsulfony fluoride and homogenized. The homogenate is centrifuged at 1770 g for 6 min at 4° C. The resulting postnuclear supernatant is applied to the top of sucrose gradients (0.4, 0.6, 0.8, 1.0, 1.4, 1.8 M) in 10 mM HEPES, pH 7.2, and 2 mM EGTA, and centrifuged at 55,000 g for 2 hours at 4° C. The fractions are collected from the top to bottom, precipitated with 15% trichloroacetic acid, and subjected to immunoblot analysis using anti-Flag antibody(Sigma), anti-Rim2 antibody (Synaptic Systems), and anti-VAMP2 antibody (Calbiochem).

Example 8

Polymorphism Analysis for Early Diagnosis of Type 1 Diabetes and Type 2 Diabetes

[0075] PBK1 is localized on chromosome 11p15.5, an IDDM2 diabetic locus. Sequence analysis of the 3'-untranslated region revealed a polymorphism, described in Miura et al. (18), which can be used to predict the onset of type 2 diabetes. The polymorphism will be investigated by analyzing the patterns of DNA fragments from HhaI/BstUI digestion of genomic DNAs of the 3' untranslated region of PBK1 gene from diabetic and non-diabetic subjects. The genomic DNAs from diabetic and non-diabetic subjects will be amplified by PCR reactions (35 cycles of 94° C. for 30 seconds, 59° C. for 30 seconds, and 72° C. for 90 seconds) using a primer pair: 5'-GTCACCTGACCCCTCAGCAA-3' (SEQ ID NO: 13) and 5'-CACCAGCTCTGTCCTCAGAG-3' (SEQ ID NO: 14). The PCR product will be purified by Qiaquick PCR purification kit (Qiagen), digested with HhaI/BstUI, and separated on a 6% polyacrylamide gel.

Example 9

Early Diagnosis of Type 1 Diabetes

[0076] Type 1 diabetes is caused by autoimmune destruction of pancreatic beta cells, which generally occurs over a period of several years leading to the eventual onset of overt diabetes mellitus. During this extended pre-clinical phase, the characteristic circulation of autoantibodies against a variety of islet cell antigens including glutamic acid decarboxylase (GAD65), IA-2 (a tyrosine phosphatase-like protein), and insulin provides early markers of autoimmune disease activity as well as early diagnosis of type 1 diabetes. The present invention comprehends PBK1 as an autoantigen in type 1 diabetes, and that the autoantigen can be used as a marker for early diagnosis of type 1 diabetes by using enzyme-linked immunosorbent assay (ELISA), radioimmuno assays (RIA), and the like. In the ELISA assay, purified PBK1 protein will be used to detect the presence of antoantibodies to PBK1 from serum of type 1 diabetic patients. Methods for detecting presence or absence of autoantigens are known to the ordinarily skilled artisan. For example, such methods have been applied to detect presence or absence of GAD65 and other autoantigens (23).

Example 10

Application to Type 2 Diabetes

[0077] PBK1 can be used as a drug target to screen activator compounds for the treatment of diabetes, obesity, diabetic

retinopathy, and other metabolic diseases. The screening process illustratively comprehends binding assay, kinase assay, and functional assay. PBK1 activators can be identified by measuring the binding potency of the compounds to the kinase ATP binding site through competition with a conjugated binding probe as described in reference 24. PBK1 activators are expected to stimulate kinase activity in a kinase assay. In the PBK1 kinase assay, purified PBK1 from *E. coli* or sf-9 insect cells are incubated in a kinase buffer (Cell Signaling Technology, USA) in the presence of 0.5 mM LNR peptide (KKLNRTLSFAEPG) (SEQ ID NO: 15) in the presence of 10 μM ATP, and 1 μl [γ-^{32}P]-ATP (5 μCi). After incubation at 30° C. for 30 min, 15 μl aceticacidglacial is added to stop the reaction. The kinase activity is quantified by radioactivity incorporated into the LNR peptide using phosphocellulose P-81 filter squares. For high throughput kinase assay for PBK1 activators, purified PBK1 protein is incubated with sepharose beads coupled with LNR peptide in the kinase buffer in the presence of 0.2 mM of [γ-^{32}P]-ATP for 30 min, and then washed three times before scintillation counting of radioactivity. In the functional assay, PBK1 activators are expected to potentiate glucose stimulated insulin secretion from isolated islet beta cells or cultured pancreatic beta cell lines, such as Min-6 cells. In this assay, addition of a PBK1 activator potentiates glucose stimulated insulin secretion in a glucose-dependent manner, but is ineffective in the absence of glucose, as demonstrated by FIG. **3**.

