US 20250255912A1

(19) United States
(12) Patent Application Publication
Barbon et al.
(10) Pub. No.: US 2025/0255912 A1
(43) Pub. Date: Aug. 14, 2025

(54) **LENTIVIRAL VECTOR**

(71) Applicant: **Genespire S.r.l.**, Milan (IT)

(72) Inventors: **Elena Barbon**, Cernusco sul Naviglio (Milano) (IT); **Alessio Cantore**, Milano (IT)

(21) Appl. No.: **18/856,436**

(22) PCT Filed: **Apr. 14, 2023**

(86) PCT No.: **PCT/EP2023/059848**

§ 371 (c)(1),
(2) Date: **Oct. 11, 2024**

(30) **Foreign Application Priority Data**

Apr. 14, 2022 (GB) 2205606.3

Publication Classification

(51) **Int. Cl.**

A61K 35/76	(2015.01)
A61K 48/00	(2006.01)
C07K 14/47	(2006.01)
C12N 15/86	(2006.01)

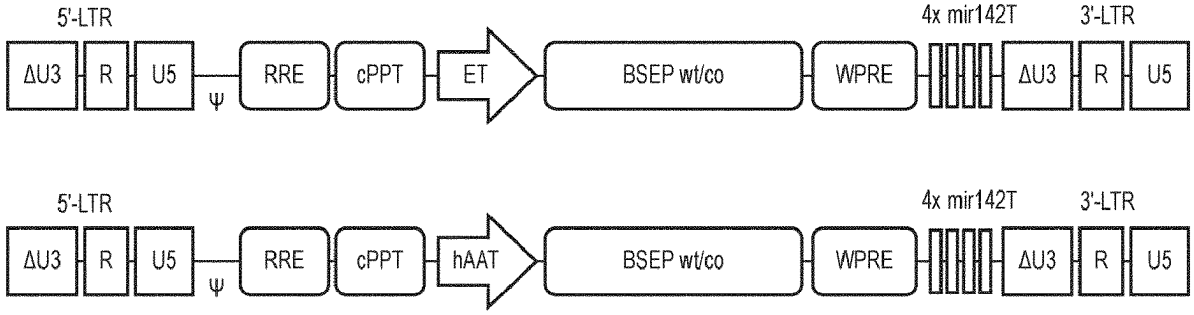
(52) **U.S. Cl.**

CPC ***A61K 35/76*** (2013.01); ***A61K 48/005*** (2013.01); ***C07K 14/47*** (2013.01); ***C12N 15/86*** (2013.01); *C12N 2740/16043* (2013.01); *C12N 2830/008* (2013.01)

(57) **ABSTRACT**

The present invention relates to lentiviral vectors encoding a progressive familial intrahepatic cholestasis-associated polypeptide. The present invention also relates to cells and pharmaceutical compositions comprising said lentiviral vectors and to uses of said lentiviral vectors in treating progressive familial intrahepatic cholestasis.

Specification includes a Sequence Listing.



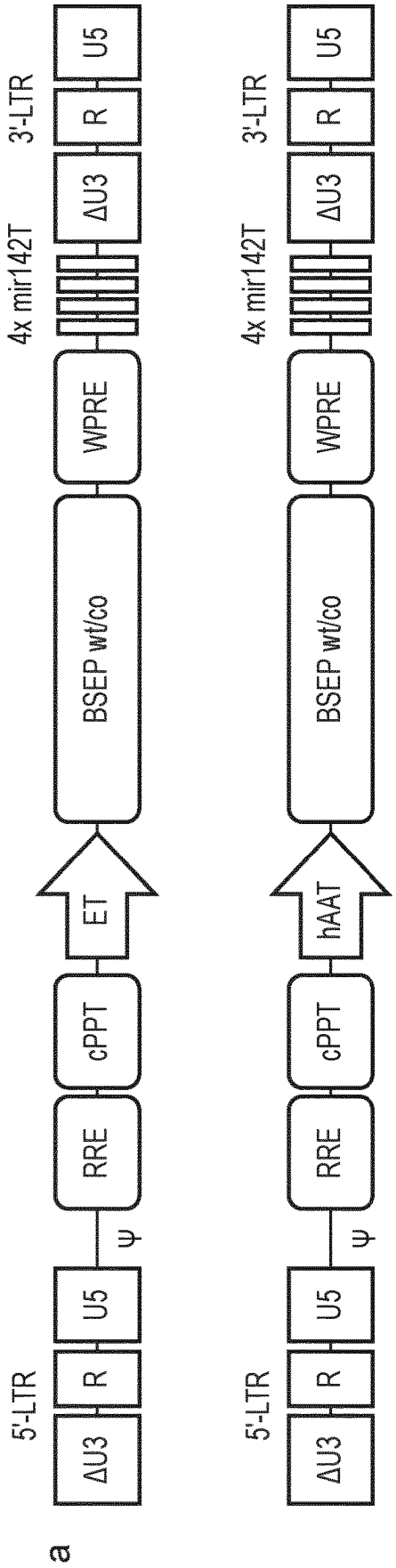
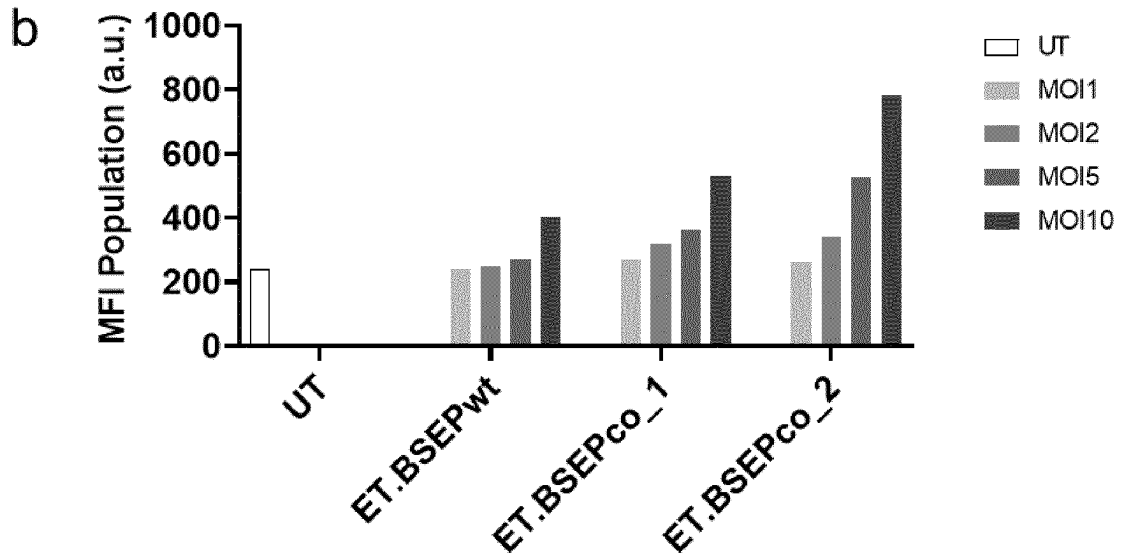

a
5'-LTR
ΔU3
R
U5
ψ
RRE
cPPT
ET
BSEP wt/co
WPRE
4x mir142T
ΔU3
R
U5
3'-LTR
5'-LTR
ΔU3
R
U5
ψ
RRE
cPPT
hAAT
BSEP wt/co
WPRE
4x mir142T
ΔU3
R
U5
3'-LTR

FIG. 1



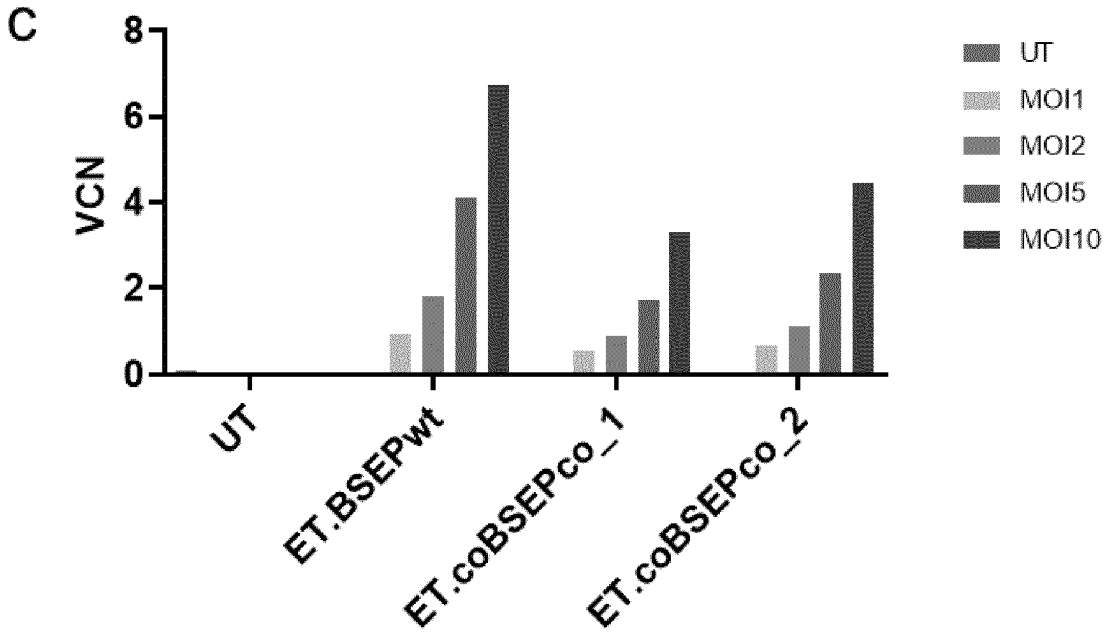
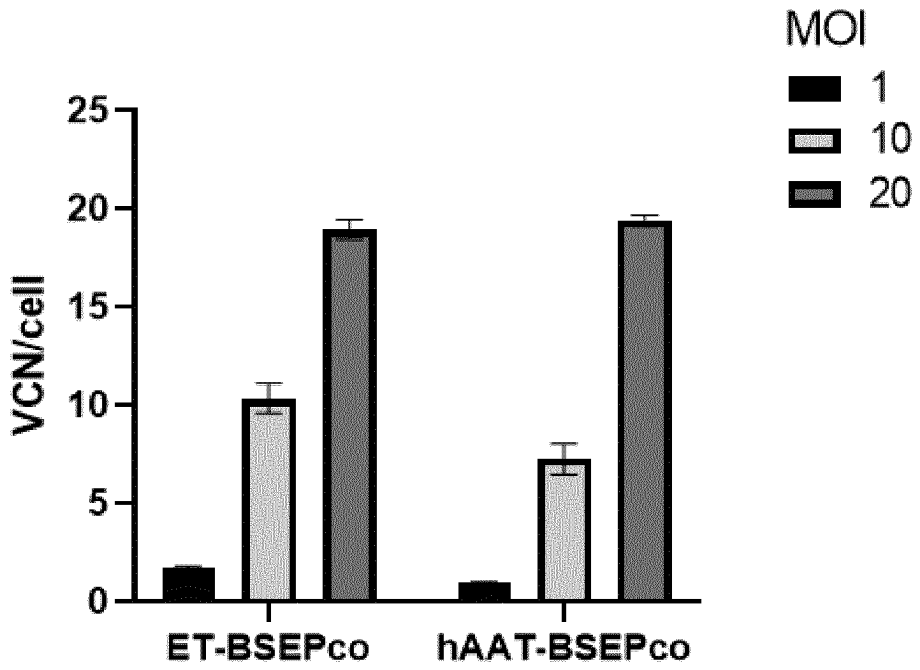


FIG. 1 (continued)

d



e

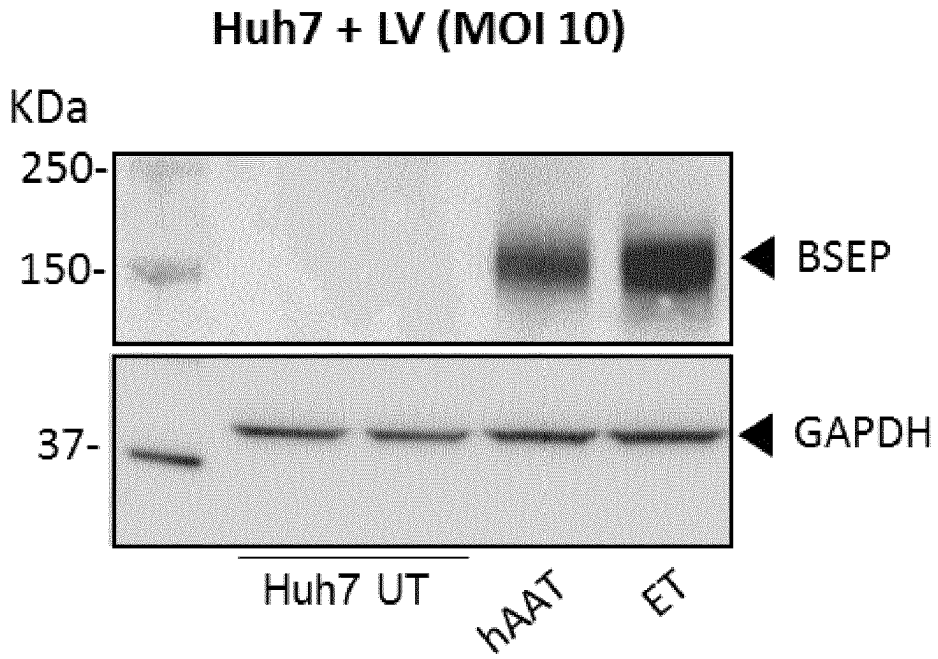


FIG. 1 (continued)

f

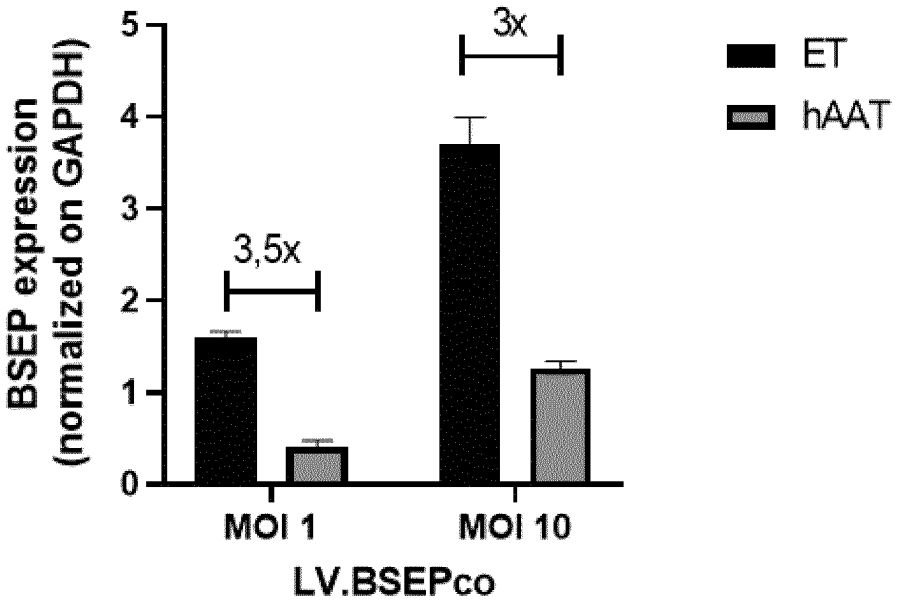


FIG. 1 (continued)

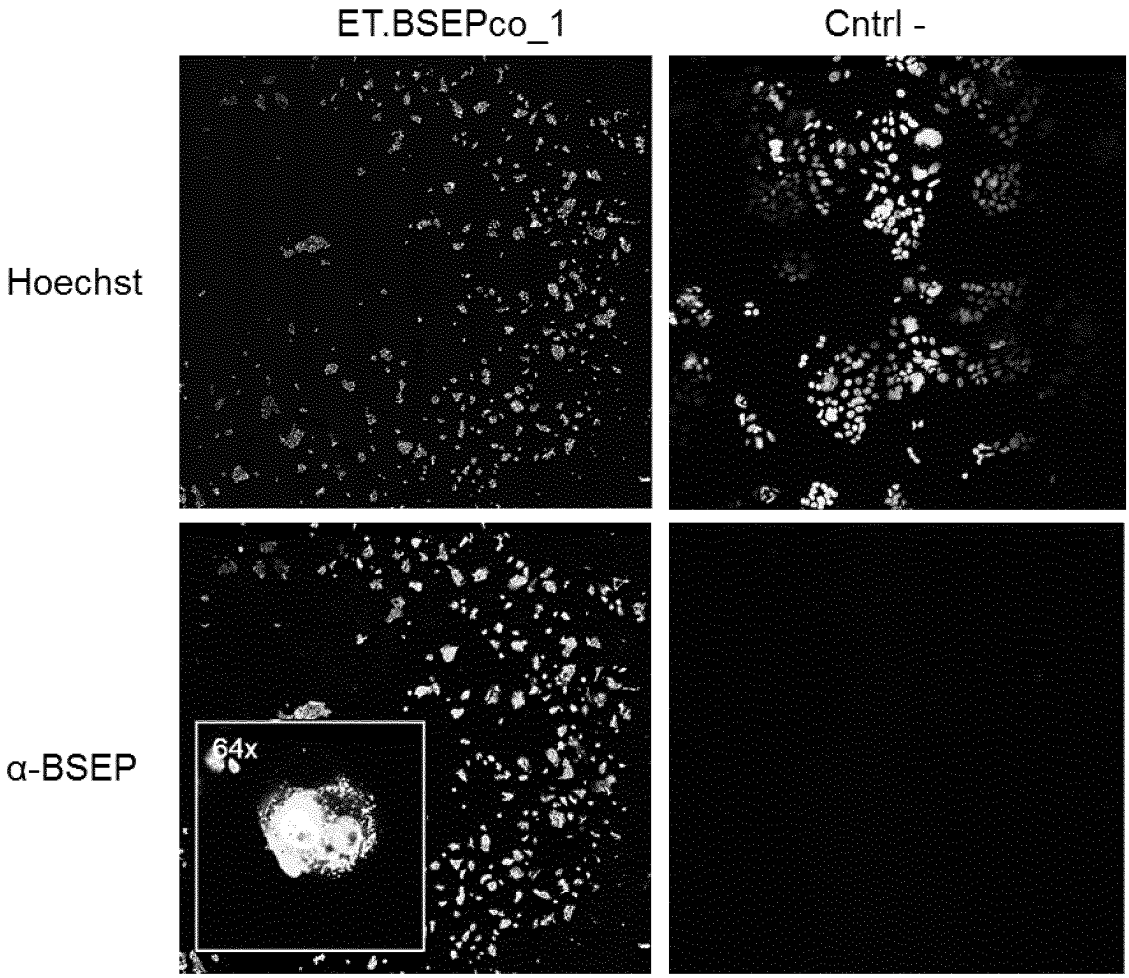


FIG. 2

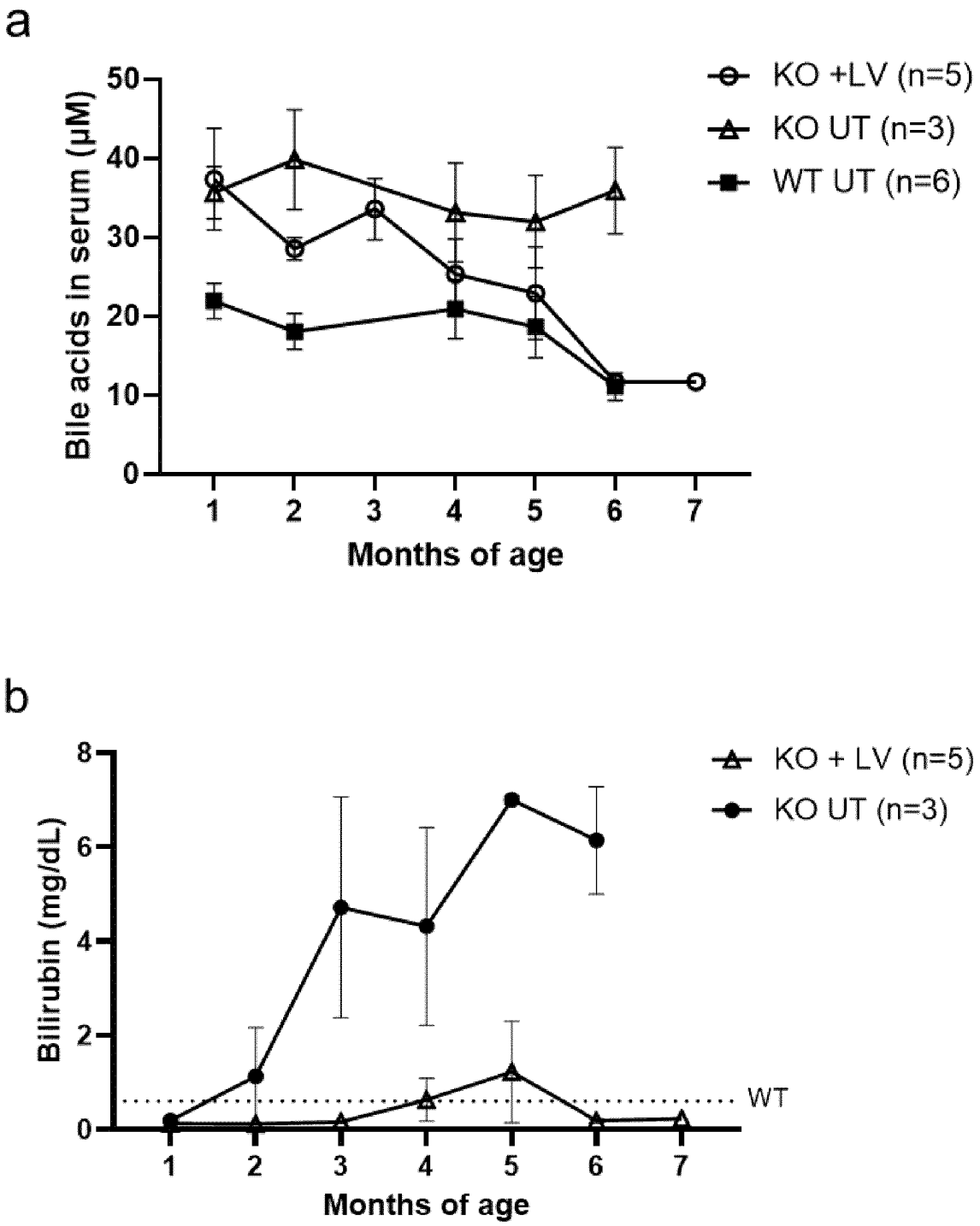

a
Bile acids in serum (μM)
50
40
30
20
10
0
1
2
3
4
5
6
7
Months of age
KO +LV (n=5)
KO UT (n=3)
WT UT (n=6)
b
Bilirubin (mg/dL)
8
6
4
2
0
WT
KO + LV (n=5)
KO UT (n=3)

FIG. 3

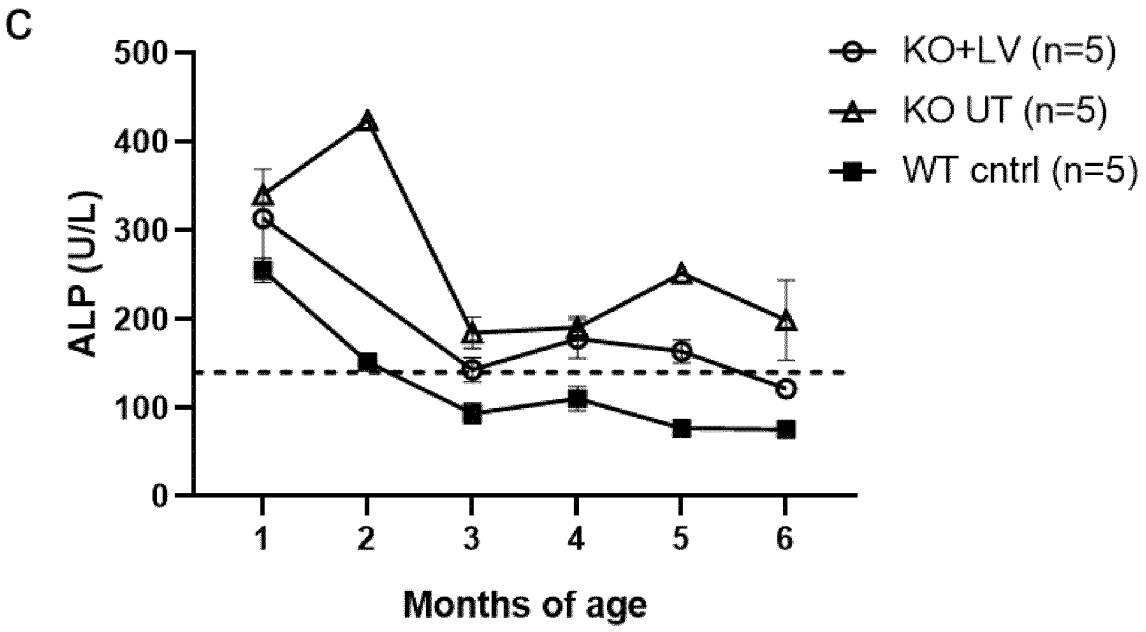


FIG. 3 (continued)

LENTIVIRAL VECTOR

FIELD OF THE INVENTION

[0001] The present invention relates to lentiviral vectors encoding a progressive familial intrahepatic cholestasis-associated polypeptide. The present invention also relates to cells and pharmaceutical compositions comprising said lentiviral vectors and to uses of said lentiviral vectors in treating progressive familial intrahepatic cholestasis.

BACKGROUND TO THE INVENTION

[0002] Progressive familial intrahepatic cholestasis (PFIC) is a group of rare autosomal recessive liver disorders of childhood characterized by mutations in genes encoding proteins involved in the hepatocellular bile transport system (see e.g. Baker, A., et al., 2019. Clinics and research in hepatology and gastroenterology, 43(1), pp. 20-36).

[0003] Three main subtypes of PFIC (PFIC-1, PFIC-2, and PFIC-3) have been identified. PFIC-1 is caused by mutations in the ATPase phospholipid transporting 8B1 gene (ATP8B1), which encodes a phospholipid-transporting transmembrane P-type adenosine triphosphatase (FIC1). PFIC-2 is caused by mutations in the ATP binding cassette subfamily B member 11 gene (ABCB11), which encodes the bile salt export pump (BSEP), the main transporter of bile acids from hepatocytes to the canalicular lumen. PFIC-3 is caused by mutations in the ATP binding cassette subfamily B member 4 gene (ABCB4), which encodes multidrug-resistance protein 3 (MDR3).

[0004] Patients with PFIC face debilitating symptoms and poor prognosis. The main clinical features of PFIC include cholestasis, jaundice and pruritus, with symptoms typically appearing in infancy or early childhood. PFIC is associated with a range of potentially fatal complications of the liver, including portal hypertension, liver failure, cirrhosis and hepatocellular carcinoma, as well as extrahepatic manifestations (see e.g. Srivastava, A., 2014. Journal of clinical and experimental hepatology, 4(1), pp. 25-36).

[0005] For example, PFIC-2 patients display cholestasis, jaundice and severe itching and usually develop liver fibrosis and end-stage liver disease before adulthood due to massive bile acid accumulation in the hepatic tissue. Current treatment consists of diet control, pharmacological or surgical treatments such as biliary diversions aiming at interrupting the enterohepatic circulation and decreasing bile acid blood concentration. However, most patients ultimately undergo liver transplantation as the only curative option.

[0006] There is a demand for new approaches for treating or preventing PFIC, for example PFIC-2.

SUMMARY OF THE INVENTION

[0007] The present inventors have developed a gene therapy for treating and/or preventing PFIC, for example PFIC-2. The lentiviral vector mediated gene therapy described herein may allow for a stable gene transfer even in paediatric patients at the first disease stages by virtue of lentiviral vector genomic integration and may therefore alleviate symptoms and limit progressive damage to the hepatocytes.

[0008] The present inventors have surprisingly shown that the lentiviral vector mediated gene therapy described herein is safe and efficacious in a model of PFIC-2. The present inventors produced a lentiviral vector encoding a human codon optimized BSEP-coding sequence under the control of a hepatocyte-specific cassette. Administration of the lentiviral vector resulted in a significant decrease of serum bile acids, and a significant decrease in serum bilirubin and ALP biomarkers, indicating prevention of progressive cholestatic damage.

[0009] In one aspect, the present invention provides a lentiviral vector comprising a nucleotide sequence encoding a progressive familial intrahepatic cholestasis (PFIC)-associated polypeptide. The lentiviral vector may be an immune-shielded lentiviral vector.

[0010] The PFIC-associated polypeptide may selected from familial intrahepatic cholestasis type 1 (FIC1), or a fragment thereof; bile salt export pump (BSEP), or a fragment thereof; multiple drug resistance 3 (MDR3), or a fragment thereof; tight junction protein 2 (TJP2), or a fragment thereof; farnesoid X receptor (FXR), or a fragment thereof; and Myosin-Vb (MYO5B), or a fragment thereof. In some embodiments, the PFIC-associated polypeptide is selected from BSEP, or a fragment thereof; FXR, or a fragment thereof; and MYO5B, or a fragment thereof. In preferred embodiments, the PFIC-associated polypeptide is BSEP, or a fragment thereof.

[0011] Suitably, the PFIC-associated polypeptide comprises or consists of an amino acid sequence which is at least 70% identical to one of SEQ ID NOs: 37, 39, 43, 45, 47, 48 or 50, or a fragment thereof. In some embodiments, the PFIC-associated polypeptide comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 39 or a fragment thereof.

[0012] Suitably, the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to one of SEQ ID NOs: 38, 40, 41, 42, 44, 46, 49 or 51, or a fragment thereof. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to one of SEQ ID NOs: 40-42, or a fragment thereof. Suitably, the nucleotide sequence encoding a PFIC-associated polypeptide is codon-optimised. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 41 or 42, or a fragment thereof. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 41, or a fragment thereof.

[0013] The lentiviral vector may be a $CD47^{high}$ lentiviral vector. Suitably, the lentiviral vector is obtained from a $CD47^{high}$ host cell, preferably wherein the host cell is genetically engineered to increase expression of CD47 on the cell surface. Suitably, the lentiviral vector has at least about 2-fold more CD47 on its surface than a lentiviral vector obtained from an unmodified host cell.

[0014] The lentiviral vector may be a $MHC\text{-}I^{free}$ lentiviral vector. Suitably, the lentiviral vector is obtained from a $MHC\text{-}I^{free}$ host cell, preferably wherein the host cell is genetically engineered to disrupt expression of MHC-I on the cell surface. Suitably, the lentiviral vector has less than about 50% of the number of surface-exposed MHC-I molecules that are displayed on a lentiviral vector obtained from an unmodified host cell. Suitably, MHC-I is not detectable on the surface of the lentiviral vector.

[0015] The lentiviral vector may be a CD47^{high}/MHC-I^{free} lentiviral vector. Suitably, the lentiviral vector is obtained from a CD47^{high}/MHC-I^{free} host cell. Suitably, the lentiviral vector has at least about 2-fold more CD47 on its surface than a lentiviral vector obtained from an unmodified host cell and MHC-I is not detectable on the surface of the lentiviral vector.

[0016] The nucleotide sequence encoding a PFIC-associated polypeptide may be operably linked to one or more miRNA target sequences. In some embodiments, the one or more miRNA target sequences suppress transgene expression in one or more cells other than hepatocytes. In some embodiments, the one or more miRNA target sequence suppress transgene expression in hematopoietic-lineage cells and/or antigen-presenting cells.

[0017] In some embodiments, the one or more miRNA target sequences are selected from miR-181, miR-142, miR-223, and miR-155 target sequences. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to one or more mir-142 target sequence, two or more mir-142 target sequences, three or more mir-142 target sequences, or four or more mir-142 target sequences. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to four mir-142 target sequences. In some embodiments, the one or more miRNA target sequence comprise or consist of a nucleotide sequence which is at least 90% identical to SEQ ID NO: 17. In some embodiments, the one or more miRNA target sequence comprise or consist of a nucleotide sequence which is at least 90% identical to SEQ ID NO: 18.

[0018] The nucleotide sequence encoding a PFIC-associated polypeptide may be operably linked to a liver-specific promoter. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a hepatocyte-specific promoter. Suitably, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a transthyretin (TTR) promoter, an alpha-1-antitrypsin (AAT) promoter, a thyroxine-binding globulin (TBG) promoter, an APoE/hAAT promoter, a HCR-hAAT promoter, a LP1 promoter, or a HLP promoter. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a transthyretin (TTR) promoter. In preferred embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to an Enh1mTTR (ET) promoter. In some embodiments, the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a promoter which comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 19.

[0019] The lentiviral vector may be pseudotyped. Suitably, the lentiviral vector is VSV.G-pseudotyped. The lentiviral vector may be a self-inactivating (SIN) lentiviral vector. Suitably, the lentiviral vector comprises self-inactivating (SIN) LTRs which comprise or consist of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 23, or a fragment thereof. The lentiviral vector may be an integrating lentiviral vector and/or a replication-defective lentiviral vector. The lentiviral vector may be HIV-derived.

[0020] In some embodiments, the lentiviral vector comprises a nucleotide sequence which is at least 70% identical to SEQ ID NO: 36.

[0021] In one aspect, the present invention provides a method of producing a lentiviral vector according to the present invention.

[0022] In one aspect, the present invention provides a kit or system for producing a lentiviral vector of the present invention.

[0023] In one aspect, the present invention provides a cell comprising a lentiviral vector according to the present invention. The cell may be an isolated cell.

[0024] In one aspect, the present invention provides a pharmaceutical composition comprising a lentiviral vector or a cell according to the present invention, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

[0025] In one aspect, the present invention provides a lentiviral vector, a cell, or a pharmaceutical composition according to the present invention, for use as a medicament.

[0026] In one aspect, the present invention provides use of a lentiviral vector, a cell, or a pharmaceutical composition according to the present invention, for the manufacture of a medicament.

[0027] In one aspect, the present invention provides a method comprising administering a therapeutically effective amount of a lentiviral vector, a cell, or a pharmaceutical composition according to the present invention, to a subject in need thereof.

[0028] In one aspect, the present invention provides a lentiviral vector, a cell, or a pharmaceutical composition according to the present invention, for use in preventing or treating progressive familial intrahepatic cholestasis (PFIC).

[0029] In one aspect, the present invention provides use of a lentiviral vector, a cell, or a pharmaceutical composition according to the present invention, for the manufacture of a medicament for preventing or treating progressive familial intrahepatic cholestasis (PFIC).

[0030] In one aspect, the present invention provides a method of preventing or treating progressive familial intrahepatic cholestasis (PFIC), comprising administering a therapeutically effective amount of a lentiviral vector, a cell, or a pharmaceutical composition according to the present invention, to a subject in need thereof.

[0031] Suitably, the PFIC-associated polypeptide is FIC1, or a fragment thereof, and the PFIC is PFIC type 1 (PFIC-1); the PFIC-associated polypeptide is a BSEP, or a fragment thereof, and the PFIC is PFIC type 2 (PFIC-2); the PFIC-associated polypeptide is MDR3, or a fragment thereof, and the PFIC is PFIC type 3 (PFIC-3); the PFIC-associated polypeptide is TJP2, or a fragment thereof, and the PFIC is PFIC type 4 (PFIC-4); the PFIC-associated polypeptide is FXR, or a fragment thereof, and the PFIC is PFIC type 5 (PFIC-5); or the PFIC-associated polypeptide is MYO5B, or a fragment thereof, and the PFIC is PFIC type 6 (PFIC-6). In some embodiments, the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC-2; the PFIC-associated polypeptide is FXR, or a fragment thereof, and the PFIC is PFIC-5; or the PFIC-associated polypeptide is MYO5B, or a fragment thereof, and the PFIC is PFIC-6. In preferred embodiments, the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC-2.

[0032] The lentiviral vector, cell, or pharmaceutical composition may be administered to any subject in need thereof. In some embodiments, the subject is a human subject. In some embodiments, the subject is a juvenile. In some

embodiments, the subject is a paediatric patient. In some embodiments, the subject is a neonatal patient or an infantile patient.

[0033] The lentiviral vector, cell, or pharmaceutical composition may be administered by any suitable route. In some embodiments, the lentiviral vector, cell, or pharmaceutical composition is administered systemically (e.g. by intravenous injection or intraperitoneal injection). In some embodiments, the lentiviral vector, cell, or pharmaceutical composition is administered locally (e.g. by direct injection, intra-arterial injection, or intraportal injection). In some embodiments, the lentiviral vector, cell, or pharmaceutical composition is administered locally to the liver (e.g. by intrahepatic injection, intrahepatic arterial injection, or intraportal injection).

[0034] The lentiviral vector, cell, or pharmaceutical composition may be administered in any suitable dose. In some embodiments, the lentiviral vector is administered at a dose of at least about 10^8 TU/kg, at least about 10^9 TU/kg, or at least about 10^{10} TU/kg. In some embodiments, the lentiviral vector is administered in a dose of from about 10^8 to about 10^{11} TU/kg, from about 10^8 to about 10^{10} TU/kg, or from about 10^9 to about 10^{10} TU/kg.

[0035] The lentiviral vector may integrate into the genome of liver cells and be maintained as the liver cells duplicate. Suitably, the lentiviral vector integrates into the genome of hepatocytes and is maintained as the hepatocytes duplicate.

[0036] Following administration, the subject's serum bile acid levels may be reduced and/or normalised; the subject's liver function may be improved (e.g. the serum level of bilirubin and/or one or more liver enzymes may be reduced and/or normalised); and/or the formation of liver fibrosis in the subject may be slowed and/or reduced.