Example 11

Sequence Data

[0078] SEQ ID NO.'s: 1 and 2 are with reference to: Accession NO. NM_003957; 3506 bp; *Homo sapiens* BR serine/threonine kinase 2 (BRSK2) setting forth:

```
/translation =
"MTSTGKDGGAQHAQYVGPYRLEKTLGKGQTGLVKLGVHCVTCQK

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LFDYLVKKGRLTPKEARKFFRQIISALDFCHSHSICHRDLKPENLLLDEKNNIRIADF

GMASLQVGDSLLETSCGSPHYACPEVIRGEKYDGRKADVWSCGVILFALLVGALPFDD

DNLRQLLEKVKRGVFHMPHFIPPDCQSLLRGMIEVDAARRLTLEHIQKHIWYIGGKNE

PEPEQPIPRKVQIRSLPSLEDIDPDVLDSMHSLGCFRDRNKLLQDLLSEEENQEKMIY

FLLLDRKERYPSQEDEDLPPRNEIDPPRKRVDSPMLNRHGKRRPERKSMEVLSVTDGG

SPVPARRAIEMAQHGQRSRSISGASSGLSTSPLSSPRVTPHPSPRGSPLPTPKGTPVH

TPKESPAGTPNPTPPSSPSVGGVPWRARLNSIKNSFLGSPRFHRRKLQVPTPEEMSNL

TPESSPELAKKSWFGNFISLEKEEQIFVVIKDKPLSSIKADIVHAFLSIPSLSHSVIS

QTSFRAEYKATGGPAVFQKPVKFQVDITYTEGGEAQKENGIYSVTFTLLSGPSRRFKR

VVETIQAQLLSTHDPPAAQHLSDTTNCMEMMTGRLSKCGIIPKS"

ORIGIN
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 121 cggcggcgga agccaggtgc cccgcccgc cctgtcctct cgacgaggcg gaggcgtcgc

 181 cgcgggccag gcctcggact gccgcgtcgg agtggacgcg gggggcggcg gcgcgggcgg

 241 acgcgggcgg cgcgaagcag cggggcccgc gggggcgccc cggccgggtc ggcgcggacg

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[0107] Any patents or publications mentioned in this specification are incorporated herein by reference to the same extent as if each individual publication is specifically and individually indicated to be incorporated by reference. Amino acid and nucleic acid sequences for PBK1 are shown in appended pages marked “Appendix” which are considered part of this application and which are incorporated herein by reference in their entirety. Additionally U.S. Patent Application Publications 2003/0092036 and 2005/0125852 are incorporated herein by reference in their entirety.

[0108] The compositions and methods described herein are presently representative of preferred embodiments, exemplary, and not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art. Such changes and other uses can be made without departing from the scope of the invention as set forth in the claims. All numerical ranges described herein include all integers and decimal values within the range and are also inclusive of the endpoints.

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Arg Glu Ile Ala Ile Leu Lys Leu Ile Glu His Pro His Val Leu Lys
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Leu His Asp Val Tyr Glu Asn Lys Lys Tyr Leu Tyr Leu Val Leu Glu
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His Val Ser Gly Gly Glu Leu Phe Asp Tyr Leu Val Lys Lys Gly Arg
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Leu Thr Pro Lys Glu Ala Arg Lys Phe Phe Arg Gln Ile Ile Ser Ala
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Glu Asn Leu Leu Leu Asp Glu Lys Asn Asn Ile Arg Ile Ala Asp Phe
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Asp Gly Arg Lys Ala Asp Val Trp Ser Cys Gly Val Ile Leu Phe Ala
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Ser Met His Ser Leu Gly Cys Phe Arg Asp Arg Asn Lys Leu Leu Gln
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Leu Leu Asp Arg Lys Glu Arg Tyr Pro Ser Gln Glu Asp Glu Asp Leu
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Pro Pro Arg Asn Glu Ile Asp Pro Pro Arg Lys Arg Val Asp Ser Pro
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His Pro Ser Pro Arg Gly Ser Pro Leu Pro Thr Pro Lys Gly Thr Pro
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Val His Thr Pro Lys Glu Ser Pro Ala Gly Thr Pro Asn Pro Thr Pro
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Pro Ser Ser Pro Ser Val Gly Gly Val Pro Trp Arg Ala Arg Leu Asn
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Ser Ile Lys Asn Ser Phe Leu Gly Ser Pro Arg Phe His Arg Arg Lys
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Ser Pro Glu Leu Ala Lys Lys Ser Trp Phe Gly Asn Phe Ile Ser Leu
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Glu Lys Glu Glu Gln Ile Phe Val Val Ile Lys Asp Lys Pro Leu Ser
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Ser Ile Lys Ala Asp Ile Val His Ala Phe Leu Ser Ile Pro Ser Leu
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Ser His Ser Val Ile Ser Gln Thr Ser Phe Arg Ala Glu Tyr Lys Ala
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Thr Gly Gly Pro Ala Val Phe Gln Lys Pro Val Lys Phe Gln Val Asp
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Ser Val Thr Phe Thr Leu Leu Ser Gly Pro Ser Arg Arg Phe Lys Arg
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Val Val Glu Thr Ile Gln Ala Gln Leu Leu Ser Thr His Asp Pro Pro
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        35                  40                  45

Lys Leu Gly Val His Cys Ile Thr Gly Gln Lys Val Ala Ile Lys Ile
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Val Asn Arg Glu Lys Leu Ser Glu Ser Val Leu Met Lys Val Glu Arg
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Glu Ile Ala Ile Leu Lys Leu Ile Glu His Pro His Val Leu Lys Leu
                85                  90                  95

His Asp Val Tyr Glu Asn Lys Lys Tyr Leu Tyr Leu Val Leu Glu His
            100                 105                 110

Val Ser Gly Gly Glu Leu Phe Asp Tyr Leu Val Lys Lys Gly Arg Leu
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Thr Pro Lys Glu Ala Arg Lys Phe Phe Arg Gln Ile Val Ser Ala Leu
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Ser Pro His Tyr Ala Cys Pro Glu Val Ile Lys Gly Glu Lys Tyr Asp
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Gly Arg Arg Ala Asp Met Trp Ser Cys Gly Val Ile Leu Phe Ala Leu
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Leu Val Gly Ala Leu Pro Phe Asp Asp Asp Asn Leu Arg Gln Leu Leu
225                 230                 235                 240

Glu Lys Val Lys Arg Gly Val Phe His Met Pro His Phe Ile Pro Pro
                245                 250                 255

Asp Cys Gln Ser Leu Leu Arg Gly Met Ile Glu Val Glu Pro Glu Lys
            260                 265                 270

Arg Leu Ser Leu Glu Gln Ile Gln Lys His Pro Trp Tyr Leu Gly Gly
        275                 280                 285

Lys His Glu Pro Asp Pro Cys Leu Glu Pro Ala Pro Gly Arg Arg Val
    290                 295                 300

Ala Met Arg Ser Leu Pro Ser Asn Gly Glu Leu Asp Pro Asp Val Leu
305                 310                 315                 320

Glu Ser Met Ala Ser Leu Gly Cys Phe Arg Asp Arg Glu Arg Leu His
                325                 330                 335

Arg Glu Leu Arg Ser Glu Glu Glu Asn Gln Glu Lys Met Ile Tyr Tyr
            340                 345                 350

Leu Leu Leu Asp Arg Lys Glu Arg Tyr Pro Ser Cys Glu Asp Gln Asp
        355                 360                 365

Leu Pro Pro Arg Asn Asp Val Asp Pro Pro Arg Lys Arg Val Asp Ser
    370                 375                 380

Pro Met Leu Ser Arg His Gly Lys Arg Arg Pro Glu Arg Lys Ser Met
385                 390                 395                 400

Glu Val Leu Ser Ile Thr Asp Ala Gly Gly Gly Ser Pro Val Pro
                405                 410                 415

Thr Arg Arg Ala Leu Glu Met Ala Gln His Ser Gln Arg Ser Arg Ser
            420                 425                 430

Val Ser Gly Ala Ser Thr Gly Leu Ser Ser Ser Pro Leu Ser Ser Pro
        435                 440                 445

Arg Ser Pro Val Phe Ser Phe Ser Pro Glu Pro Gly Ala Gly Asp Glu
    450                 455                 460

Ala Arg Gly Gly Gly Ser Pro Thr Ser Lys Thr Gln Thr Leu Pro Ser
465                 470                 475                 480

Arg Gly Pro Arg Gly Gly Gly Ala Gly Glu Gln Pro Pro Pro Pro Ser
                485                 490                 495

Ala Arg Ser Thr Pro Leu Pro Gly Pro Pro Gly Ser Pro Arg Ser Ser
            500                 505                 510

Gly Gly Thr Pro Leu His Ser Pro Leu His Thr Pro Arg Ala Ser Pro
        515                 520                 525

Thr Gly Thr Pro Gly Thr Thr Pro Pro Pro Ser Pro Gly Gly Gly Val
    530                 535                 540

Gly Gly Ala Ala Trp Arg Ser Arg Leu Asn Ser Ile Arg Asn Ser Phe
545                 550                 555                 560
```

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```
Leu Gly Ser Pro Arg Phe His Arg Arg Lys Met Gln Val Pro Thr Ala
                565                 570                 575

Glu Glu Met Ser Ser Leu Thr Pro Glu Ser Ser Pro Glu Leu Ala Lys
            580                 585                 590

Arg Ser Trp Phe Gly Asn Phe Ile Ser Leu Asp Lys Glu Glu Gln Ile
        595                 600                 605

Phe Leu Val Leu Lys Asp Lys Pro Leu Ser Ser Ile Lys Ala Asp Ile
    610                 615                 620

Val His Ala Phe Leu Ser Ile Pro Ser Leu Ser His Ser Val Leu Ser
625                 630                 635                 640

Gln Thr Ser Phe Arg Ala Glu Tyr Lys Ala Ser Gly Gly Pro Ser Val
                645                 650                 655

Phe Gln Lys Pro Val Arg Phe Gln Val Asp Ile Ser Ser Ser Glu Gly
            660                 665                 670

Pro Glu Pro Ser Pro Arg Arg Asp Gly Ser Gly Gly Gly Gly Ile Tyr
        675                 680                 685

Ser Val Thr Phe Thr Leu Ile Ser Gly Pro Ser Arg Arg Phe Lys Arg
    690                 695                 700

Val Val Glu Thr Ile Gln Ala Gln Leu Leu Ser Thr His Asp Gln Pro
705                 710                 715                 720

Ser Val Gln Ala Leu Ala Asp Glu Lys Asn Gly Ala Gln Thr Arg Pro
                725                 730                 735

Ala Gly Ala Pro Pro Arg Ser Leu Gln Pro Pro Pro Gly Arg Pro Asp
            740                 745                 750

Pro Glu Leu Ser Ser Ser Pro Arg Arg Gly Pro Pro Lys Asp Lys Lys
        755                 760                 765

Leu Leu Ala Thr Asn Gly Thr Pro Leu Pro
    770                 775
```

<210> SEQ ID NO 5
<211> LENGTH: 3576
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