[0037] In one aspect, the present invention provides an immune-shielded lentiviral vector for use in a method of therapy, wherein the method comprises administration of the immune-shielded lentiviral vector to a juvenile or paediatric subject. In one aspect, the present invention provides a cell for use in a method of therapy, wherein the cell comprises an immune-shielded lentiviral vector, and wherein the method comprises administration of the cell to a juvenile or paediatric subject.

[0038] In one aspect, the present invention provides use of an immune-shielded lentiviral vector for the manufacture of a medicament for treatment or prevention of a disease, wherein the treatment or prevention comprises administration of the immune-shielded lentiviral vector to a juvenile or paediatric subject. In one aspect, the present invention provides use of a cell for the manufacture of a medicament for treatment or prevention of a disease, wherein the cell comprises an immune-shielded lentiviral vector, and wherein the treatment or prevention comprises administration of the cell to a juvenile or paediatric subject.

[0039] In one aspect, the present invention provides a method of treatment, wherein the method comprises administration of an immune-shielded lentiviral vector to a juvenile or paediatric subject. In one aspect, the present invention provides a method of treatment, wherein the method comprises administration of a cell to a juvenile or paediatric subject, wherein the cell comprises an immune-shielded lentiviral vector.

[0040] In one aspect, the present invention provides an immune-shielded lentiviral vector for use in a method of treatment of a juvenile or paediatric subject. In one aspect, the present invention provides a cell for use in a method of treatment of a juvenile or paediatric subject, wherein the cell comprises an immune-shielded lentiviral vector.

[0041] In one aspect, the present invention provides use of an immune-shielded lentiviral vector for the manufacture of a medicament for treatment or prevention of a disease in a juvenile or paediatric subject. In one aspect, the present invention provides use of a cell for the manufacture of a medicament for treatment or prevention of a disease in a juvenile or paediatric subject, wherein the cell comprises an immune-shielded lentiviral vector.

[0042] In one aspect, the present invention provides a method of treatment of a juvenile or paediatric subject, wherein the method comprises administration of an immune-shielded lentiviral vector to the juvenile or paediatric subject in need thereof. In one aspect, the present invention provides a method of treatment of a juvenile or paediatric subject, wherein the method comprises administration of a cell to the juvenile or paediatric subject in need thereof, wherein the cell comprises an immune-shielded lentiviral vector.

[0043] In some embodiments, the subject is a neonatal subject or an infantile subject.

[0044] In some embodiments, the method comprises transduction of a liver cell with the immune-shielded lentiviral vector

[0045] In some embodiments, the immune-shielded lentiviral vector comprises a nucleotide sequence encoding a progressive familial intrahepatic cholestasis (PFIC)-associated polypeptide.

DESCRIPTION OF DRAWINGS

[0046] FIG. **1**—Design and testing of a lentiviral vector (LV) encoding a BSEP transgene (a) Schematic of the third-generation lentiviral vectors (provirus). LTRs, long terminal repeats: the 5' LTR and the 3' LTR each have an almost complete deletion of the U3 region (ΔU3); ψ, the psi packaging sequence; RRE, Rev response element; cPPT, central polypurine tract; ET, enhanced transthyretin promoter; BSEP wt/co, BSEP coding sequences (wild-type or codon optimised); WPRE, posttranscriptional element from the genome of the woodchuck hepatitis virus; 4×mir142T, four copies of miR-142 target sequence in tandem. (b) Flow cytometry analysis on Huh7 cells transduced with LV-ET.BSEPwt, LV-ET.BSEPco_1 or LV-ET.BSEPco_2 at different MOI. UT, untransduced Huh7 cells. MFI, mean fluorescence intensity. (c) Histogram reporting the vector copy number (VCN) per cell (VCN/cell) in Huh7 transduced with LV-ET.BSEPwt, LV-ET.BSEPco_1 or LV-ET.BSEPco_2 at different MOI. (d) Histogram reporting the vector copy number (VCN) per cell (VCN/cell) in Huh7 transduced with LV-ET.BSEPco_1 or LV-hAAT.BSEPco_1 at different MOI. (e) Western Blot analysis of BSEP protein expression on Huh7 cell lysate. UT, untransduced Huh7; hAAT, cells transduced with LV-hAAT.BSEPco; ET, cells transduced with LV-ET.BSEPco. (f) BSEP protein expression as determined by WB densitometry analysis and normalized on GAPDH housekeeping gene in Huh7 cells transduced with LV-ET.BSEPco or LV-hAAT.BSEPco.

[0047] FIG. **2**—Immunofluorescence analysis of LV-ET.BSEPco_1 in transduced Huh7 cells Left upper panel, Huh7 cells transduced with LV-ET.BSEPco_1 and stained with Hoechst. Right upper panel, Huh7 cells untransduced (Cntrl-) and stained with Hoechst. Left lower panel, Huh7 cells

transduced with LV-ET.BSEPco_1 and stained with an anti-BSEP antibody. Right lower panel, Huh7 cells untransduced (Cntrl−) and stained with an anti-BSEP antibody.

[0048] FIG. **3**—Evaluation of LV gene therapy in Abcb11−/− mice (a) Serum bile acids in Abcb11−/− animals treated with LV-ET.BSEPco (KO+LV) compared to Abcb11−/− untreated animals (KO UT) and to WT untreated animals (WT UT). (b) Serum total bilirubin in Abcb11−/− animals treated with LV-ET.BSEPco (KO+LV) compared to Abcb11−/− untreated animals (KO UT). Dotted line indicates the upper limit of the bilirubin levels (mg/dL) measured in WT control mice. (c) Serum alkaline phosphatase (ALP) levels (IU/L) in Abcb11−/− animals treated with LV-ET.BSEPco (KO+LV) compared to Abcb11−/− untreated animals (KO UT) and to WT untreated animals (WT UT). Dotted line indicates the upper limit of the ALP levels measured in a cohort of WT control mice.

DETAILED DESCRIPTION

[0049] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

[0050] The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or method steps. The terms "comprising", "comprises" and "comprised of" also include the term "consisting of".

[0051] Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

[0052] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto.

[0053] The skilled person will understand that they can combine all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

Lentiviral Vector

[0054] In one aspect, the present invention provides a lentiviral vector comprising a protein-coding sequence, wherein the protein-coding sequence is a nucleotide sequence encoding a progressive familial intrahepatic cholestasis (PFIC)-associated polypeptide.

[0055] A "lentiviral vector" may refer to an enveloped lentiviral genome (i.e. a lentiviral particle). For example, the pharmaceutical composition of the present invention preferably comprises the lentiviral vector in the form of a lentiviral particle and the lentiviral vector is preferably administered in the form of a lentiviral particle. In other embodiments, a "lentiviral vector" may comprise a lentiviral genome, optionally wherein the lentiviral genome is enveloped. As used herein, a "lentiviral genome" may refer to a genome that comprises at least one element derived or derivable from a lentivirus genome. Suitably, a lentiviral genome comprises at least one element that is involved in the mechanisms by which a lentivirus infects cells, expresses genes, and/or is replicated.

[0056] Lentivirus is a genus of retroviruses, which contain an RNA genome that is converted to DNA in the transduced cell by a virally encoded reverse transcriptase. Lentiviral vectors can transduce a wide range of cell types and integrate into the host genome in both dividing and post-mitotic cells, resulting in long-term expression of the protein-coding sequence both in vitro and in vivo (see e.g. Tiscornia, G., et al., 2006. Nature protocols, 1(1), pp. 241-245).

[0057] The basic genes required for lentivirus survival and function are the gag, pol, and env genes: gag encodes structural proteins; po/encodes enzymes required for reverse transcription and integration into the host cell genome; and env encodes the viral envelope glycoprotein (see e.g. Milone, M.C. and O'Doherty, U., 2018. Leukemia, 32(7), pp. 1529-1541). Lentiviruses may also have additional cis-acting elements, such as a rev response element (RRE), which enables the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell; a retroviral psi packaging element, which is involved in regulating the essential process of packaging the retroviral RNA genome into the viral capsid during replication; a primer binding site (PBS), where reverse transcription is initiated; the TAT activation region (TAR); splice donor and acceptor sites; and central and terminal polypurine tracts, which allow initiation of plus-strand synthesis.

[0058] In a lentivirus genome, these elements are typically flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for integration and transcription. LTRs may also serve as enhancer-promoter sequences and can control the expression of the lentiviral genes. The LTRs themselves are identical or near-identical sequences that can typically be divided into three regions: U3, R and U5. LTRs may be naturally occurring or may be modified. For example, U3 and U5 modifications are described in Iwakuma, T., et al., 1999. Virology, 261(1), pp. 120-132.

[0059] The lentiviral vector of the present invention may comprise a minimal lentiviral genome. As used herein, a "minimal lentiviral genome" may mean that the lentiviral genome has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell (see e.g. Kim, V. N., et al., 1998. Journal of virology, 72(1), pp. 811-816 and Sertkaya, H., et al., 2021. Scientific reports, 11(1), pp. 1-15).

[0060] A lentiviral genome may comprise from 5' to 3': a 5' LTR, one or more lentiviral-derived cis-acting elements, and a 3' LTR. Suitably, a lentiviral genome may comprise from 5' to 3': a 5' LTR, a RRE, and a 3' LTR. Suitably, a lentiviral genome may comprise from 5' to 3': a 5' LTR, a retroviral psi packaging element, a RRE, and a 3' LTR. Suitably, a lentiviral genome may comprise from 5' to 3': a 5' LTR, a retroviral psi packaging element, a RRE, a cPPT, and a 3' LTR. Suitably, a lentiviral genome may comprise from 5' to 3': a 5' LTR, a PBS, a retroviral psi packaging element, a RRE, a cPPT, and a 3' LTR.

[0061] A lentiviral genome may further comprise a protein-coding sequence and, optionally, one or more regulatory elements (e.g. operably linked to the protein-coding sequence). Suitably, a lentiviral genome may comprise from

5' to 3': a 5' LTR, a RRE, a protein-coding sequence, and a 3' LTR. Suitably, a lentiviral genome may comprise from 5' to 3': a 5' LTR, a retroviral psi packaging element, a RRE, a protein-coding sequence, and a 3' LTR. Suitably, a lentiviral genome may comprise from 5' to 3': a 5' LTR, a retroviral psi packaging element, a RRE, a cPPT, a protein-coding sequence, and a 3' LTR. Suitably, lentiviral genome may comprise from 5' to 3': a 5' LTR, a PBS, a retroviral psi packaging element, a RRE, a cPPT, a protein-coding sequence, and a 3' LTR.

[0062] The lentiviral vector of the present invention may be replication-defective. Typically, at least part of one or more protein coding regions essential for replication may be removed from the lentiviral genome. This makes the lentiviral vector "replication-defective" or "replication-incompetent". Suitably, one or more of gag, pol, rev, and env genes are deleted (at least partially) in a replication-defective lentiviral vector. Suitably, each of the gag, pol, rev, and env genes are deleted (at least partially) in a replication-defective lentiviral vector. Optionally, the lentiviral vector lacks a functional gag-pol and/or env gene and/or other genes essential for replication.

[0063] The lentiviral vector of the present invention may be derived from any lentivirus. As used herein "lentivirus-derived" or "lentivirus-based" may mean that the lentiviral genome comprises one or more elements from said lentivirus. For example, the coding regions of viral proteins may be deleted, but one or more cis-acting element may be retained from said lentivirus.

[0064] The lentiviral vector may be derived from a primate lentivirus. Examples of "primate" lentiviruses include, but are not limited to, human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV). The lentiviral vector may be derived from a non-primate lentivirus (i.e. derived from a lentivirus which does not primarily infect primates, especially humans). Examples of "non-primate" lentiviruses include, but are not limited to, the prototype "slow virus" visna/maedi virus (VMV), caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV), feline immunodeficiency virus (FIV), and bovine immunodeficiency virus (BIV).

[0065] Suitably, the lentiviral vector of the present invention is a HIV-derived lentiviral vector. As used herein "HIV-derived" or "HIV-based" may mean that the lentiviral genome comprises one or more element from HIV. For example, the coding regions of HIV viral proteins may be deleted, and one or more HIV cis-acting element may retained in the lentiviral genome (see e.g. Johnson, N. M., et al., 2021. Molecular Therapy-Methods & Clinical Development, 21, pp. 451-465). A HIV-derived lentiviral genome may comprise from 5' to 3': a 5' LTR, one or more HIV-derived cis-acting elements (e.g. RRE and/or cPPT), and a 3' LTR.

[0066] Suitably, the lentiviral vector of the present invention is a HIV-1-derived lentiviral vector. The prototype lentiviral vector system is based on HIV-1 (see e.g. Merten, O. W., et al., 2016. Molecular Therapy-Methods & Clinical Development, 3, p. 16017). It has been shown that sequences that extend into the gag open reading frame may be important for packaging of HIV-1. Therefore, HIV-1 vectors often contain the relevant portion of gag in which the translational initiation codon has been mutated. In addition, HIV-1 vectors often also contain a portion of the env gene that includes the RRE. Rev binds to RRE, which permits the transport of full-length or singly spliced mRNAs from the nucleus to the cytoplasm. In the absence of rev and/or a RRE, full-length HIV-1 RNAs may accumulate in the nucleus. Alternatively, a constitutive transport element from certain simple retroviruses such as Mason-Pfizer monkey virus can be used to relieve the requirement for rev and a RRE. A HIV-1-derived lentiviral genome may comprise from 5' to 3': a 5' LTR, one or more HIV-1-derived cis-acting elements (e.g. a PBS, a retroviral psi packaging element, a RRE and/or a cPPT), and a 3' LTR.

[0067] The lentiviral vector of the present invention may be a self-inactivating lentiviral vector. As used herein, "self-inactivating" or "SIN" lentiviral vectors may comprise lentiviral genomes in which the lentiviral enhancer and promoter sequences have been deleted (see e.g. Zufferey, R., et al., 1998. Journal of virology, 72(12), pp. 9873-9880 and Miyoshi, H., et al., 1998. Journal of virology, 72(10), pp. 8150-8157). SIN lentiviral vectors can be generated and transduce non-dividing cells in vivo with an efficacy similar to that of wild-type vectors. The transcriptional inactivation of the long terminal repeat (LTR) in the SIN provirus can prevent mobilisation by replication-competent virus. This can also enable the regulated expression of genes from internal promoters by eliminating any cis-acting effects of the LTR.

[0068] The lentiviral vector of the present invention may be integration competent. As used herein, an "integration competent" lentiviral vector is capable of integrating into the genome of a host cell. In contrast to integration competent lentiviral vectors, integration defective lentiviral vectors can be produced, for example, either by packaging the lentiviral vector with catalytically inactive integrase (such as an HIV integrase bearing the D64V mutation in the catalytic site) or by modifying or deleting essential att sequences from the lentiviral genome LTR, or by a combination of the above (see e.g. Wanisch, K. and Yáñez-Muñoz, R. J., 2009. Molecular Therapy, 17(8), pp. 1316-1332).

[0069] The lentiviral vector of the present invention may be replication-defective and integrating. The lentiviral vector of the present invention may be replication-defective, integrating, and self-inactivating. The lentiviral vector of the present invention may be replication-defective, integrating, self-inactivating, and HIV-derived.

[0070] The lentiviral vector of the present invention may be a lentiviral particle. A "lentiviral particle" may refer to an enveloped lentiviral genome. Lentiviral particles may be generated by co-transfection of a plasmid containing a lentiviral genome (e.g. a "transfer vector") with helper plasmids (e.g. "packaging vectors" encoding gag-pol and/or rev and "envelope vectors" encoding env) into host cells and harvesting of the lentivirus-containing supernatant afterwards.

[0071] The lentiviral vector of the present invention may be pseudotyped. Pseudotyping lentiviral vectors with naturally occurring or engineered lentiviral envelopes can allow targeted transduction of specific cell types (see e.g. Joglekar, A. V. and Sandoval, S., 2017. Human Gene Therapy Methods, 28(6), pp. 291-301). Suitably, the lentiviral vector is pseudotyped to allow transduction of liver cells (e.g. hepatocytes).

[0072] The lentiviral vector of the present invention may be VSV-G pseudotyped. Vesicular stomatitis virus G protein (VSV-G) is a commonly used envelope protein for pseudotyping. VSV-G is a trimeric protein that binds phosphati-

dylserine and low-density lipoprotein receptors on a cell surface to endocytose into the cell. VSV-G pseudotyped lentiviral vectors may efficiently transduce liver cells (e.g. hepatocytes).

[0073] The lentiviral vector of the present invention may be replication-defective, integrating, and VSV-G pseudotyped. The lentiviral vector of the present invention may be replication-defective, integrating, self-inactivating, and VSV-G pseudotyped. The lentiviral vector of the present invention may be replication-defective, integrating self-inactivating, HIV-derived, and VSV-G pseudotyped.

Immune-Shielded Lentiviral Vector

[0074] The lentiviral vector of the present invention may be an immune-shielded lentiviral vector.

[0075] As used herein, an "immune shielded lentiviral vector" may refer to a lentiviral vector which is modified to reduce immune responses following administration. For example, the immune-shielding may reduce activation of acute inflammatory response after administration.

[0076] In some embodiments, the lentiviral vector of the present invention is: (i) a CD47^{high} lentiviral vector; (ii) a MHC-I^{free} lentiviral vector; and/or (iii) comprises one or more miRNA target sequences (e.g. which suppress transgene expression in antigen-presenting cells). In preferred embodiments, the lentiviral vector of the present invention is a CD47^{high}/MHC-I^{free} lentiviral vector and comprises one or more miRNA target sequences (e.g. which suppress transgene expression in antigen-presenting cells). Each of these modifications may act to reduce immune responses following administration.

[0077] An immune-shielded lentiviral vector may be "phagocytosis-shielded" to reduce uptake by professional phagocytes. In some embodiments, the lentiviral vector is a phagocytosis-shielded lentiviral vector. For example, the lentiviral vector may be a CD47^{high} lentiviral vector.

CD47^{high} Lentiviral Vectors

[0078] The lentiviral vector of the present invention may be a CD47^{high} lentiviral vector. As used herein, a "CD47^{high} lentiviral vector" may refer to a lentiviral vector with increased levels of CD47 (or a fragment thereof) on its surface. A CD47^{high} lentiviral vector may have reduced uptake by professional phagocytes.

[0079] CD47 (Cluster of Differentiation 47) also known as integrin associated protein (IAP) is a transmembrane protein that in humans is encoded by the CD47 gene. Phagocytosis is physiologically inhibited by CD47, which is a ubiquitously expressed ligand of signal regulatory protein α (SIRP-α) receptor, that is expressed by professional phagocytes. CD47 may be incorporated into lentiviral vectors when they bud from producer cells.

[0080] The lentiviral vector of the present invention may comprise one or more CD47 polypeptides (or a fragment thereof) on its surface. The amount of CD47 (or a fragment thereof) on the surface may be enough to reduce uptake by professional phagocytes. Any suitable assay to quantify the amount of CD47 polypeptides (or fragments thereof) present on the surface of the lentiviral vector may be used.

[0081] In some embodiments, the density of CD47 polypeptides (or fragments thereof) may be determined by immunostaining for CD47 and total internal reflection fluorescence microscopy, e.g. as described in US2010/0316570A1. The CD47 polypeptides (or fragments thereof) may be present in a density of at least about 20 molecules/μm^2, at least about 25 molecules/μm^2, at least about 30 molecules/μm^2, at least about 35 molecules/μm^2, at least about 40 molecules/μm^2, at least about 45 molecules/μm^2, at least about 50 molecules/μm^2, at least about 60 molecules/μm^2, at least about 70 molecules/μm^2, at least about 80 molecules/μm^2, at least about 90 molecules/μm^2, at least about 100 molecules/μm^2, at least about 150 molecules/μm^2, at least about 200 molecules/μm^2, at least about 250 molecules/μm^2, at least about 300 molecules/μm^2, at least about 350 molecules/μm^2, at least about 400 molecules/μm^2, at least about 450 molecules/μm^2, at least about 500 molecules/μm^2, at least about 600 molecules/μm^2, at least about 700 molecules/μm^2, at least about 800 molecules/μm^2, at least about 900 molecules/μm^2, or at least about 1000 molecules/μm^2. The CD47 polypeptides (or fragments thereof) may be present in a density of about 1000 molecules/μm^2 or less, about 500 molecules/μm^2 or less, or about 250 molecules/μm^2 or less. The CD47 polypeptides (or fragments thereof) may be present in a density of from about 20 molecules/μm^2 to about 1000 molecules/μm^2, from about 20 molecules/μm^2 to about 500 molecules/μm^2, or from about 20 molecules/μm^2 to about 250 molecules/μm^2.

[0082] In some embodiments, the amount of CD47 polypeptides (or fragments thereof) may be determined by immunostaining for CD47 and electron microscopy, as described in Milani, M., et al., 2019. Science Translational Medicine, 11(493), p.eaav7325. The CD47 polypeptides (or fragments thereof) may be detected in an amount of at least about 10 gold particles/lentiviral particle, at least about 15 gold particles/lentiviral particle, or at least about 20 gold particles/lentiviral particle. The CD47 polypeptides (or fragments thereof) may be detected in an amount of about 100 gold particles/lentiviral particle or less, about 80 gold particles/lentiviral particle or less, or about 60 gold particles/lentiviral particle or less. The CD47 polypeptides (or fragments thereof) may be detected in an amount of from about 10 to about 100 gold particles/lentiviral particle, from about 15 to about 80 gold particles/lentiviral particle, or from about 20 to about 60 gold particles/lentiviral particle.

[0083] The lentiviral vector of the present invention may be obtained from a CD47^{high} host cell. As used herein, a "CD47^{high} host cell" may refer to a host cell with increased levels of CD47 (or a fragment thereof) on its surface.

[0084] A CD47^{high} host cell may be genetically engineered to increase expression of CD47 (or a fragment thereof) on the cell surface. For example, the host cell may comprise a vector encoding CD47 (or a fragment thereof) or may be edited to introduce a nucleotide sequence encoding CD47 (or a fragment thereof) into its genome. Suitably, the host cell is transduced with a viral vector encoding a CD47 polypeptide (or a fragment thereof).

[0085] A CD47^{high} host cell may have a higher concentration of CD47 (or a fragment thereof) on its surface than an unmodified host cell (e.g. an unmodified producer cell or packaging cell, as described herein). Suitably, the host cell has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, or at least about 30-fold more CD47 on its cell surface than an unmodified host cell. Suitably, the host cell has from about 5-fold to about 30-fold more CD47 (or a fragment thereof) on its cell surface than an unmodified host cell.

[0086] Suitably, the lentiviral vector of the present has a higher concentration of CD47 (or a fragment thereof) on its surface than a lentiviral vector obtained from an unmodified host cell (e.g. an unmodified producer cell or packaging cell, as described herein). Suitably, the lentiviral vector has at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, or at least about 50-fold more CD47 (or a fragment thereof) on its surface than a lentiviral vector obtained from an unmodified host cell. Suitably, the lentiviral vector has from about 5-fold to about 30-fold more CD47 (or a fragment thereof) on its surface than a lentiviral vector obtained from an unmodified host cell.

[0087] CD47 is a member of the immunoglobulin (lg) superfamily of membrane proteins, with a single IgV-like domain at its N-terminus, a highly hydrophobic stretch with five membrane-spanning segments and an alternatively spliced cytoplasmic C-terminus ranging in length from 3 to 36 amino acids. Mouse, rat, bovine and human CD47 molecules have been cloned and show about 70% overall amino acid identity (see e.g. Brown, E. J. and Frazier, W. A., 2001. Trends in cell biology, 11(3), pp. 130-135).

[0088] The CD47 polypeptide (or a fragment thereof) may be a human CD47 polypeptide (or a fragment thereof). A CD47 polypeptide may have an amino acid sequence of UniProtKB Q08722.

[0089] Exemplary CD47 polypeptides are provided by SEQ ID NOs: 1-4. Suitably, a CD47 polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to any of SEQ ID NOs: 1-4. Suitably, a CD47 polypeptide comprises or consists of the amino acid sequence of any of SEQ ID NOs: 1-4.

```
Exemplary CD47 polypeptide
                                        (SEQ ID NO: 1)
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQN
TTEVYVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKM
DKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNENILIVIFPI
FAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILFVPG
EYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYI
LAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQ
PPRNN

Exemplary CD47 polypeptide
                                        (SEQ ID NO: 2)
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQN
TTEVYVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKM
DKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNENILIVIFPI
FAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILFVPG
EYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYI
LAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQ
PPRKAVEEPLNAFKESKGMMNDE

Exemplary CD47 polypeptide
                                        (SEQ ID NO: 3)
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQN
TTEVYVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKM
DKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNENILIVIFPI
FAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILFVPG
EYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYI
LAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFV
```

-continued

```
Exemplary CD47 polypeptide
                                        (SEQ ID NO: 4)
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQN
TTEVYVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKM
DKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPNENILIVIFPI
FAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVIVGAILFVPG
EYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIAILVIQVIAYI
LAVVGLSLCIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQ
PPRKAVEEPLN
```

[0090] Exemplary CD47 polypeptides excluding the signal peptide are provided by SEQ ID NOs: 5-8. Suitably, a CD47 polypeptide comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to any of SEQ ID NOs: 5-8. Suitably, a CD47 polypeptide comprises or consists of the amino acid sequence of any of SEQ ID NOs: 5-8.

```
Exemplary CD47 polypeptide excluding signal peptide
                                        (SEQ ID NO: 5)
QLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTF
DGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTE
LTREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYR
SGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTG
ILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHG
PLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRNN

Exemplary CD47 polypeptide excluding signal peptide
                                        (SEQ ID NO: 6)
QLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTF
DGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTE
LTREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYR
SGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTG
ILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHG
PLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKG
MMNDE

Exemplary CD47 polypeptide excluding signal peptide
                                        (SEQ ID NO: 7)
QLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTF
DGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTE
LTREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYR
SGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTG
ILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHG
PLLISGLSILALAQLLGLVYMKFV

Exemplary CD47 polypeptide excluding signal peptide
                                        (SEQ ID NO: 8)
QLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTF
DGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTE
LTREGETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYR
SGGMDEKTIALLVAGLVITVIVIVGAILFVPGEYSLKNATGLGLIVTSTG
ILILLHYYVFSTAIGLTSFVIAILVIQVIAYILAVVGLSLCIAACIPMHG
PLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLN
```

[0091] A person skilled in the art would be able to generate variants and/or fragments based on conservative substitutions and/or the known structural and functional features of CD47. These are described, for instance in Fenalti, G., et al., 2021. Nature communications, 12(1), pp. 1-14.

[0092] Suitably, a fragment of CD47 and/or CD47 variant retains the ability to inhibit phagocytosis. Suitably, a CD47 fragment and/or CD47 variant may comprise the extracellular domain of CD47. The extracellular domain of human CD47 may interact with SIRP-α and inhibit phagocytosis. Optionally, a CD47 fragment and/or CD47 variant comprises the transmembrane domain of CD47. The domains may be linked by inter-domain linker(s). The fragment

and/or variant may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of a full-length CD47 polypeptide.

[0093] Suitably, a variant of SEQ ID NO: 1 may comprise one or more variation selected from V5I, C14W, C15R, F22L, S27F, F30L, F32Y, T36S, V38L, V38I, F42V, T44A, N50S, T51A, T52S, T52A, V56I, R63K, A71T, S75Y, T76A, P78L, P78S, P78A, S82R, S82N, S83T, K85N, K85E, V88A, V88L, V88I, Q90R, L91F, K93N, M100I, M100V, D101G, K102R, K102T, S107L, I126F, I127V, K130Q, R132H, S138F, V146I, I150V, I153V, S169A, G170R, G170S, G171S, D173Y, I177V, A178G, V181I, V185A, I186V, V188A, I189T, I191V, V198I, A207S, T215I, I219M, Y226C, A231S, T235A, S236F, A240V, A240T, I241V, V243I, I244T, V246L, Y249F, A252S, A252T, V254A, S257T, I264M, I264L, M266I, M266T, M266V, V287I, V292A, N295S, N295D, Q296L, P302S, N304S, and N304D. These are considered to be tolerated, benign, and/or likely benign variations as predicted by SIFT, PolyPhen, CADD, REVEL, and MetaLR.

[0094] Suitably, a variant of SEQ ID NO: 2 may comprise one or more variation selected from P3L, A6P, F22L, S27F, F30L, F32Y, T36S, V38L, V38I, F42V, N50S, T51A, T52S, T52A, V56I, R63K, A71T, S75Y, T76A, P78L, P78S, P78A, S82R, S82N, S83T, K85N, K85E, V88A, V88L, V88I, Q90R, L91F, K93N, M100I, M100V, D101G, K102R, K102T, S107L, I126F, I127V, K130Q, R132H, S138F, V146I, I150V, I153V, S169A, G170R, G170S, G171S, I177V, A178G, V181I, I186V, V188A, I189T, I191V, V198I, A207S, T215I, I219M, Y226C, A231S, T235A, A240V, A240T, I241V, V243I, I244T, V246L, Y249F, A252S, A252T, V254A, I264M, I264L, M266I, M266T, M266V, V287I, V292A, N295S, N295D, and Q296L. These are considered to be tolerated, benign, and/or likely benign variations as predicted by SIFT, PolyPhen, CADD, REVEL, and MetaLR.

[0095] An exemplary CD47 fragment is provided by SEQ ID NO: 9. Suitably, a CD47 fragment comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to SEQ ID NO: 9. Suitably, a CD47 fragment comprises or consists of the amino acid sequence of SEQ ID NO: 9.

```
Exemplary CD47 fragment
                                        (SEQ ID NO: 9)
MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQN

TTEVYVKWKFKGRDIYTFDGALNKSTVPTDFSSAKIEVSQLLKGDASLKM

DKSDAVSHTGNYTCEVTELTREGETIIELKYRVVSWFSPN
```

[0096] An exemplary CD47 fragment excluding the signal peptide is provided by SEQ ID NO: 10. Suitably, a CD47 fragment comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to SEQ ID NO: 10. Suitably, a CD47 fragment comprises or consists of the amino acid sequence of SEQ ID NO: 10.

```
Exemplary CD47 fragment excluding signal peptide
                                        (SEQ ID NO: 10)
QLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFKGRDIYTF

DGALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTE

LTREGETIIELKYRVVSWFSPN
```

MHC-I^{low} or MHC-I^{free} Lentiviral Vectors

[0097] The lentiviral vector of the present invention may be a MHC-I^{low} lentiviral vector or a MHC-I^{free} lentiviral vector. In preferred embodiments, the lentiviral vector of the present invention is a MHC-I^{free} lentiviral vector.

[0098] As used herein, a "MHC-I^{low} lentiviral vector" may refer to a lentiviral vector with reduced levels of one or more MHC-I molecules on its surface (i.e. reduced levels of surface-exposed MHC-I molecules). The number of surface-exposed MHC-I molecules may be reduced such that the immune response to the MHC-I is decreased to a therapeutically relevant degree.

[0099] As used herein, a "MHC-I^{free} lentiviral vector" may refer to a lentiviral vector which is substantially devoid of (or free of) one or more MHC-I molecules on its surface (i.e. substantially devoid of (or free of) surface-exposed MHC-I molecules).

[0100] The major histocompatibility complex class I (MHC-I) is a heterodimeric membrane protein that is displayed on the outer leaflet of the cell membrane (see e.g. Penn, D. J. and Ilmonen, P., 2005. Major histocompatibility complex (MHC). eLS). MHC-I functions to bind and display peptide fragments of proteins to the extracellular environment where they may be recognised by CD8+ cytotoxic T cells. Peptide fragments generated from normal cellular proteins will not activate cytotoxic T cells due to central and peripheral tolerance mechanisms. However, foreign peptides (e.g. those originating from viral proteins) will cause activation of an immune response to destroy the cell. An allogeneic MHC-I protein itself may be recognised by the immune system. For example, antibodies may bind MHC-I epitopes directly. As a result, lentiviral vectors that comprise MHC-I molecules originating from an allogeneic source may be targeted and neutralised by the immune system.

[0101] The term "MHC-I molecules" may refer to human MHC-I molecules. Human MHC-I is also referred to as human leukocyte antigen class I (HLA-I) and is expressed on almost all nucleated cells. HLA-I consists of two polypeptide chains, an HLA-I heavy chain (α chain) and β2 microglobulin (β2M or β chain). The HLA-I α chain and β2M are linked non-covalently. The HLA-I α chain is polymorphic. Six HLA-I α chains have been identified to date, including three classical, highly polymorphic α chains (HLA-A, HLA-B and HLA-C) and three non-classical, less polymorphic (HLA-E, HLA-F and HLA-G) a chains. The MHC-I molecules may comprise or consist of HLA-A, HLA-B, and HLA-C molecules, which comprise an invariant β2M sequence.

[0102] The term "MHC-I molecules" may also include variant MHC-I sequences, such as polymorphisms of HLA-I α chain sequences and/or β2M sequences. For example, variant MHC-I sequences may include HLA-I α chain sequences and/or β2M sequences with single nucleotide polymorphisms (SNPs) or multiple SNPs.

[0103] Any suitable assay to quantify the amount of MHC-I molecules present on the surface of the lentiviral vector may be used.

[0104] In some embodiments, the amount of MHC-I molecules may be determined by immunostaining for MHC-I and electron microscopy, as described in Milani, M., et al., 2017. EMBO molecular medicine, 9(11), pp. 1558-1573. The MHC-I molecules may be detected in an amount of less than about 10 gold particles/lentiviral particle, less than about 9 gold particles/lentiviral particle, less than about 8 gold particles/lentiviral particle, less than about 7 gold particles/lentiviral particle, less than about 6 gold particles/lentiviral particle, less than about 5 gold particles/lentiviral particle, less than about 4 gold particles/lentiviral particle, less than about 3 gold particles/lentiviral particle, less than about 2 gold particles/lentiviral particle, less than about 1 gold particle/lentiviral particle, or about 0 gold particles/lentiviral particle. The MHC-I molecules may be undetectable (e.g. the amount of gold particles detected may not be significantly higher than background levels).

[0105] The lentiviral vector of the present invention may be obtained from a MHC-I^{low} host cell or a MHC-I^{free} host cell. In preferred embodiments, the lentiviral vector of the present invention is obtained from a MHC-I^{free} host cell. As used herein, a "MHC-I^{low} host cell" may refer to a host cell with reduced levels of one or more MHC-I molecule on its surface. As used herein, a "MHC-I^{free} host cell" may refer to a host cell which is substantially devoid of or free of one or more MHC-I molecule on its surface.

[0106] A MHC-I^{low} or MHC-I^{free} host cell may be genetically engineered to decrease expression of MHC-I on the cell surface. For example, the cell may comprise a genetically engineered disruption of a gene encoding β2-microglobulin and/or a genetically engineered disruption of a gene encoding an MHC-I α chain.

[0107] Methods for genetic engineering to decrease protein expression are known in the art. For example, this may be achieved by targeted gene knockout. To decrease protein expression, the gene encoding the protein itself or its regulatory sequence (e.g. its promoter) may be knocked out. Knockout may be achieved by deletion of a section of the coding nucleic acid sequence, which may delete a section of the protein essential for expression or stability, or alter the reading frame of the coding sequence or by base-editing. Suitable methods for targeted gene knockout include use of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR/Cas-based RNA-guided nucleases (see e.g. Gaj, T. et al., 2013. Trends Biotechnol, 31, pp. 397-405). For example, the CRISPR/Cas9 RNA-guided nuclease may be used to catalyse a double strand break at a specific locus in the genome if provided with appropriate RNA guides designed to bind that locus. Cas9 and the guide RNA may be delivered to a target cell by transfection of vectors encoding the protein and RNA. Cells attempt to repair any double strand breaks in their DNA using the non-homologous end joining (NHEJ) pathway. This is an error-prone mechanism which inserts random nucleotides and often disrupts the reading frame of the targeted gene. Alternatively, the genetic engineering to decrease protein expression may be accomplished using RNAi techniques, microRNA or antisense RNA to suppress expression of the target gene.

[0108] Once the targeted gene knockout or suppression of expression approach has been carried out, the resulting population of cells may be screened to select and enrich for those cells exhibiting the phenotype of interest, for example decreased expression of surface-exposed MHC-I. Suitable techniques for screening and enrichment are known in the art and include flow cytometry and fluorescence-activated cell sorting (FACS).

[0109] In some embodiments, the host cell comprises a genetically engineered disruption of a gene encoding β2-microglobulin. β2-microglobulin stabilises MHC-I, thus cells deficient in β2-microglobulin will exhibit decreased expression of MHC-I on the surface of the cell. The cell may comprise genetically engineered disruptions in all copies of the gene encoding β2-microglobulin.

[0110] In another embodiment, the cell comprises a genetically engineered disruption of one or more gene encoding an MHC-I α chain. The cell may comprise genetically engineered disruptions in all copies of the gene encoding an MHC-I α chain.

[0111] The cell may comprise both genetically engineered disruptions of genes encoding β2-microglobulin and genetically engineered disruptions of genes encoding an MHC-I α chain.

[0112] Decreased expression of MHC-I on the surface of the cell may refer to a decrease in the number of MHC-I molecules that are expressed on the surface of the cell that has been genetically engineered, in comparison to the number of MHC-I molecules that are expressed on the surface of a cell lacking the genetic engineering, but under otherwise substantially identical conditions. The expression of MHC-I on the surface of the cell may be decreased such that the number of surface-exposed MHC-I molecules is, for example, less than about 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% of the number of surface-exposed MHC-I molecules that are displayed in the absence of the genetic engineering. In some embodiments, the expression of MHC-I on the surface of the cell is decreased such that the number of surface-exposed MHC-I molecules is 0% of the number of surface-exposed MHC-I molecules that are displayed in the absence of the genetic engineering.