```
agtctcaggc tggctagttc ctccttcctg gtcactgagc cagccttgct gaggggagag     60

cgggttctgg acgtgctctg agcttccttc ctcacagcct tgctcctggg ccagatcagc    120

aggaaagcag ccagtgcccc gccatggcct gcccgggtgg ggtcctgaag ctggggccgg    180

agcagggggc acagttctgc cccatctggc cctagtttgg ggagggagcc tggtagggca    240

ccagcctcac cccatgagcc ctgagggcca ccccagccga tgggcacgtc cccgccggcc    300

ctgcatctgt ccttcctccc tctgctcccc aagagagccc aggtctggcc cagcggtggg    360

caggggaggg gccgcacatc acagagtgcc agctggccac actcccggcc cacagctgct    420

ccagccgcac ctccaccttc ctcaaggcca gacctggctc tgcctgcagc ccagcccagc    480

aggtctggtg aagctggggg ttcactgcgt cacctgccag aaggtggcca tcaagatcgt    540

caaccgtgag aagctcagcg agtcggtgct gatgaaggtg gagcgggaga tcgcgatcct    600

gaagctcatt gagcaccccc acgtcctaaa gctgcacgac gtttatgaaa acaaaaaata    660

tttgtacctg gtgctagaac acgtgtcagg tggtgagctc ttcgactacc tggtgaagaa    720

ggggaggctg acgcctaagg aggctcggaa gttcttccgg cagatcatct ctgcgctgga    780
```

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```
cttctgccac agccactcca tatgccacag ggatctgaaa cctgaaaacc tcctgctgga    840
cgagaagaac aacatccgca tcgcagactt tggcatggcg tccctgcagg ttggcgacag    900
cctgttggag accagctgtg ggtcccccca ctacgcctgc cccgaggtga tccgggggga    960
gaagtatgac ggccggaagg cggacgtgtg gagctgcggc gtcatcctgt tcgccttgct   1020
ggtgggggct ctgcccttcg acgatgacaa cttgcgacag ctgctggaga aggtgaagcg   1080
gggcgtgttc cacatgccgc actttatccc gcccgactgc cagagtctgc tacggggcat   1140
gatcgaggtg gacgccgcac gccgcctcac gctagagcac attcagaaac acatatggta   1200
tatagggggc aagaatgagc ccgaaccaga gcagcccatt cctcgcaagg tgcagatccg   1260
ctcgctgccc agcctggagg acatcgaccc cgacgtgctg gacagcatgc actcactggg   1320
ctgcttccga gaccgcaaca agctgctgca ggacctgctg tccgaggagg agaaccagga   1380
gaagatgatt tacttcctcc tcctggaccg gaaagaaagg tacccgagcc aggaggatga   1440
ggacctgccc ccccggaacg agatagaccc tccccggaag cgtgtggact ccccgatgct   1500
gaaccggcac ggcaagcggc ggccagaacg caaatccatg gaggtgctca gcgtgacgga   1560
cggcggctcc ccggtgcctg cgcggcgggc cattgagatg gcccagcacg gccagaggtc   1620
tcggtccatc agcggtgcct cctcaggcct ttccaccagc ccactcagca gcccccgggt   1680
gacccctcac ccctcaccaa ggggcagtcc cctccccacc cccaagggga cacctgtcca   1740
cacgccaaag gagagcccgg ctggcacgcc caaccccacg cccccgtcca gccccagcgt   1800
cggagggggtg ccctggaggg cgcggctcaa ctccatcaag aacagctttc tgggctcacc   1860
ccgcttccac cgccggaaac tgcaagttcc gacgccggag gagatgtcca acctgacacc   1920
agagtcgtcc ccagagctgg cgaagaagtc ctggtttggg aacttcatca gcctggagaa   1980
ggaggagcag atcttcgtgg tcatcaaaga caaacctctg agctccatca aggctgacat   2040
cgtgcacgcc ttcctgtcga ttcccagtct cagccacagc gtcatctccc aaacgagctt   2100
ccgggccgag tacaaggcca cgggggggcc agccgtgttc cagaagccgg tcaagttcca   2160
ggttgatatc acctacacgg agggtgggga ggcgcagaag gagaacggca tctactccgt   2220
caccttcacc ctgctctcag gccccagccg tcgcttcaag agggtggtgg agaccatcca   2280
ggcccagctg ctgagcacac acgacccgcc tgcggcccag cacttgtcag acaccactaa   2340
ctgtatggaa atgatgacgg ggcggctttc caaatgtgac gagaagaacg ggcaggcggc   2400
ccaggccccc agcacgcccg ccaagcggag tgcccacggc ccactcggtg actccgcggc   2460
cgctggccct ggccccggag ggacgccga gtacccaacg ggcaaggaca cggccaagat   2520
gggcccgccc accgcccgcc gcgagcagcc ttagacacac tagcccccc ccccagcaca   2580
gcactgacag cggctgcctc gccgcccgcc gcccgccctg ccccgagtgg acccgcggcc   2640
gcgccgcccg tccgtccaga ctgttctcag agcctgggag gaaaggaaag ggcgttggg   2700
gccggcctgt gggctgcgcc acccgcgccc gctctctttt ctctctgtct ctgcctctgc   2760
ctgtctctga cagcatcgct tgtttccact ctgataccag gaattatccc gaaaagttaa   2820
catgtcacct ccacgaggcc atcctctgtg accgaaggca gctgctgcgg acccgccctc   2880
cctccgctcc tgctgttgct gccgggcagt gaggcccagc ccagcgcccc gtccaccccg   2940
cggcagctcc tcgcctcagc tccgcacggc ccgtgggagg aaggccaggc tcgggggagc   3000
ctcctccagc ccggccgacc cggactcccg gtcacctgac ccctcagcaa gaacagcctg   3060
```

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```
cctggtggcc ttctggggcc aggacccctg gtgggcaacg tagccacagg aacaggcccc   3120

gtccaccgcc tccacgccgc acctggaggc ctcctcgcag gcccgtgccc cgcctccctg   3180

gccgcgccgc ctccgtgtag tcttggcctc ctcaggctgc ctcccgtcct ctcgtctcac   3240

ccgcgcctcc cttgcctcat ctggggcggc tgtgggctct ggcgctcctc tctggctgag   3300

gtggaaacag agacaccctg cggcaccaga gccttcccag caggccaggc cgctgggctg   3360

ggatcagtgt tatttatttg ccgttttaat ttatggattc tccgcacctc tgttcaggga   3420

agggcggcgg ccacatcccc tgccgtctgc gtgtctcagg cagtgggggg gctggggcca   3480

gggcgccctc tgaggacaga gctggtgggg cgcggggggg ctggcgagct actgtaaact   3540

ttaaagaatt cctgcaagat atttttataa actttt                             3576
```

<210> SEQ ID NO 6
<211> LENGTH: 766
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

```
Met Ser Pro Glu Gly His Pro Ser Arg Trp Ala Arg Pro Arg Arg Pro
1               5                   10                  15

Cys Ile Cys Pro Ser Ser Leu Cys Ser Pro Arg Glu Pro Arg Ser Gly
            20                  25                  30

Pro Ala Val Gly Arg Gly Gly Ala Ala His His Arg Val Pro Ala Gly
        35                  40                  45

His Thr Pro Gly Pro Gln Leu Leu Gln Pro His Leu His Leu Pro Gln
    50                  55                  60

Gly Gln Thr Trp Leu Cys Leu Gln Pro Ser Pro Ala Gly Leu Val Lys
65                  70                  75                  80

Leu Gly Val His Cys Val Thr Cys Gln Lys Val Ala Ile Lys Ile Val
                85                  90                  95

Asn Arg Glu Lys Leu Ser Glu Ser Val Leu Met Lys Val Glu Arg Glu
            100                 105                 110

Ile Ala Ile Leu Lys Leu Ile Glu His Pro His Val Leu Lys Leu His
        115                 120                 125

Asp Val Tyr Glu Asn Lys Lys Tyr Leu Tyr Leu Val Leu Glu His Val
    130                 135                 140