[0113] The expression of MHC-I on the surface of the cell is preferably decreased such that the cell is substantially devoid of surface-exposed MHC-I molecules. In this context, "substantially devoid" may mean that there is a substantial decrease in the number of MHC-I molecules that are expressed on the surface of the cell that has been genetically engineered, in comparison to the number of MHC-I molecules that are expressed on the surface of a cell lacking the genetic engineering, such that the immune response to MHC-I on lentiviral vectors produced by the cell is decreased to a therapeutically useful degree.

[0114] Suitably, the lentiviral vector of the present invention has a lower concentration of MHC-I molecules on its surface than a lentiviral vector obtained from an unmodified host cell (e.g. an unmodified producer cell or packaging cell, as described herein). Suitably, the lentiviral vector has less than about 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% of the number of surface-exposed MHC-I molecules that are displayed on a lentiviral vector obtained from an unmodified host cell. In some embodiments, the lentiviral vector has less than about 20% of the number of surface-exposed MHC-I molecules that are displayed on a lentiviral vector obtained from an unmodified host cell.

[0115] In some embodiments, the lentiviral vector of the present invention is substantially devoid of MHC-I molecules on its surface. In this context, "substantially devoid" may mean that there is no detectable immune response due to the molecules on the surface of the lentiviral vector.

[0116] In some embodiments, the lentiviral vector of the present invention is free of MHC-I molecules on its surface. In this context, "free" may mean that there are no detectable molecules (e.g. by immunostaining and electron microscopy) on the surface of the lentiviral vector. As used herein, "not detectable" may refer to levels which are not statistically significantly different compared to background levels.

[0117] In some embodiments, the lentiviral vector of the present invention has decreased HLA-A, HLA-B, and/or HLA-C molecules on its surface. Suitably, the lentiviral vector has less than about 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% of the number of surface-exposed HLA-A molecules that are displayed on a lentiviral vector obtained from an unmodified host cell. Suitably, the lentiviral vector has less than about 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% of the number of surface-exposed HLA-B molecules that are displayed on a lentiviral vector obtained from an unmodified host cell. Suitably, the lentiviral vector has less than about 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2% or 1% of the number of surface-exposed HLA-C molecules that are displayed on a lentiviral vector obtained from an unmodified host cell.

[0118] In some embodiments, the lentiviral vector of the present invention is substantially devoid of HLA-A, HLA-B, and/or HLA-C molecules on its surface. In some embodiments, the lentiviral vector of the present invention is substantially devoid of HLA-A, HLA-B, and HLA-C molecules on its surface. In some embodiments, the lentiviral vector of the present invention is free of HLA-A, HLA-B, and/or HLA-C molecules on its surface. In some embodiments, the lentiviral vector of the present invention is free of HLA-A, HLA-B, and HLA-C molecules on its surface.

[0119] As described above, an HLA-I molecule consists of two polypeptide chains, an HLA-I heavy chain (α chain) and β2 microglobulin (β2M or β chain). The HLA-I α chain and β2M are linked non-covalently.

[0120] The skilled person would readily be able to determine amino acid and nucleic acid sequences of HLA-I α chains. For example, the HLA-I α chains may be identified in a genome sequence using their location within the major histocompatibility complex region of the chromosome (see e.g. Penn, D. J. and Ilmonen, P., 2005. Major histocompatibility complex (MHC). eLS).

[0121] HLA-A alpha chains may have an amino acid sequence of UniProtKB P04439. Exemplary HLA-A alpha chains are provided by SEQ ID NOs: 11 and 12. Suitably, an HLA-A alpha chain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to SEQ ID NO: 11 or 12. Suitably, a HLA-A alpha chain comprises or consists of the amino acid sequence of SEQ ID NO: 11 or 12.

Exemplary HLA-A alpha chain

(SEQ ID NO: 11)

MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFFTSVSRPGRGEPRFIAVG
YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDQETRNVKAQSQTDRV
DLGTLRGYYNQSEAGSHTIQIMYGCDVGSDGRFLRGYRQDAYDGKDYIAL
NEDLRSWTAADMAAQITKRKWEAAHEAEQLRAYLDGTCVEWLRRYLENGK
ETLQRTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQ
DTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEL
SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSDRKGGSYTQAASS
DSAQGSDVSLTACKV

-continued

Exemplary HLA-A alpha chain

(SEQ ID NO: 12)

MAVMAPRTLLLLLSGALALTQTWAGSHSMRYFFTSVSRPGRGEPRFIAVG
YVDDTQFVRFDSDAASQRMEPRAPWIEQEGPEYWDQETRNVKAQSQTDRV
DLGTLRGYYNQSEAGSHTIQIMYGCDVGSDGRFLRGYRQDAYDGKDYIAL
NEDLRSWTAADMAAQITKRKWEAAHAAEQQRAYLEGRCVEWLRRYLENGK
ETLQRTDPPKTHMTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQ
DTELVETRPAGDGTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEL
SSQPTIPIVGIIAGLVLLGAVITGAVVAAVMWRRKSSGGEGVKDRKGGSY
TQAASSDSAQGSDVSLTACKV

[0122] HLA-B alpha chains may have an amino acid sequence of UniProtKB P01889. An exemplary HLA-B alpha chain is provided by SEQ ID NO: 13. Suitably, an HLA-B alpha chain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to SEQ ID NO: 13. Suitably, a HLA-B alpha chain comprises or consists of the amino acid sequence of SEQ ID NO: 13.

Exemplary HLA-B alpha chain

(SEQ ID NO: 13)

MLVMAPRTVLLLLSAALALTETWAGSHSMRYFYTSVSRPGRGEPRFISVG

YVDDTQFVRFDSDAASPREEPRAPWIEQEGPEYWDRNTQIYKAQAQTDRE

SLRNLRGYYNQSEAGSHTLQSMYGCDVGPDGRLLRGHDQYAYDGKDYIAL

NEDLRSWTAADTAAQITQRKWEAAREAEQRRAYLEGECVEWLRRYLENGK

DKLERADPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQRDGEDQTQ

DTELVETRPAGDRTFQKWAAVVVPSGEEQRYTCHVQHEGLPKPLTLRWEP

SSQSTVPIVGIVAGLAVLAVVVIGAVVAAVMCRRKSSGGKGGSYSQAACS

DSAQGSDVSLTA

[0123] HLA-C alpha chains may have an amino acid sequence of UniProtKB P10321. Exemplary HLA-C alpha chains are provided by SEQ ID NOs: 14 and 15. Suitably, an HLA-C alpha chain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to SEQ ID NO: 14 or 15. Suitably, a HLA-C alpha chain comprises or consists of the amino acid sequence of SEQ ID NO: 14 or 15.

Exemplary HLA-C alpha chain

(SEQ ID NO: 14)

MRVMAPRALLLLLSGGLALTETWACSHSMRYFDTAVSRPGRGEPRFISVG
YVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYWDRETQKYKRQAQADRV
SLRNLRGYYNQSEDGSHTLQRMSGCDLGPDGRLLRGYDQSAYDGKDYIAL
NEDLRSWTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGK
ETLQRAEPPKTHVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQ
DTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHMQHEGLQEPLTLSWEP
SSQPTIPIMGIVAGLAVLVVLAVLGAVVTAMMCRRKSSGGKGGSCSQAAC
SNSAQGSDESLITCKA

Exemplary HLA-C alpha chain

(SEQ ID NO: 15)

MRVMAPRALLLLLSGGLALTETWACSHSMRYFDTAVSRPGRGEPRFISVG
YVDDTQFVRFDSDAASPRGEPRAPWVEQEGPEYWDRETQKYKRQAQADRV
SLRNLRGYYNQSEDGSHTLQRMSGCDLGPDGRLLRGYDQSAYDGKDYIAL
NEHLRSCTAADTAAQITQRKLEAARAAEQLRAYLEGTCVEWLRRYLENGK
ETLQRAEPPKTHVTHHPLSDHEATLRCWALGFYPAEITLTWQRDGEDQTQ
DTELVETRPAGDGTFQKWAAVVVPSGQEQRYTCHMQHEGLQEPLTLRWGG
KGGSCSQAACSNSAQGSDESLITCKA

[0124] Amino acid and nucleic acid sequences encoding β2M are also known in the art. For example, a nucleic acid sequence of a human β2M is deposited as GenBank Accession No. NM_004048.

[0125] An HLA β chain may be that of UniProtKB P61769. An exemplary HLA β chain is provided by SEQ ID NO: 16. Suitably, a HLA β chain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identity to SEQ ID NO: 16. Suitably, a HLA β chain comprises or consists of the amino acid sequence of SEQ ID NO: 16.

```
Exemplary HLA beta chain
                                        (SEQ ID NO: 16)
MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGF

HPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYAC

RVNHVTLSQPKIVKWDRDM
```

[0126] The lentiviral vector of the present invention may be a CD47^{high}/MHC-I^{free} lentiviral vector or a CD47^{high}/MHC-I^{low} lentiviral vector. In preferred embodiments, the lentiviral vector of the present invention is a CD47^{high}/MHC-I^{free} lentiviral vector.

[0127] The lentiviral vector of the present invention may be obtained from a CD47^{high}/MHC-I^{free} host cell or a CD47^{high}/MHC-I^{low} host cell. In preferred embodiments, the lentiviral vector of the present invention is obtained from a CD47^{high}/MHC-I^{free} host cell.

miRNA Target Sequence

[0128] The lentiviral vector of the present invention may comprise one or more miRNA target sequences. The one or more miRNA target sequences may be operably linked to the protein-coding sequence. The term "operably linked" may mean that the components described are in a relationship permitting them to function in their intended manner.

[0129] MicroRNA (miRNA) genes are scattered across all human chromosomes, except for the Y chromosome. Similar to protein-coding genes, miRNAs are usually transcribed from polymerase-II promoters, generating a so-called primary miRNA transcript (pri-miRNA). From the pri-miRNA, a stem loop of about 60 nucleotides in length, called miRNA precursor (pre-miRNA), is excised leaving a 5' phosphate and a 2 bp long, 3' overhang. The pre-miRNA is then actively transported from the nucleus to the cytoplasm. Then, Dicer performs a double strand cut at the other end of the stem loop, generating a 19-24 bp duplex, which is composed of the mature miRNA and the opposite strand of the duplex, called miRNA*. One strand of the duplex is selectively loaded into the RNA-induced silencing complex (RISC), and accumulates as the mature microRNA. This strand is usually the one whose 5' end is less tightly paired to its complement. However, there are some miRNAs that support accumulation of both duplex strands to similar extent.

[0130] Once loaded into RISC, the guide strand of the mature microRNA interacts with mRNA target sequences preferentially found in the 3' untranslated region (3'UTR) of protein-coding genes. If the whole guide strand sequence is perfectly complementary to the mRNA target, the mRNA is endonucleolytically cleaved. If only the seed sequence (i.e. nucleotides 2-8 counted from the 5' end of the miRNA) is perfectly complementary to the target mRNA, RNAi may act through alternative mechanisms leading to translational repression.

[0131] Expression of the protein from the protein-coding sequence (i.e. "transgene expression") may be regulated by one or more endogenous miRNAs using one or more corresponding miRNA target sequences. Using this method, one or more miRNAs endogenously expressed in a cell prevent or reduce transgene expression in that cell by interacting with its corresponding miRNA target sequence positioned in the lentiviral genome (see e.g. Brown, B. D. et al. (2007) Nat Biotechnol 25:1457-1467).

[0132] Suitable miRNA target sequences which suppress transgene expression in specific cells will be known to the skilled person. Determining a miRNA with the desired expression profile may be achieved using techniques known to those skilled in the art. For example, a mammalian microRNA expression atlas is described in Landgraf, P., et al., 2007. Cell, 129(7), pp. 1401-1414 and the distribution of miRNA expression across human tissues is described in Ludwig, N., et al., 2016. Nucleic acids research, 44(8), pp. 3865-3877. Once a miRNA has been identified, the corresponding target sequence can readily be determined using, for example, a microRNA database, such as miRBase (Griffiths-Jones, S., et al., 2007. Nucleic acids research, 36 (suppl_1), pp. D154-D158).

[0133] A miRNA target sequence may be fully or partially complementary to the corresponding miRNA. The term "fully complementary", as used herein, may mean that the target sequence has a nucleic acid sequence which is 100% complementary to the sequence of the miRNA which recognises it. The term "partially complementary", as used herein, may mean that the target sequence is only in part complementary to the sequence of the miRNA which recognises it, whereby the partially complementary sequence is still recognised by the miRNA. In other words, a partially complementary target sequence in the context of the present invention is effective in recognising the corresponding miRNA and effecting prevention or reduction of transgene expression in cells expressing that miRNA. Suitably, a partially complementary miRNA target sequence may be fully complementary to the miRNA seed sequence.

[0134] Including more than one copy of a miRNA target sequence in a lentiviral vector may increase the effectiveness of the system. Also, different miRNA target sequences can be included. For example, the protein-coding sequence may be operably linked to more than one miRNA target sequence, which may or may not be different. The miRNA target sequences may be in tandem, but other arrangements are envisaged. The lentiviral vector may, for example, comprise 1, 2, 3, 4, 5, 6, 7 or 8 copies of the same or different miRNA target sequences. Suitably, the lentiviral vector comprises 4 miRNA target sequences of each miRNA target sequence.

[0135] Copies of miRNA target sequences may be separated by a spacer sequence. A spacer sequence may comprise, for example, at least one, at least two, at least three, at least four or at least five nucleotide bases.

[0136] Suitably, the lentiviral vector comprises one or more miRNA target sequence, two or more miRNA target sequences, three or more miRNA target sequences, or four or more miRNA target sequences. Suitably, the protein-coding sequence is operably linked to one or more miRNA target sequence, two or more miRNA target sequences, three

or more miRNA target sequences, or four or more miRNA target sequences. In some embodiments, the protein-coding sequence is operably linked to four miRNA target sequences.

[0137] The miRNA target sequence may be a human miRNA target sequence. Suitably, the miRNA target sequence is a -5p or -3p miRNA target sequence.

[0138] The one or more miRNA target sequence may suppress transgene expression in one or more cells other than liver cells (e.g. hepatocytes).

[0139] The one or more miRNA target sequence may suppress transgene expression in hematopoietic-lineage cells. Hematopoietic stem cells give rise to different types of blood cells, in lines called myeloid and lymphoid. As used herein, "hematopoietic-lineage cells" may include myeloid cells and lymphoid cells. Myeloid cells may include monocytes, macrophages, neutrophils, basophils, and eosinophils. Lymphoid cells may include T cells, B cells, natural killer cells, and innate lymphoid cells.

[0140] The one or more miRNA target sequences may suppress transgene expression in antigen-presenting cells. As used herein, an "antigen presenting cell" (APC) may refer to a cell that displays antigen bound by major histocompatibility complex (MHC) proteins on its surface. APCs may be hematopoietic-lineage cells. The antigen-presenting cells may be professional antigen-presenting cells. Professional APCs specialize in presenting antigens to T cells and may include macrophages, B cells and dendritic cells. Suitably, the APCs are splenic and/or hepatic APCs.

[0141] The one or more miRNA target sequences may suppress transgene expression in hematopoietic-lineage antigen-presenting cells.

[0142] By preventing transgene expression in antigen-presenting cells, while permitting high levels of expression in other cells, miRNA regulation may enable strong and stable gene transfer in the absence of an immune response.

[0143] As used herein, the term "suppress expression" may refer to a reduction of expression in the relevant cell type(s) of a transgene to which the one or more miRNA target sequence is operably linked as compared to transgene expression in the absence of the one or more miRNA target sequence, but under otherwise substantially identical conditions. In some embodiments, transgene expression is suppressed by at least 50%. In some embodiments, transgene expression is suppressed by at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%. In some embodiments, transgene expression is substantially prevented, e.g. not detectable.

[0144] The miRNA-mediated approach for restricting gene expression has several advantages over other strategies of regulating transgenes. Although using tissue-specific promoters can successfully limit expression to target cells, leaky expression in a fraction of non-target cells is observed. This occurs because the reconstituted promoter, modified for inclusion into a vector system, often loses some of its cell specificity and also because vector integration near active promoters and enhancers can activate the tissue-specific promoter and drive transgene expression. In contrast, because miRNA-mediated silencing occurs at the post-transcriptional level, promoter and enhancer trapping is irrelevant. As such, miRNA-regulation can be used to effectively de-target transgene expression from a particular cell type, while still allowing for broad tissue expression. miRNA regulation may also be used as in combination with tissue-specific promoter/enhancers. By including the miRNA target sequence in expression cassettes already under the control of a tissue-specific promoter, an additional layer of regulation is added which may eliminate off-target expression.

[0145] Exemplary miRNA target sequences which suppress transgene expression in hematopoietic-lineage cells and/or antigen-presenting cells, include, but are not limited to, miR-181, miR-142, miR-223, and miR-155 target sequences. Other miRNA target sequences which suppress transgene expression in hematopoietic-lineage cells and/or antigen-presenting cells are known in the art (see e.g. Ghafouri-Fard, S., et al., 2021. Non-coding RNA research, 6(1), pp. 8-14). miRNAs which are expressed in hematopoietic-lineage cells and/or antigen-presenting cells interact with the corresponding miRNA target sequence and reduce the expression of the target gene (see e.g. Brown, B. D., et al., 2006. Nature medicine, 12(5), pp. 585-591 and Brown, B. D., et al., 2007. Nature biotechnology, 25(12), pp. 1457-1467).

[0146] Further miRNA target sequences that suppress transgene expression in hematopoietic-lineage cells and/or antigen-presenting cells can be identified by any suitable method, for example miRNA expression analysis as described in Monticelli, S., et al., 2005. Genome biology, 6(8), pp. 1-15.

[0147] Suitably, the one or more miRNA target sequence comprise or consist of: (i) one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8) miR-142 target sequence; (ii) one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8) miR-181 target sequence; (iii) one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8) miR-223 target sequence; and/or (iv) one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8) miR-155 target sequence.

[0148] In some embodiments, the one or more miRNA target sequence comprise or consist of: (i) two or more miR-142 target sequences; (ii) two or more miR-181 target sequences; (iii) two or more miR-223 target sequences; and/or (iv) two or more miR-155 target sequences. In some embodiments, the one or more miRNA target sequence comprise or consist of: (i) at least four miR-142 target sequences; (ii) at least four miR-181 target sequences; (iii) at least four miR-223 target sequences; and/or (iv) at least four miR-155 target sequences. In some embodiments, the one or more miRNA target sequence comprise or consist of: (i) four miR-142 target sequences; (ii) four miR-181 target sequences; (iii) four miR-223 target sequences; and/or (iv) four miR-155 target sequences. Suitably, the target sequences are separated by spacer sequences.

[0149] In some embodiments, the one or more miRNA target sequence comprise or consist of one or more (e.g. 1, 2, 3, 4, 5, 6, 7, 8) miR-142 target sequence. In some embodiments, the one or more miRNA target sequence comprise or consist of two or more miR-142 target sequences. In some embodiments, the one or more miRNA target sequence comprise or consist of three or more miR-142 target sequences. In some embodiments, the one or more miRNA target sequence comprise or consist of four or more miR-142 target sequences. In some embodiments, the one or more miRNA target sequence comprise or consist of four miR-142 target sequences. Suitably, the target sequences are separated by spacer sequences.

[0150] The miR-142 target sequence may be a human miRNA target sequence. Suitably, the miR-142 target sequence is a miR-142-5p or miR-142-3p miRNA target

sequence. In some embodiments, the miR-142 target sequence is a miR-142-3p miRNA target sequence.

[0151] In some embodiments, the miR-142 target sequence comprises or consists of a nucleotide sequence which is at least 80% identical to SEQ ID NO: 17 or a fragment thereof. Suitably, the miR-142 target sequence comprises or consists of a nucleotide sequence which is at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 17 or a fragment thereof.

[0152] In some embodiments, the miR-142 target sequence comprises or consists of the nucleotide sequence SEQ ID NO: 17 or a fragment thereof.

```
Exemplary miR-142 target sequence
                                    (SEQ ID NO: 17)
TCCATAAAGTAGGAAACACTACA
```

[0153] In some embodiments, the one or more miRNA target sequence comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 18 or a fragment thereof. Suitably, the one or more miRNA target sequence comprises or consists of a nucleotide sequence which is at least 75%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 18 or a fragment thereof.

[0154] In some embodiments, the one or more miRNA target sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 18 or a fragment thereof.

```
Exemplary 4x miR-142 target sequence
                                    (SEQ ID NO: 18)
TCCATAAAGTAGGAAACACTACACGATTCCATAAAGTAGGAAACACTACA

ACCGGTTCCATAAAGTAGGAAACACTACATCACTCCATAAAGTAGGAAAC

ACTACA
```

PFIC-Associated Polypeptide

[0155] The protein-coding sequence delivered by the lentiviral vector of the present invention is a nucleotide sequence encoding a progressive familial intrahepatic cholestasis (PFIC)-associated polypeptide. Once delivered to a cell, the protein encoded by the protein-coding sequence may be expressed in the cell (i.e. "transgene expression" may occur).

[0156] As used herein, a "PFIC-associated polypeptide" may be any polypeptide associated with progressive familial intrahepatic cholestasis (PFIC). As described above, PFIC is a group of rare autosomal recessive liver disorders characterized by mutations in hepatocellular transport system genes involved in bile formation. A PFIC-associated polypeptide may be any polypeptide associated with bile formation from hepatocytes (e.g. FIC1, BSEP, MDR3, or FXR). A deficiency of the bile acid export pump (BSEP) activity impairing bile salt handling is seen in several forms of PFIC. A PFIC-associated polypeptide may refer to a hepatic bile acid transporter (e.g. BSEP) or a protein essential for expression and/or function of a hepatic bile acid transporter (e.g. FXR, MYO5B).

[0157] Suitably, the PFIC-associated polypeptide is selected from familial intrahepatic cholestasis type 1 (FIC1), or a fragment and/or variant thereof; bile salt export pump (BSEP), or a fragment and/or variant thereof; multiple drug resistance 3 (MDR3), or a fragment and/or variant thereof; tight junction protein 2 (TJP2), or a fragment and/or variant thereof; farnesoid X receptor (FXR), or a fragment and/or variant thereof; and Myosin-Vb (MYO5B), or a fragment and/or variant thereof.

[0158] The protein-coding sequence may be codon-optimised. For example, the protein-coding sequence may be codon-optimised for expression in a mammalian (e.g. human) cell.

[0159] Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available. Codon usage tables are known in the art for mammalian cells (e.g. humans), as well as for a variety of other organisms.

FIC1

[0160] In some embodiments, the PFIC-associated polypeptide is FIC1 or a fragment and/or variant thereof.

[0161] "FIC1" is the abbreviated name of the polypeptide encoded by the ATP8B1 gene and is also known as familial intrahepatic cholestasis type 1 protein, phospholipid-transporting ATPase IC, ATPase class I type 8B member 1, and 4-ATPase flippase complex alpha subunit ATP8B1. FIC1 is a member of the P_4-subfamily of P-type adenosine triphosphatases (ATPases) and transports phospholipids (e.g. phosphatidylserine) from the outer to the inner leaflet of the plasma membrane. FIC1 is localized on the apical membrane of epithelial cells, including the canalicular membrane of hepatocytes (see e.g. Amer, S. and Hajira, A., 2014. Gastroenterology Research, 7(2), pp. 39-43).

[0162] A fragment and/or variant of FIC1 may retain FIC1 activity (see e.g. EC 7.6.2.1). For example, a fragment and/or variant of FIC1 may localise on the canalicular membrane of hepatocytes and transport phospholipids from the outer to the inner leaflet of the plasma membrane. Suitably, a fragment and/or variant of FIC1 may have the same or similar activity to FIC1, e.g. may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of FIC1.

[0163] A person skilled in the art would be able to generate fragments and/or variants using conservative substitutions, based on the known structural and functional features of FIC1 (see e.g. Klomp, L. W., et al., 2004. Hepatology, 40(1), pp. 27-38 and Shin, H. W. and Takatsu, H., 2019. The FASEB Journal, 33(3), pp. 3087-3096), and/or based on known variants (see e.g. NCBI Gene ID: 5205 and NCBI HomoloGene: 21151).

[0164] The ATP8B1 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. The FIC1 may be a human FIC1. Suitably, the FIC1 may comprise or consist of a polypeptide sequence of UniProtKB accession 043520, or a fragment and/or variant thereof.

[0165] In some embodiments, the FIC1 comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 37 or a fragment thereof. Suitably, the FIC1 comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least

90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 37 or a fragment thereof.

[0166] In some embodiments, the FIC1 comprises or consists of SEQ ID NO: 37 or a fragment thereof.

Exemplary FIC1

(SEQ ID NO: 37)

MSTERDSETTFDEDSQPNDEVVPYSDDETEDELDDQGSAVEPEQNRVNRE

AEENREPFRKECTWQVKANDRKYHEQPHFMNTKFLCIKESKYANNAIKTY

KYNAFTFIPMNLFEQFKRAANLYFLALLILQAVPQISTLAWYTTLVPLLV

VLGVTAIKDLVDDVARHKMDKEINNRTCEVIKDGRFKVAKWKEIQVGDVI

RLKKNDFVPADILLLSSSEPNSLCYVETAELDGETNLKFKMSLEITDQYL

QREDTLATFDGFIECEEPNNRLDKFTGTLFWRNTSFPLDADKILLRGCVI

RNTDFCHGLVIFAGADTKIMKNSGKTRFKRTKIDYLMNYMVYTIFVVLIL

LSAGLAIGHAYWEAQVGNSSWYLYDGEDDTPSYRGFLIFWGYIIVLNTMV

PISLYVSVEVIRLGQSHFINWDLQMYYAEKDTPAKARTTTLNEQLGQIHY

IFSDKTGTLTQNIMTFKKCCINGQIYGDHRDASQHNHNKIEQVDFSWNTY

ADGKLAFYDHYLIEQIQSGKEPEVRQFFFLLAVCHTVMVDRTDGQLNYQA

ASPDEGALVNAARNFGFAFLARTQNTITISELGTERTYNVLAILDFNSDR

KRMSIIVRTPEGNIKLYCKGADTVIYERLHRMNPTKQETQDALDIFANET

LRTLCLCYKEIEEKEFTEWNKKEMAASVASTNRDEALDKVYEEIEKDLIL

LGATAIEDKLQDGVPETISKLAKADIKIWVLTGDKKETAENIGFACELLT

EDTTICYGEDINSLLHARMENQRNRGGVYAKFAPPVQESFFPPGGNRALI

ITGSWLNEILLEKKTKRNKILKLKFPRTEEERRMRTQSKRRLEAKKEQRQ

KNFVDLACECSAVICCRVTPKQKAMVVDLVKRYKKAITLAIGDGANDVNM

IKTAHIGVGISGQEGMQAVMSSDYSFAQFRYLQRLLLVHGRWSYIRMCKF

LRYFFYKNFAFTLVHFWYSFFNGYSAQTAYEDWFITLYNVLYTSLPVLLM

GLLDQDVSDKLSLRFPGLYIVGQRDLLFNYKRFFVSLLHGVLTSMILFFI

PLGAYLQTVGQDGEAPSDYQSFAVTIASALVITVNFQIGLDTSYWTFVNA

FSIFGSIALYFGIMFDFHSAGIHVLFPSAFQFTGTASNALRQPYIWLTII

LAVAVCLLPVVAIRFLSMTIWPSESDKIQKHRKRLKAEEQWQRRQQVFRR

GVSTRRSAYAFSHQRGYADLISSGRSIRKKRSPLDAIVADGTAEYRRTGDS

[0167] Suitably, a FIC1 variant may comprise one or more variation selected from: N45T, H78Q, I393V, I577V, S580N, M647T, K814N, R833Q, R930Q, R952Q, and A1152T. These are considered to be benign (or likely benign) variations based on clinical data.

[0168] Suitably, a nucleotide sequence encoding FIC1, or a fragment and/or variant thereof, may comprise or consist of a nucleotide sequence of NCBI reference sequence NM_005603, NM_001374385 or NM_001374386, or a fragment and/or variant thereof.

[0169] In some embodiments, the nucleotide sequence encoding FIC1, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 38 or a fragment thereof. Suitably, the nucleotide sequence encoding FIC1, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 38 or a fragment thereof.

[0170] In some embodiments, the nucleotide sequence encoding FIC1, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 38 or a fragment thereof.

Exemplary nucleotide encoding FIC1

(SEQ ID NO: 38)

ATGAGTACAGAAAGAGACTCAGAAACGACATTTGACGAGGATTCTCAGCC

TAATGACGAAGTGGTTCCCTACAGTGATGATGAAACAGAAGATGAACTTG

ATGACCAGGGGTCTGCTGTTGAACCAGAACAAAACCGAGTCAACAGGGAA

GCAGAGGAGAACCGGGAGCCATTCAGAAAAGAATGTACATGGCAAGTCAA

AGCAAACGATCGCAAGTACCACGAACAACCTCACTTTATGAACACAAAAT

TCTTGTGTATTAAGGAGAGTAAATATGCGAATAATGCAATTAAAACATAC

AAGTACAACGCATTTACCTTTATACCAATGAATCTGTTTGAGCAGTTTAA

GAGAGCAGCCAATTTATATTTCCTGGCTCTTCTTATCTTACAGGCAGTTC

CTCAAATCTCTACCCTGGCTTGGTACACCACACTAGTGCCCCTGCTTGTG

GTGCTGGGCGTCACTGCAATCAAAGACCTGGTGGACGATGTGGCTCGCCA

TAAAATGGATAAGGAAATCAACAATAGGACGTGTGAAGTCATTAAGGATG

GCAGGTTCAAAGTTGCTAAGTGGAAAGAAATTCAAGTTGGAGACGTCATT

CGTCTGAAAAAAAATGATTTTGTTCCAGCTGACATTCTCCTGCTGTCTAG

CTCTGAGCCTAACAGCCTCTGCTATGTGGAAACAGCAGAACTGGATGGAG

AAACCAATTTAAAATTTAAGATGTCACTTGAAATCACAGACCAGTACCTC

CAAAGAGAAGATACATTGGCTACATTTGATGGTTTTATTGAATGTGAAGA

ACCCAATAACAGACTAGATAAGTTTACAGGAACACTATTTTGGAGAAACA

CAAGTTTTCCTTTGGATGCTGATAAAATTTTGTTACGTGGCTGTGTAATT

AGGAACACCGATTTCTGCCACGGCTTAGTCATTTTTGCAGGTGCTGACAC

TAAAATAATGAAGAATAGTGGGAAAACCAGATTTAAAAGAACTAAAATTG

ATTACTTGATGAACTACATGGTTTACACGATCTTTGTTGTTCTTATTCTG

CTTTCTGCTGGTCTTGCCATCGGCCATGCTTATTGGGAAGCACAGGTGGG

CAATTCCTCTTGGTACCTCTATGATGGAGAAGACGATACACCCTCCTACC

GTGGATTCCTCATTTTCTGGGGCTATATCATTGTTCTCAACACCATGGTA

CCCATCTCTCTCTATGTCAGCGTGGAAGTGATTCGTCTTGGACAGAGTCA

CTTCATCAACTGGGACCTGCAAATGTACTATGCTGAGAAGGACACACCCG

CAAAAGCTAGAACCACCACACTCAATGAACAGCTCGGGCAGATCCATTAT

ATCTTCTCTGATAAGACGGGGACACTCACACAAAATATCATGACCTTTAA

AAAGTGCTGTATCAACGGGCAGATATATGGGGACCATCGGGATGCCTCTC

AACACAACCACAACAAAATAGAGCAAGTTGATTTTAGCTGGAATACATAT

GCTGATGGGAAGCTTGCATTTTATGACCACTATCTTATTGAGCAAATCCA

GTCAGGGAAAGAGCCAGAAGTACGACAGTTCTTCTTCTTGCTCGCAGTTT

GCCACACAGTCATGGTGGATAGGACTGATGGTCAGCTCAACTACCAGGCA

-continued

GCCTCTCCCGATGAAGGTGCCCTGGTAAACGCTGCCAGGAACTTTGGCTT

TGCCTTCCTCGCCAGGACCCAGAACACCATCACCATCAGTGAACTGGGCA

CTGAAAGGACTTACAATGTTCTTGCCATTTTGGACTTCAACAGTGACCGG

AAGCGAATGTCTATCATTGTAAGAACCCCAGAAGGCAATATCAAGCTTTA

CTGTAAAGGTGCTGACACTGTTATTTATGAACGGTTACATCGAATGAATC

CTACTAAGCAAGAAACACAGGATGCCCTGGATATCTTTGCAAATGAAACT

CTTAGAACCCTATGCCTTTGCTACAAGGAAATTGAAGAAAAAGAATTTAC

AGAATGGAATAAAAAGTTTATGGCTGCCAGTGTGGCCTCCACCAACCGGG

ACGAAGCTCTGGATAAAGTATATGAGGAGATTGAAAAAGACTTAATTCTC

CTGGGAGCTACAGCTATTGAAGACAAGCTACAGGATGGAGTTCCAGAAAC

CATTTCAAAACTTGCAAAAGCTGACATTAAGATCTGGGTGCTTACTGGAG

ACAAAAAGGAAACTGCTGAAAATATAGGATTTGCTTGTGAACTTCTGACT

GAAGACACCACCATCTGCTATGGGGAGGATATTAATTCTCTTCTTCATGC

AAGGATGGAAAACCAGAGGAATAGAGGTGGCGTCTACGCAAAGTTTGCAC

CTCCTGTGCAGGAATCTTTTTTTCCACCCGGTGGAAACCGTGCCTTAATC

ATCACTGGTTCTTGGTTGAATGAAATTCTTCTCGAGAAAAAGACCAAGAG

AAATAAGATTCTGAAGCTGAAGTTCCCAAGAACAGAAGAAGAAAGACGGA

TGCGGACCCAAAGTAAAAGGAGGCTAGAAGCTAAGAAAGAGCAGCGGCAG

AAAAACTTTGTGGACCTGGCCTGCGAGTGCAGCGCAGTCATCTGCTGCCG

CGTCACCCCCAAGCAGAAGGCCATGGTGGTGGACCTGGTGAAGAGGTACA

AGAAAGCCATCACGCTGGCCATCGGAGATGGGGCCAATGACGTGAACATG

ATCAAAACTGCCCACATTGGCGTTGGAATAAGTGGACAAGAAGGAATGCA

AGCTGTCATGTCGAGTGACTATTCCTTTGCTCAGTTCCGATATCTGCAGA

GGCTACTGCTGGTGCATGGCCGATGGTCTTACATAAGGATGTGCAAGTTC

CTACGATACTTCTTTTACAAAAACTTTGCCTTTACTTTGGTTCATTTCTG

GTACTCCTTCTTCAATGGCTACTCTGCGCAGACTGCATACGAGGATTGGT

TCATCACCCTCTACAACGTGCTGTACACCAGCCTGCCCGTGCTCCTCATG

GGGCTGCTCGACCAGGATGTGAGTGACAAACTGAGCCTCCGATTCCCTGG

GTTATACATAGTGGGACAAAGAGACTTACTATTCAACTATAAGAGATTCT

TTGTAAGCTTGTTGCATGGGGTCCTAACATCGATGATCCTCTTCTTCATA

CCTCTTGGAGCTTATCTGCAAACCGTAGGGCAGGATGGAGAGGCACCTTC

CGACTACCAGTCTTTTGCCGTCACCATTGCCTCTGCTCTTGTAATAACAG

TCAATTTCCAGATTGGCTTGGATACTTCTTATTGGACTTTTGTGAATGCT

TTTTCAATTTTTGGAAGCATTGCACTTTATTTTGGCATCATGTTTGACTT

TCATAGTGCTGGAATACATGTTCTCTTTCCATCTGCATTTCAATTTACAG

GCACAGCTTCAAACGCTCTGAGACAGCCATACATTTGGTTAACTATCATC

CTGGCTGTTGCTGTGTGCTTACTACCCGTCGTTGCCATTCGATTCCTGTC

AATGACCATCTGGCCATCAGAAAGTGATAAGATCCAGAAGCATCGCAAGC

GGTTGAAGGCGGAGGAGCAGTGGCAGCGACGGCAGCAGGTGTTCCGCCGG

-continued

GGCGTGTCAACGCGGCGCTCGGCCTACGCCTTCTCGCACCAGCGGGGCTA

CGCGGACCTCATCTCCTCCGGGCGCAGCATCCGCAAGAAGCGCTCGCCGC

TTGATGCCATCGTGGCGGATGGCACCGCGGAGTACAGGCGCACCGGGGAC

AGCTGA

BSEP

[0171] In some embodiments, the PFIC-associated polypeptide is BSEP or a fragment and/or variant thereof.

[0172] "BSEP" is the abbreviated name of the polypeptide encoded by the ABCB11 gene and is also known as Bile salt export pump, ATP-binding cassette sub-family B member 11, ABCB11, ABC16, BRIC2, PFIC-2, PFIC2, PGY4, and SPGP. BSEP catalyses the transport of the major hydrophobic bile salts, such as taurine and glycine-conjugated cholic acid across the canalicular membrane of hepatocytes in an ATP-dependent manner. BSEP therefore participates in hepatic bile acid homeostasis and consequently to lipid homeostasis through regulation of biliary lipid secretion in a bile salts dependent manner.