Ser Gly Gly Glu Leu Phe Asp Tyr Leu Val Lys Lys Gly Arg Leu Thr
145                 150                 155                 160

Pro Lys Glu Ala Arg Lys Phe Phe Arg Gln Ile Ile Ser Ala Leu Asp
                165                 170                 175

Phe Cys His Ser His Ser Ile Cys His Arg Asp Leu Lys Pro Glu Asn
            180                 185                 190

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

Ala Ser Leu Gln Val Gly Asp Ser Leu Leu Glu Thr Ser Cys Gly Ser
    210                 215                 220

Pro His Tyr Ala Cys Pro Glu Val Ile Arg Gly Glu Lys Tyr Asp Gly
225                 230                 235                 240

Arg Lys Ala Asp Val Trp Ser Cys Gly Val Ile Leu Phe Ala Leu Leu
                245                 250                 255

Val Gly Ala Leu Pro Phe Asp Asp Asp Asn Leu Arg Gln Leu Leu Glu
            260                 265                 270
```

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```
Lys Val Lys Arg Gly Val Phe His Met Pro His Phe Ile Pro Pro Asp
        275                 280                 285

Cys Gln Ser Leu Leu Arg Gly Met Ile Glu Val Asp Ala Ala Arg Arg
    290                 295                 300

Leu Thr Leu Glu His Ile Gln Lys His Ile Trp Tyr Ile Gly Gly Lys
305                 310                 315                 320

Asn Glu Pro Glu Pro Glu Gln Pro Ile Pro Arg Lys Val Gln Ile Arg
                325                 330                 335

Ser Leu Pro Ser Leu Glu Asp Ile Asp Pro Asp Val Leu Asp Ser Met
            340                 345                 350

His Ser Leu Gly Cys Phe Arg Asp Arg Asn Lys Leu Leu Gln Asp Leu
        355                 360                 365

Leu Ser Glu Glu Glu Asn Gln Glu Lys Met Ile Tyr Phe Leu Leu Leu
    370                 375                 380

Asp Arg Lys Glu Arg Tyr Pro Ser Gln Glu Asp Glu Asp Leu Pro Pro
385                 390                 395                 400

Arg Asn Glu Ile Asp Pro Pro Arg Lys Arg Val Asp Ser Pro Met Leu
                405                 410                 415

Asn Arg His Gly Lys Arg Arg Pro Glu Arg Lys Ser Met Glu Val Leu
            420                 425                 430

Ser Val Thr Asp Gly Gly Ser Pro Val Pro Ala Arg Arg Ala Ile Glu
        435                 440                 445

Met Ala Gln His Gly Gln Arg Ser Arg Ser Ile Ser Gly Ala Ser Ser
    450                 455                 460

Gly Leu Ser Thr Ser Pro Leu Ser Ser Pro Arg Val Thr Pro His Pro
465                 470                 475                 480

Ser Pro Arg Gly Ser Pro Leu Pro Thr Pro Lys Gly Thr Pro Val His
                485                 490                 495

Thr Pro Lys Glu Ser Pro Ala Gly Thr Pro Asn Pro Thr Pro Pro Ser
            500                 505                 510

Ser Pro Ser Val Gly Gly Val Pro Trp Arg Ala Arg Leu Asn Ser Ile
        515                 520                 525

Lys Asn Ser Phe Leu Gly Ser Pro Arg Phe His Arg Arg Lys Leu Gln
    530                 535                 540

Val Pro Thr Pro Glu Glu Met Ser Asn Leu Thr Pro Glu Ser Ser Pro
545                 550                 555                 560

Glu Leu Ala Lys Lys Ser Trp Phe Gly Asn Phe Ile Ser Leu Glu Lys
                565                 570                 575

Glu Glu Gln Ile Phe Val Val Ile Lys Asp Lys Pro Leu Ser Ser Ile
            580                 585                 590

Lys Ala Asp Ile Val His Ala Phe Leu Ser Ile Pro Ser Leu Ser His
        595                 600                 605

Ser Val Ile Ser Gln Thr Ser Phe Arg Ala Glu Tyr Lys Ala Thr Gly
    610                 615                 620

Gly Pro Ala Val Phe Gln Lys Pro Val Lys Phe Gln Val Asp Ile Thr
625                 630                 635                 640

Tyr Thr Glu Gly Gly Glu Ala Gln Lys Glu Asn Gly Ile Tyr Ser Val
                645                 650                 655

Thr Phe Thr Leu Leu Ser Gly Pro Ser Arg Arg Phe Lys Arg Val Val
            660                 665                 670
```

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```
Glu Thr Ile Gln Ala Gln Leu Leu Ser Thr His Asp Pro Pro Ala Ala
        675                 680                 685

Gln His Leu Ser Asp Thr Thr Asn Cys Met Glu Met Met Thr Gly Arg
    690                 695                 700

Leu Ser Lys Cys Asp Glu Lys Asn Gly Gln Ala Ala Gln Ala Pro Ser
705                 710                 715                 720

Thr Pro Ala Lys Arg Ser Ala His Gly Pro Leu Gly Asp Ser Ala Ala
                725                 730                 735

Ala Gly Pro Gly Pro Gly Gly Asp Ala Glu Tyr Pro Thr Gly Lys Asp
            740                 745                 750

Thr Ala Lys Met Gly Pro Pro Thr Ala Arg Arg Glu Gln Pro
        755                 760                 765