[0173] A fragment and/or variant of BSEP may retain BSEP activity. For example, a fragment and/or variant of BSEP may transport hydrophobic bile salts (e.g. taurine and glycine-conjugated cholic acid) across the canalicular membrane of hepatocytes. Suitably, a fragment and/or variant of BSEP may have the same or similar activity to BSEP, e.g. may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of BSEP. Assays to determine BSEP activity (e.g. bile acid transport function) are described, for instance in Hayashi, H., et al., 2005. Hepatology, 41(4), pp. 916-924 and Noé, J., et al., 2002. Gastroenterology, 123(5), pp. 1659-1666.

[0174] A "fragment of BSEP" may refer to a portion or region of full-length BSEP that has the same or similar activity as full-length BSEP, i.e. the fragment may be a functional fragment (e.g. the fragment may transport hydrophobic bile salts across the canalicular membrane of hepatocytes). The fragment may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of full-length BSEP.

[0175] A "BSEP variant" may include an amino acid sequence or a nucleotide sequence which may be at least 50%, at least 55%, at least 65%, at least 70%, at least 75%, at least 75%, at least 80%, at least 85% or at least 90% identical, optionally at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a wild-type BSEP. BSEP variants may have the same or similar activity to wild-type BSEP (e.g. BSEP variants may transport hydrophobic bile salts across the canalicular membrane of hepatocytes). BSEP variants may have at least at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of a wild-type BSEP.

[0176] A person skilled in the art would be able to generate a fragment of BSEP and/or BSEP variants using conservative substitutions, based on the known structural and functional features of BSEP (see e.g. Wang, L., et al., 2020. Cell Research, 30(7), pp. 623-625 and Pauli-Magnus, C., et al.,

2004. Hepatology, 39(3), pp. 779-791) and/or based on known variants (see e.g. NCBI Gene ID: 8647 and NCBI HomoloGene: 74509).

[0177] Suitably, a fragment of BSEP and/or a BSEP variant comprises two homologous halves, each comprising a hydrophobic membrane-anchoring domain (e.g. an ABC transporter transmembrane region) and an ATP binding cassette (ABC) domain. The domains/sequences may be linked by inter-domain linker(s).

[0178] The ABCB11 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. The BSEP may be a human BSEP. Suitably, the BSEP may comprise or consist of a polypeptide sequence of UniProtKB accession 095342, or a fragment and/or variant thereof.

[0179] In some embodiments, the BSEP comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 39 or a fragment thereof. Suitably, the BSEP comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 39 or a fragment thereof.

[0180] In some embodiments, the BSEP comprises or consists of SEQ ID NO: 39 or a fragment thereof.

Exemplary BSEP

(SEQ ID NO: 39)

MSDSVILRSIKKFGEENDGFESDKSYNNDKKSRLQDEKKGDGVRVGFFQL

FRFSSSTDIWLMFVGSLCAFLHGIAQPGVLLIFGTMTDVFIDYDVELQEL

QIPGKACVNNTIVWTNSSLNQNMTNGTRCGLLNIESEMIKFASYYAGIAV

AVLITGYIQICFWVIAAARQIQKMRKFYFRRIMRMEIGWFDCNSVGELNT

RFSDDINKINDAIADQMALFIQRMTSTICGFLLGFFRGWKLTLVIISVSP

LIGIGAATIGLSVSKFTDYELKAYAKAGVVADEVISSMRTVAAFGGEKRE

VERYEKNLVFAQRWGIRKGIVMGFFTGFVWCLIFLCYALAFWYGSTLVLD

EGEYTPGTLVQIFLSVIVGALNLGNASPCLEAFATGRAAATSIFETIDRK

PIIDCMSEDGYKLDRIKGEIEFHNVTFHYPSRPEVKILNDLNMVIKPGEM

TALVGPSGAGKSTALQLIQRFYDPCEGMVTVDGHDIRSLNIQWLRDQIGI

VEQEPVLFSTTIAENIRYGREDATMEDIVQAAKEANAYNFIMDLPQQFDT

LVGEGGGQMSGGQKQRVAIARALIRNPKILLLDMATSALDNESEAMVQEV

LSKIQHGHTIISVAHRLSTVRAADTIIGFEHGTAVERGTHEELLERKGVY

FTLVTLQSQGNQALNEEDIKDATEDDMLARTFSRGSYQDSLRASIRQRSK

SQLSYLVHEPPLAVVDHKSTYEEDRKDKDIPVQEEVEPAPVRRILKFSAP

EWPYMLVGSVGAAVNGTVTPLYAFLFSQILGTFSIPDKEEQRSQINGVCL

/LFVAMGCVSLFTQFLQGYAFAKSGELLTKRLRKFGFRAMLGQDIAWFDD

LRNSPGALTTRLATDASQVQGAAGSQIGMIVNSFTNVTVAMIIAFSFSWK

LSLVILCFFPFLALSGATQTRMLTGFASRDKQALEMVGQITNEALSNIRT

VAGIGKERRFIEALETELEKPFKTAIQKANIYGFCFAFAQCIMFIANSAS

YRYGGYLISNEGLHFSYVFRVISAVVLSATALGRAFSYTPSYAKAKISAA

RFFQLLDRQPPISVYNTAGEKWDNFQGKIDFVDCKFTYPSRPDSQVLNGL

SVSISPGQTLAFVGSSGCGKSTSIQLLERFYDPDQGKVMIDGHDSKKVNV

-continued

QFLRSNIGIVSQEPVLFACSIMDNIKYGDNTKEIPMERVIAAAKQAQLHD

FVMSLPEKYETNVGSQGSQLSRGEKQRIAIARAIVRDPKILLLDEATSAL

DTESEKTVQVALDKAREGRTCIVIAHRLSTIQNADIIAVMAQGVVIEKGT

HEELMAQKGAYYKLVTTGSPIS

[0181] Suitably, a BSEP variant may comprise one or more variation selected from R8Q, K12E, F13S, F13Y, E15Q, D18H, G19D, G19R, E21D, E21Q, S22P, D23H, D23N, K24E, Y26H, Y26N, D29G, K31T, G40V, G40D, G40S, V43F, V43I, R44T, V45I, S56L, S56P, T57A, I59L, V64A, I74T, V79M, V95A, V95I, I102F, G104E, V108M, N120H, Q121R, Q121K, M123R, M123T, M123L, M123V, T124K, R128H, N133S, I139T, S143I, A151V, L153F, I160V, I165T, I171T, M174L, N193S, F202L, I206V, N207S, A218S, A218T, M224L, F236S, I245V, I254M, T258A, S262T, K265R, M288I, M288V, R299K, V301A, V301L, V301I, K306E, V309A, R313H, R313S, I320L, V321A, M322L, F324L, V329M, C336S, G352E, G352R, T355A, I362V, L364F, N375S, A384T, I393V, R399S, R399T, I402T, I402V, R415Q, H428R, E434D, V444A, P447S, M450T, M478T, M478K, E521K, D522N, T524S, M525I, V529I, D543G, D543A, D543N, Q546K, G557S, V5671, K578R, M5961, M596V, E599Q, I610T, V613I, I627T, A634V, R637S, R637K, T639N, E641K, E642D, E645G, R646K, T652A, Q659R, Q662L, Q662E, A663D, A663T, L664F, L664V, E666K, I669M, K670N, D675G, M677V, A679V, A679E, R680K, R680G, T681I, T681N, F6821, S686R, Q688R, A693T, R698H, E709K, P710L, P711A, L712S, A713V, A713S, V714F, V715L, V715I, D716G, D716A, H717R, H717N, T720S, T720A, Y721C, E722D, E722G, D724A, R725T, R725G, D727E, D729V, I730S, I730T, I730L, V732A, V732L, V732M, P740S, V741F, V741I, A749V, M755I, M755L, M755L, F774L, F774I, Q778E, Q778K, I785V, P786L, P786S, K788N, E790Q, N796D, A804V, F834L, A838T, G841R, R851K, S853N, 1879M, V886I, M890I, F908V, A916V, T917A, N941S, S945G, R958Q, I960V, T965S, T965A, E966K, K969N, K969E, F971L, F971Y, Q976R, 1991V, I1007V, N1009K, N1009S, N1009H, P1060S, V1063A, Y1064F, A1067G, E1069D, N1073S, K1077N, K1077E, V1081I, K1084T, K1136R, V1147L, V1147I, K1181R, E1182D, P1184S, M1185K, A1190T, S1203A, N1211D, V1259I, K1263E, M1289I, A1290T, V1293M, T1299S, E1301A, E1302D, Q1306R. These are considered to be tolerated, benign, and/or likely benign variations as predicted by SIFT, PolyPhen, CADD, REVEL, and MetaLR.

[0182] Suitably, a BSEP variant may comprise one or more variation selected from: V95I, I206V, N375S, V444A, Q546K, N591S, R646K, A663T, M677V, R698H, S701P, A865V, R1057Q, and E1186K. These are considered to be benign (or likely benign) variations based on clinical data.

[0183] Suitably, a nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, may comprise or consist of a nucleotide sequence of NCBI reference sequence NM_003742, or a fragment and/or variant thereof.

[0184] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 40 or a fragment thereof. Suitably, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of a

nucleotide sequence which is at least 75%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 40 or a fragment thereof.

[0185] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 40 or a fragment thereof.

Exemplary nucleotide encoding BSEP

(SEQ ID NO: 40)

ATGTCTGACTCAGTAATTCTTCGAAGTATAAAGAAATTTGGAGAGGAGAATGATGGTTTTGAGTCAGAT

AAATCATATAATAATGATAAGAAATCAAGGTTACAAGATGAGAAGAAAGGTGATGGCGTTAGAGTTGGC

TTCTTTCAATTGTTTCGGTTTTCTTCATCAACTGACATTTGGCTGATGTTTGTGGGAAGTTTGTGTGCA

TTTCTCCATGGAATAGCCCAGCCAGGCGTGCTACTCATTTTTGGCACAATGACAGATGTTTTTATTGAC

TACGACGTTGAGTTACAAGAACTCCAGATTCCAGGAAAAGCATGTGTGAATAACACCATTGTATGGACT

AACAGTTCCCTCAACCAGAACATGACAAATGGAACACGTTGTGGGTTGCTGAACATCGAGAGCGAAATG

ATCAAATTTGCCAGTTACTATGCTGGAATTGCTGTCGCAGTACTTATCACAGGATATATTCAAATATGC

TTTTGGGTCATTGCCGCAGCTCGTCAGATACAGAAAATGAGAAAATTTTACTTTAGGAGAATAATGAGA

ATGGAAATAGGGTGGTTTGACTGCAATTCAGTGGGGGAGCTGAATACAAGATTCTCTGATGATATTAAT

AAAATCAATGATGCCATAGCTGACCAAATGGCCCTTTTCATTCAGCGCATGACCTCGACCATCTGTGGT

TTCCTGTTGGGATTTTTCAGGGGTTGGAAACTGACCTTGGTTATTATTTCTGTCAGCCCTCTCATTGGG

ATTGGAGCAGCCACCATTGGTCTGAGTGTGTCCAAGTTTACGGACTATGAGCTGAAGGCCTATGCCAAA

GCAGGGGTGGTGGCTGATGAAGTCATTTCATCAATGAGAACAGTGGCTGCTTTTGGTGGTGAGAAAAGA

GAGGTTGAAAGGTATGAGAAAAATCTTGTGTTCGCCCAGCGTTGGGGAATTAGAAAAGGAATAGTGATG

GGATTCTTTACTGGATTCGTGTGGTGTCTCATCTTTTTGTGTTATGCACTGGCCTTCTGGTACGGCTCC

ACACTTGTCCTGGATGAAGGAGAATATACACCAGGAACCCTTGTCCAGATTTTCCTCAGTGTCATAGTA

GGAGCTTTAAATCTTGGCAATGCCTCTCCTTGTTTGGAAGCCTTTGCAACTGGACGTGCAGCAGCCACC

AGCATTTTTGAGACAATAGACAGGAAACCCATCATTGACTGCATGTCAGAAGATGGTTACAAGTTGGAT

CGAATCAAGGGTGAAATTGAGTTCCATAATGTGACCTTCCATTATCCTTCCAGACCAGAGGTGAAGATT

CTAAATGACCTCAACATGGTCATTAAACCAGGGGAAATGACAGCTCTGGTAGGACCCAGTGGAGCTGGA

AAAAGTACAGCACTGCAACTCATTCAGCGATTCTATGACCCCTGTGAAGGAATGGTGACCGTGGATGGC

CATGACATTCGCTCTCTTAACATTCAGTGGCTTAGAGATCAGATTGGGATAGTGGAGCAAGAGCCAGTT

CTGTTCTCTACCACCATTGCAGAAAATATTCGCTATGGCAGAGAAGATGCAACAATGGAAGACATAGTC

CAAGCTGCCAAGGAGGCCAATGCCTACAACTTCATCATGGACCTGCCACAGCAATTTGACACCCTTGTT

GGAGAAGGAGGAGGCCAGATGAGTGGTGGCCAGAAACAAAGGGTAGCTATCGCCAGAGCCCTCATCCGA

AATCCCAAGATTCTGCTTTTGGACATGGCCACCTCAGCTCTGGACAATGAGAGTGAAGCCATGGTGCAA

GAAGTGCTGAGTAAGATTCAGCATGGGCACACAATCATTTCAGTTGCTCATCGCTTGTCTACGGTCAGA

GCTGCAGATACCATCATTGGTTTTGAACATGGCACTGCAGTGGAAAGAGGGACCCATGAAGAATTACTG

GAAAGGAAAGGTGTTTACTTCACTCTAGTGACTTTGCAAAGCCAGGGAAATCAAGCTCTTAATGAAGAG

GACATAAAGGATGCAACTGAAGATGACATGCTTGCGAGGACCTTTAGCAGAGGGAGCTACCAGGATAGT

TTAAGGGCTTCCATCCGGCAACGCTCCAAGTCTCAGCTTTCTTACCTGGTGCACGAACCTCCATTAGCT

GTTGTAGATCATAAGTCTACCTATGAAGAAGATAGAAAGGACAAGGACATTCCTGTGCAGGAAGAAGTT

GAACCTGCCCCAGTTAGGAGGATTCTGAAATTCAGTGCTCCAGAATGGCCCTACATGCTGGTAGGGTCT

GTGGGTGCAGCTGTGAACGGGACAGTCACACCCTTGTATGCCTTTTTATTCAGCCAGATTCTTGGGACT

TTTTCAATTCCTGATAAAGAGGAACAAAGGTCACAGATCAATGGTGTGTGCCTACTTTTTGTAGCAATG

-continued

GGCTGTGTATCTCTTTTCACCCAATTTCTACAGGGATATGCCTTTGCTAAATCTGGGGAGCTCCTAACA

AAAAGGCTACGTAAATTTGGTTTCAGGGCAATGCTGGGGCAAGATATTGCCTGGTTTGATGACCTCAGA

AATAGCCCTGGAGCATTGACAACAAGACTTGCTACAGATGCTTCCCAAGTTCAAGGGGCTGCCGGCTCT

CAGATCGGGATGATAGTCAATTCCTTCACTAACGTCACTGTGGCCATGATCATTGCCTTCTCCTTTAGC

TGGAAGCTGAGCCTGGTCATCTTGTGCTTCTTCCCCTTCTTGGCTTTATCAGGAGCCACACAGACCAGG

ATGTTGACAGGATTTGCCTCTCGAGATAAGCAGGCCCTGGAGATGGTGGGACAGATTACAAATGAAGCC

CTCAGTAACATCCGCACTGTTGCTGGAATTGGAAAGGAGAGGCGGTTCATTGAAGCACTTGAGACTGAG

CTGGAGAAGCCCTTCAAGACAGCCATTCAGAAAGCCAATATTTACGGATTCTGCTTTGCCTTTGCCCAG

TGCATCATGTTTATTGCGAACTCTGCTTCCTACAGATATGGAGGTTACTTAATCTCCAATGAGGGGCTC

CATTTCAGCTATGTGTTCAGGGTGATCTCTGCAGTTGTACTGAGTGCAACAGCTCTTGGAAGAGCCTTC

TCTTACACCCCAAGTTATGCAAAAGCTAAAATATCAGCTGCACGCTTTTTTCAACTGCTGGACCGACAA

CCCCCAATCAGTGTATACAATACTGCAGGTGAAAAATGGGACAACTTCCAGGGGAAGATTGATTTTGTT

GATTGTAAATTTACATATCCTTCTCGACCTGACTCGCAAGTTCTGAATGGTCTCTCAGTGTCGATTAGT

CCAGGGCAGACACTGGCGTTTGTTGGGAGCAGTGGATGTGGCAAAAGCACTAGCATTCAGCTGTTGGAA

CGTTTCTATGATCCTGATCAAGGGAAGGTGATGATAGATGGTCATGACAGCAAAAAAGTAAATGTCCAG

TTCCTCCGCTCAAACATTGGAATTGTTTCCCAGGAACCAGTGTTGTTTGCCTGTAGCATAATGGACAAT

ATCAAGTATGGAGACAACACCAAAGAAATTCCCATGGAAAGAGTCATAGCAGCTGCAAAACAGGCTCAG

CTGCATGATTTTGTCATGTCACTCCCAGAGAAATATGAAACTAACGTTGGGTCCCAGGGGTCTCAACTC

TCTAGAGGGGAGAAACAACGCATTGCTATTGCTCGGGCCATTGTACGAGATCCTAAAATCTTGCTACTA

GATGAAGCCACTTCTGCCTTAGACACAGAAAGTGAAAAGACGGTGCAGGTTGCTCTAGACAAAGCCAGA

GAGGGTCGGACCTGCATTGTCATTGCCCATCGCTTGTCCACCATCCAGAACGCGGATATCATTGCTGTC

ATGGCACAGGGGGTGGTGATTGAAAAGGGGACCCATGAAGAACTGATGGCCCAAAAAGGAGCCTACTAC

AAACTAGTCACCACTGGATCCCCCATCAGTTGA

[0186] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, is codon optimised. Exemplary codon-optimised sequences are provided in SEQ ID NOs: 41 and 42.

[0187] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 41 or a fragment thereof. Suitably, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 41 or a fragment thereof.

[0188] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 41 or a fragment thereof.

Exemplary codon-optimised nucleotide encoding BSEP

(SEQ ID NO: 41)

ATGAGCGACTCCGTGATCCTGCGGAGCATCAAGAAGTTTGGCGAGGAGAACGACGGCTTCGAGTCTGAT

AAGAGCTACAACAATGATAAGAAGTCCAGACTGCAGGACGAGAAGAAGGGCGATGGCGTGCGGGTGGGC

TTCTTTCAGCTGTTCCGGTTCAGCAGCAGCACCGACATCTGGCTGATGTTTGTGGGCTCCCTGTGCGCC

TTCCTGCACGGAATCGCACAGCCAGGCGTGCTGCTGATCTTTGGCACCATGACAGACGTGTTCATCGAC

TATGATGTGGAGCTGCAGGAGCTGCAGATCCCTGGCAAGGCCTGCGTGAACAATACCATCGTGTGGACA

AATAGCTCCCTGAACCAGAATATGACCAACGGCACAAGATGTGGCCTGCTGAATATCGAGTCTGAGATG

ATCAAGTTTGCCAGCTACTATGCAGGAATCGCAGTGGCCGTGCTGATCACCGGCTACATCCAGATCTGC

TTCTGGGTCATCGCAGCAGCAAGGCAGATCCAGAAGATGAGAAAGTTCTATTTTCGGAGAATCATGCGG

-continued

ATGGAGATCGGCTGGTTTGACTGTAACTCTGTGGGCGAGCTGAATACAAGATTCAGCGACGACATCAAC

AAGATCAATGACGCCATCGCCGATCAGATGGCCCTGTTTATCCAGCGGATGACCAGCACAATCTGTGGC

TTCCTGCTGGGCTTCTTTAGAGGCTGGAAGCTGACCCTGGTCATCATCTCTGTGAGCCCACTGATCGGA

ATCGGAGCAGCAACAATCGGCCTGTCCGTGTCTAAGTTCACCGACTACGAGCTGAAGGCATATGCAAAG

GCAGGAGTGGTGGCAGATGAAGTGATCAGCAGCATGAGGACCGTGGCAGCCTTTGGCGGAGAGAAGAGG

GAGGTGGAGCGGTACGAGAAGAACCTGGTGTTCGCCCAGCGGTGGGGCATCAGAAAGGGCATCGTGATG

GGCTTCTTTACAGGCTTCGTGTGGTGCCTGATCTTCCTGTGCTACGCCCTGGCCTTTTGGTATGGCTCC

ACCCTGGTGCTGGACGAGGGAGAGTATACCCCTGGCACACTGGTGCAGATCTTCCTGAGCGTGATCGTG

GGCGCCCTGAACCTGGGAAATGCCTCCCCATGCCTGGAGGCCTTTGCCACAGGCAGGGCAGCAGCCACC

TCCATCTTCGAGACAATCGACCGCAAGCCCATCATCGACTGTATGTCTGAGGATGGCTACAAGCTGGAC

AGGATCAAGGGCGAGATCGAGTTTCACAATGTGACCTTCCACTATCCCAGCCGCCCTGAGGTGAAGATC

CTGAACGATCTGAATATGGTCATCAAGCCAGGAGAGATGACAGCCCTGGTGGGACCCTCTGGAGCAGGC

AAGAGCACCGCCCTGCAGCTGATCCAGCGGTTTTACGACCCTTGCGAGGGAATGGTGACCGTGGACGGA

CACGACATCAGGTCCCTGAACATCCAGTGGCTGCGCGATCAGATCGGCATCGTGGAGCAGGAGCCAGTG

CTGTTCTCTACCACAATCGCCGAGAATATCAGATACGGCCGCGAGGATGCCACAATGGAGGACATCGTG

CAGGCCGCCAAGGAGGCCAACGCCTATAACTTCATCATGGATCTGCCCCAGCAGTTCGACACCCTGGTG

GGAGAGGGAGGAGGACAGATGTCCGGAGGCCAGAAGCAGAGAGTGGCCATCGCCAGAGCCCTGATCCGC

AACCCTAAGATCCTGCTGCTGGATATGGCCACAAGCGCCCTGGACAATGAGTCCGAGGCCATGGTGCAG

GAGGTGCTGAGCAAGATCCAGCACGGCCACACCATCATCTCTGTGGCACACCGGCTGAGCACAGTGAGA

GCAGCCGACACCATCATCGGCTTTGAGCACGGAACAGCAGTGGAGAGGGGAACCCACGAGGAGCTGCTG

GAGAGGAAGGGCGTGTACTTCACCCTGGTGACACTGCAGAGCCAGGGCAACCAGGCCCTGAATGAGGAG

GACATCAAGGATGCCACAGAGGACGATATGCTGGCCCGGACCTTCTCCAGAGGCTCTTATCAGGATTCT

CTGCGGGCCAGCATCAGGCAGCGCAGCAAGTCCCAGCTGAGCTACCTGGTGCACGAGCCACCTCTGGCC

GTGGTGGACCACAAGTCCACCTATGAGGAGGATAGAAAGGACAAGGACATCCCCGTGCAGGAGGAGGTG

GAGCCTGCCCCAGTGAGGCGCATCCTGAAGTTTTCCGCCCCAGAGTGGCCCTACATGCTGGTGGGATCT

GTGGGAGCAGCAGTGAACGGCACCGTGACACCACTGTATGCCTTCCTGTTTTCCCAGATCCTGGGCACC

TTCTCTATCCCCGACAAGGAGGAGCAGAGGTCCCAGATCAATGGCGTGTGCCTGCTGTTTGTGGCCATG

GGCTGCGTGAGCCTGTTTACACAGTTCCTGCAGGGCTACGCCTTCGCCAAGAGCGGCGAGCTGCTGACC

AAGCGGCTGAGAAAGTTCGGCTTTCGCGCAATGCTGGGACAGGACATCGCATGGTTTGACGATCTGCGG

AACAGCCCAGGCGCCCTGACCACAAGACTGGCCACAGATGCCTCTCAGGTGCAGGGAGCAGCAGGCAGC

CAGATCGGCATGATCGTGAACTCCTTCACCAATGTGACAGTGGCCATGATCATCGCCTTCTCTTTTAGC

TGGAAGCTGTCTCTGGTCATCCTGTGCTTCTTCCCCTTTCTGGCCCTGAGCGGAGCAACCCAGACAAGG

ATGCTGACCGGCTTCGCCTCCAGAGACAAGCAGGCCCTGGAGATGGTGGGCCAGATCACAAACGAGGCC

CTGAGCAATATCAGGACCGTGGCAGGAATCGGCAAGGAGCGGCGGTTCATCGAGGCCCTGGAGACAGAG

CTGGAGAAGCCTTTCAAGACCGCCATCCAGAAGGCCAACATCTACGGCTTCTGCTTTGCCTTCGCCCAG

TGTATCATGTTCATCGCCAACTCCGCCTCTTACAGATATGGCGGCTACCTGATCAGCAATGAGGGCCTG

CACTTTTCCTACGTGTTCAGAGTGATCAGCGCCGTGGTGCTGTCTGCCACAGCCCTGGGAAGGGCCTTC

TCCTACACCCCATCTTATGCCAAGGCCAAGATCTCTGCCGCCAGGTTCTTTCAGCTGCTGGACCGCCAG

CCACCCATCAGCGTGTACAACACAGCCGGCGAGAAGTGGGATAATTTCCAGGGCAAGATCGACTTTGTG

-continued

GATTGCAAGTTCACCTATCCTAGCAGGCCAGACTCCCAGGTGCTGAACGGCCTGAGCGTGTCCATCTCT

CCAGGCCAGACACTGGCCTTTGTGGGCTCCTCTGGCTGTGGCAAGAGCACCTCCATCCAGCTGCTGGAG

AGGTTCTACGACCCCGATCAGGGCAAAGTGATGATCGACGGCCACGATTCTAAGAAGGTGAACGTGCAG

TTTCTGCGCTCCAATATCGGCATCGTGTCTCAGGAGCCTGTGCTGTTCGCCTGCAGCATCATGGATAAC

ATCAAGTACGGCGACAATACAAAGGAGATCCCAATGGAGAGAGTGATCGCAGCAGCAAAGCAGGCACAG

CTGCACGATTTCGTGATGTCCCTGCCCGAGAAGTATGAGACAAACGTGGGCAGCCAGGGCTCCCAGCTG

TCTAGGGGCGAGAAGCAGAGGATCGCAATCGCCAGGGCCATCGTGCGCGATCCCAAGATCCTGCTGCTG

GACGAGGCCACCAGCGCCCTGGATACAGAGTCCGAGAAGACCGTGCAGGTGGCCCTGGACAAGGCCCGG

GAGGGAAGAACATGTATCGTGATCGCCCACCGCCTGAGCACCATCCAGAATGCCGACATCATCGCCGTG

ATGGCCCAGGGCGTGGTCATCGAGAAGGGCACCCACGAGGAGCTGATGGCCCAGAAGGGCGCCTACTAT

AAGCTGGTGACCACAGGCAGCCCTATCTCCTGA

[0189] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 42 or a fragment thereof. Suitably, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 42 or a fragment thereof.

[0190] In some embodiments, the nucleotide sequence encoding BSEP, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 42 or a fragment thereof.

Exemplary codon-optimised nucleotide encoding BSEP

(SEQ ID NO: 42)

ATGAGCGATTCTGTGATCCTGCGGAGCATCAAGAAGTTCGGCGAGGAAAACGACGGCTTCGAGAGCGAC

AAGAGCTACAACAACGACAAGAAGTCCCGGCTGCAGGACGAGAAGAAAGGCGACGGTGTCAGAGTGGGC

TTCTTCCAGCTGTTCCGGTTCAGCAGCAGCACCGACATCTGGCTGATGTTCGTGGGCAGCCTGTGCGCC

TTTCTGCACGGAATTGCTCAGCCTGGCGTGCTGCTGATCTTCGGCACCATGACCGACGTGTTCATCGAC

TACGACGTGGAACTGCAAGAGCTGCAGATCCCTGGCAAGGCCTGCGTGAACAACACCATCGTGTGGACC

AACAGCAGCCTGAACCAGAACATGACCAACGGCACCAGATGCGGCCTGCTGAATATCGAGTCCGAGATG

ATCAAGTTCGCCAGCTACTACGCCGGAATCGCCGTGGCCGTTCTGATCACCGGCTACATCCAGATCTGC

TTTTGGGTTATCGCCGCTGCCAGACAGATCCAGAAGATGCGGAAGTTCTACTTCCGGCGGATCATGCGG

ATGGAAATCGGATGGTTCGACTGCAACAGCGTGGGCGAGCTGAACACCAGATTCAGCGACGACATCAAC

AAGATCAACGACGCCATTGCCGACCAGATGGCCCTGTTCATCCAGCGGATGACCTCTACCATCTGCGGC

TTTCTGCTGGGCTTTTTCAGAGGCTGGAAGCTGACCCTGGTCATCATCAGCGTGTCCCCACTGATCGGA

ATCGGAGCCGCCACAATCGGCCTGAGCGTGTCCAAGTTCACCGATTACGAGCTGAAGGCCTACGCCAAA

GCTGGCGTGGTGGCCGATGAAGTGATCAGCTCCATGAGAACCGTGGCCGCCTTTGGCGGCGAGAAGAGA

GAGGTGGAACGCTACGAGAAAAACCTGGTGTTCGCCCAGAGATGGGGCATCAGAAAGGGCATCGTGATG

GGATTCTTCACCGGCTTCGTGTGGTGCCTGATCTTCCTGTGTTACGCCCTGGCCTTTTGGTACGGCAGC

ACCCTGGTTCTGGATGAGGGCGAGTATACCCCTGGAACTCTGGTGCAGATCTTTCTGAGCGTGATCGTG

GGCGCCCTGAACCTGGGAAATGCCTCTCCATGCCTGGAAGCCTTTGCCACAGGCAGAGCCGCCGCTACC

AGCATCTTCGAGACAATCGACAGAAAGCCCATCATCGACTGCATGAGCGAGGACGGCTACAAGCTGGAC

AGAATCAAGGGCGAGATCGAGTTCCACAACGTGACCTTTCACTACCCCAGCAGACCCGAAGTGAAGATC

CTGAACGACCTGAACATGGTCATCAAGCCCGGCGAGATGACAGCCCTCGTTGGACCTTCTGGCGCCGGA

AAATCTACAGCCCTGCAGCTGATCCAGCGGTTCTACGATCCTTGCGAAGGCATGGTCACCGTGGACGGC

-continued

CACGACATCAGATCTCTGAACATCCAGTGGCTGCGGGACCAGATCGGCATCGTGGAACAAGAGCCTGTG

CTGTTCAGCACCACAATCGCCGAGAACATCAGATACGGCCGCGAGGATGCCACCATGGAAGATATTGTG

CAGGCCGCTAAAGAGGCCAACGCCTACAACTTCATCATGGACCTGCCTCAGCAGTTCGACACCCTCGTC

GGAGAAGGCGGAGGACAAATGTCTGGCGGCCAGAAACAGAGAGTGGCCATTGCTAGAGCCCTGATCAGA

AACCCCAAGATCCTGCTGCTGGACATGGCCACAAGCGCCCTGGATAATGAGAGCGAGGCCATGGTGCAA

GAGGTGCTGAGCAAGATCCAGCACGGCCACACCATCATCAGTGTGGCCCACAGACTGAGCACCGTGCGG

GCTGCCGATACCATCATCGGATTTGAGCACGGCACCGCCGTGGAAAGAGGCACACACGAGGAACTGCTG

GAACGGAAGGGCGTGTACTTCACCCTCGTGACACTGCAGAGCCAGGGCAATCAGGCCCTGAACGAAGAG

GACATCAAGGACGCCACCGAGGACGACATGCTGGCCAGAACCTTTAGCAGAGGCAGCTACCAGGACAGC

CTGCGGGCCTCTATCAGACAGAGAAGCAAGAGCCAGCTGAGCTACCTGGTGCACGAACCTCCACTGGCT

GTGGTGGATCACAAGAGCACCTACGAGGAAGATCGGAAGGACAAGGACATCCCCGTGCAAGAAGAAGTG

GAACCCGCTCCTGTGCGGCGGATCCTGAAATTTTCTGCCCCTGAGTGGCCCTACATGCTCGTGGGATCT

GTGGGAGCCGCCGTGAATGGAACAGTGACCCCTCTGTACGCCTTCCTGTTCTCCCAGATCCTGGGCACC

TTCAGCATCCCCGACAAAGAGGAACAGCGGAGCCAGATCAATGGCGTGTGCCTGCTGTTTGTGGCTATG

GGCTGCGTGTCCCTGTTTACCCAGTTCCTGCAGGGATACGCCTTCGCCAAGAGCGGAGAGCTGCTGACC

AAGCGGCTGAGAAAGTTCGGCTTCAGAGCCATGCTCGGCCAGGATATCGCTTGGTTTGACGACCTGAGA

AACAGCCCTGGCGCTCTGACAACCAGACTGGCCACAGATGCTTCTCAGGTGCAAGGCGCTGCCGGCTCT

CAGATCGGAATGATCGTGAACAGCTTCACCAATGTGACCGTGGCCATGATCATTGCCTTTAGCTTCAGC

TGGAAACTGAGCCTCGTGATCCTGTGCTTCTTCCCATTTCTGGCCCTGTCCGGCGCTACCCAGACCAGA

ATGCTTACAGGCTTCGCCAGCAGAGACAAGCAGGCCCTGGAAATGGTCGGACAGATCACCAACGAGGCC

CTGAGCAACATCAGAACAGTGGCCGGCATCGGCAAAGAGCGGCGGTTTATTGAGGCTCTGGAAACCGAG

CTGGAAAAACCCTTCAAGACCGCCATCCAGAAGGCCAATATCTACGGCTTCTGCTTCGCCTTCGCTCAG

TGCATCATGTTTATCGCCAACAGCGCCAGCTATAGATACGGCGGCTACCTGATCAGCAATGAGGGCCTG

CACTTCAGCTACGTGTTCCGCGTGATCTCTGCCGTGGTGCTGTCTGCCACAGCTCTGGGCAGAGCATTC

AGCTACACACCCAGCTACGCCAAGGCCAAGATTAGCGCCGCCAGATTCTTTCAGCTGCTGGATAGACAG

CCTCCTATCTCCGTGTACAACACCGCTGGCGAGAAGTGGGACAACTTCCAGGGCAAGATCGACTTCGTG

GATTGCAAGTTCACATACCCCTCTCGGCCCGACAGCCAGGTGCTGAATGGACTGTCCGTGTCTATCAGC

CCCGGACAGACACTGGCCTTTGTGGGAAGCTCTGGCTGCGGCAAAAGCACCAGCATCCAGCTGCTCGAG

AGATTCTACGACCCCGACCAGGGCAAAGTGATGATCGACGGACACGACAGCAAGAAAGTGAACGTGCAG

TTCCTGAGATCCAACATCGGGATCGTGTCTCAAGAACCCGTGCTGTTTGCCTGCTCCATCATGGATAAC

ATTAAGTACGGCGACAACACCAAAGAAATCCCCATGGAAAGAGTGATCGCTGCCGCCAAACAGGCCCAG

CTGCACGATTTCGTGATGAGCCTGCCTGAGAAGTACGAGACAAACGTGGGCTCTCAGGGCAGCCAGCTG

TCTAGAGGCGAAAAGCAGCGGATCGCTATCGCCAGAGCCATCGTGCGGGATCCTAAGATTCTGCTCCTG

GATGAAGCCACCAGCGCACTGGACACCGAGTCCGAAAAGACAGTGCAAGTGGCCCTGGACAAAGCCAGA

GAGGGCAGAACCTGTATCGTGATCGCCCACCGGCTGAGCACAATCCAGAACGCCGATATTATCGCCGTG

ATGGCCCAGGGCGTCGTGATCGAAAAGGGAACCCACGAAGAACTGATGGCTCAGAAGGGCGCCTACTAC

AAGCTCGTGACAACAGGCAGCCCTATCTCCTGA

MDR3

[0191] In some embodiments, the PFIC-associated polypeptide is MDR3 or a fragment and/or variant thereof.

[0192] "MDR3" is the abbreviated name of the polypeptide encoded by the ABCB4 gene and is also known as Multidrug resistance protein 3, Phosphatidylcholine translocator ABCB4, and ATP-binding cassette sub-family B member 4. MDR3 is a floppase that translocates specifically phosphatidylcholine (PC) from the inner to the outer leaflet of the canalicular membrane bilayer. Translocation of PC makes the biliary phospholipids available for extraction into the canaliculi lumen by bile salt mixed micelles and therefore protects the biliary tree from the detergent activity of bile salts.

[0193] A fragment and/or variant of MDR3 may retain MDR3 activity (see e.g. EC 7.6.2.1). For example, a fragment and/or variant of MDR3 may translocate phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane bilayer. Suitably, a fragment and/or variant of MDR3 may have the same or similar activity to MDR3, e.g. may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of MDR3.

[0194] A person skilled in the art would be able to generate fragments and/or variants using conservative substitutions, based on the known structural and functional features of MDR3 (see e.g. Oude Elferink, R. P. and Paulusma, C. C., 2007. Pflügers Archiv-European Journal of Physiology, 453 (5), pp. 601-610; Olsen, J. A., et al., 2020. Nature structural & molecular biology, 27(1), pp. 62-70; and Nosol, K., et al., 2021. PNAS, 118(33), e2106702118), and/or based on known variants (see e.g. NCBI Gene ID: 5244 and NCBI HomoloGene: 136368).