<210> SEQ ID NO 7
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 7

cgatgacatc gacggggaag gac                                              23


<210> SEQ ID NO 8
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 8

gatggcctcg tggaggtgac atg                                              23


<210> SEQ ID NO 9
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 9

gccaccatgg attacaagga tgacgacgat aagacatcga cggggaagga cggcggc        57


<210> SEQ ID NO 10
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 10

gatggcctcg tggaggtgac atg                                              23


<210> SEQ ID NO 11
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 11
```

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ccagaaggtg gccatcatga tcgtcaaccg tgag 34

<210> SEQ ID NO 12
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 12

cgccgcacgc cgcctcgcgc tagagcacat tcag 34

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 13

gtcacctgac ccctcagcaa 20

<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA primer

<400> SEQUENCE: 14

caccagctct gtcctcagag 20

<210> SEQ ID NO 15
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: enzyme substrate

<400> SEQUENCE: 15

Lys Lys Leu Asn Arg Thr Leu Ser Phe Ala Glu Pro Gly
1 5 10

<210> SEQ ID NO 16
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

agcgagagat tgccatcttg a 21

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 17

gccagagaac ctgctgttgg a 21

<210> SEQ ID NO 18
<211> LENGTH: 3117
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 18

```
tgttcggctc agctgcacgg ctcggctcgg ctcggctcgg ctcggctgcg cggccgctga     60
cgggcgtgcg ctgggggcgc ggggcgcggg gcgcgggcct cggcggcggc ggcggcggcg    120
gcggcggaag ccaggtgccc ccgcccgccc tgtcctctcg acgaggcgga ggcgtcgccg    180
cgggccaggc ctcggactgc cgcgtcggag tggacgcggg gggcggcggc gcgggcggac    240
gcgggcggcg cgaagcagcg gggcccgcgg gggcgccccg gccgggtcgg cgcggacggc    300
actcggcgga cgcgggcgga cgctgggcgg cccctccctg cccgcgcgcc cgggcgcccc    360
tggccggcgc tgggccccag agcgatgaca tcgacgggga aggacggcgg cgcgcagcac    420
gcgcagtatg ttgggcccta ccggctggag aagacgctgg gcaaggggca gacaggtctg    480
gtgaagctgg gggttcactg cgtcacctgc cagaaggtgg ccatcaagat cgtcaaccgt    540
gagaagctca gcgagtcggt gctgatgaag gtggagcggg agatcgcgat cctgaagctc    600
attgagcacc cccacgtcct aaagctgcac gacgtttatg aaaacaaaaa atatttgtac    660
ctggtgctag aacacgtgtc aggtggtgag ctcttcgact acctggtgaa gaaggggagg    720
ctgacgccta aggaggctcg gaagttcttc cggcagatca tctctgcgct ggacttctgc    780
cacagccact ccatatgcca cagggatctg aaacctgaaa acctcctgct ggacgagaag    840
aacaacatcc gcatcgcaga ctttggcatg gcgtccctgc aggttggcga cagcctgttg    900
gagaccagct gtgggtcccc ccactacgcc tgccccgagg tgatccgggg ggagaagtat    960
gacggccgga aggcggacgt gtggagctgc ggcgtcatcc tgttcgcctt gctggtgggg   1020
gctctgccct tcgacgatga caacttgcga cagctgctgg agaaggtgaa gcggggcgtg   1080
ttccacatgc cgcactttat cccgcccgac tgccagagtc tgctacgggg catgagcgag   1140
gtggacgccg cacgccgcct cacgctagag cacattcaga aacacatatg gtatataggg   1200
ggcaagaatg agcccgaacc agagcagccc attcctcgca aggtgcagat ccgctcgctg   1260
cccagcctgg aggacatcga ccccgacgtg ctggacagca tgcactcact gggctgcttc   1320
cgagaccgca acaagctgct gcaggacctg ctgtccgagg aggagaacca ggagaagatg   1380
atttacttcc tcctcctgga ccggaaagaa aggtacccga gccaggagga tgaggacctg   1440
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cacggcaagc ggcggccaga acgcaaatcc atggaggtgc tcagcgtgac ggacggcggc   1560
tccccggtgc ctgcgcggcg ggccattgag atggcccagc acggccagag gtctcggtcc   1620
atcagcggtg cctcctcagg cctttccacc agcccactca gcagcccccg ggtgacccct   1680
cacccctcac caaggggcag tcccctcccc acccccaagg ggacacctgt ccacacgcca   1740
aaggagagcc cggctggcac gcccaacccc acgcccccgt ccagccccag cgtcggaggg   1800
gtgccctgga gggcgcggct caactccatc aagaacagct ttctgggctc accccgcttc   1860
caccgccgaa aactgcaagt tccgacgccg gaggagatgt ccaacctgac accagagtcg   1920
tccccagagc tggcgaagaa gtcctggttt gggaacttca tcagcctgga gaaggaggag   1980
cagatcttcg tggtcatcaa agacaaacct ctgagctcca tcaaggctga catcgtgcac   2040
gccttcctgt cgattcccag tctcagccac agcgtcatct cccaaacgag cttccgggcc   2100
gagtacaagg ccacgggggg gccagccgtg ttccagaagc cggtcaagtt ccaggttgat   2160
atcacctaca cggagggtgg ggaggcgcag aaggagaacg gcatctactc cgtcaccttc   2220
```

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```
accctgctct caggccccag ccgtcgcttc aagagggtgg tggagaccat ccaggcccag   2280

ctgctgagca cacacgaccc gcctgcggcc cagcacttgt cagacaccac taactgtatg   2340

gaaatgatga cggggcggct ttccaaatgt ggaattatcc cgaaaagtta acatgtcacc   2400

tccacgaggc catcctctgt gaccgaaggc agctgctgcg gacccgccct ccctccgctc   2460

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cttctggggc caggaccccc ggtgggcaac gtagccacag gaacaggccc cgtccaccgc   2700

ctccacgccg cacctggagg cctcctgcag gcccgtgccc cgcctccctg gccgcgccgc   2760

ctccgtgtag tcttggcctc ctcaggctgc ctcccgtcct ctcgtctcac ccgcgcctcc   2820

cttgcctcat ctggggaggc tgtgggctct ggcgctcctc tctggctgag gtggaaacag   2880

agacaccctg cggcaccaga gccttcccag caggccaggc cgctgggctg ggatcagtgt   2940

tatttatttg ccgttttcca atttatggat tctccgcacc tctgttcagg gaagggcggc   3000

ggccacatcc cctgccgtct gcgtgtctca ggcagtgggg gggctggggc cagggcgccc   3060

tctgaggaca gagctggtgg ggcgcggggg ggctggcgag ctactgtaaa ctttaaa      3117

&lt;210&gt; SEQ ID NO 19
&lt;211&gt; LENGTH: 668
&lt;212&gt; TYPE: PRT
&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 19

Met Thr Ser Thr Gly Lys Asp Gly Gly Ala Gln His Ala Gln Tyr Val