[0195] Suitably, a fragment of MDR3 and/or a MDR3 variant comprises two homologous halves, each comprising a hydrophobic membrane-anchoring domain (e.g. an ABC transporter transmembrane region) and an ATP binding cassette (ABC) domain. The domains/sequences may be linked by inter-domain linker(s).

[0196] The ABCB4 gene is conserved in chimpanzee, dog, cow, mouse, and rat. The MDR3 may be a human MDR3. Suitably, the MDR3 may comprise or consist of a polypeptide sequence of UniProtKB accession P21439, or a fragment and/or variant thereof.

[0197] In some embodiments, the MDR3 comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 43 or a fragment thereof. Suitably, the MDR3 comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 43 or a fragment thereof.

[0198] In some embodiments, the MDR3 comprises or consists of SEQ ID NO: 43 or a fragment thereof.

Exemplary MDR3

(SEQ ID NO: 43)

MDLEAAKNGTAWRPTSAEGDFELGISSKQKRKKTKTVKMIGVLTLFRYSD

WQDKLFMSLGTIMAIAHGSGLPLMMIVFGEMTDKFVDTAGNFSFPVNFSL

SLLNPGKILEEEMTRYAYYYSGLGAGVLVAAYIQVSFWTLAAGRQIRKIR

-continued

QKFFHAILRQEIGWFDINDTTELNTRLTDDISKISEGIGDKVGMFFQAVA

TFFAGFIVGFIRGWKLTLVIMAISPILGLSAAVWAKILSAFSDKELAAYA

KAGAVAEEALGAIRTVIAFGGQNKELERYQKHLENAKEIGIKKAISANIS

MGIAFLLIYASYALAFWYGSTLVISKEYTIGNAMTVFFSILIGAFSVGQA

APCIDAFANARGAAYVIFDIIDNNPKIDSFSERGHKPDSIKGNLEFNDVH

FSYPSRANVKILKGLNLKVQSGQTVALVGSSGCGKSTTVQLIQRLYDPDE

GTINIDGQDIRNENVNYLREIIGVVSQEPVLESTTIAENICYGRGNVTMD

EIKKAVKEANAYEFIMKLPQKFDTLVGERGAQLSGGQKQRIAIARALVRN

PKILLLDEATSALDTESEAEVQAALDKAREGRTTIVIAHRLSTVRNADVI

AGFEDGVIVEQGSHSELMKKEGVYFKLVNMQTSGSQIQSEEFELNDEKAA

TRMAPNGWKSRLFRHSTQKNLKNSQMCQKSLDVETDGLEANVPPVSFLKV

LKLNKTEWPYFVVGTVCAIANGGLQPAFSVIFSEIIAIFGPGDDAVKQQK

CNIFSLIFLFLGIISFFTFFLQGFTFGKAGEILTRRLRSMAFKAMLRQDM

SWFDDHKNSTGALSTRLATDAAQVQGATGTRLALIAQNIANLGTGIIISF

IYGWQLTLLLLAVVPIIAVSGIVEMKLLAGNAKRDKKELEAAGKIATEAI

ENIRTVVSLTQERKFESMYVEKLYGPYRNSVQKAHIYGITFSISQAFMYF

SYAGCFRFGAYLIVNGHMRFRDVILVESAIVFGAVALGHASSFAPDYAKA

KLSAAHLFMLFERQPLIDSYSEEGLKPDKFEGNITFNEVVFNYPTRANVP

VLQGLSLEVKKGQTLALVGSSGCGKSTVVQLLERFYDPLAGTVFVDFGFQ

LLDGQEAKKLNVQWLRAQLGIVSQEPILFDCSIAENIAYGDNSRVVSQDE

IVSAAKAANIHPFIETLPHKYETRVGDKGTQLSGGQKQRIAIARALIRQP

QILLLDEATSALDTESEKVVQEALDKAREGRTCIVIAHRLSTIQNADLIV

VFQNGRVKEHGTHQQLLAQKGIYFSMVSVQAGTQNL

[0199] Suitably, a MDR3 variant may comprise one or more variation selected from: E4D, T175A, L238V, E528D, R652G, and R788Q. These are considered to be benign (or likely benign) variations based on clinical data. Suitably, a fragment of MDR3 and/or a MDR3 variant may comprise a deletion of aa 1094-1100 and/or aa 929-975.

[0200] Suitably, a nucleotide sequence encoding MDR3, or a fragment and/or variant thereof, may comprise or consist of a nucleotide sequence of NCBI reference sequence NM_018849, NM_000443 or NM_018850, or a fragment and/or variant thereof.

[0201] In some embodiments, the nucleotide sequence encoding MDR3, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 44 or a fragment thereof. Suitably, the nucleotide sequence encoding MDR3, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 44 or a fragment thereof.

[0202] In some embodiments, the nucleotide sequence encoding MDR3, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 44 or a fragment thereof.

Exemplary nucleotide encoding MDR3

(SEQ ID NO: 44)

ATGGATCTTGAGGCGGCAAAGAACGGAACAGCCTGGCGCCCCACGAGCGCGGAGGGCGACTTTGAACTG

GGCATCAGCAGCAAACAAAAAAGGAAAAAAACGAAGACAGTGAAAATGATTGGAGTATTAACATTGTTT

CGATACTCCGATTGGCAGGATAAATTGTTTATGTCGCTGGGTACCATCATGGCCATAGCTCACGGATCA

GGTCTCCCCCTCATGATGATAGTATTTGGAGAGATGACTGACAAATTTGTTGATACTGCAGGAAACTTC

TCCTTTCCAGTGAACTTTTCCTTGTCGCTGCTAAATCCAGGCAAAATTCTGGAAGAAGAAATGACTAGA

TATGCATATTACTACTCAGGATTGGGTGCTGGAGTTCTTGTTGCTGCCTATATACAAGTTTCATTTTGG

ACTTTGGCAGCTGGTCGACAGATCAGGAAAATTAGGCAGAAGTTTTTTCATGCTATTCTACGACAGGAA

ATAGGATGGTTTGACATCAACGACACCACTGAACTCAATACGCGGCTAACAGATGACATCTCCAAAATC

AGTGAAGGAATTGGTGACAAGGTTGGAATGTTCTTTCAAGCAGTAGCCACGTTTTTTGCAGGATTCATA

GTGGGATTCATCAGAGGATGGAAGCTCACCCTTGTGATAATGGCCATCAGCCCTATTCTAGGACTCTCT

GCAGCCGTTTGGGCAAAGATACTCTCGGCATTTAGTGACAAAGAACTAGCTGCTTATGCAAAAGCAGGC

GCCGTGGCAGAAGAGGCTCTGGGGGCCATCAGGACTGTGATAGCTTTCGGGGGCCAGAACAAAGAGCTG

GAAAGGTATCAGAAACATTTAGAAAATGCCAAAGAGATTGGAATTAAAAAAGCTATTTCAGCAAACATT

TCCATGGGTATTGCCTTCCTGTTAATATATGCATCATATGCACTGGCCTTCTGGTATGGATCCACTCTA

GTCATATCAAAAGAATATACTATTGGAAATGCAATGACAGTTTTTTTTTCAATCCTAATTGGAGCTTTC

AGTGTTGGCCAGGCTGCCCCATGTATTGATGCTTTTGCCAATGCAAGAGGAGCAGCATATGTGATCTTT

GATATTATTGATAATAATCCTAAAATTGACAGTTTTTCAGAGAGAGGACACAAACCAGACAGCATCAAA

GGGAATTTGGAGTTCAATGATGTTCACTTTTCTTACCCTTCTCGAGCTAACGTCAAGATCTTGAAGGGC

CTCAACCTGAAGGTGCAGAGTGGGCAGACGGTGGCCCTGGTTGGAAGTAGTGGCTGTGGGAAGAGCACA

ACGGTCCAGCTGATACAGAGGCTCTATGACCCTGATGAGGGCACAATTAACATTGATGGGCAGGATATT

AGGAACTTTAATGTAAACTATCTGAGGGAAATCATTGGTGTGGTGAGTCAGGAGCCGGTGCTGTTTTCC

ACCACAATTGCTGAAAATATTTGTTATGGCCGTGGAAATGTAACCATGGATGAGATAAAGAAAGCTGTC

AAAGAGGCCAACGCCTATGAGTTTATCATGAAATTACCACAGAAATTTGACACCCTGGTTGGAGAGAGA

GGGGCCCAGCTGAGTGGTGGGCAGAAGCAGAGGATCGCCATTGCACGTGCCCTGGTTCGCAACCCCAAG

ATCCTTCTGCTGGATGAGGCCACGTCAGCATTGGACACAGAAAGTGAAGCTGAGGTACAGGCAGCTCTG

GATAAGGCCAGAGAAGGCCGGACCACCATTGTGATAGCACACCGACTGTCTACGGTCCGAAATGCAGAT

GTCATCGCTGGGTTTGAGGATGGAGTAATTGTGGAGCAAGGAAGCCACAGCGAACTGATGAAGAAGGAA

GGGGTGTACTTCAAACTTGTCAACATGCAGACATCAGGAAGCCAGATCCAGTCAGAAGAATTTGAACTA

AATGATGAAAAGGCTGCCACTAGAATGGCCCCAAATGGCTGGAAATCTCGCCTATTTAGGCATTCTACT

CAGAAAAACCTTAAAAATTCACAAATGTGTCAGAAGAGCCTTGATGTGGAAACCGATGGACTTGAAGCA

AATGTGCCACCAGTGTCCTTTCTGAAGGTCCTGAAACTGAATAAAACAGAATGGCCCTACTTTGTCGTG

GGAACAGTATGTGCCATTGCCAATGGGGGGCTTCAGCCGGCATTTTCAGTCATATTCTCAGAGATCATA

GCGATTTTTGGACCAGGCGATGATGCAGTGAAGCAGCAGAAGTGCAACATATTCTCTTTGATTTTCTTA

TTTCTGGGAATTATTTCTTTTTTTACTTTCTTCCTTCAGGGTTTCACGTTTGGGAAAGCTGGCGAGATC

CTCACCAGAAGACTGCGGTCAATGGCTTTTAAAGCAATGCTAAGACAGGACATGAGCTGGTTTGATGAC

CATAAAAACAGTACTGGTGCACTTTCTACAAGACTTGCCACAGATGCTGCCCAAGTCCAAGGAGCCACA

GGAACCAGGTTGGCTTTAATTGCACAGAATATAGCTAACCTTGGAACTGGTATTATCATATCATTTATC

TACGGTTGGCAGTTAACCCTATTGCTATTAGCAGTTGTTCCAATTATTGCTGTGTCAGGAATTGTTGAA

-continued

```
ATGAAATTGTTGGCTGGAAATGCCAAAAGAGATAAAAAAGAACTGGAAGCTGCTGGAAAGATTGCAACA

GAGGCAATAGAAAATATTAGGACAGTTGTGTCTTTGACCCAGGAAAGAAAATTTGAATCAATGTATGTT

GAAAAATTGTATGGACCTTACAGGAATTCTGTGCAGAAGGCACACATCTATGGAATTACTTTTAGTATC

TCACAAGCATTTATGTATTTTTCCTATGCCGGTTGTTTTCGATTTGGTGCATATCTCATTGTGAATGGA

CATATGCGCTTCAGAGATGTTATTCTGGTGTTTTCTGCAATTGTATTTGGTGCAGTGGCTCTAGGACAT

GCCAGTTCATTTGCTCCAGACTATGCTAAAGCTAAGCTGTCTGCAGCCCACTTATTCATGCTGTTTGAA

AGACAACCTCTGATTGACAGCTACAGTGAAGAGGGGCTGAAGCCTGATAAATTTGAAGGAAATATAACA

TTTAATGAAGTCGTGTTCAACTATCCCACCCGAGCAAACGTGCCAGTGCTTCAGGGGCTGAGCCTGGAG

GTGAAGAAAGGCCAGACACTAGCCCTGGTGGGCAGCAGTGGCTGTGGGAAGAGCACGGTGGTCCAGCTC

CTGGAGCGGTTCTACGACCCCTTGGCGGGGACAGTGTTTGTGGACTTTGGTTTTCAGCTTCTCGATGGT

CAAGAAGCAAAGAAACTCAATGTCCAGTGGCTCAGAGCTCAACTCGGAATCGTGTCTCAGGAGCCTATC

CTATTTGACTGCAGCATTGCCGAGAATATTGCCTATGGAGACAACAGCCGGGTTGTATCACAGGATGAA

ATTGTGAGTGCAGCCAAAGCTGCCAACATACATCCTTTCATCGAGACGTTACCCCACAAATATGAAACA

AGAGTGGGAGATAAGGGGACTCAGCTCTCAGGAGGTCAAAAACAGAGGATTGCTATTGCCCGAGCCCTC

ATCAGACAACCTCAAATCCTCCTGTTGGATGAAGCTACATCAGCTCTGGATACTGAAAGTGAAAAGGTT

GTCCAAGAAGCCCTGGACAAAGCCAGAGAAGGCCGCACCTGCATTGTGATTGCTCACCGCCTGTCCACC

ATCCAGAATGCAGACTTAATAGTGGTGTTTCAGAATGGGAGAGTCAAGGAGCATGGCACGCATCAGCAG

CTGCTGGCACAGAAAGGCATCTATTTTTCAATGGTCAGTGTCCAGGCTGGGACACAGAACTTATGA
```

TJP2

[0203] In some embodiments, the PFIC-associated polypeptide is TJP2 or a fragment and/or variant thereof.

[0204] "TJP2" is the abbreviated name of the polypeptide encoded by the TJP2 gene and is also known as Tight junction protein ZO-2, Tight junction protein 2, Zona occludens protein 2, and Zonula occludens protein 2. TJP2 is a multi-domain protein that consists of an SH3 domain, a GK domain and three copies of a PDZ domain that plays a role in tight junctions and adherens junctions. TJP2 is a cytosolic component of several classes of cell-cell junctions (see e.g. Fanning, A. S., et al., 2012. Molecular biology of the cell, 23(4), pp. 577-590).

[0205] A fragment and/or variant of TJP2 may retain TJP2 activity. For example, a fragment and/or variant of TJP2 may bind to the cytoplasmic C termini of junctional transmembrane proteins and link them to the actin cytoskeleton. Suitably, a fragment and/or variant of TJP2 may have the same or similar activity to TJP2, e.g. may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of TJP2.

[0206] A person skilled in the art would be able to generate fragments and/or variants using conservative substitutions, based on the known structural and functional features of TJP2 (see e.g. in Sambrotta, M., et al., 2014. Nature genetics, 46(4), pp. 326-328 and Chen, H., et al., 2009. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun., 65(4), pp. 327-330), and/or based on known variants (see e.g. NCBI Gene ID: 9414 and NCBI HomoloGene: 3541).

[0207] Suitably, a fragment of TJP2 and/or a TJP2 variant comprises three PDZ domains, a SH3 domain, and a nucleoside/nucleotide kinase domain. The domains/sequences may be linked by inter-domain linker(s).

[0208] The TJP2 gene is conserved in chimpanzee, Rhesus monkey, dog, mouse, and rat. The TJP2 may be a human TJP2. Suitably, the TJP2 may comprise or consist of a polypeptide sequence of UniProtKB accession Q9UDY2, or a fragment and/or variant thereof.

[0209] In some embodiments, the TJP2 comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 45 or a fragment thereof. Suitably, the TJP2 comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 45 or a fragment thereof.

[0210] In some embodiments, the TJP2 comprises or consists of SEQ ID NO: 45 or a fragment thereof.

Exemplary TJP2

(SEQ ID NO: 45)

```
MPVRGDRGFPPRRELSGWLRAPGMEELIWEQYTVTLQKDSKRGFGIAVSG

GRDNPHFENGETSIVISDVLPGGPADGLLQENDRVVMVNGTPMEDVLHSF

AVQQLRKSGKVAAIVVKRPRKVQVAALQASPPLDQDDRAFEVMDEFDGRS

FRSGYSERSRLNSHGGRSRSWEDSPERGRPHERARSRERDLSRDRSRGRS

LERGLDQDHARTRDRSRGRSLERGLDHDFGPSRDRDRDRSRGRSIDQDYE

RAYHRAYDPDYERAYSPEYRRGARHDARSRGPRSRSREHPHSRSPSPEPR

GRPGPIGVLLMKSRANEEYGLRLGSQIFVKEMTRTGLATKDGNLHEGDII

LKINGTVTENMSLTDARKLIEKSRGKLQLVVLRDSQQTLINIPSLNDSDS

EIEDISEIESNRSFSPEERRHQYSDYDYHSSSEKLKERPSSREDTPSRLS
```

-continued

RMGATPTPFKSTGDIAGTVVPETNKEPRYQEDPPAPQPKAAPRTFLRPSP

EDEAIYGPNTKMVRFKKGDSVGLRLAGGNDVGIFVAGIQEGTSAEQEGLQ

EGDQILKVNTQDFRGLVREDAVLYLLEIPKGEMVTILAQSRADVYRDILA

CGRGDSFFIRSHFECEKETPQSLAFTRGEVFRVVDTLYDGKLGNWLAVRI

GNELEKGLIPNKSRAEQMASVQNAQRDNAGDRADFWRMRGQRSGVKKNLR

KSREDLTAVVSVSTKFPAYERVLLREAGFKRPVVLFGPIADIAMEKLANE

LPDWFQTAKTEPKDAGSEKSTGVVRLNTVRQIIEQDKHALLDVTPKAVDL

LNYTQWFPIVIFFNPDSRQGVKTMRQRLNPTSNKSSRKLFDQANKLKKTC

AHLFTATINLNSANDSWFGSLKDTIQHQQGEAVWVSEGKMEGMDDDPEDR

MSYLTAMGADYLSCDSRLISDFEDTDGEGGAYTDNELDEPAEEPLVSSIT

RSSEPVQHEESIRKPSPEPRAQMRRAASSDQLRDNSPPPAFKPEPPKAKT

QNKEESYDESKSYEYKSNPSAVAGNETPGASTKGYPPPVAAKPTFGRSIL

KPSTPIPPQEGEEVGESSEEQDNAPKSVLGKVKIFEKMDHKARLQRMQEL

QEAQNARIEIAQKHPDIYAVPIKTHKPDPGTPQHTSSRPPEPQKAPSRPY

QDTRGSYGSDAEEEEYRQQLSEHSKRGYYGQSARYRDTEL

[0211] Suitably, a TJP2 variant may comprise one or more variation selected from: L15V, Q128K, R215H, R223Q, S296N, G355R, T364M, D482E, R493K, M668I, S711P, Q879R, L937P, P940L, S1010F, and V1064L. These are considered to be benign (or likely benign) variations based on clinical data. Suitably, a fragment of TJP2 and/or a TJP2 variant may comprise a deletion of aa 1-23 and/or aa 961-1107,

[0212] Suitably, a nucleotide sequence encoding TJP2, or a fragment and/or variant thereof, may comprise or consist of a nucleotide sequence of NCBI reference sequence NM_004817, NM_001170414, NM_001170415, NM_001170416, NM_001369870, NM_001369871, NM_001369872, NM_001369873, NM_001369874, NM_001369875 or NM_201629.3, or a fragment and/or variant thereof.

[0213] In some embodiments, the nucleotide sequence encoding TJP2, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 46 or a fragment thereof. Suitably, the nucleotide sequence encoding TJP2, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 46 or a fragment thereof.

[0214] In some embodiments, the nucleotide sequence encoding TJP2, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 46 or a fragment thereof.

Exemplary nucleotide encoding TJP2

(SEQ ID NO: 46)

ATGCCGGTGCGAGGAGACCGCGGGTTTCCACCCCGGCGGGAGCTGTCAGGTTGGCTCCGCGCCCCAGGC

ATGGAAGAGCTGATATGGGAACAGTACACTGTGACCCTACAAAAGGATTCCAAAAGAGGATTTGGAATT

GCAGTGTCCGGAGGCAGAGACAACCCCCACTTTGAAAATGGAGAAACGTCAATTGTCATTTCTGATGTG

CTCCCGGGTGGGCCTGCTGATGGGCTGCTCCAAGAAAATGACAGAGTGGTCATGGTCAATGGCACCCCC

ATGGAGGATGTGCTTCATTCGTTTGCAGTTCAGCAGCTCAGAAAAAGTGGGAAGGTCGCTGCTATTGTG

GTCAAGAGGCCCCGGAAGGTCCAGGTGGCCGCACTTCAGGCCAGCCCTCCCCTGGATCAGGATGACCGG

GCTTTTGAGGTGATGGACGAGTTTGATGGCAGAAGTTTCCGGAGTGGCTACAGCGAGAGGAGCCGGCTG

AACAGCCATGGGGGGCGCAGCCGCAGCTGGGAGGACAGCCCGGAAAGGGGGCGTCCCCATGAGCGGGCC

CGGAGCCGGGAGCGGGACCTCAGCCGGGACCGGAGCCGTGGCCGGAGCCTGGAGCGGGGCCTGGACCAA

GACCATGCGCGCACCCGAGACCGCAGCCGTGGCCGGAGCCTGGAGCGGGGCCTGGACCACGACTTTGGG

CCATCCCGGGACCGGGACCGTGACCGCAGCCGCGGCCGGAGCATTGACCAGGACTACGAGCGAGCCTAT

CACCGGGCCTACGACCCAGACTACGAGCGGGCCTACAGCCCGGAGTACAGGCGCGGGGCCCGCCACGAT

GCCCGCTCTCGGGGACCCCGAAGCCGCAGCCGCGAGCACCCGCACTCACGGAGCCCCAGCCCCGAGCCT

AGGGGGCGGCCGGGGCCCATCGGGGTCCTCCTGATGAAAAGCAGAGCGAACGAAGAGTATGGTCTCCGG

CTTGGGAGTCAGATCTTCGTAAAGGAAATGACCCGAACGGGTCTGGCAACTAAAGATGGCAACCTTCAC

GAAGGAGACATAATTCTCAAGATCAATGGGACTGTAACTGAGAACATGTCTTTAACGGATGCTCGAAAA

TTGATAGAAAAGTCAAGAGGAAAACTACAGCTAGTGGTGTTGAGAGACAGCCAGCAGACCCTCATCAAC

ATCCCGTCATTAAATGACAGTGACTCAGAAATAGAAGATATTTCAGAAATAGAGTCAAACCGATCATTT

TCTCCAGAGGAGAGACGTCATCAGTATTCTGATTATGATTATCATTCCTCAAGTGAGAAGCTGAAGGAA

AGGCCAAGTTCCAGAGAGGACACGCCGAGCAGATTGTCCAGGATGGGTGCGACACCCACTCCCTTTAAG

-continued

TCCACAGGGGATATTGCAGGCACAGTTGTCCCAGAGACCAACAAGGAACCCAGATACCAAGAGGACCCC

CCAGCTCCTCAACCAAAAGCAGCCCCGAGAACTTTTCTTCGTCCTAGTCCTGAAGATGAAGCAATATAT

GGCCCTAATACCAAAATGGTAAGGTTCAAGAAGGGAGACAGCGTGGGCCTCCGGTTGGCTGGTGGCAAT

GATGTCGGGATATTTGTTGCTGGCATTCAAGAAGGGACCTCGGCGGAGCAGGAGGGCCTTCAAGAAGGA

GACCAGATTCTGAAGGTGAACACACAGGATTTCAGAGGATTAGTGCGGGAGGATGCCGTTCTCTACCTG

TTAGAAATCCCTAAAGGTGAAATGGTGACCATTTTAGCTCAGAGCCGAGCCGATGTGTATAGAGACATC

CTGGCTTGTGGCAGAGGGGATTCGTTTTTTATAAGAAGCCACTTTGAATGTGAGAAGGAAACTCCACAG

AGCCTGGCCTTCACCAGAGGGGAGGTCTTCCGAGTGGTAGACACACTGTATGACGGCAAGCTGGGCAAC

TGGCTGGCTGTGAGGATTGGGAACGAGTTGGAGAAAGGCTTAATCCCCAACAAGAGCAGAGCTGAACAA

ATGGCCAGTGTTCAAAATGCCCAGAGAGACAACGCTGGGGACCGGGCAGATTTCTGGAGAATGCGTGGC

CAGAGGTCTGGGGTGAAGAAGAACCTGAGGAAAAGTCGGGAAGACCTCACAGCTGTTGTGTCTGTCAGC

ACCAAGTTCCCAGCTTATGAGAGGGTTTTGCTGCGAGAAGCTGGTTTCAAGAGACCTGTGGTCTTATTC

GGCCCCATAGCTGATATAGCAATGGAAAAATTGGCTAATGAGTTACCTGACTGGTTTCAAACTGCTAAA

ACGGAACCAAAAGATGCAGGATCTGAGAAATCCACTGGAGTGGTCCGGTTAAATACCGTGAGGCAAATT

ATTGAACAGGATAAGCATGCACTACTGGATGTGACTCCGAAAGCTGTGGACCTGTTGAATTACACCCAG

TGGTTCCCAATTGTGATTTTTTTCAACCCAGACTCCAGACAAGGTGTCAAAACCATGAGACAAAGGTTA

AATCCAACGTCCAACAAAAGTTCTCGAAAGTTATTTGATCAAGCCAACAAGCTTAAAAAAACGTGTGCA

CACCTTTTTACAGCTACAATCAACCTAAATTCAGCCAATGATAGCTGGTTTGGCAGCTTAAAGGACACT

ATTCAGCATCAGCAAGGAGAAGCGGTTTGGGTCTCTGAAGGAAAGATGGAAGGGATGGATGATGACCCC

GAAGACCGCATGTCCTACTTAACCGCCATGGGCGCGGACTATCTGAGTTGCGACAGCCGCCTCATCAGT

GACTTTGAAGACACGGACGGTGAAGGAGGCGCCTACACTGACAATGAGCTGGATGAGCCAGCCGAGGAG

CCGCTGGTGTCGTCCATCACCCGCTCCTCGGAGCCGGTGCAGCACGAGGAGAGCATAAGGAAACCCAGC

CCAGAGCCACGAGCTCAGATGAGGAGGGCTGCTAGCAGCGATCAACTTAGGGACAATAGCCCGCCCCCA

GCATTCAAGCCAGAGCCGCCCAAGGCCAAAACCCAGAACAAAGAAGAATCCTATGACTTCTCCAAATCC

TATGAATATAAGTCAAACCCCTCTGCCGTTGCTGGTAATGAAACTCCTGGGGCATCTACCAAAGGTTAT

CCTCCTCCTGTTGCAGCAAAACCTACCTTTGGGCGGTCTATACTGAAGCCCTCCACTCCCATCCCTCCT

CAAGAGGGTGAGGAGGTGGGAGAGAGCAGTGAGGAGCAAGATAATGCTCCCAAATCAGTCCTGGGCAAA

GTCAAAATATTTGAGAAGATGGATCACAAGGCCAGGTTACAGAGAATGCAGGAGCTCCAGGAAGCACAG

AATGCAAGGATCGAAATTGCCCAGAAGCATCCTGATATCTATGCAGTTCCAATCAAAACGCACAAGCCA

GACCCTGGCACGCCCCAGCACACGAGTTCCAGACCCCCTGAGCCACAGAAAGCTCCTTCCAGACCTTAT

CAGGATACCAGAGGAAGTTATGGCAGTGATGCCGAGGAGGAGGAGTACCGCCAGCAGCTGTCAGAACAC

TCCAAGCGCGGTTACTATGGCCAGTCTGCCCGATACCGGGACACAGAATTATAG

FXR

[0215] In some embodiments, the PFIC-associated polypeptide is FXR or a fragment and/or variant thereof.

[0216] "FXR" is the abbreviated name of the polypeptide encoded by the NR1H4 gene and is also known as Bile acid receptor, Farnesoid X-activated receptor, Farnesol receptor HRR-1, Nuclear receptor subfamily 1 group H member 4, and Retinoid X receptor-interacting protein 14. FXR is a receptor for bile acids such as chenodeoxycholic acid, lithocholic acid, deoxycholic acid and alcoholic acid and plays an essential role in bile acid homeostasis.

[0217] A fragment and/or variant of FXR may retain FXR activity. For example, a fragment and/or variant of FXR may sense bile acid levels (e.g. by bile acid binding and subsequent activation). Suitably, a fragment and/or variant of FXR may have the same or similar activity to a FXR, e.g. may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of FXR.

[0218] A person skilled in the art would be able to generate fragments and/or variants using conservative substitutions, based on the known structural and functional features of FXR (see e.g. Mi, L. Z., et al., 2003. Molecular cell, 11(4), pp. 1093-1100 and Gomez-Ospina, N., et al., 2016. Nature communications, 7(1), pp. 1-8), and/or based on known variants (see e.g. NCBI Gene ID: 9971 and NCBI HomoloGene: 3760).

[0219] Suitably, a fragment of FXR and/or a FXR variant comprises a nuclear receptor DNA-binding domain and a nuclear receptor ligand-binding domain. The domains/sequences may be linked by inter-domain linker(s).

[0220] The NR1H4 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. The FXR may be a human FXR. Suitably, the FXR may comprise or consist of a polypeptide sequence of UniProtKB accession Q96RI1, or a fragment and/or variant thereof.

[0221] In some embodiments, the FXR comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 47 or a fragment thereof. Suitably, the FXR comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 47 or a fragment thereof.

[0222] In some embodiments, the FXR comprises or consists of SEQ ID NO: 47 or a fragment thereof.

Exemplary FXR

(SEQ ID NO: 47)

MVMQFQGLENPIQISPHCSCTPSGFFMEMMSMKPAKGVLTEQVAGPLGQN

LEVEPYSQYSNVQFPQVQPQISSSSYYSNLGFYPQQPEEWYSPGIYELRR

MPAETLYQGETEVAEMPVTKKPRMGASAGRIKGDELCVVCGDRASGYHYN

ALTCEGCKGFFRRSITKNAVYKCKNGGNCVMDMYMRRKCQECRLRKCKEM

GMLAECMYTGLLTEIQCKSKRLRKNVKQHADQTVNEDSEGRDLRQVTSTT

KSCREKTELTPDQQTLLHFIMDSYNKQRMPQEITNKILKEEFSAEENFLI

LTEMATNHVQVLVEFTKKLPGFQTLDHEDQIALLKGSAVEAMFLRSAEIF

NKKLPSGHSDLLEERIRNSGISDEYITPMFSFYKSIGELKMTQEEYALLT

AIVILSPDRQYIKDREAVEKLQEPLLDVLQKLCKIHQPENPQHFACLLGR

LTELRTFNHHHAEMLMSWRVNDHKFTPLLCEIWDVQ

[0223] Suitably, a FXR variant may comprise one or more variation selected from V381 and M183T. These are considered to be benign (or likely benign) variations based on clinical data. Suitably, a fragment of FXR and/or a FXR variant may comprise a deletion of aa 207-210 or aa 159-209.

[0224] In some embodiments, the FXR comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 48 or a fragment thereof. Suitably, the FXR comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 48 or a fragment thereof.

[0225] In some embodiments, the FXR comprises or consists of SEQ ID NO: 48 or a fragment thereof.

Exemplary FXR

(SEQ ID NO: 48)

MGSKMNLIEHSHLPTTDEFSFSENLFGVLTEQVAGPLGQNLEVEPYSQYS

NVQFPQVQPQISSSSYYSNLGFYPQQPEEWYSPGIYELRRMPAETLYQGE

TEVAEMPVTKKPRMGASAGRIKGDELCVVCGDRASGYHYNALTCEGCKGF

FRRSITKNAVYKCKNGGNCVMDMYMRRKCQECRLRKCKEMGMLAECMYTG

LLTEIQCKSKRLRKNVKQHADQTVNEDSEGRDLRQVTSTTKSCREKTELT

PDQQTLLHFIMDSYNKQRMPQEITNKILKEEFSAEENFLILTEMATNHVQ

VLVEFTKKLPGFQTLDHEDQIALLKGSAVEAMFLRSAEIFNKKLPSGHSD

LLEERIRNSGISDEYITPMFSFYKSIGELKMTQEEYALLTAIVILSPDRQ

YIKDREAVEKLQEPLLDVLQKLCKIHQPENPQHFACLLGRLTELRTFNHH

HAEMLMSWRVNDHKFTPLLCEIWDVQ

[0226] Suitably, a FXR variant may comprise one or more variation selected from V28I and M173T. These are considered to be benign (or likely benign) variations based on clinical data. Suitably, a fragment of FXR and/or a FXR variant may comprise a deletion of aa 197-200 or aa 149-199.

[0227] Suitably, the nucleotide sequence encoding FXR, or a fragment and/or variant thereof, may comprise or consist of a nucleotide sequence of NCBI reference sequence NM_001206993, NM_001206977, NM_001206978, NM_001206979, NM_001206992 or NM_005123.4, or a fragment and/or variant thereof.

[0228] In some embodiments, the nucleotide sequence encoding FXR, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 49 or a fragment thereof. Suitably, the nucleotide sequence encoding FXR, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 49 or a fragment thereof.

[0229] In some embodiments, the nucleotide sequence encoding FXR, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 49 or a fragment thereof.

Exemplary nucleotide encoding FXR

(SEQ ID NO: 49)

ATGGTAATGCAGTTTCAGGGGTTAGAAAATCCAATTCAAATTAGTCCTCA

CTGCAGCTGTACGCCGTCAGGATTTTTCATGGAAATGATGAGTATGAAGC

CCGCGAAAGGTGTTTTAACAGAACAAGTGGCAGGTCCTCTGGGACAGAAC

CTGGAAGTGGAACCATACTCGCAATACAGCAATGTTCAGTTTCCCCAAGT

TCAACCACAGATTTCCTCGTCATCCTATTATTCCAACCTGGGTTTCTACC

CCCAGCAGCCTGAAGAGTGGTACTCTCCTGGAATATATGAACTCAGGCGT

ATGCCAGCTGAGACTCTCTACCAGGGAGAAACTGAGGTAGCAGAGATGCC

TGTAACAAAGAAGCCCCGCATGGGCGCGTCAGCAGGGAGGATCAAAGGGG

ATGAGCTGTGTGTTGTTTGTGGAGACAGAGCCTCTGGATACCACTATAAT

GCACTGACCTGTGAGGGGTGTAAAGGTTTCTTCAGGAGAAGCATTACCAA

-continued

```
AAACGCTGTGTACAAGTGTAAAAACGGGGGCAACTGTGTGATGGATATGT
ACATGCGAAGAAAGTGTCAAGAGTGTCGACTAAGGAAATGCAAAGAGATG
GGAATGTTGGCTGAATGTATGTATACAGGCTTGTTAACTGAAATTCAGTG
TAAATCTAAGCGACTGAGAAAAAATGTGAAGCAGCATGCAGATCAGACCG
TGAATGAAGACAGTGAAGGTCGTGACTTGCGACAAGTGACCTCGACAACA
AAGTCATGCAGGGAGAAAACTGAACTCACCCCAGATCAACAGACTCTTCT
ACATTTTATTATGGATTCATATAACAAACAGAGGATGCCTCAGGAAATAA
CAAATAAAATTTTAAAAGAAGAATTCAGTGCAGAAGAAAATTTTCTCATT
TTGACGGAAATGGCAACCAATCATGTACAGGTTCTTGTAGAATTCACAAA
AAAGCTACCAGGATTTCAGACTTTGGACCATGAAGACCAGATTGCTTTGC
TGAAAGGGTCTGCGGTTGAAGCTATGTTCCTTCGTTCAGCTGAGATTTTC
AATAAGAAACTTCCGTCTGGGCATTCTGACCTATTGGAAGAAAGAATTCG
AAATAGTGGTATCTCTGATGAATATATAACACCTATGTTTAGTTTTTATA
AAAGTATTGGGGAACTGAAAATGACTCAAGAGGAGTATGCTCTGCTTACA
GCAATTGTTATCCTGTCTCCAGATAGACAATACATAAAGGATAGAGAGGC
AGTAGAGAAGCTTCAGGAGCCACTTCTTGATGTGCTACAAAAGTTGTGTA
AGATTCACCAGCCTGAAAATCCTCAACACTTTGCCTGTCTCCTGGGTCGC
CTGACTGAATTACGGACATTCAATCATCACCACGCTGAGATGCTGATGTC
ATGGAGAGTAAACGACCACAAGTTTACCCCACTTCTCTGTGAAATCTGGG
ACGTGCAGTGA
```

MYO5B

[0230] In some embodiments, the PFIC-associated polypeptide is MYO5B or a fragment and/or variant thereof.

[0231] "MYO5B" is the abbreviated name of the polypeptide encoded by the MYO5B gene and is also known as myosin VB. MYO5B is involved in vesicular trafficking and interacts with rab11. This allows for proper functioning of polarized epithelial cells, including hepatocytes (see e.g. Gonzales, E., et al., 2017. Hepatology, 65(1), pp. 164-173).

[0232] A fragment and/or variant of MYO5B may retain MYO5B activity. For example, a fragment and/or variant of MYO5B may interact with rab11 and traffic vesicles. Suitably, a fragment and/or variant of MYO5B may have the same or similar activity to MYO5B, e.g. may have at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the activity of MYO5B.

[0233] A person skilled in the art would be able to generate fragments and/or variants using conservative substitutions, based on the known structural and functional features of MYO5B (see e.g. Müller, T., et al., 2008. Nature genetics, 40(10), pp. 1163-1165; Velvarska, H. and Niessing, D., 2013. PLoS One, 8(12), p. e82065; and Nascimento, A. F., et al., 2013. Journal of Biological Chemistry, 288(47), pp. 34131-34145), and/or based on known variants (see e.g. NCBI Gene ID: 4645 and NCBI HomoloGene: 49481).

[0234] Suitably, a fragment of MYO5B and/or a MYO5B variant comprises a Myosin and Kinesin motor domain, an IQ calmodulin-binding motif, and a DIL domain. The domains/sequences may be linked by inter-domain linker(s).

[0235] The MYO5B gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, and rat. The MYO5B may be a human MYO5B. Suitably, the MYO5B may comprise or consist of a polypeptide sequence of UniProtKB accession Q9ULV0, or a fragment and/or variant thereof.

[0236] In some embodiments, the MYO5B comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 50 or a fragment thereof. Suitably, the MYO5B comprises or consists of an amino acid sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 50 or a fragment thereof.

[0237] In some embodiments, the MYO5B comprises or consists of SEQ ID NO: 50 or a fragment thereof.

Exemplary MYO5B

(SEQ ID NO: 50)

```
MSVGELYSQCTRVWIPDPDEVWRSAELTKDYKEGDKSLQLRLEDETILEY
PIDVQRNQLPFLRNPDILVGENDLTALSYLHEPAVLHNLKVRFLESNHIY
TYCGIVLVAINPYEQLPIYGQDVIYTYSGQNMGDMDPHIFAVAEEAYKQM
ARDEKNQSIIVSGESGAGKTVSAKYAMRYFATVGGSASETNIEEKVLASS
PIMEAIGNAKTTRNDNSSRFGKYIQIGEDKRYHIIGANMRTYLLEKSRVV
FQADDERNYHIFYQLCAAAGLPEFKELALTSAEDFFYTSQGGDTSIEGVD
DAEDFEKTRQAFTLLGVKESHQMSIFKIIASILHLGSVAIQAERDGDSCS
ISPQDVYLSNFCRLLGVEHSQMEHWLCHRKLVTTSETYVKTMSLQQVINA
RNALAKHIYAQLFGWIVEHINKALHTSLKQHSFIGVLDIYGFETFEVNSF
EQFCINYANEKLQQQFNSHVFKLEQEEYMKEQIPWTLIDFYDNQPCIDLI
EAKLGILDLLDEECKVPKGTDQNWAQKLYDRHSSSQHFQKPRMSNTAFII
VHFADKVEYLSDGFLEKNRDTVYEEQINILKASKFPLVADLFHDDKDPVP
ATTPGKGSSSKISVRSARPPMKVSNKEHKKTVGHQFRTSLHLLMETLNAT
TPHYVRCIKPNDEKLPFHFDPKRAVQQLRACGVLETIRISAAGYPSRWAY
HDFFNRYRVLVKKRELANTDKKAICRSVLENLIKDPDKFQFGRTKIFFRA
GQVAYLEKLRADKFRTATIMIQKTVRGWLQKVKYHRLKGATLTLQRYCRG
HLARRLAEHLRRIRAAVVLQKHYRMQRARQAYQRVRRAAVVIQAFTRAMF
VRRTYRQVLMEHKATTIQKHVRGWMARRHFQRLRDAAIVIQCAFRMLKAR
RELKALRIEARSAEHLKRLNVGMENKVVQLQRKIDEQNKEFKTLSEQLSV
TTSTYTMEVERLKKELVHYQQSPGEDTSLRLQEEVESLRTELQRAHSERK
ILEDAHSREKDELRKRVADLEQENALLKDEKEQLNNQILCQSKDEFAQNS
VKENLMKKELEEERSRYQNLVKEYSQLEQRYDNLRDEMTIIKQTPGHRRN
PSNQSSLESDSNYPSISTSEIGDTEDALQQVEEIGLEKAAMDMTVFLKLQ
KRVRELEQERKKLQVQLEKREQQDSKKVQAEPPQTDIDLDPNADLAYNSL
KRQELESENKKLKNDLNELRKAVADQATQNNSSHGSPDSYSLLLNQLKLA
HEELEVRKEEVLILRTQIVSADQRRLAGRNAEPNINARSSWPNSEKHVDQ
EDAIEAYHGVCQTNSKTEDWGYLNEDGELGLAYQGLKQVARLLEAQLQAQ
```

-continued

SLEHEEEVEHLKAQLEALKEEMDKQQQTFCQTLLLSPEAQVEFGVQQEIS

RLTNENLDLKELVEKLEKNERKLKKQLKIYMKKAQDLEAAQALAQSERKR

HELNRQVTVQRKEKDFQGMLEYHKEDEALLIRNLVTDLKPQMLSGTVPCL

PAYILYMCIRHADYTNDDLKVHSLLTSTINGIKKVLKKHNDDFEMTSFWL

SNTCRLLHCLKQYSGDEGFMTQNTAKQNEHCLKNEDLTEYRQVLSDLSIQ

IYQQLIKIAEGVLQPMIVSAMLENESIQGLSGVKPTGYRKRSSSMADGDN

SYCLEAIIRQMNAFHTVMCDQGLDPEIILQVFKQLFYMINAVTLNNLLLR

KDVCSWSTGMQLRYNISQLEEWLRGRNLHQSGAVQTMEPLIQAAQLLQLK

KKTQEDAEAICSLCTSLSTQQIVKILNLYTPLNEFEERVTVAFIRTIQAQ

LQERNDPQQLLLDAKHMFPVLFPFNPSSLTMDSIHIPACLNLEFLNEV

[0238] Suitably, a MYO5B variant may comprise one or more variation selected from C10G, E49Q, R56L, L59P, F61L, T126A, 1296V, K307N, R344H, S385L, V516I, P598L, R743H, I769N, K781N, R901Q, R911L, R918H, D1011N, P1191L, G1321E, T1382M, S1494L, H1558Q, K1683R, and M1688V. These are considered to be benign (or likely benign) variations based on clinical data. Suitably, a fragment of MYO5B and/or a MYO5B variant may comprise a deletion of aa 1 to 859 and/or aa 1315-1340, or aa 1-1430.

[0239] Suitably, the nucleotide sequence encoding MYO5B, or a fragment and/or variant thereof, may comprise or consist of a nucleotide sequence of NCBI reference sequence NM_001080467, or a fragment and/or variant thereof.

[0240] In some embodiments, the nucleotide sequence encoding MYO5B, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 51 or a fragment thereof. Suitably, the nucleotide sequence encoding MYO5B, or a fragment and/or variant thereof, comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to SEQ ID NO: 51 or a fragment thereof.

[0241] In some embodiments, the nucleotide sequence encoding MYO5B, or a fragment and/or variant thereof, comprises or consists of the nucleotide sequence SEQ ID NO: 51 or a fragment thereof.

Exemplary nucleotide encoding MYO5B

(SEQ ID NO: 51)

ATGTCGGTGGGCGAGCTCTACAGCCAGTGCACAAGGGTCTGGATCCCTGACCCTGATGAGGTATGGCGC

TCAGCTGAGTTAACCAAGGACTACAAAGAAGGAGACAAGAGCCTACAGCTCAGACTGGAGGATGAAACG

ATTCTGGAATACCCAATTGATGTACAACGCAACCAGCTGCCCTTCTTACGGAATCCAGATATCTTGGTG

GGAGAAAATGACCTGACTGCCCTTAGCTATCTTCATGAGCCTGCAGTTTTGCATAATTTGAAGGTCCGT

TTCCTGGAGTCCAACCATATCTACACTTACTGTGGTATCGTACTTGTTGCCATTAATCCTTATGAACAG

TTGCCAATCTATGGACAAGATGTCATCTATACCTACAGTGGCCAAAACATGGGAGACATGGACCCCCAC

ATCTTTGCTGTGGCAGAAGAAGCCTACAAGCAGATGGCCAGAGATGAGAAGAATCAGTCCATCATAGTC

AGTGGGGAGTCTGGAGCCGGGAAGACGGTATCAGCCAAGTATGCCATGCGCTATTTCGCCACCGTTGGT

GGCTCGGCCAGTGAAACCAACATCGAAGAGAAGGTGCTGGCATCCAGTCCCATCATGGAGGCCATTGGA

AATGCCAAGACCACCCGCAATGACAACAGCAGCCGTTTTGGCAAGTACATCCAGATTGGCTTTGACAAA

AGGTACCACATCATCGGGGCCAACATGAGGACTTACCTCTTGGAGAAGTCCAGAGTGGTCTTCCAGGCA

GATGATGAGAGGAATTACCACATCTTTTACCAGCTCTGTGCTGCTGCCGGTCTTCCAGAATTTAAAGAG

CTTGCACTAACAAGTGCAGAGGACTTTTTCTATACATCACAGGGAGGAGACACTTCCATCGAGGGTGTG

GACGATGCTGAGGACTTTGAGAAGACTCGACAAGCCTTCACACTCCTCGGAGTGAAAGAGTCCCATCAG

ATGAGCATTTTTAAGATAATTGCTTCTATCTTGCACCTTGGAAGTGTGGCGATTCAGGCTGAGCGTGAT

GGTGATTCCTGTAGTATATCACCCCAGGATGTATACCTAAGCAACTTCTGCCGACTGCTAGGGGTGGAG

CACAGTCAGATGGAGCACTGGCTGTGTCATCGCAAGCTGGTCACCACCTCGGAGACCTACGTCAAGACC

ATGTCCCTGCAGCAGGTGATCAATGCGCGCAACGCCCTGGCGAAGCACATCTATGCCCAGTTGTTCGGC

TGGATTGTGGAGCACATCAACAAGGCCCTGCACACCTCCCTCAAGCAGCACTCCTTCATCGGGGTCCTG

GACATCTATGGGTTTGAGACATTTGAGGTAAACAGCTTTGAGCAGTTCTGTATCAACTATGCAAATGAA

AAGCTCCAGCAGCAGTTCAACTCGCATGTTTTCAAACTGGAGCAAGAAGAATACATGAAGGAACAGATC

CCTTGGACCCTGATTGATTTTTATGATAACCAACCTTGTATCGACCTCATTGAAGCCAAGCTGGGTATC

TTGGACCTGTTGGATGAAGAATGTAAGGTCCCCAAAGGAACTGACCAGAACTGGGCTCAGAAGCTCTAT

-continued

GACCGGCACTCCAGCAGCCAGCACTTCCAGAAGCCCCGCATGTCCAACACGGCCTTCATCATCGTCCAC

TTTGCAGACAAGGTGGAGTACCTCTCTGATGGTTTTCTGGAGAAAAACAGAGACACGGTGTATGAAGAG

CAGATCAATATCCTGAAGGCCAGCAAGTTCCCACTAGTGGCTGACTTGTTTCATGATGACAAGGACCCT

GTTCCTGCCACCACCCCTGGGAAGGGGTCATCTTCGAAGATCAGCGTCCGTTCTGCCAGACCCCCCATG

AAAGTCTCCAACAAGGAGCACAAGAAAACCGTTGGCCACCAGTTCCGTACCTCCCTGCATCTGCTCATG

GAGACCCTGAATGCCACGACACCTCACTATGTCCGCTGCATCAAGCCCAACGATGAGAAGCTCCCCTTT

CACTTTGACCCAAAGAGAGCAGTGCAGCAACTCAGAGCCTGCGGGGTGTTGGAGACGATTCGAATCAGT

GCAGCTGGCTACCCATCCAGGTGGGCCTACCATGACTTTTTCAACCGGTATCGGGTGCTGGTCAAGAAG

AGAGAGCTCGCCAACACAGACAAAAAGGCCATCTGCAGGTCTGTCCTGGAGAACCTCATCAAGGACCCC

GACAAGTTCCAGTTTGGCCGCACCAAGATCTTCTTTCGAGCAGGCCAGGTGGCCTACCTGGAGAAGCTG

CGGGCTGACAAGTTCCGGACAGCCACCATCATGATCCAGAAAACTGTCCGGGGATGGCTGCAGAAGGTG

AAATATCACAGGCTGAAGGGGGCTACCTTAACCCTGCAGAGGTACTGCCGGGGACACCTGGCCCGCAGG

CTGGCTGAGCACCTGCGGAGGATCAGAGCGGCTGTGGTGCTCCAGAAACATTACCGCATGCAGAGGGCC

CGCCAGGCCTACCAGAGGGTCCGCAGAGCTGCCGTTGTTATCCAGGCCTTCACCCGGGCCATGTTTGTG

CGGAGAACCTACCGCCAGGTCCTCATGGAGCACAAGGCCACCACCATCCAGAAGCACGTGCGGGGCTGG

ATGGCACGCAGGCACTTCCAGCGGCTGCGGGATGCAGCCATTGTCATCCAGTGTGCCTTCCGGATGCTC

AAGGCCAGGCGGGAGCTGAAGGCCCTCAGGATTGAGGCCCGCTCAGCAGAGCATCTGAAACGTCTCAAC

GTGGGCATGGAGAACAAGGTGGTCCAGCTGCAGCGGAAGATCGATGAGCAGAACAAAGAGTTCAAGACA

CTTTCAGAGCAGTTGTCCGTGACCACCTCAACATACACCATGGAGGTAGAGCGGCTGAAGAAGGAGCTG

GTGCACTACCAGCAGAGCCCAGGTGAGGACACCAGCCTCAGGCTGCAGGAGGAGGTGGAGAGCCTGCGC

ACAGAGCTGCAGAGGGCCCACTCGGAGCGCAAGATCTTGGAGGACGCCCACAGCAGGGAGAAAGATGAG

CTGAGGAAGCGAGTTGCAGACCTGGAGCAAGAAAATGCTCTCTTGAAAGATGAGAAAGAACAGCTCAAC

AACCAAATCCTGTGCCAGTCTAAAGATGAATTTGCCCAGAACTCTGTGAAGGAAAATCTCATGAAGAAA

GAACTGGAGGAGGAGCGATCCCGGTACCAGAACCTTGTGAAGGAATATTCACAGTTGGAGCAGAGATAC

GACAACCTTCGGGATGAAATGACCATCATAAAGCAAACTCCAGGTCATAGGCGGAACCCATCAAACCAA

AGTAGCTTAGAATCTGACTCCAATTACCCCTCCATCTCCACATCTGAGATCGGAGACACTGAGGATGCC

CTCCAGCAGGTGGAGGAAATTGGCCTGGAGAAGGCAGCCATGGACATGACGGTCTTCCTGAAGCTGCAG

AAGAGAGTACGGGAGCTGGAGCAGGAGAGGAAAAAGCTGCAAGTGCAGCTGGAGAAGAGAGAACAGCAG

GACAGCAAGAAAGTCCAGGCGGAACCACCACAGACTGACATAGATTTGGACCCGAATGCAGATCTGGCC

TACAATAGTCTGAAGAGGCAAGAGCTGGAGTCAGAGAACAAAAAGCTGAAGAATGACCTGAATGAGCTG

AGGAAAGCCGTGGCCGACCAAGCCACGCAGAATAACTCCAGCCACGGCTCCCCAGATAGCTACAGCCTC

CTGCTGAACCAGCTCAAGCTGGCCCACGAGGAGCTCGAGGTGCGCAAGGAGGAGGTGCTCATCCTCAGG

ACCCAGATCGTGAGCGCCGACCAGCGGCGACTCGCCGGCAGGAACGCGGAGCCGAACATTAATGCCAGA

TCAAGTTGGCCTAACAGTGAAAAGCATGTTGACCAGGAGGATGCCATTGAGGCCTATCACGGGGTCTGC

CAGACAAACAGCAAGACTGAGGATTGGGGATATTTAAATGAAGATGGAGAACTCGGCTTGGCCTACCAA

GGCCTAAAGCAAGTTGCCAGGCTGCTGGAGGCTCAGCTGCAGGCCCAGAGCCTGGAGCATGAGGAGGAG

GTGGAGCATCTCAAGGCTCAGCTCGAGGCCCTGAAGGAGGAGATGGACAAACAGCAGCAGACCTTCTGC

CAGACGCTACTGCTCTCCCCAGAGGCCCAGGTGGAATTCGGCGTTCAGCAGGAAATATCCCGGCTGACC

AACGAGAATCTGGACCTTAAAGAACTGGTAGAAAAGCTGGAAAAGAATGAGAGGAAGCTCAAAAAGCAA

-continued

CTGAAGATTTACATGAAGAAAGCCCAGGACCTAGAAGCTGCCCAGGCATTGGCCCAGAGTGAGAGGAAG

CGCCATGAGCTCAACAGGCAGGTCACGGTCCAGCGGAAAGAGAAGGATTTCCAGGGCATGCTGGAGTAC

CACAAAGAGGACGAGGCCCTCCTCATCCGGAACCTGGTGACAGACTTGAAGCCCCAGATGCTGTCGGGC

ACAGTGCCCTGTCTCCCCGCCTACATCCTCTACATGTGCATCCGGCACGCGGACTACACCAACGACGAT

CTCAAGGTGCACTCCCTGCTGACCTCCACCATCAACGGCATTAAGAAAGTCCTGAAAAAGCACAATGAT

GACTTTGAGATGACGTCATTCTGGTTATCCAACACCTGCCGCCTTCTTCACTGTCTGAAGCAGTACAGC

GGGGATGAGGGCTTCATGACTCAGAACACTGCAAAGCAGAATGAACACTGTCTTAAGAATTTTGACCTC

ACCGAATACCGTCAGGTGCTGAGTGACCTTTCCATTCAGATCTACCAGCAGCTCATTAAAATTGCCGAG

GGCGTGTTACAGCCGATGATAGTTTCTGCCATGTTGGAAAATGAGAGCATTCAGGGTCTATCTGGTGTG

AAGCCCACCGGCTACCGGAAGCGCTCCTCCAGCATGGCAGATGGGGATAACTCATACTGCCTGGAAGCT

ATCATCCGCCAGATGAATGCCTTTCATACAGTCATGTGTGACCAGGGCTTGGACCCTGAGATCATCCTG

CAGGTATTCAAACAGCTCTTCTACATGATCAACGCAGTGACTCTTAACAACCTGCTCTTGCGGAAGGAC

GTCTGCTCTTGGAGCACAGGCATGCAACTCAGGTACAATATAAGTCAGCTTGAGGAGTGGCTTCGGGGA

AGAAACCTTCACCAGAGTGGAGCAGTTCAGACCATGGAACCTCTGATCCAAGCAGCCCAGCTCCTGCAA

TTAAAGAAGAAAACCCAGGAGGACGCAGAGGCTATCTGCTCCCTGTGTACCTCCCTCAGCACCCAGCAG

ATTGTCAAAATTTTAAACCTTTATACTCCCCTGAATGAATTTGAAGAACGGGTAACAGTGGCCTTTATA

CGAACAATCCAGGCACAACTACAAGAGCGGAATGACCCTCAGCAACTGCTATTAGATGCCAAGCACATG

TTTCCTGTTTTGTTTCCATTTAATCCATCTTCTCTAACCATGGACTCAATCCACATCCCAGCGTGTCTC

AATCTGGAATTCCTCAATGAAGTCTGA

Regulatory Elements

[0242] The lentiviral vector of the present invention may further comprise one or more regulatory elements which may act pre- or post-transcriptionally. Suitably, the protein-coding sequence is operably linked to one or more regulatory elements which may act pre- or post-transcriptionally. The one or more regulatory elements may facilitate expression of the transgene in liver cells (e.g. hepatocytes).

[0243] As used herein, a "regulatory element" may refer any nucleotide sequence that facilitates expression of a polypeptide, e.g. acts to increase expression of a transcript or to enhance mRNA stability. Suitable regulatory elements include for example promoters, enhancer elements, post-transcriptional regulatory elements, polyadenylation sites, and Kozak sequences.

Promoter

[0244] The lentiviral vector of the present invention may comprise a promoter, preferably a liver-specific (e.g. hepatocyte-specific) promoter. Suitably, the protein-coding sequence is operably linked to a promoter, preferably a liver-specific (e.g. hepatocyte-specific) promoter.

[0245] A "promoter" may refer to a region of DNA that leads to initiation of transcription of a gene. Promoters are located near the transcription start sites of genes, upstream on the DNA (towards the 5' region of the sense strand).

[0246] As used herein, a "tissue-specific promoter" may refer to a promoter which preferentially facilitates expression of a transgene in a specific type of cells or tissue. Suitably, a tissue-specific promoter may facilitate higher expression of a transgene in one cell-type as compared to other cell-types. Higher expression may be measured for example by measuring the expression of a transgene, e.g. green fluorescence protein (GFP), operably linked to the promoter, wherein expression of the transgene correlates with the ability of the promoter to facilitate expression of a gene. For example, a tissue-specific promoter may be a promoter which facilitates transgene expression levels at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 100% higher, at least 200% higher, at least 300% higher, at least 400% higher, at least 500% higher, or at least 1000% higher in one cell-type as compared to expression levels in other cell-types.

[0247] In some embodiments, the promoter is a liver-specific promoter. In some embodiments, the promoter is a hepatocyte-specific promoter.

[0248] Suitably, the promoter may be (or may be derived from) a promoter associated with a gene with selective expression in human liver cells (e.g. hepatocytes). Suitably, the promoter may be (or may be derived from) a promoter associated with a gene with selective expression in human hepatocyte cells. Methods to identify promoters associated with genes will be well known to those of skill in the art.

[0249] Exemplary liver-specific and/or hepatocyte-specific promoters are described in Kattenhorn, L. M., et al., 2016. Human gene therapy, 27(12), pp. 947-961 and include transthyretin (TTR) promoters, alpha-1-antitrypsin (AAT) promoters, thyroxine-binding globulin (TBG) promoters, APoE/hAAT promoters, HCR-hAAT promoters, LP1 promoters, and HLP promoters.

[0250] An engineered promoter variant derived from any of these promoters may be used, provided that the variant

retains the capacity to drive liver-specific and/or hepatocyte-specific expression of a transgene which is operably coupled to the promoter. A skilled person will be arrive at such variants using methods known in the art. The variant may have at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to any of the promoters.

[0251] A fragment of any of these promoters (or variants thereof) may be used, provided that the fragment retains the capacity to drive liver-specific and/or hepatocyte-specific expression of a transgene which is operably coupled to the promoter. A skilled person will be able to arrive at such fragments using methods known in the art. The fragment may be, for example, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, or at least 1000 nucleotides in length.

[0252] In some embodiments, the promoter is selected from the group consisting of: a transthyretin (TTR) promoter, an alpha-1-antitrypsin (AAT) promoter, a thyroxine-binding globulin (TBG) promoter, an APoE/hAAT promoter, a HCR-hAAT promoter, a LP1 promoter, and a HLP promoter.

[0253] In some embodiments, the promoter is a TTR promoter, or a variant and/or fragment thereof. In some embodiments, the promoter is an Enh1mTTR (ET) promoter, or a variant and/or fragment thereof.

[0254] An exemplary ET promoter is provided in GenBank accession number AY661265. In some embodiments, the ET promoter comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 19 or a fragment thereof. Suitably, the ET promoter comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 19 or a fragment thereof.

[0255] In some embodiments, the ET promoter comprises or consists of the nucleotide sequence SEQ ID NO: 19 or a fragment thereof.

Exemplary ET promoter

(SEQ ID NO: 19)

CGCGAGTTAATAATTACCAGCGCGGGCCAAATAAATAATCCGCGAGGGGC

AGGTGACGTTTGCCCAGCGCGCGCTGGTAATTATTAACCTCGCGAATATT

GATTCGAGGCCGCGATTGCCGCAATCGCGAGGGGCAGGTGACCTTTGCCC

AGCGCGCGTTCGCCCCGCCCCGGACGGTATCGATAAGCTTAGGAGCTTGG

GCTGCAGGTCGAGGGCACTGGGAGGATGTTGAGTAAGATGGAAAACTACT

GATGACCCTTGCAGAGACAGAGTATTAGGACATGTTTGAACAGGGGCCGG

GCGATCAGCAGGTAGCTCTAGAGGATCCCCGTCTGTCTGCACATTTCGTA

GAGCGAGTGTTCCGATACTCTAATCTCCCTAGGCAAGGTTCATATTTGTG

TAGGTTACTTATTCTCCTTTTGTTGACTAAGTCAATAATCAGAATCAGCA

GGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTGGAAGGAGGGG

GTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCACAAGCTC

CTG

[0256] In some embodiments, the promoter is an AAT promoter, or a variant and/or fragment thereof. In some embodiments, the promoter is a human AAT (hAAT) promoter, or a variant and/or fragment thereof.

[0257] In some embodiments, the hAAT promoter comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 20 or a fragment thereof. Suitably, the hAAT promoter comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 20 or a fragment thereof.

[0258] In some embodiments, the hAAT promoter comprises or consists of the nucleotide sequence SEQ ID NO: 20 or a fragment thereof.

Exemplary hAAT promoter

(SEQ ID NO: 20)

GATCTTGCTACCAGTGGAACAGCCACTAAGGATTCTGCAGTGAGAGCAGA

GGGCCAGCTAAGTGGTACTCTCCCAGAGACTGTCTGACTCACGCCACCCC

CTCCACCTTGGACACAGGACGCTGTGGTTTCTGAGCCAGGTACAATGACT

CCTTTCGGTAAGTGCAGTGGAAGCTGTACACTGCCCAGGCAAAGCGTCCG

GGCAGCGTAGGCGGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCTGT

TTGCTCCTCCGATAACTGGGGTGACCTTGGTTAATATTCACCAGCAGCCT

CCCCCGTTGCCCCTCTGGATCCACTGCTTAAATACGGACGAGGACAG

[0259] The promoter may be a constitutive promoter. As used herein, a "constitutive promoter" is a promoter which is always active.

[0260] Alternatively, the promoter may be an inducible promoter. As used herein, an "inducible promoter" is a promoter which is only active under specific conditions. For example, expression of the transgene may be induced by a small molecule or drug (e.g. which binds to a promoter, regulatory sequence or to a transcriptional repressor or activator molecule) or by using an environmental trigger. Types of inducible promoter include chemically-inducible promoters (e.g. a Tet-on system); temperature-inducible promoters (e.g. Hsp70 or Hsp90-derived promoters); and light-inducible promoters. Suitably, the promoter is chemically-inducible. Any suitable method for engineering an inducible promoter may be used.

Enhancer Elements

[0261] The lentiviral vector of the present invention may comprise an enhancer, preferably a liver-specific (e.g. hepatocyte-specific) enhancer. Suitably, the protein-coding sequence is operably linked to an enhancer, preferably a liver-specific (e.g. hepatocyte-specific) enhancer.

[0262] An "enhancer" or "enhancer element" may refer a region of DNA that can be bound by proteins (activators) to increase the likelihood that transcription of a particular gene will occur. Enhancers are cis-acting. They can be located up to 1 Mbp (1,000,000 bp) away from the gene, upstream or downstream from the start site.

[0263] As used herein, a "tissue-specific enhancer" is an enhancer which preferentially facilitates expression of a gene in specific cells or tissues. Suitably, a tissue-specific enhancer may facilitate higher expression of a gene in specific cells-types as compared to other cell-types. Higher expression may be measured for example by measuring the expression of a transgene, e.g. green fluorescence protein (GFP), operably linked to the enhancer, wherein expression of the transgene correlates with the ability of the enhancer to

facilitate expression of a gene. For example, a tissue-specific enhancer may be an enhancer which facilitates gene expression levels at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 100% higher, at least 200% higher, at least 300% higher, at least 400% higher, at least 500% higher, or at least 1000% higher in a specific cell-type compared to expression levels in other cell types.

[0264] Suitable tissue-specific enhancers will be well known to those of skill in the art. The enhancer may be a liver-specific enhancer, preferably a hepatocyte-specific enhancer.

[0265] Suitably, the enhancer may be (or may be derived from) an enhancer associated with a gene with selective expression in human liver cells (e.g. hepatocytes). Suitably, the enhancer may be (or may be derived from) an enhancer associated with a gene with selective expression in human hepatocyte cells. Methods to identify the enhancer regions associated with genes will be well known to those of skill in the art.

[0266] Exemplary liver-specific and/or hepatocyte-specific enhancers are described in Kramer, M. G., et al., 2003. Molecular therapy, 7(3), pp. 375-385, and include enhancer regions of the albumin, α1-antitrypsin, hepatitis B virus core protein, and hemopexin genes. Other liver-specific and/or hepatocyte-specific enhancers include apolipoprotein E (APoE) enhancers, hepatic control region (HCR) enhancers, and alpha-1-antitrypsin (AAT) enhancers.

[0267] An engineered enhancer variant derived from any of these enhancers may be used, provided that the variant retains the capacity to drive liver-specific and/or hepatocyte-specific expression of a transgene which is operably coupled to the enhancer. A skilled person will be arrive at such variants using methods known in the art. The variant may have at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to any of the enhancers.

[0268] A fragment of any of these enhancers (or variants thereof) may be used, provided that the fragment retains the capacity to drive liver-specific and/or hepatocyte-specific expression of a transgene which is operably coupled to the enhancer. A skilled person will be able to arrive at such fragments using methods known in the art. The fragment may be at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, at least 500 nucleotides, or at least 1000 nucleotides in length.

[0269] The vector of the present invention may comprise a liver-specific promoter and/or a liver-specific enhancer, i.e. a liver-specific promoter and/or enhancer. Suitably, the protein-coding sequence is operably linked to a liver-specific promoter and/or enhancer. Suitably, the protein-coding sequence is operably linked to a hepatocyte-specific promoter and/or enhancer. The promoter and enhancer may be a combination of any of the above, for example a hAAT promoter and an ApoE or HCR enhancer.

Post-Transcriptional Regulatory Elements

[0270] The lentiviral vector of the present invention may comprise one or more further post-transcriptional regulatory elements (e.g. in addition to one or more miRNA target sequence). Suitably, the protein-coding sequence is operably linked to one or more further post-transcriptional regulatory elements. The further post-transcriptional regulatory element may improve gene expression.

[0271] The lentiviral vector of the present invention may comprise a Woodchuck Hepatitis Virus Post-transcriptional Regulatory Element (WPRE). Suitably, the protein-coding sequence is operably linked to a WPRE.

[0272] Suitable WPRE sequences will be well known to those of skill in the art (see e.g. Zufferey, R., et al., 1999. Journal of virology, 73(4), pp. 2886-2892; and Zanta-Boussif, M. A. et al., 2009. Gene therapy, 16(5), pp. 605-619). Suitably, the WPRE is a wild-type WPRE or is a mutant WPRE. For example, the WPRE may be mutated to abrogate translation of the woodchuck hepatitis virus X protein (WHX) e.g. by mutating the WHX ORF translation start codon.

[0273] In some embodiments, the WPRE comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 21 or a fragment thereof. Suitably, the WPRE comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 21 or a fragment thereof.

[0274] In some embodiments, the WPRE comprises or consists of the nucleotide sequence SEQ ID NO: 21 or a fragment thereof.

Exemplary WPRE

(SEQ ID NO: 21)

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAA

CTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGT

ATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAA

TCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACG

TGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCA

TTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCT

ATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGG

GGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCAT

CGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGG

ACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTC

CCGC

Polyadenylation Sequence

[0275] The lentiviral vector of the present invention may comprise a polyadenylation sequence. Suitably, the protein-coding sequence is operably linked to a polyadenylation sequence. A polyadenylation sequence may be inserted after the protein-coding sequence to improve transgene expression.

[0276] A polyadenylation sequence typically comprises a polyadenylation signal, a polyadenylation site and a downstream element: the polyadenylation signal comprises the sequence motif recognised by the RNA cleavage complex; the polyadenylation site is the site of cleavage at which a poly-A tails is added to the mRNA; the downstream element is a GT-rich region which usually lies just downstream of the polyadenylation site, which is important for efficient processing.

[0277] Suitable polyadenylation sequences will be well known to those of skill in the art (see e.g. Schambach, A., et

al., 2007. Molecular Therapy, 15(6), pp. 1167-1173; and Choi, J. H. et al., 2014. Molecular brain, 7(1), pp. 1-10). Exemplary polyadenylation sequences include the bGH poly (A) signal sequence and SV40pA signal sequence.

[0278] Suitably, the polyadenylation sequence may be present in the 3' LTR (i.e. the lentiviral vector does not comprise an additional polyadenylation sequence).

Kozak Sequence

[0279] The lentiviral vector of the present invention may comprise a Kozak sequence. Suitably, the protein-coding sequence is operably linked to a Kozak sequence. A Kozak sequence may be inserted before the start codon to improve the initiation of translation.

[0280] Suitable Kozak sequences will be well known to those of skill in the art (see e.g. Kozak, M., 1987. Nucleic acids research, 15(20), pp. 8125-8148).

[0281] In some embodiments, the Kozak sequence comprises or consists of a nucleotide sequence which is at least 80% identical to SEQ ID NO: 22 or a fragment thereof.

[0282] In some embodiments, the Kozak sequence comprises or consists of the nucleotide sequence SEQ ID NO: 22 or a fragment thereof.

```
(SEQ ID NO: 22)
GCCACC
Exemplary Kozak sequence
```

Other Cis-Acting Elements

[0283] The lentiviral vector of the present invention may comprise any other suitable cis-acting elements, such as one or more of a rev response element (RRE); a retroviral psi packaging element; a primer binding site (PBS); a TAT activation region (TAR); splice donor and acceptor sites; and central and terminal polypurine tracts.

Long Terminal Repeats (LTRs)

[0284] The lentiviral vector of the present invention may comprise one or more long terminal repeat (LTR). As described above, LTRs are responsible for proviral integration and transcription. Typically, a naturally occurring LTR comprises U3, R, and U5 regions.

[0285] The lentiviral vector may comprise a 5' LTR and/or a 3' LTR. The lentiviral vector may comprise a 5' LTR and a 3' LTR. Suitably, a 5' LTR comprises R and U5 regions, and optionally comprise a U3 region. Suitably, a 3' LTR comprises U3, R, and U5 regions.

[0286] Suitable LTR sequences will be well known to those of skill in the art (see e.g. Frech, K., et al., 1996. Virology, 224(1), pp. 256-267).

[0287] In some embodiments, a LTR comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 23 or a fragment thereof. Suitably, a LTR comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 23 or a fragment thereof.

[0288] In some embodiments, a LTR comprises or consists of the nucleotide sequence SEQ ID NO: 23 or a fragment thereof.

```
(SEQ ID NO: 23)
TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATCTGCTTTTTGCTTG

TACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGC

TAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGC

TTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATC

CCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAG
Exemplary LTR
```

[0289] The lentiviral vector of the present invention may comprise one or more self-inactivating long terminal repeat (SIN-LTR). A "SIN-LTR" may comprise a deletion that abolishes transcription of the full-length virus after it has incorporated into a host cell. For example, a 3' SIN-LTR may comprise a deletion in the U3 region removing the promoter/enhancer elements (see e.g. Zufferey, R., et al., 1998. Journal of virology, 72(12), pp. 9873-9880). This deletion is copied into the 5' LTR after reverse transcription, thereby making the gene expression in target cells dependent on an internal promoter of choice.

[0290] Suitable SIN-LTR sequences will be well known to those of skill in the art (see e.g. Zufferey, R., et al., 1998. Journal of virology, 72(12), pp. 9873-9880 and Miyoshi, H., et al., 1998. Journal of virology, 72(10), pp. 8150-8157).

[0291] In some embodiments, the 5' LTR comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 24 or a fragment thereof. Suitably, the 5' LTR comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 24 or a fragment thereof.

[0292] In some embodiments, the 5' LTR comprises or consists of the nucleotide sequence SEQ ID NO: 24 or a fragment thereof.

```
(SEQ ID NO: 24)
GGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAAC

TAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCA

AGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTC

AGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAG
Exemplary 5' LTR
```

[0293] In some embodiments, the 5' LTR and/or the 3' LTR comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 25 or a fragment thereof. Suitably, the 5' LTR and/or the 3' LTR comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 25 or a fragment thereof.

[0294] In some embodiments, the 5' LTR and/or the 3' LTR comprises or consists of the nucleotide sequence SEQ ID NO: 25 or a fragment thereof.

[0295] In some embodiments, the 5' LTR and the 3' LTR comprise or consist of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 25 or a fragment thereof. Suitably, the 5' LTR and the 3' LTR comprise or consist of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 25 or a fragment thereof.

[0296] In some embodiments, the 5' LTR and the 3' LTR comprise or consist of the nucleotide sequence SEQ ID NO: 25 or a fragment thereof.

```
                                        (SEQ ID NO: 25)
TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATCTGCTTTTTGCTTG

TACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGC

TAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGC

TTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATC

CCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAG
Exemplary 3' LTR
```

Primer Binding Site (PBS)

[0297] The lentiviral vector of the present invention may comprise a primer binding site (PBS). A PBS is a cis-acting element where a primer may bind to initiate reverse transcription of the RNA genome (see e.g. Lanchy, J. M., et al., 1998. Journal of Biological Chemistry, 273(38), pp. 24425-24432).

[0298] Suitable retroviral PBSs will be well known to those of skill in the art.

[0299] In some embodiments, a PBS comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 26 or a fragment thereof. Suitably, a PBS comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 26 or a fragment thereof.

[0300] In some embodiments, a PBS comprises or consists of the nucleotide sequence SEQ ID NO: 26 or a fragment thereof.

```
                                        (SEQ ID NO: 26)
TGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGCTCT

CTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGG

GCGGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAG

GAGAGAG
Exemplary primer binding site
```

[0301] In some embodiments, a PBS comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 27 or a fragment thereof. Suitably, a PBS comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 27 or a fragment thereof.

[0302] In some embodiments, a PBS comprises or consists of the nucleotide sequence SEQ ID NO: 27 or a fragment thereof.

```
                                        (SEQ ID NO: 27)
TGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTC

GACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCG

GCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAG

AGAG
Exemplary primer binding site
```

Retroviral Psi Packaging Element

[0303] The lentiviral vector of the present invention may comprise a retroviral psi packaging element. As described above, a retroviral psi packaging element is a cis-acting element which is involved in regulating the essential process of packaging the retroviral RNA genome into the viral capsid during replication (see e.g. McBride, M. S., et al., 1997. Journal of virology, 71(6), pp. 4544-4554). A retroviral psi packaging element may form part of the 5' region of the gag gene.