1               5                   10                  15

Gly Pro Tyr Arg Leu Glu Lys Thr Leu Gly Lys Gly Gln Thr Gly Leu
            20                  25                  30

Val Lys Leu Gly Val His Cys Val Thr Cys Gln Lys Val Ala Ile Lys
        35                  40                  45

Ile Val Asn Arg Glu Lys Leu Ser Glu Ser Val Leu Met Lys Val Glu
    50                  55                  60

Arg Glu Ile Ala Ile Leu Lys Leu Ile Glu His Pro His Val Leu Lys
65                  70                  75                  80

Leu His Asp Val Tyr Glu Asn Lys Lys Tyr Leu Tyr Leu Val Leu Glu
                85                  90                  95

His Val Ser Gly Gly Glu Leu Phe Asp Tyr Leu Val Lys Lys Gly Arg
            100                 105                 110

Leu Thr Pro Lys Glu Ala Arg Lys Phe Phe Arg Gln Ile Ile Ser Ala
        115                 120                 125

Leu Asp Phe Cys His Ser His Ser Ile Cys His Arg Asp Leu Lys Pro
    130                 135                 140

Glu Asn Leu Leu Leu Asp Glu Lys Asn Asn Ile Arg Ile Ala Asp Phe
145                 150                 155                 160

Gly Met Ala Ser Leu Gln Val Gly Asp Ser Leu Leu Glu Thr Ser Cys
                165                 170                 175

Gly Ser Pro His Tyr Ala Cys Pro Glu Val Ile Arg Gly Glu Lys Tyr
            180                 185                 190

Asp Gly Arg Lys Ala Asp Val Trp Ser Cys Gly Val Ile Leu Phe Ala
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        195                 200                 205

Leu Leu Val Gly Ala Leu Pro Phe Asp Asp Asp Asn Leu Arg Gln Leu
    210                 215                 220

Leu Glu Lys Val Lys Arg Gly Val Phe His Met Pro His Phe Ile Pro
225                 230                 235                 240

Pro Asp Cys Gln Ser Leu Leu Arg Gly Met Ser Glu Val Asp Ala Ala
                245                 250                 255

Arg Arg Leu Thr Leu Glu His Ile Gln Lys His Ile Trp Tyr Ile Gly
            260                 265                 270

Gly Lys Asn Glu Pro Glu Pro Glu Gln Pro Ile Pro Arg Lys Val Gln
        275                 280                 285

Ile Arg Ser Leu Pro Ser Leu Glu Asp Ile Asp Pro Asp Val Leu Asp
    290                 295                 300

Ser Met His Ser Leu Gly Cys Phe Arg Asp Arg Asn Lys Leu Leu Gln
305                 310                 315                 320

Asp Leu Leu Ser Glu Glu Glu Asn Gln Glu Lys Met Ile Tyr Phe Leu
                325                 330                 335

Leu Leu Asp Arg Lys Glu Arg Tyr Pro Ser Gln Glu Asp Glu Asp Leu
            340                 345                 350

Pro Pro Arg Asn Glu Ile Asp Pro Pro Arg Lys Arg Val Asp Ser Pro
        355                 360                 365

Met Leu Asn Arg His Gly Lys Arg Arg Pro Glu Arg Lys Ser Met Glu
    370                 375                 380

Val Leu Ser Val Thr Asp Gly Gly Ser Pro Val Pro Ala Arg Arg Ala
385                 390                 395                 400

Ile Glu Met Ala Gln His Gly Gln Arg Ser Arg Ser Ile Ser Gly Ala
                405                 410                 415

Ser Ser Gly Leu Ser Thr Ser Pro Leu Ser Ser Pro Arg Val Thr Pro
            420                 425                 430

His Pro Ser Pro Arg Gly Ser Pro Leu Pro Thr Pro Lys Gly Thr Pro
        435                 440                 445

Val His Thr Pro Lys Glu Ser Pro Ala Gly Thr Pro Asn Pro Thr Pro
    450                 455                 460

Pro Ser Ser Pro Ser Val Gly Gly Val Pro Trp Arg Ala Arg Leu Asn
465                 470                 475                 480

Ser Ile Lys Asn Ser Phe Leu Gly Ser Pro Arg Phe His Arg Arg Lys
                485                 490                 495

Leu Gln Val Pro Thr Pro Glu Glu Met Ser Asn Leu Thr Pro Glu Ser
            500                 505                 510

Ser Pro Glu Leu Ala Lys Lys Ser Trp Phe Gly Asn Phe Ile Ser Leu
        515                 520                 525

Glu Lys Glu Glu Gln Ile Phe Val Val Ile Lys Asp Lys Pro Leu Ser
    530                 535                 540

Ser Ile Lys Ala Asp Ile Val His Ala Phe Leu Ser Ile Pro Ser Leu
545                 550                 555                 560

Ser His Ser Val Ile Ser Gln Thr Ser Phe Arg Ala Glu Tyr Lys Ala
                565                 570                 575

Thr Gly Gly Pro Ala Val Phe Gln Lys Pro Val Lys Phe Gln Val Asp
            580                 585                 590

Ile Thr Tyr Thr Glu Gly Gly Glu Ala Gln Lys Glu Asn Gly Ile Tyr
        595                 600                 605
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Ser Val Thr Phe Thr Leu Leu Ser Gly Pro Ser Arg Arg Phe Lys Arg
    610                 615                 620

Val Val Glu Thr Ile Gln Ala Gln Leu Leu Ser Thr His Asp Pro Pro
625                 630                 635                 640

Ala Ala Gln His Leu Ser Asp Thr Thr Asn Cys Met Glu Met Met Thr
                645                 650                 655

Gly Arg Leu Ser Lys Cys Gly Ile Ile Pro Lys Ser
            660                 665
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1. A method of detecting type 1 diabetes in a patient, comprising:

reacting a patient sample with a PBK1 protein or a fragment thereof under conditions that promote antibody-antigen binding; and

detecting the presence of an autoantibody in said sample.

2. The method of claim **1** wherein the PBK1 protein is immobilized on a solid support.