[0304] Suitable retroviral psi packaging elements will be well known to those of skill in the art.

[0305] In some embodiments, a retroviral psi packaging element comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 28 or a fragment thereof. Suitably, a retroviral psi packaging element comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 28 or a fragment thereof.

[0306] In some embodiments, a retroviral psi packaging element comprises or consists of the nucleotide sequence SEQ ID NO: 28 or a fragment thereof.

```
                                        (SEQ ID NO: 28)
ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATG

GGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAA

CATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTG

GCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACA

ACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAATACA

GTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCA

AGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCAC

CGCACAGCAAGCGGCCGCTGAT
Exemplary retroviral psi packaging element
```

Rev Response Element (RRE)

[0307] The lentiviral vector of the present invention may comprise a rev response element (RRE). As described above, a RRE is a cis-acting element that enables the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell (see e.g. Pollard, V. W. and Malim, M. H., 1998. Annual review of microbiology, 52(1), pp. 491-532).

[0308] Suitable RRE sequences will be well known to those of skill in the art.

[0309] In some embodiments, a RRE comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 29 or a fragment thereof. Suitably, a RRE comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 29 or a fragment thereof.

[0310] In some embodiments, a RRE comprises or consists of the nucleotide sequence SEQ ID NO: 29 or a fragment thereof.

(SEQ ID NO: 29)
GGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCG

CAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTAT

AGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCAT

CTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCC

TGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTT
Exemplary rev response element

[0311] In some embodiments, a RRE comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 30 or a fragment thereof. Suitably, a RRE comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 30 or a fragment thereof.

[0312] In some embodiments, a RRE comprises or consists of the nucleotide sequence SEQ ID NO: 30 or a fragment thereof.

(SEQ ID NO: 30)
GGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCG

CAGCCTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTAT

AGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCAT

CTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCC

TGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTT
Exemplary rev response element

Central Polypurine Tract (cPPT)

[0313] The lentiviral vector of the present invention may comprise a central polypurine tract (cPPT). As described above, a cPPT may allow initiation of plus-strand synthesis (see e.g. Follenzi, A. et al., 2000. Nature genetics, 25(2), pp. 217-222).

[0314] Suitable cPPT sequences will be well known to those of skill in the art.

[0315] In some embodiments, a cPPT comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 31 or a fragment thereof. Suitably, a cPPT comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 31 or a fragment thereof.

[0316] In some embodiments, a cPPT comprises or consists of the nucleotide sequence SEQ ID NO: 31 or a fragment thereof.

(SEQ ID NO: 31)
AACTTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGA

ATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAAC

AAATTACAAAAATTCAAAATTTTATC
Exemplary central polypurine tract

Other Elements

[0317] The lentiviral vector of the present invention may comprise any other suitable elements.

[0318] In some embodiments, the lentiviral vector of the present invention comprises an element comprising or consisting of a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 32 or a fragment thereof. In some embodiments, the lentiviral vector of the present invention comprises an element comprising or consisting of the nucleotide sequence of SEQ ID NO: 32 or a fragment thereof.

(SEQ ID NO: 32)
TCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATT

ATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACC

AAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATA
Exemplary delta ENV1

[0319] In some embodiments, the lentiviral vector of the present invention comprises an element comprising or consisting of a nucleotide sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 33 or a fragment thereof. In some embodiments, the lentiviral vector of the present invention comprises an element comprising or consisting of the nucleotide sequence of SEQ ID NO: 33 or a fragment thereof.

(SEQ ID NO: 33)
GGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAATGCT

AGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACGACCTGGA

TGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACACTCCTT

AATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGAATTATTG

GAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATT

GGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCTTGGTAGG

TTTAAGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAG

GGATATTCACCATTATCGTTTCAGACCCACCTCCCAACCCCGAGGGGAC

CCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGA

CAGATCCATTCGATTAGTGAACGGATC
Exemplary delta ENV2

Exemplary Cis-Acting Elements

[0320] The lentiviral vector of the present invention may comprise a cis-acting element comprising a PBS, a retroviral psi packaging element, and a rev response element (RRE).

[0321] In some embodiments, a cis-acting element comprising a PBS, a retroviral psi packaging element, and a RRE comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 34 or a fragment thereof. Suitably, a cis-acting element comprising a PBS, a retroviral psi packaging element, and a RRE comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 34 or a fragment thereof.

[0322] In some embodiments, a cis-acting element comprising a PBS, a retroviral psi packaging element, and a RRE comprises or consists of the nucleotide sequence SEQ ID NO: 34 or a fragment thereof.

(SEQ ID NO: 34)

```
TGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTC

GACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCG

GCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAG

AGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGC

GATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATT

AAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAAT

CCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGC

TACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAA

TACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGAC

ACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGA

CCACCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATA

TGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAAT

TGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAG

AGAGAAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGG

GAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACGGTACA

GGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTG

AGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCA

TCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGA

TCAACAGCTCCTGGGGATTT
```

Exemplary cis-acting element comprising a PBS, a retroviral psi packaging element, and a RRE

[0323] The lentiviral vector of the present invention may comprise a cis-acting element comprising a PBS, a retroviral psi packaging element, a rev response element (RRE), and a central polypurine tract (cPPT).

[0324] In some embodiments, a cis-acting element comprising a PBS, a retroviral psi packaging element, a RRE, and a cPPT comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 35 or a fragment thereof. Suitably, a cis-acting element comprising a PBS, a retroviral psi packaging element, a RRE, and a cPPT comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 35 or a fragment thereof.

[0325] In some embodiments, a cis-acting element comprising a PBS, a retroviral psi packaging element, a RRE, and a cPPT comprises or consists of the nucleotide sequence SEQ ID NO: 35 or a fragment thereof.

(SEQ ID NO: 35)

```
TGGCGCCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTC

GACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCG

GCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAG

AGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGC

GATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATT

AAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAAT
```

-continued

```
CCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTGGGACAGC

TACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAA

TACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGAC

ACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGA

CCACCGCACAGCAAGCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATA

TGAGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAAT

TGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAG

AGAGAAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGG

GAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACGGTACA

GGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTG

AGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCA

TCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGA

TCAACAGCTCCTGGGGATTTGGGGTTGCTCTGGAAAACTCATTTGCACC

ACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGAACAGA

TTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAATTA

CACAAGCTTAATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAA

AAGAATGAACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGA

ATTGGTTTAACATAACAAATTGGCTGTGGTATATAAAATTATTCATAAT

GATAGTAGGAGGCTTGGTAGGTTTAAGAATAGTTTTTGCTGTACTTTCT

ATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCC

ACCTCCCAACCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGA

AGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCT

CGACGGTATCGGTTAACTTTTAAAAGAAAAGGGGGGATTGGGGGGTACA

GTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAA

AGAATTACAAAAACAAATTACAAAAATTCAAAATTTTATC
```

Exemplary cis-acting element comprising a PBS, a retroviral psi packaging element, a RRE, and a cPPT

Exemplary Lentiviral Genomes

[0326] The lentiviral genome of the present invention may comprise from 5' to 3': a 5' LTR, one or more cis-acting elements, a protein-coding sequence, optionally one or more post-transcriptional regulatory sequences, and a 3' LTR.

[0327] For example, the lentiviral genome of the present invention may comprise from 5' to 3': a 5' LTR, a PBS, a retroviral psi packaging element, a RRE, a cPPT, a liver-specific promoter, a protein-coding sequence, a WPRE, one or more miRNA target sequence, and a 3' LTR.

[0328] The lentiviral genome of the present invention may further comprise any other suitable elements, such as any other elements described herein or one or more spacer sequence. The spacer sequence(s) may comprise, for example, at least one (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10), at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten nucleotide bases.

[0329] In some embodiments, the lentiviral genome comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 36 or a fragment thereof. Suitably, the lentiviral genome comprises or consists of a nucleotide sequence which is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 36 or a fragment thereof.

[0330] In some embodiments, the lentiviral genome comprises or consists of the nucleotide sequence SEQ ID NO: 36 or a fragment thereof.

(SEQ ID NO: 36)

GGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAAC

TAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCA

AGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTC

AGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAG

GGACCTGAAAGCGAAAGGGAAACCAGAGCTCTCTCGACGCAGGACTCGG

CTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTA

CGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGA

GAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATT

CGGTTAAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTAT

GGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGA

AACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTT

CAGACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAACCC

TCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTT

AGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCACCGCACAGCAA

GCGGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGG

AGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAG

TAGCACCCACCAAGGCAAAGAGAAGAGTGGTGCAGAGAGAAAAAAGAGC

AGTGGGAATAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC

ACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCAGACAATTAT

TGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGC

GCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAG

GCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGG

GGATTTGGGGTTGCTCTGGAAAACTCATTTGCACCACTGCTGTGCCTTG

GAATGCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATCACACG

ACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATAC

ACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGAACAAGA

ATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATA

ACAAATTGGCTGTGGTATATAAAATTATTCATAATGATAGTAGGAGGCT

TGGTAGGTTTAAGAATAGTTTTTGCTGTACTTTCTATAGTGAATAGAGT

TAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAACCCCG

AGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAG

-continued

ACAGAGACAGATCCATTCGATTAGTGAACGGATCTCGACGGTATCGGTT

AACTTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGA

ATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAAC

AAATTACAAAAATTCAAAATTTTATCGATCACGAGACTAGCCTCGAGCA

CGCGAGTTAATAATTACCAGCGCGGGCCAAATAAATAATCCGCGAGGGG

CAGGTGACGTTTGCCCAGCGCGCGCTGGTAATTATTAACCTCGCGAATA

TTGATTCGAGGCCGCGATTGCCGCAATCGCGAGGGGCAGGTGACCTTTG

CCCAGCGCGCGTTCGCCCCGCCCCGGACGGTATCGATAAGCTTAGGAGC

TTGGGCTGCAGGTCGAGGGCACTGGGAGGATGTTGAGTAAGATGGAAAA

CTACTGATGACCCTTGCAGAGACAGAGTATTAGGACATGTTTGAACAGG

GGCCGGGCGATCAGCAGGTAGCTCTAGAGGATCCCCGTCTGTCTGCACA

TTTCGTAGAGCGAGTGTTCCGATACTCTAATCTCCCTAGGCAAGGTTCA

TATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGTCAATAATCA

GAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTG

GAAGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGA

TCCACAAGCTCCTGGCTAGCGTACGCCACCATGAGCGACTCCGTGATCC

TGCGGAGCATCAAGAAGTTTGGCGAGGAGAACGACGGCTTCGAGTCTGA

TAAGAGCTACAACAATGATAAGAAGTCCAGACTGCAGGACGAGAAGAAG

GGCGATGGCGTGCGGGTGGGCTTCTTTCAGCTGTTCCGGTTCAGCAGCA

GCACCGACATCTGGCTGATGTTTGTGGGCTCCCTGTGCGCCTTCCTGCA

CGGAATCGCACAGCCAGGCGTGCTGCTGATCTTTGGCACCATGACAGAC

GTGTTCATCGACTATGATGTGGAGCTGCAGGAGCTGCAGATCCCTGGCA

AGGCCTGCGTGAACAATACCATCGTGTGGACAAATAGCTCCCTGAACCA

GAATATGACCAACGGCACAAGATGTGGCCTGCTGAATATCGAGTCTGAG

ATGATCAAGTTTGCCAGCTACTATGCAGGAATCGCAGTGGCCGTGCTGA

TCACCGGCTACATCCAGATCTGCTTCTGGGTCATCGCAGCAGCAAGGCA

GATCCAGAAGATGAGAAAGTTCTATTTTCGGAGAATCATGCGGATGGAG

ATCGGCTGGTTTGACTGTAACTCTGTGGGCGAGCTGAATACAAGATTCA

GCGACGACATCAACAAGATCAATGACGCCATCGCCGATCAGATGGCCCT

GTTTATCCAGCGGATGACCAGCACAATCTGTGGCTTCCTGCTGGGCTTC

TTTAGAGGCTGGAAGCTGACCCTGGTCATCATCTCTGTGAGCCCACTGA

TCGGAATCGGAGCAGCAACAATCGGCCTGTCCGTGTCTAAGTTCACCGA

CTACGAGCTGAAGGCATATGCAAAGGCAGGAGTGGTGGCAGATGAAGTG

ATCAGCAGCATGAGGACCGTGGCAGCCTTTGGCGGAGAGAAGAGGGAGG

TGGAGCGGTACGAGAAGAACCTGGTGTTCGCCCAGCGGTGGGGCATCAG

AAAGGGCATCGTGATGGGCTTCTTTACAGGCTTCGTGTGGTGCCTGATC

TTCCTGTGCTACGCCCTGGCCTTTTGGTATGGCTCCACCCTGGTGCTGG

ACGAGGGAGAGTATACCCCTGGCACACTGGTGCAGATCTTCCTGAGCGT

GATCGTGGGCGCCCTGAACCTGGGAAATGCCTCCCCATGCCTGGAGGCC

-continued

TTTGCCACAGGCAGGGCAGCAGCCACCTCCATCTTCGAGACAATCGACC
GCAAGCCCATCATCGACTGTATGTCTGAGGATGGCTACAAGCTGGACAG
GATCAAGGGCGAGATCGAGTTTCACAATGTGACCTTCCACTATCCCAGC
CGCCCTGAGGTGAAGATCCTGAACGATCTGAATATGGTCATCAAGCCAG
GAGAGATGACAGCCCTGGTGGGACCCTCTGGAGCAGGCAAGAGCACCGC
CCTGCAGCTGATCCAGCGGTTTTACGACCCTTGCGAGGGAATGGTGACC
GTGGACGGACACGACATCAGGTCCCTGAACATCCAGTGGCTGCGCGATC
AGATCGGCATCGTGGAGCAGGAGCCAGTGCTGTTCTCTACCACAATCGC
CGAGAATATCAGATACGGCCGCGAGGATGCCACAATGGAGGACATCGTG
CAGGCCGCCAAGGAGGCCAACGCCTATAACTTCATCATGGATCTGCCCC
AGCAGTTCGACACCCTGGTGGGAGAGGGAGGAGGACAGATGTCCGGAGG
CCAGAAGCAGAGAGTGGCCATCGCCAGAGCCCTGATCCGCAACCCTAAG
ATCCTGCTGCTGGATATGGCCACAAGCGCCCTGGACAATGAGTCCGAGG
CCATGGTGCAGGAGGTGCTGAGCAAGATCCAGCACGGCCACACCATCAT
CTCTGTGGCACACCGGCTGAGCACAGTGAGAGCAGCCGACACCATCATC
GGCTTTGAGCACGGAACAGCAGTGGAGAGGGGAACCCACGAGGAGCTGC
TGGAGAGGAAGGGCGTGTACTTCACCCTGGTGACACTGCAGAGCCAGGG
CAACCAGGCCCTGAATGAGGAGGACATCAAGGATGCCACAGAGGACGAT
ATGCTGGCCCGGACCTTCTCCAGAGGCTCTTATCAGGATTCTCTGCGGG
CCAGCATCAGGCAGCGCAGCAAGTCCCAGCTGAGCTACCTGGTGCACGA
GCCACCTCTGGCCGTGGTGGACCACAAGTCCACCTATGAGGAGGATAGA
AAGGACAAGGACATCCCCGTGCAGGAGGAGGTGGAGCCTGCCCCAGTGA
GGCGCATCCTGAAGTTTTCCGCCCCAGAGTGGCCCTACATGCTGGTGGG
ATCTGTGGGAGCAGCAGTGAACGGCACCGTGACACCACTGTATGCCTTC
CTGTTTTCCCAGATCCTGGGCACCTTCTCTATCCCCGACAAGGAGGAGC
AGAGGTCCCAGATCAATGGCGTGTGCCTGCTGTTTGTGGCCATGGGCTG
CGTGAGCCTGTTTACACAGTTCCTGCAGGGCTACGCCTTCGCCAAGAGC
GGCGAGCTGCTGACCAAGCGGCTGAGAAAGTTCGGCTTTCGCGCAATGC
TGGGACAGGACATCGCATGGTTTGACGATCTGCGGAACAGCCCAGGCGC
CCTGACCACAAGACTGGCCACAGATGCCTCTCAGGTGCAGGGAGCAGCA
GGCAGCCAGATCGGCATGATCGTGAACTCCTTCACCAATGTGACAGTGG
CCATGATCATCGCCTTCTCTTTTAGCTGGAAGCTGTCTCTGGTCATCCT
GTGCTTCTTCCCCTTTCTGGCCCTGAGCGGAGCAACCCAGACAAGGATG
CTGACCGGCTTCGCCTCCAGAGACAAGCAGGCCCTGGAGATGGTGGGCC
AGATCACAAACGAGGCCCTGAGCAATATCAGGACCGTGGCAGGAATCGG
CAAGGAGCGGCGGTTCATCGAGGCCCTGGAGACAGAGCTGGAGAAGCCT
TTCAAGACCGCCATCCAGAAGGCCAACATCTACGGCTTCTGCTTTGCCT
TCGCCCAGTGTATCATGTTCATCGCCAACTCCGCCTCTTACAGATATGG
CGGCTACCTGATCAGCAATGAGGGCCTGCACTTTTCCTACGTGTTCAGA

-continued

GTGATCAGCGCCGTGGTGCTGTCTGCCACAGCCCTGGGAAGGGCCTTCT
CCTACACCCCATCTTATGCCAAGGCCAAGATCTCTGCCGCCAGGTTCTT
TCAGCTGCTGGACCGCCAGCCACCCATCAGCGTGTACAACACAGCCGGC
GAGAAGTGGGATAATTTCCAGGGCAAGATCGACTTTGTGGATTGCAAGT
TCACCTATCCTAGCAGGCCAGACTCCCAGGTGCTGAACGGCCTGAGCGT
GTCCATCTCTCCAGGCCAGACACTGGCCTTTGTGGGCTCCTCTGGCTGT
GGCAAGAGCACCTCCATCCAGCTGCTGGAGAGGTTCTACGACCCCGATC
AGGGCAAAGTGATGATCGACGGCCACGATTCTAAGAAGGTGAACGTGCA
GTTTCTGCGCTCCAATATCGGCATCGTGTCTCAGGAGCCTGTGCTGTTC
GCCTGCAGCATCATGGATAACATCAAGTACGGCGACAATACAAAGGAGA
TCCCAATGGAGAGAGTGATCGCAGCAGCAAAGCAGGCACAGCTGCACGA
TTTCGTGATGTCCCTGCCCGAGAAGTATGAGACAAACGTGGGCAGCCAG
GGCTCCCAGCTGTCTAGGGGCGAGAAGCAGAGGATCGCAATCGCCAGGG
CCATCGTGCGCGATCCCAAGATCCTGCTGCTGGACGAGGCCACCAGCGC
CCTGGATACAGAGTCCGAGAAGACCGTGCAGGTGGCCCTGGACAAGGCC
CGGGAGGGAAGAACATGTATCGTGATCGCCCACCGCCTGAGCACCATCC
AGAATGCCGACATCATCGCCGTGATGGCCCAGGGCGTGGTCATCGAGAA
GGGCACCCACGAGGAGCTGATGGCCCAGAAGGGCGCCTACTATAAGCTG
GTGACCACAGGCAGCCCTATCTCCTGATGAGTCGACTCGACAATCAACC
TCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTT
GCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATG
CTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTG
GTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGC
GTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTG
CCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTAT
TGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGG
GCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCAT
CGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGG
GACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCT
TCCCGCGGCCTGCTGCCGGCTCTAGATAATCCATAAAGTAGGAAACACT
ACACGATTCCATAAAGTAGGAAACACTACAACCGGTTCCATAAAGTAGG
AAACACTACATCACTCCATAAAGTAGGAAACACTACACCCGGGTCGAGC
TCGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCC
ACTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACG
AAGACAAGATCTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAG
ATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGC
CTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTT
GTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGG
AAAATCTCTAGCA

Exemplary lentiviral genome

Variants, Derivatives, Analogues, and Fragments

[0331] In addition to the specific polypeptides and polynucleotides mentioned herein, the invention also encompasses variants, derivatives, and fragments thereof.

[0332] In the context of the invention, a "variant" of any given sequence is a sequence in which the specific sequence of residues (whether amino acid or nucleic acid residues) has been modified in such a manner that the polypeptide or polynucleotide in question retains at least one or all of its endogenous functions. A variant sequence can be obtained by addition, deletion, substitution, modification, replacement and/or variation of at least one residue present in the naturally occurring polypeptide or polynucleotide.

[0333] The term "derivative" as used herein in relation to proteins or polypeptides of the invention includes any substitution of, variation of, modification of, replacement of, deletion of and/or addition of one (or more) amino acid residues from or to the sequence, providing that the resultant protein or polypeptide retains at least one or all of its endogenous functions.

[0334] Typically, amino acid substitutions may be made, for example from 1, 2 or 3, to 10 or 20 substitutions, provided that the modified sequence retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

[0335] Polypeptides used in the invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent polypeptide. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues as long as the endogenous function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include asparagine, glutamine, serine, threonine and tyrosine.

[0336] Conservative substitutions may be made, for example according to the table below. Amino acids in the same block in the second column and in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P I L V
	Polar - uncharged	C S T M N Q
	Polar - charged	D E K R H
AROMATIC		F W Y

[0337] The effect of additions, deletions, substitutions, modifications, replacements and/or variations may be predicted using any suitable prediction tool e.g. SIFT (Vaser, R., et al., 2016. Nature protocols, 11(1), pp. 1-9), PolyPhen-2 (Adzhubei, I., et al., 2013. Current protocols in human genetics, 76(1), pp. 7-20), CADD (Rentzsch, P., et al., 2021. Genome medicine, 13(1), pp. 1-12), REVEL (Ioannidis, N. M., et al., 2016. The American Journal of Human Genetics, 99(4), pp. 877-885), MetaLR (Dong, C., et al., 2015. Human molecular genetics, 24(8), pp. 2125-2137), and/or MutationAssessor (Reva, B., et al., 2011. Nucleic acids research, 39(17), pp. e118-e118) or based on clinical data e.g. ClinVar (Landrum, M. J., et al., 2016. Nucleic acids research, 44(D1), pp. D862-D868). Suitable additions, deletions, substitutions, modifications, replacements and/or variations may be considered tolerated, benign, and/or likely benign.

[0338] Typically, a variant may have a certain identity with the wild type amino acid sequence or the wild type nucleotide sequence.

[0339] In the present context, a variant sequence is taken to include an amino acid sequence which may be at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85% or at least 90% identical, suitably at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the subject sequence. Although a variant can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express in terms of sequence identity.

[0340] In the present context, a variant sequence is taken to include a nucleotide sequence which may be at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85% or at least 90% identical, suitably at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the subject sequence. Although a variant can also be considered in terms of similarity, in the context of the present invention it is preferred to express it in terms of sequence identity.

[0341] Suitably, reference to a sequence which has a percent identity to any one of the SEQ ID NOs detailed herein refers to a sequence which has the stated percent identity over the entire length of the SEQ ID NO referred to.

[0342] Sequence identity comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percent identity between two or more sequences.

[0343] Percent identity may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid or nucleotide in one sequence is directly compared with the corresponding amino acid or nucleotide in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

[0344] Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion in the amino acid or nucleotide sequence may cause the following residues or codons to be put out of alignment, thus potentially resulting in a large reduction in percent identity when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalizing unduly the overall identity score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local identity.

[0345] However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids or nucleotides, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the

most commonly used gap scoring system. High gap penalties will produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is −12 for a gap and −4 for each extension.

[0346] Calculation of maximum percent identity therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (see e.g. Devereux, J., et al., 1984. Nucleic acids research, 12(1), pp. 387-395). Examples of other software that can perform sequence comparisons include, but are not limited to, the BLAST package (see e.g. Altschul, S. F., et al., 1990. Journal of molecular biology, 215(3), pp. 403-410), BLAST 2 (see e.g. Tatusova, T. A. and Madden, T. L., 1999. FEMS microbiology letters, 174(2), pp. 247-250), FASTA (see e.g. Pearson, W. R. and Lipman, D. J., 1988. PNAS, 85(8), pp. 2444-2448.), EMBOSS Needle (Madeira, F., et al., 2019. Nucleic acids research, 47(W1), pp. W636-W641) and the GENEWORKS suite of comparison tools. For some applications, it is preferred to use EMBOSS Needle.

[0347] Although the final percent identity can be measured, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix.

[0348] Once the software has produced an optimal alignment, it is possible to calculate percent sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result. The percent sequence identity may be calculated as the number of identical residues as a percentage of the total residues in the SEQ ID NO referred to.

[0349] "Fragments" are also variants and the term typically refers to a selected region of the polypeptide or polynucleotide that is of interest either functionally or, for example, in an assay. "Fragment" thus refers to an amino acid or nucleic acid sequence that is a portion of a full-length polypeptide or polynucleotide.

[0350] Such variants, derivatives, and fragments may be prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site may be made. The flanking regions will contain convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the synthetic DNA ligated into the cut. The DNA is then expressed in accordance with the invention to make the encoded polypeptide. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used.

Method of Production

[0351] In one aspect, the present invention provides a method of producing a lentiviral vector according to the present invention.

[0352] Suitable methods to produce lentiviral vectors will be well known to those of skill in the art (see e.g. Merten, O. W., et al., 2016. Molecular Therapy-Methods & Clinical Development, 3, p. 16017).

[0353] The method of production may comprise: (a) introducing a transfer vector and one or more helper vectors into a host cell; (b) culturing the host cell under conditions suitable to produce lentiviral vectors according to the present invention; and (c) obtaining the lentiviral vectors from the host cell.

[0354] As used herein, a "transfer vector" may encode the lentiviral genome of the present invention. Suitably, the transfer vector used to produce the lentiviral viral genome within a host cell/packaging cell will have sufficient lentiviral genetic information to allow packaging of an RNA genome, in the presence of packaging components (e.g. gag-pol, rev, env), into a viral particle which is capable of infecting a target cell, but is incapable of independent replication to produce infectious viral particles within the final target cell.

[0355] The transfer vector used to produce the viral genome within a host cell/packaging cell may include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a host cell/packaging cell. These regulatory sequences may be the natural sequences associated with the transcribed viral sequence (i.e. the 5' U3 region), or they may be a heterologous promoter, such as another viral promoter (e.g. the CMV promoter). The transfer vector may be a plasmid.

[0356] As used herein, a "helper vector" may encode one or more packaging components (e.g. gag-pol, rev, env). The nucleotide sequence encoding the packaging component(s) may be operably linked to a promoter (e.g. a CMV promoter or a RSV promoter) and/or a polyadenylation signal. The term "helper vector" may include "packaging vectors" (e.g. encoding gag-pol or rev) and "envelope vectors" (e.g. encoding an env gene, such as VSV-g). The helper vectors, packaging vectors, and/or envelope vectors may be plasmids.

[0357] The transfer vector and one or more helper vectors may be introduced into the host cell by any suitable technique known in the art, such as transfection, transduction and/or transformation. Suitably, the helper vectors may be transiently transfected or transduced into the host cell or may be stably maintained (e.g. stably integrated into the cell genome or episomally maintained) within the host cell. Alternatively, a combination of transient transfection or transduction and stable maintenance may be used to introduce the helper vectors into the host cell.

[0358] Suitably, the transfer vector and/or the helper vectors may be plasmids and introduced by transfection. Suitably, a four plasmid system may be used consisting of a transfer plasmid and three helper plasmids. The three helper plasmids may consist of: a first helper plasmid encoding a gag-pol gene; a second helper plasmid encoding a rev gene; and a third helper plasmid encoding an env gene. Alternatively, a three plasmid system may be used which consists of a transfer plasmid, one helper plasmid encoding a gag-pol gene and a rev gene; and one helper plasmid encoding an env gene. Alternatively, a two plasmid system may be used

in which all helper functions (e.g. gag-pol, rev, and env) are encoded by one helper plasmid.

[0359] Any suitable host cell may be used to produce the lentiviral vector. Suitable host cells include producer cells and packaging cells, such as those described below (e.g. HEK 293, or derivatives thereof). Suitable conditions for culturing the host cell will be well known to the skilled person. For example, the host cells may be incubated in chemically defined medium for from about 1 day to about 5 days (e.g. about 48 hours, about 72 hours, or about 96 hours).

[0360] The lentiviral vector may be obtained using in any suitable methods known in the art. For example the culture supernatant may be harvested and lentiviral vector subsequently purified from the culture supernatant (e.g. by centrifugation, membrane filtration and/or chromatography). The method of production may further comprise any other suitable process steps e.g. DNA reduction, concentration, formulation and/or sterilization.

Vectors, Kits and Systems

[0361] In one aspect, the present invention provides a vector encoding the lentiviral genome of the present invention. The vector may be a transfer vector, as described herein. For example, the vector may be a plasmid and/or the lentiviral genome may be operably linked to a promoter (e.g. a viral promoter, such as a CMV promoter).

[0362] In one aspect, the present invention provides a kit or system for producing the lentiviral vector of the present invention.

[0363] The kit or system may be a lentivirus packaging kit or system or a lentivirus production kit or system. As used herein, a "lentivirus packaging kit or system" may comprise one or more components, and optionally instructions, for packaging the lentiviral vector of the present invention. As used herein, a "lentivirus production kit or system" may comprise one or more components, and optionally instructions, for producing the lentiviral vector of the present invention.

[0364] The kit or system may comprise a transfer vector encoding the lentivirus genome of the present invention and optionally one or more helper vectors. The kit or system may further comprise host cells (e.g. packaging cells or producer cells) and/or other reagents (e.g. transfection reagent, culture medium, etc.). The kit or system may further comprise any other suitable components, and optionally instructions for packaging and/or producing the lentiviral vector of the present invention.

Cells

[0365] In one aspect, the present invention provides a cell comprising the lentiviral vector of the present invention. The cell may be an isolated cell. Suitably, the cell is a mammalian cell, for example a human cell. The cell may be an isolated human cell.

[0366] Suitably, the cell may be a producer cell. The term "producer cell" includes a cell that produces viral particles, after transient transfection, stable transfection or vector transduction of all the elements necessary to produce the viral particles or any cell engineered to stably comprise the elements necessary to produce the viral particles. Suitable producer cells will be well known to those of skill in the art and may include HEK293, COS-1, COS-7, CV-1, HeLa, CHO, and A549 cell lines. In some embodiments, the producer cell is a HEK293 cell, or a derivative thereof (e.g. a HEK293T cell, a HEK293T Lenti-X, a HEK293T-Rex cell, a HEK293FT cell, a HEK293SF-3F6 cell, a HEK293SF-3F9 cell, a HEK293-EBNA1 cell, or a SJ293TS cell).

[0367] Suitably, the cell may be a packaging cell. The term "packaging cell" includes a cell which contains some or all of the elements necessary for packaging a recombinant virus genome. Typically, such packaging cells contain one or more vectors which are capable of expressing viral structural proteins (e.g. gag-pol, rev, env) and/or one or more genes encoding the viral structural proteins have been integrated into the genome of the packaging cell. Cells comprising only some of the elements required for the production of enveloped viral particles are useful as intermediate reagents in the generation of viral particle producer cell lines, through subsequent steps of transient transfection, transduction or stable integration of each additional required element. These intermediate reagents are encompassed by the term "packaging cell". Suitable packaging cells will be well known to those of skill in the art (see e.g. Merten, O. W., et al., 2016. Molecular Therapy-Methods & Clinical Development, 3, p. 16017).

[0368] Suitably, the cell may be a liver cell, for example a hepatocyte. Suitably, the cell may be an immortalized liver cell, for example an immortalized hepatocyte. Suitable cell lines will be well known to those of skill in the art, for example HepG2, Hep3B, HBG, and HepaRG cell lines. Methods to generate immortalized liver cells (e.g. immortalized hepatocytes) will be well known to those of skill in the art (see e.g. Ramboer, E., et al., 2015. Methods Mol Biol, 1250, pp. 53-76). Suitably, the cell may be a stem cell.

Pharmaceutical Compositions

[0369] In one aspect, the present invention provides pharmaceutical composition comprising the lentiviral vector or cell of the present invention. In preferred embodiments, the pharmaceutical composition comprises the lentiviral vector of the present invention in the form of a lentiviral particle.

[0370] A pharmaceutical composition is a composition that comprises or consists of a therapeutically effective amount of a pharmaceutically active agent e.g. the lentiviral vector. A pharmaceutical composition preferably includes a pharmaceutically acceptable carrier, diluent or excipient (including combinations thereof).

[0371] By "pharmaceutically acceptable" is included that the formulation is sterile and pyrogen free. The carrier, diluent, and/or excipient must be "acceptable" in the sense of being compatible with the lentiviral vector and not deleterious to the recipients thereof. Typically, the carriers, diluents, and excipients will be saline or infusion media which will be sterile and pyrogen free, however, other acceptable carriers, diluents, and excipients may be used.

[0372] Acceptable carriers, diluents, and excipients for therapeutic use are well known in the pharmaceutical art. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s) or solubilising agent(s).

[0373] Examples of pharmaceutically acceptable carriers include, for example, water, salt solutions, alcohol, silicone, waxes, petroleum jelly, vegetable oils, polyethylene glycols, propylene glycol, liposomes, sugars, gelatin, lactose, amylose, magnesium stearate, talc, surfactants, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethylcellulose, polyvinylpyrrolidone, and the like.

[0374] The lentiviral vector, cell, or pharmaceutical composition according to the present invention may be administered in a manner appropriate for treating and/or preventing the diseases described herein. Suitable administration routes will be known to the skilled person (see e.g. Fumoto, S., et al., 2013. Novel Gene Therapy Approaches, pp. 3-31). The quantity and frequency of administration may be determined by the skilled person, for example depending by such factors as the condition of the subject, and the type and severity of the subject's disease. The pharmaceutical composition may be formulated accordingly.

[0375] The lentiviral vector, cell or pharmaceutical composition according to the present invention may be administered parenterally, (e.g. intravenous, intra-arterial, intramuscular, intrathecal, subcutaneous), or by infusion techniques. The lentiviral vector, cell or pharmaceutical composition may be administered in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solution may be suitably buffered (preferably to a pH of from 3 to 9). The pharmaceutical composition may be formulated accordingly. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

[0376] The lentiviral vector, cell or pharmaceutical composition according to the present invention may be administered systemically, for example by intravenous injection or intraperitoneal injection. In some embodiments, the lentiviral vector, cell or pharmaceutical composition according to the present invention is administered by intravenous injection. The pharmaceutical composition may be formulated accordingly.

[0377] The lentiviral vector, cell or pharmaceutical composition according to the present invention may be administered locally, for example by direct injection, intra-arterial injection, or intraportal injection. In some embodiments, the lentiviral vector, cell or pharmaceutical composition according to the present invention is administered locally to the liver. In some embodiments, the lentiviral vector, cell or pharmaceutical composition according to the present invention is administered by intrahepatic injection, intrahepatic arterial injection, or intraportal injection. The pharmaceutical composition may be formulated accordingly.

[0378] The pharmaceutical compositions may comprise lentiviral vectors or cells of the invention in infusion media, for example sterile isotonic solution. The pharmaceutical composition may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0379] The lentiviral vector, cell or pharmaceutical composition may be administered in a single or in multiple doses. Suitably, the lentiviral vector, cell or pharmaceutical composition may be administered in a single, one off dose. The pharmaceutical composition may be formulated accordingly.

[0380] The lentiviral vector, cell or pharmaceutical composition may be administered at varying doses (e.g. measured in Transducing Units (TU) per kg). The physician in any event may determine the actual dosage which will be most suitable for any individual subject and the dosage may, for example, vary with the age, weight and response of the particular subject. Suitably, the lentiviral vector of the present invention is administered at a dose of at least about 10^8 TU/kg, at least about 10^9 TU/kg, or at least about 10^{10} TU/kg. Suitably, the lentiviral vector of the present invention is administered at a dose of about 10^{13} TU/kg or less, about 10^{12} TU/kg or less, or about 10^{11} TU/kg or less. Suitably, the lentiviral vector of the present invention is administered in a dose of from about 10^8 to about 10^{13} TU/kg, from about 10^9 to about 10^{13} TU/kg, or from about 10^{10} to about 10^{13} TU/kg. Suitably, the lentiviral vector of the present invention is administered in a dose of from about 10^8 to about 10^{12} TU/kg, from about 10^9 to about 10^{12} TU/kg, or from about 10^{10} to about 10^{12} TU/kg. Suitably, the lentiviral vector of the present invention is administered in a dose of from about 10^8 to about 10^{11} TU/kg, from about 10^9 to about 10^{11} TU/kg, or from about 10^{10} to about 10^{11} TU/kg. In some embodiments, the lentiviral vector of the present invention is administered in a dose of from about 10^8 to about 10^{11} TU/kg, from about 10^8 to about 10^{10} TU/kg, or from about 10^9 to about 10^{10} TU/kg. In some embodiments, the lentiviral vector of the present invention is administered in a dose of from about 10^9 to about 10^{10} TU/kg. The pharmaceutical composition may be formulated accordingly.

[0381] The pharmaceutical composition may further comprise one or more other therapeutic agents.