3. The method of claim **1** wherein the patient sample is selected from the group consisting of: blood, serum, plasma, and a biopsy tissue.

4. The method of claim **1** wherein the detecting of the autoantibody comprises reacting the autoantibody with an antibody having a detectable label.

5. A mammalian pancreas-derived cell comprising a recombinant nucleic acid encoding a PBK1 protein.

6. The mammalian pancreas-derived cell of claim **5** wherein the cell is a cell of a mammalian pancreas-derived cell line.

7. The pancreas-derived cell of claim **5** wherein the cell is a Min6 cell transfected with a recombinant nucleic acid encoding a PBK1 protein.

8. A method of identifying a PBK1 modulator, comprising one or more of:

a) contacting a PBK1 protein with a test agent under conditions that promote kinase activity of said PBK1 protein, and detecting an increase in the kinase activity; and

b) contacting a cell that expresses a PBK1 protein with a test agent under conditions that promote glucose-stimulated insulin secretion from said cell, and detecting an increase in the glucose-stimulated insulin secretion from the cell; wherein

said increase in a) or b) each indicates that the test agent is a PBK1 modulator.

9. The method of claim **8** further comprising determining that the increase in the glucose-stimulated insulin secretion comprises potentiation of secretagogue-stimulated insulin secretion from a cell that expresses a PBK1 protein.

10. The method of claim **8** wherein the cell is a mammalian pancreas-derived cell.

11. The method of claim **8** wherein the cell is a MIN6 cell.

12. The method of claim **8** wherein the cell contains a recombinant nucleic acid encoding a PBK1 protein.

13. A method of screening for an agent that treats a metabolic disease, said method comprising one or more of:

a) contacting a PBK1 protein with a test agent under conditions that promote binding of said test agent to said PBK1 protein, and detecting binding of said test agent to said PBK protein;

b) contacting a PBK1 protein with a test agent under conditions that promote kinase activity of said PBK1 protein, and detecting an increase in the kinase activity; and

c) contacting a cell that expresses a PBK protein with a test agent under conditions that promote glucose-stimulated insulin secretion from said cell, and detecting an increase in the glucose-stimulated insulin secretion from the cell; wherein

said disorder is selected from diabetes, type 2 diabetes, obesity, and diabetic retinopathy.

14. The method of claim **13**, further comprising designating said test agent as a compound that treats said disease.

15. The method of claim **13**, further comprising designating said test agent as a compound that treats said disease.

16. The method of claim **13**, further comprising designating said test agent as a compound that treats type 2 diabetes.

17. The method of claim **13**, wherein said PBK1 protein is selected from a protein having a polypeptide sequence that has at least 73%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the protein having a polypeptide sequence set forth in SEQ ID NO: 2.

18. The method of claim **17**, wherein said PBK1 protein is selected from a protein having a polypeptide sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 6

19. The method of claim **13** wherein a) is performed prior to c).

20. The method of claim **13** wherein b) is performed prior to c).

21. The method of claim **13** wherein a) is performed prior to b), and b) is performed prior to c).

22. The method of claim **13** further comprising determining that the increase in the glucose-stimulated insulin secretion comprises potentiation of secretagogue-stimulated insulin secretion from a cell that expresses a PBK1 protein.

23. The method of claim **13** wherein the cell is a mammalian pancreas-derived cell.

24. The method of claim **13** wherein the cell is a MIN6 cell.

25. The method of claim **13** wherein the cell contains a recombinant nucleic acid encoding a PBK1 protein.

26. A method for stimulating cell differentiation and/or regeneration in a pancreatic beta cell comprising delivering PBK-1 DNA to said cell.

27. The method of claim **26** wherein said DNA is operably linked to a promoter that drives expression of said DNA in said cell.

28. A method for in vivo screening for an agent that treats diabetes, said method comprising:

a) subjecting a mammal comprising a PBK1 knockout genome to a diet that promotes diabetes;

b) treating said mammal with a test agent;

c) determining blood glucose level of said mammal; and

d) determining whether said mammal is glucose tolerant.

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专利名称(译)	与PBK1有关的方法和组合物		
公开(公告)号	US20100146642A1	公开(公告)日	2010-06-10
申请号	US12/154296	申请日	2008-05-22
申请(专利权)人(译)	宾夕法尼亚州立大学		
当前申请(专利权)人(译)	宾州州立大学研究基金会		
[标]发明人	SHI YUGUANG YE GUANGMING		
发明人	SHI, YUGUANG YE, GUANGMING		
IPC分类号	A61K49/00 G01N33/567 C12N5/071 G01N33/53 C12N9/12 A61P3/10		
CPC分类号	C12Q1/485 C12Q1/6883 G01N33/507 G01N33/5088 C12Q2600/158 G01N2800/042 C12Q2600/136 C12Q2600/156 G01N33/564		
优先权	60/939462 2007-05-22 US		
其他公开文献	US8163505		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及包含和利用PBK1蛋白和DNA的方法的组合物，包括检测1型糖尿病的方法;哺乳动物胰腺来源的细胞，其包含编码PBK1蛋白的重组核酸;一种鉴定PBK1调节剂的方法;用于治疗受试者的2型糖尿病的药物组合物;一种筛选治疗代谢疾病的药剂的方法;将PI3K-1 DNA递送至受试者以刺激胰腺β细胞分化和/或再生;一种刺激胰腺β细胞中细胞分化和/或再生的方法;使用具有靶向缺失或过表达PI3K-1基因的转基因小鼠来测试PI3K-1调节剂化合物的功效和特异性。