[0382] The invention further includes kits comprising the lentiviral vector, cell and/or pharmaceutical composition of the present invention. Preferably said kits are for use in the methods and used as described herein, e.g., the therapeutic methods as described herein. Preferably said kits comprise instructions for use of the kit components.

Methods for Treating and/or Preventing Disease

[0383] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use as a medicament.

[0384] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament.

[0385] In one aspect, the present invention provides a method of administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

[0386] The lentiviral vector mediated gene therapy described herein may allow for a stable gene transfer even in paediatric patients at the first disease stages by virtue of lentiviral vector genomic integration.

[0387] Following administration of the lentiviral vector of the present invention, the lentiviral vector may integrate into the genome of liver cells (e.g. hepatocyte cells). Subsequently, the lentiviral vector may be maintained in the genome of liver cells (e.g. hepatocyte cells) as they duplicate. The integration of the lentiviral vector in the genome of liver cells may be determined by integration site (IS) analysis (e.g. quantitative high-throughput vector IS analy-

sis). Suitable methods are known in the art (see e.g. Cantore, A., et al., 2015. Science translational medicine, 7(277), p. 277ra28).

[0388] The lentiviral vector, cell or pharmaceutical composition may be administered to any subject in need thereof. The subject may be a mammal (e.g. a human). In some embodiments, the subject is male. In some embodiments, the subject is female. In preferred embodiments, the lentiviral vector is administered in the form of a lentiviral particle.

[0389] In some embodiments, the subject is a juvenile, an adolescent, or a child. The term "juvenile" may refer to an individual that has not yet reached adulthood. The term "adolescent" may refer to an individual during the period from the onset of puberty to adulthood. The term "child" may refer an individual between the stages of birth and puberty.

[0390] In some embodiments, the subject is a young child, a toddler, on an infant. The term "young child" may refer to a human subject aged from 3 years to 5 years. The term "toddler" may refer to a human subject aged from 1 year to 3 years. The term "infant" may mean refer to a human subject under the age of 12 months.

[0391] In some embodiments, the subject is a paediatric patient. The term "paediatric patient" may refer to a human subject until about 18-21 years of age (see e.g. Sawyer, S. M., et al., 2019. The Lancet Child & Adolescent Health, 3(11), pp. 822-830).

[0392] In some embodiments, the subject is a neonatal patient or an infantile patient. The term "neonatal patient" may refer to a human subject who is aged about 4 weeks old or younger. The term "infantile patient" may refer to a human subject who is aged from about 4 weeks to about 1 year.

[0393] In other embodiments, the subject is an adult. Human liver is expected to completely renew every 5 years in humans, so integrating vectors are expected to be more persisting compared to mostly episomal vectors (e.g. AAV).

Progressive Familial Intrahepatic Cholestasis (PFIC)

[0394] The vector, cell or pharmaceutical composition according to the present invention may be used to prevent and/or treat progressive familial intrahepatic cholestasis (PFIC).

[0395] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating progressive familial intrahepatic cholestasis (PFIC).

[0396] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating progressive familial intrahepatic cholestasis (PFIC).

[0397] In one aspect, the present invention provides a method of preventing or treating progressive familial intrahepatic cholestasis (PFIC), the method comprising administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

[0398] As described above, PFIC is a group of rare autosomal recessive liver disorders characterized by mutations in genes encoding proteins involved in the hepatocellular transport system (e.g. bile secretion from hepatocyte to canaliculi). PFIC is characterised by an early onset of cholestasis (usually during infancy) with pruritus and malabsorption, which rapidly progresses.

[0399] Progressive liver fibrosis, cirrhosis, and end stage liver disease may eventually develop (see e.g. Davit-Spraul, A., et al., 2009. Progressive familial intrahepatic cholestasis. Orphanet journal of rare diseases, 4(1), pp. 1-12; Jacquemin, E., 2012. Clinics and research in hepatology and gastroenterology, 36, pp. S26-S35; and Gunaydin, M. and Cil, A. T. B., 2018. Hepatic Medicine: Evidence and Research, 10, pp. 95-104).

[0400] PFICs may be sub-grouped according to the genetic defect, clinical presentation, laboratory findings, and liver histology. PFIC-1, PFIC-2, and PFIC-3 are the main subtypes of PFIC. Other subtypes may include PFIC-4, PFIC-5, and PFIC-6 (see e.g. Bull, L. N. and Thompson, R. J., 2018. Clinics in liver disease, 22(4), pp. 657-669). Suitably, PFIC refers to PFIC-1, PFIC-2, PFIC-3, PFIC-4, PFIC-5, and PFIC-6. Suitably, PFIC refers to PFIC-1, PFIC-2, PFIC-3.

[0401] In some embodiments, the PFIC is selected from PFIC-2, PFIC-5, PFIC-6. In preferred embodiments, the PFIC is PFIC-2.

[0402] Following administration of the lentiviral vector of the present invention to a subject in need thereof, serum bile acid levels may be prevented from increasing or reduced. Suitably, serum bile acid levels may be reduced by at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%. Suitably, following administration, the subject's serum bile acid level may be from about 20 to about 30 μmol/L, or from about 10 to about 20 μmol/L. Following administration of the lentiviral vector of the present invention to a subject in need thereof, serum bile acid levels may be normalised. A normal serum bile acid level may be from about 4.5 to about 19.2 μmol/L (see e.g. Tibesar, E., et al., 2014. Case Reports in Pediatrics, 2014, 185923). Serum bile acid levels may be determined by any method known in the art (see e.g. Barnes, S., et al., 1975. Journal of clinical pathology, 28(6), pp. 506-509).

[0403] Following administration of the lentiviral vector of the present invention to a subject in need thereof the liver function of the subject may be improved. Liver function may be determined by any suitable method known in the art, for example by measuring biochemical markers such as serum bilirubin, alanine amino transferase, aspartate amino transferase, ratio of aminotransferases, alkaline phosphatase, gamma glutamyl transferase, 5' nucleotidase, ceruloplasmin, and α-fetoprotein (see e.g. Gowda, S., et al., 2009. The Pan African Medical Journal, 3, p. 17; Hoekstra, L. T., et al., 2013. Annals of surgery, 257(1), pp. 27-36; and Limdi, J. K. and Hyde, G. M., 2003. Postgraduate medical journal, 79(932), pp. 307-312).

[0404] Following administration of the lentiviral vector of the present invention to a subject in need thereof, serum bilirubin levels (e.g. total and/or direct) may be prevented from increasing or reduced. Suitably, serum bilirubin levels may be reduced by at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%. Suitably, following administration serum bilirubin levels may be less than about 1 mg/dL. Following administration of the lentiviral vector of the present invention to a subject in need thereof, serum bilirubin levels may be normalised. A normal bilirubin level may be from about 2 to about 17 μmol/L (see e.g. Limdi, J. K. and Hyde, G. M., 2003. Postgraduate medical journal,

79(932), pp. 307-312). Serum bilirubin levels may be determined by any method known in the art (see e.g. Lathe, G. H. and Ruthven, C. R. J., 1958. Journal of clinical pathology, 11(2), p. 155).

[0405] Following administration of the lentiviral vector of the present invention to a subject in need thereof, the serum level of one or more liver enzymes may be prevented from increasing or reduced. Suitable liver enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Suitably, one or more liver enzymes may be reduced by at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%. Suitably, following administration ALP levels may be less than about 150 IU/L. Following administration of the lentiviral vector of the present invention to a subject in need thereof, the serum level of one or more liver enzymes may be normalised. A normal ALT level may be from about 0 to about 45 IU/L, a normal AST level may be from about 0 to about 35 IU/L, and a normal ALP level may be from about 30 to about 120 IU/L (see e.g. Limdi, J. K. and Hyde, G. M., 2003. Postgraduate medical journal, 79(932), pp. 307-312). The serum level of liver enzymes may be determined by any method known in the art.

[0406] Following administration of the lentiviral vector of the present invention to a subject in need thereof, the formation of liver fibrosis may be slowed and/or reduced. Suitably, the extent of fibrosis is reduced by at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%. The extent of liver fibrosis may be determined by any method known in the art (see e.g. Standish, R. A., et al., 2006. Gut, 55(4), pp. 569-578).

PFIC-1

[0407] Progressive familial intrahepatic cholestasis type 1 (PFIC-1, MCID: CHL132, OMIM: 211600) is also called Fic1 deficiency or Byler disease and is caused by a variety of mutations in ATP8B1, the gene coding for FIC-1.

[0408] In some embodiments, the PFIC is PFIC-1 and the PFIC-associated polypeptide is FIC1 or fragment and/or variant thereof.

[0409] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating PFIC-1.

[0410] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating PFIC-1.

[0411] In one aspect, the present invention provides a method of preventing or treating PFIC-1, the method comprising administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

PFIC-2

[0412] Progressive familial intrahepatic cholestasis type X (PFIC-2, MCID: CHL136, OMIM: 601847) is also called Bsep Deficiency or Severe Abcb11 Deficiency and is caused by a variety of mutations in ABCB11, the gene coding for BSEP.

[0413] In some embodiments, the PFIC is PFIC-2 and the PFIC-associated polypeptide is BSEP or fragment and/or variant thereof.

[0414] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating PFIC-2.

[0415] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating PFIC-2.

[0416] In one aspect, the present invention provides a method of preventing or treating PFIC-2, the method comprising administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

PFIC-3

[0417] Progressive familial intrahepatic cholestasis type 3 (PFIC-3, MCID: CHL137, OMIM: 602347) is also called Mdr3 Deficiency and is caused by a variety of mutations in ABCB4, the gene coding for MDR3.

[0418] In some embodiments, the PFIC is PFIC-3 and the PFIC-associated polypeptide is MDR3 or fragment and/or variant thereof.

[0419] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating PFIC-3.

[0420] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating PFIC-3.

[0421] In one aspect, the present invention provides a method of preventing or treating PFIC-3, the method comprising administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

PFIC-4

[0422] Progressive familial intrahepatic cholestasis type 4 (PFIC-4, MCID: CHL143, OMIM: 615878) is also called Tjp2 Deficit and is caused by a variety of mutations in TJP2, the gene coding for TJP2 (see e.g. Sambrotta, M., et al., 2014. Nature genetics, 46(4), pp. 326-328).

[0423] In some embodiments, the PFIC is PFIC-4 and the PFIC-associated polypeptide is TJP2 or fragment and/or variant thereof.

[0424] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating PFIC-4.

[0425] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating PFIC-4.

[0426] In one aspect, the present invention provides a method of preventing or treating PFIC-4, the method comprising administering a therapeutically effective amount of

the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

PFIC-5

[0427] Progressive familial intrahepatic cholestasis type X (PFIC-5, MCID: CHL091, OMIM: 617049) is also called Nr1h4 Deficiency and is caused by a variety of mutations in NR1H4, the gene coding for FXR (see e.g. Gomez-Ospina, N., et al., 2016. Nature communications, 7(1), pp. 1-8).

[0428] In some embodiments, the PFIC is PFIC-5 and the PFIC-associated polypeptide is FXR or fragment and/or variant thereof.

[0429] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating PFIC-5.

[0430] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating PFIC-5.

[0431] In one aspect, the present invention provides a method of preventing or treating PFIC-5, the method comprising administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

PFIC-6

[0432] Progressive familial intrahepatic cholestasis type 6 (PFIC-6, MCID: CHL 186) is caused by a variety of mutations in MYO5B, the gene coding for MYO5B (see e.g. Gonzales, E., et al., 2017. Hepatology, 65(1), pp. 164-173).

[0433] In some embodiments, the PFIC is PFIC-6 and the PFIC-associated polypeptide is MYO5B or fragment and/or variant thereof.

[0434] In one aspect, the present invention provides the lentiviral vector, cell or pharmaceutical composition according to the present invention for use in preventing or treating PFIC-6.

[0435] In one aspect, the present invention provides use of the lentiviral vector, cell or pharmaceutical composition according to the present invention in the manufacture of a medicament for preventing or treating PFIC-6.

[0436] In one aspect, the present invention provides a method of preventing or treating PFIC-6, the method comprising administering a therapeutically effective amount of the lentiviral vector, cell or pharmaceutical composition according to the present invention to a subject in need thereof.

EXAMPLES

[0437] Preferred features and embodiments of the invention will now be described by way of non-limiting examples.

Example 1—Lentiviral Vector (LV) Encoding a BSEP Transgene

[0438] We generated a lentiviral vector (LV) encoding a BSEP transgene and evaluated in vitro in hepatocyte cell lines and in vivo in a mouse model of PFIC-2, the Abcb11−/− mice.

Results

[0439] We generated and produced LV encoding a human wild type or codon optimized BSEP transgenes under the control of a hepatocyte-specific cassette based on the enhanced transthyretin promoter (ET.BSEP) or the human alpha-1 antitrypsin promoter (hAAT.BSEP) (FIG. **1***a*).

[0440] To compare between the different BSEP transgenes, we first transduced Huh7 cells with LV-ET.BSEPwt, LV-ET.BSEPco_1 or LV-ET.BSEPco_2 at increasing multiplicity of infection (MOI). We performed flow cytometry analysis 10 days after transduction, detecting BSEP overexpression in LV-ET.BSEP transduced cells. Mean fluorescence intensity increased proportionally to the MOI employed and was higher for the BSEPco_1 and BSEPco_2 transgenes compared to BSEPwt at similar vector copy number (VCN) per cell (FIG. **1***b,c*).

[0441] We then compared the activities of the ET and the hAAT promoters by transducing Huh7 cells with LV-ET.BSEPco_1 or LV-hAAT.BSEP_co1. VCN/cell was comparable in cells transduced with the two vectors at different MOI (FIG. **1***d*), while BSEP protein expression was higher in LV-ET.BSEPco_1 compared to LV-hAAT.BSEPco_1 transduced cells as detected by western blot analysis (FIG. **1***e*). By BSEP protein quantification and normalization on the GAPDH housekeeper, we confirmed a higher transgene expression (3-3.5 fold) obtained from the expression cassette driven by the ET promoter compared to the hAAT (FIG. **1***f*).

[0442] Immunofluorescence analysis on Huh7 cells transduced with LV-ET.BSEPco_1 confirmed proper BSEP membrane and cytoplasmic localization while no signal was detectable in Huh7 untransduced cells (FIG. **2**).

[0443] We administered LV-ET.BSEPco_1 to juvenile Abcb11−/− mice at a dose of 3×10^{10} transducing units (TU)/kg by i.v. injection. The treatment resulted in a significant decrease of serum BA, bilirubin and ALP biomarkers until 7 months of age, indicating prevention of the progressive cholestatic damage (FIG. **3***a-c*).

Materials and Methods

Plasmid Construction

[0444] The BSEP coding sequences (WT or codon optimized) used in the study were synthetized by GeneScript and cloned into a third-generation self-inactivating (SIN) LV transfer plasmid (Milani, M., et al., 2019. Science Translational Medicine, 11(493), p.eaav7325) under the control of the enhanced transthyretin promoter (ET) or the human alpha-1 antitrypsin promoter (hAAT).

Vector Production

[0445] Lab-grade VSV.G-pseudotyped third-generation SIN LV were produced by calcium phosphate transient transfection into 293T cells. 293T cells were transfected with a solution containing a mix of the selected LV genome transfer plasmid, the packaging plasmids pMDLg/pRRE and pCMV.REV, pMD2.G and pAdvantage, as previously described (Milani, M., et al., 2017. EMBO molecular medicine, 9(11), pp. 1558-1573; and Milani, M., et al., 2019. Science Translational Medicine, 11(493), p.eaav7325).

[0446] Medium was replaced 14-16 hours post transfection and supernatant was collected around 30 hours after medium change. LV-containing supernatants were sterilized

through a 0.22 μm filter (Millipore) and transferred into sterile poliallomer tubes (Beckman) and centrifuged at 20,000 g for 120 min at 20° C. (Beckman Optima XL-100K Ultracentrifuge). LV pellet was resuspended in the appropriate volume of PBS to allow 500-1000× concentration.

LV Titration

[0447] For LV titration, 1×10^5 293T cells were transduced with serial LV dilutions in the presence of polybrene (8 μg/ml). Genomic DNA (gDNA) was extracted 10 days after transduction, using Maxwell 16 Cell DNA Purification Kit (Promega), following manufacturer's instructions. VCN was determined by digital droplet PCR (ddPCR) starting from 5-20 ng of template gDNA using primers (HIV fw: 5'-T ACTGACGCTCTCGCACC-3'; HIV rv: 5'-TCTCGACGCAGGACTCG-3') and a probe (FAM 5'-ATCTCTCTCCTTCTAGCCTC-3') designed on the primer binding site region of LV. The amount of endogenous DNA was quantified by a primers/probe set designed on the human GAPDH gene (Applied Biosystems HS00483111_cm). The PCR reaction was performed with each primer (900 nM) and the probe (250 nM) following manufacturer's instructions (Biorad), read with QX200 reader and analyzed with QuantaSoft software (Biorad). Infectious titer, expressed as TU/mL, was calculated using the formula TU/mL=(VCN×100,000×(1/dilution factor)). LV physical particles were measured by HIV-1 Gag p24 antigen immunocapture assay (Perkin Elmer) following manufacturer's instructions. LV specific infectivity was calculated as the ratio between infectious titer and physical particles.

Cell Culture and in Vitro Transduction Experiments

[0448] HuH7 cells were maintained under 37° C., 5% CO_2 condition in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA). Cells were seeded into 12-well plates (2×10^5 cells/well) and transduced with LV variants at different MOI in the presence of polybrene (8 μg/ml). Ten days post-LV transduction, cells were harvested for molecular analyses.

Mice Experiments

[0449] All animal experiments were performed in strict accordance with good animal practices following Italian and European legislation on animal care and experimentation (2010/63/EU). Abcb11−/− mice (Jackson strain n.004125) 2-week old were used in these studies. Animals were administered with LV at a dose of 3×10^{10} TU/kg via the retro-orbital plexus. Blood samples were collected monthly throughout the duration of the study for analysis of the metabolites.

VCN Determination

[0450] DNA was extracted from cells or liver samples using Maxwell 16 Cell or Tissue DNA Purification Kits (Promega). VCN was determined in Huh7 samples as described above (see "LV titration"). VCN in murine DNA was determined by ddPCR, starting from 5-20 ng of template gDNA using a primers/probe set designed on the primer binding site region of LV (see "LV titration" above). The amount of endogenous murine DNA was quantified by a primers/probe set designed on the murine sema3a gene (Sema3A fw: 5'-ACCGATTCCAGATGATTGGC-3'; Sema3A rv: 5'-TCCATATTAATGCAGTGCTTGC-3'; Sema3A probe: HEX 5'-AGAGGCCTGTCCTGCAGCTC

[0451] ATGG-3' BHQ1). The PCR reaction was performed with each primer (900 nM) and the probe (250 nM) following manufacturer's instructions (Biorad), read with QX200 reader and analyzed with QuantaSoft software (Biorad).

Western Blot Analysis

[0452] Protein extracts from HuH7 cell or from mouse liver samples were prepared using RIPA buffer (EMD Millipore) and protease inhibitors (Roche). Protein concentration was determined using the DC Protein Assay (Bio Rad Laboratories). SDS-page electrophoresis was performed in a Bis-Tris 4-12% gradient polyacrylamide gel. After transfer, the membrane was blocked with 1×TBS+5% milk and incubated with an anti-Abcb11 antibody (Sigma-Aldrich HPA019035) or anti-GAPDH (Biolegend 649201). The membrane was incubated with an anti-Mouse and an anti-Rabbit IgG secondary antibodies (Jackson Immunoresearch 115-035-003 and 111-035-144), then with clarity Western ECL substrate (Bio Rad) and visualized by Uvitec Imaging System (Cleaver Scientific). For Western blot quantification, we used the Image J software.

BSEP Immunofluorescence Analysis

[0453] Huh7 LV-transduced cells were seeded in an 8-well slide (Nunc® Lab-Tek® Chamber Slide™ system) to reach 40-50% confluency. 24 hours later, cells were fixed in 4% PFA and permeabilized in PBS+Triton X-100 0.1% for 10 minutes at room temperature (RT). Cells were blocked in in PBST+1% BSA 1 hour at RT and stained with a rabbit anti-BSEP (ab255605, dil. 1:100) primary antibody, overnight at 4° C. After an incubation with a goat anti rabbit IgG 488 secondary antibody (Invitrogen, dil. 1:1000), the nuclear staining was performed with Hoechst (diluted at 2 ug/mL in PBS), 5 minutes at RT. The staining slide was acquired at Leica TCS SP8 SMD confocal microscope.

Bile Acids, ALP, Bilirubin Determination

[0454] Total bile acids were determined in serum samples by using the Mouse Total Bile Acid Assay Kit (Crystal Chem, 80471) according to manufacturer's instructions. Serum ALP activity was measured with an International Federation of Clinical Chemistry and Laboratory Medicine optimized kinetic UV method in a SABA chemical analyzer (Seac-Radim), and it is expressed as units per liter (U/L).

EMBODIMENTS

[0455] Various preferred features and embodiments of the present invention will now be described with reference to the following numbered paragraphs (paras).

[0456] 1. A lentiviral vector comprising a nucleotide sequence encoding a progressive familial intrahepatic cholestasis (PFIC)-associated polypeptide, optionally wherein the lentiviral vector is an immune-shielded lentiviral vector.

[0457] 2. The lentiviral vector according to para 1, wherein the PFIC-associated polypeptide is selected from familial intrahepatic cholestasis type 1 (FIC1), or a fragment thereof; bile salt export pump (BSEP), or a fragment thereof; multiple drug resistance 3 (MDR3), or a fragment thereof; tight junction protein 2 (TJP2), or a fragment thereof; farnesoid X receptor (FXR), or a fragment thereof; and Myosin-Vb (MYO5B), or a fragment thereof.

[0458] 3. The lentiviral vector according to para 1 or 2, wherein the PFIC-associated polypeptide is selected from BSEP, or a fragment thereof; FXR, or a fragment thereof; and MYO5B, or a fragment thereof; preferably wherein the PFIC-associated polypeptide is BSEP, or a fragment thereof.

[0459] 4. The lentiviral vector according to any preceding para, wherein the PFIC-associated polypeptide comprises or consists of an amino acid sequence which is at least 70% identical to one of SEQ ID NOs: 37, 39, 43, 45, 47, 48 or 50, or a fragment thereof, preferably wherein the PFIC-associated polypeptide comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 39 or a fragment thereof.

[0460] 5. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to one of SEQ ID NOs: 38, 40, 41, 42, 44, 46, 49 or 51, or a fragment thereof, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to one of SEQ ID NOs: 40-42, or a fragment thereof.

[0461] 6. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is codon-optimised, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 41 or 42, or a fragment thereof.

[0462] 7. The lentiviral vector according to any preceding para, wherein the lentiviral vector is a $CD47^{high}$ lentiviral vector.

[0463] 8. The lentiviral vector according to any preceding para, wherein the lentiviral vector is obtained from a $CD47^{high}$ host cell, optionally wherein the host cell is genetically engineered to increase expression of CD47 on the cell surface.

[0464] 9. The lentiviral vector according to any preceding para, wherein the lentiviral vector has at least about 2-fold more CD47 on its surface than a lentiviral vector obtained from an unmodified host cell.

[0465] 10. The lentiviral vector according to any preceding para, wherein the lentiviral vector is a MHC-I^{free} lentiviral vector.

[0466] 11. The lentiviral vector according to any preceding para, wherein the lentiviral vector is obtained from a MHC-I^{free} host cell, optionally wherein the host cell is genetically engineered to disrupt expression of MHC-I on the cell surface.

[0467] 12. The lentiviral vector according to any preceding para, wherein MHC-I is not detectable on the surface of the lentiviral vector.

[0468] 13. The lentiviral vector according to any preceding para, wherein the lentiviral vector is a $CD47^{high}$/MHC-I^{free} lentiviral vector.

[0469] 14. The lentiviral vector according to any preceding para, wherein the lentiviral vector is obtained from a $CD47^{high}$/MHC-I^{free} host cell.

[0470] 15. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to one or more miRNA target sequences.

[0471] 16. The lentiviral vector according to para 15, wherein the one or more miRNA target sequences suppress transgene expression in one or more cells other than hepatocytes, preferably wherein the one or more miRNA target sequence suppress transgene expression in hematopoietic-lineage cells and/or antigen-presenting cells.

[0472] 17. The lentiviral vector according to para 15 or 16, wherein the one or more miRNA target sequences are selected from miR-181, miR-142, miR-223, and miR-155 target sequences.

[0473] 18. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to one or more mir-142 target sequence, two or more mir-142 target sequences, three or more mir-142 target sequences, or four or more mir-142 target sequences.

[0474] 19. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to four mir-142 target sequences.

[0475] 20. The lentiviral vector according to any of paras 15-19, wherein the one or more miRNA target sequences comprise or consist of a nucleotide sequence which is at least 90% identical to SEQ ID NO: 17, preferably wherein the one or more miRNA target sequences comprise or consist of a nucleotide sequence which is at least 90% identical to SEQ ID NO: 18.

[0476] 21. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a liver-specific promoter, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a hepatocyte-specific promoter.

[0477] 22. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a transthyretin (TTR) promoter, an alpha-1-antitrypsin (AAT) promoter, a thyroxine-binding globulin (TBG) promoter, a APoE/hAAT promoter, a HCR-hAAT promoter, a LP1 promoter, or a HLP promoter.

[0478] 23. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a transthyretin (TTR) promoter, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a Enh1mTTR (ET) promoter.

[0479] 24. The lentiviral vector according to any preceding para, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a promoter which comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 19.

[0480] 25. The lentiviral vector according to any preceding para, wherein the lentiviral vector is pseudotyped, preferably wherein the lentiviral vector is VSV.G-pseudotyped.

[0481] 26. The lentiviral vector according to any preceding para, wherein the lentiviral vector is a self-inactivating (SIN) lentiviral vector, preferably wherein the lentiviral vector comprises self-inactivating (SIN) LTRs which comprise or consist of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 23, or a fragment thereof.

[0482] 27. The lentiviral vector according to any preceding para, wherein the lentiviral vector is an integrating lentiviral vector and/or a replication-defective lentiviral vector.

[0483] 28. The lentiviral vector according to any preceding para, wherein the lentiviral vector comprises a nucleotide sequence which is at least 70% identical to SEQ ID NO: 36.

[0484] 29. An isolated cell comprising a lentiviral vector according to any of paras 1-28.

[0485] 30. A pharmaceutical composition comprising a lentiviral vector according to any of paras 1-28, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

[0486] 31. The lentiviral vector according to any of paras 1-28 or pharmaceutical composition according to para 30, for use as a medicament.

[0487] 32. Use of a lentiviral vector according to any of paras 1-28 or a pharmaceutical composition according to para 30, for the manufacture of a medicament.

[0488] 33. A method comprising administering a therapeutically effective amount of a lentiviral vector according to any of paras 1-28 or a pharmaceutical composition according to para 30, to a subject in need thereof.

[0489] 34. The lentiviral vector according to any of paras 1-28 or pharmaceutical composition according to para 30, for use in preventing or treating progressive familial intrahepatic cholestasis (PFIC).

[0490] 35. Use of a lentiviral vector according to any of paras 1-28 or a pharmaceutical composition according to para 30, for the manufacture of a medicament for preventing or treating progressive familial intrahepatic cholestasis (PFIC).

[0491] 36. A method of preventing or treating progressive familial intrahepatic cholestasis (PFIC), comprising administering a therapeutically effective amount of a lentiviral vector according to any of paras 1-28 or a pharmaceutical composition according to para 30, to a subject in need thereof.

[0492] 37. The lentiviral vector or pharmaceutical composition for use according to para 34, the use according to para 35, or the method according to para 36, wherein the PFIC-associated polypeptide is FIC1, or a fragment thereof, and the PFIC is PFIC type 1 (PFIC-1); the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC type 2 (PFIC-2); the PFIC-associated polypeptide is MDR3, or a fragment thereof, and the PFIC is PFIC type 3 (PFIC-3); the PFIC-associated polypeptide is TJP2, or a fragment thereof, and the PFIC is PFIC type 4 (PFIC-4); the PFIC-associated polypeptide is FXR, or a fragment thereof, and the PFIC is PFIC type 5 (PFIC-5); or the PFIC-associated polypeptide is MYO5B, or a fragment thereof, and the PFIC is PFIC type 6 (PFIC-6).

[0493] 38. The lentiviral vector or pharmaceutical composition for use according to para 34 or 37, the use according to para 35 or 37, or the method according to para 36 or 37, wherein the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC-2; the PFIC-associated polypeptide is FXR, or a fragment thereof, and the PFIC is PFIC-5; or the PFIC-associated polypeptide is MYO5B, or a fragment thereof, and the PFIC is PFIC-6, preferably wherein the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC-2.

[0494] 39. The lentiviral vector or pharmaceutical composition for use according to any of paras para 31, 34, 37-38, the use according to any of paras 32, 35, 37-38, or the method according to any of paras 33, 36-39, wherein the subject is a human subject.

[0495] 40. The lentiviral vector or pharmaceutical composition for use according to any of paras para 31, 34, 37-39, the use according to any of paras 32, 35, 37-39, or the method according to any of paras 33, 36-39, wherein the subject is a juvenile.

[0496] 41. The lentiviral vector or pharmaceutical composition for use according to any of paras para 31, 34, 37-39, the use according to any of paras 32, 35, 37-39, or the method according to any of paras 33, 36-39, wherein the subject is a paediatric patient, preferably wherein the subject is a neonatal patient or an infantile patient.

[0497] 42. The lentiviral vector or pharmaceutical composition for use according to any of paras 31, 34, 37-41, the use according to any of paras 32, 35, 37-41, or the method according to any of paras 33, 36-41, wherein said lentiviral vector or said pharmaceutical composition is administered systemically, preferably wherein said lentiviral vector or said pharmaceutical composition is administered by intravenous injection or intraperitoneal injection.

[0498] 43. The lentiviral vector or pharmaceutical composition for use according to any of paras 31, 34, 37-41, the use according to any of paras 32, 35, 37-41, or the method according to any of paras 33, 36-41, wherein said lentiviral vector or said pharmaceutical composition is administered locally, preferably wherein said lentiviral vector or said pharmaceutical composition is administered by direct injection, intra-arterial injection, or intraportal injection.

[0499] 44. The lentiviral vector or pharmaceutical composition for use according to para 43, the use according to para 43, or the method according to para 43, wherein said lentiviral vector or said pharmaceutical composition is administered locally to the liver, preferably wherein said lentiviral vector or said pharmaceutical composition is administered by intrahepatic injection, intrahepatic arterial injection, or intraportal injection.

[0500] 45. The lentiviral vector or pharmaceutical composition for use according to any of paras 31, 34, 37-44, the use according to any of paras 32, 35, 37-43, or the method according to any of paras 33, 36-44, wherein the lentiviral vector is administered at a dose of at least about 10^8 TU/kg, at least about 10^9 TU/kg, or at least about 10^{10} TU/kg.

[0501] 46. The lentiviral vector or pharmaceutical composition for use according to any of paras 31, 34, 37-45, the use according to any of paras 32, 35, 37-45, or the method according to any of paras 33, 36-45, wherein the lentiviral vector is administered in a dose of from about 10^8 to about 10^{11} TU/kg, from about 10^8 to about 10^{10} TU/kg, or from about 10^9 to about 10^{10} TU/kg.

[0502] 47. The lentiviral vector or pharmaceutical composition for use according to any of paras 31, 34, 37-46, the use according to any of paras 32, 35, 37-46, or the method according to any of paras 33, 36-46, wherein the lentiviral vector integrates into the genome of liver cells and is maintained as the liver cells duplicate, preferably wherein the lentiviral vector integrates into the genome of hepatocytes and is maintained as the hepatocytes duplicate.

[0503] 48. The lentiviral vector or pharmaceutical composition for use according to any of paras 34, 37-47, the use according to any of paras 35, 37-47, or the method according to any of paras 36-47, wherein serum bile acid levels are reduced and/or normalised.

[0504] 49. The lentiviral vector or pharmaceutical composition for use according to any of paras 34, 37-48, the use according to any of paras 35, 37-48, or the method according

to any of paras 36-48, wherein the liver function is improved, preferably wherein the serum level of bilirubin and/or one or more liver enzymes is reduced and/or normalised.

[0505] 50. The lentiviral vector or pharmaceutical composition for use according to any of paras 34, 37-49, the use according to any of paras 35, 37-49, or the method according to any of paras 36-49, wherein the formation of liver fibrosis is slowed and/or reduced.

[0506] All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the disclosed vectors, cells, compositions, kits and uses of the invention will be apparent to the skilled person without departing from the scope and spirit of the invention. Although the invention has been disclosed in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the disclosed modes for carrying out the invention, which are obvious to the skilled person are intended to be within the scope of the following claims.

SEQUENCE LISTING

```
Sequence total quantity: 57
SEQ ID NO: 1            moltype = AA  length = 305
FEATURE                 Location/Qualifiers
source                  1..305
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 1
MWPLVAALLL GSACCGSAQL LFNKTKSVEF TFCNDTVVIP CFVTNMEAQN TTEVYVKWKF  60
KGRDIYTFDG ALNKSTVPTD FSSAKIEVSQ LLKGDASLKM DKSDAVSHTG NYTCEVTELT  120
REGETIIELK YRVVSWFSPN ENILIVIFPI FAILLFWGQF GIKTLKYRSG GMDEKTIALL  180
VAGLVITVIV IVGAILFVPG EYSLKNATGL GLIVTSTGIL ILLHYYVFST AIGLTSFVIA  240
ILVIQVIAYI LAVVGLSLCI AACIPMHGPL LISGLSILAL AQLLGLVYMK FVASNQKTIQ  300
PPRNN                                                              305

SEQ ID NO: 2            moltype = AA  length = 323
FEATURE                 Location/Qualifiers
source                  1..323
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 2
MWPLVAALLL GSACCGSAQL LFNKTKSVEF TFCNDTVVIP CFVTNMEAQN TTEVYVKWKF  60
KGRDIYTFDG ALNKSTVPTD FSSAKIEVSQ LLKGDASLKM DKSDAVSHTG NYTCEVTELT  120
REGETIIELK YRVVSWFSPN ENILIVIFPI FAILLFWGQF GIKTLKYRSG GMDEKTIALL  180
VAGLVITVIV IVGAILFVPG EYSLKNATGL GLIVTSTGIL ILLHYYVFST AIGLTSFVIA  240
ILVIQVIAYI LAVVGLSLCI AACIPMHGPL LISGLSILAL AQLLGLVYMK FVASNQKTIQ  300
PPRKAVEEPL NAFKESKGMM NDE                                          323

SEQ ID NO: 3            moltype = AA  length = 292
FEATURE                 Location/Qualifiers
source                  1..292
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 3
MWPLVAALLL GSACCGSAQL LFNKTKSVEF TFCNDTVVIP CFVTNMEAQN TTEVYVKWKF  60
KGRDIYTFDG ALNKSTVPTD FSSAKIEVSQ LLKGDASLKM DKSDAVSHTG NYTCEVTELT  120
REGETIIELK YRVVSWFSPN ENILIVIFPI FAILLFWGQF GIKTLKYRSG GMDEKTIALL  180
VAGLVITVIV IVGAILFVPG EYSLKNATGL GLIVTSTGIL ILLHYYVFST AIGLTSFVIA  240
ILVIQVIAYI LAVVGLSLCI AACIPMHGPL LISGLSILAL AQLLGLVYMK FV          292

SEQ ID NO: 4            moltype = AA  length = 311
FEATURE                 Location/Qualifiers
source                  1..311
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 4
MWPLVAALLL GSACCGSAQL LFNKTKSVEF TFCNDTVVIP CFVTNMEAQN TTEVYVKWKF  60
KGRDIYTFDG ALNKSTVPTD FSSAKIEVSQ LLKGDASLKM DKSDAVSHTG NYTCEVTELT  120
REGETIIELK YRVVSWFSPN ENILIVIFPI FAILLFWGQF GIKTLKYRSG GMDEKTIALL  180
VAGLVITVIV IVGAILFVPG EYSLKNATGL GLIVTSTGIL ILLHYYVFST AIGLTSFVIA  240
ILVIQVIAYI LAVVGLSLCI AACIPMHGPL LISGLSILAL AQLLGLVYMK FVASNQKTIQ  300
PPRKAVEEPL N                                                       311

SEQ ID NO: 5            moltype = AA  length = 287
FEATURE                 Location/Qualifiers
source                  1..287
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 5
QLLFNKTKSV EFTFCNDTVV IPCFVTNMEA QNTTEVYVKW KFKGRDIYTF DGALNKSTVP  60
TDFSSAKIEV SQLLKGDASL KMDKSDAVSH TGNYTCEVTE LTREGETIIE LKYRVVSWFS  120
```

-continued

```
PNENILIVIF PIFAILLFWG QFGIKTLKYR SGGMDEKTIA LLVAGLVITV IVIVGAILFV  180
PGEYSLKNAT GLGLIVTSTG ILILLHYYVF STAIGLTSFV IAILVIQVIA YILAVVGLSL  240
CIAACIPMHG PLLISGLSIL ALAQLLGLVY MKFVASNQKT IQPPRNN                287

SEQ ID NO: 6            moltype = AA  length = 305
FEATURE                 Location/Qualifiers
source                  1..305
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 6
QLLFNKTKSV EFTFCNDTVV IPCFVTNMEA QNTTEVYVKW KFKGRDIYTF DGALNKSTVP  60
TDFSSAKIEV SQLLKGDASL KMDKSDAVSH TGNYTCEVTE LTREGETIIE LKYRVVSWFS  120
PNENILIVIF PIFAILLFWG QFGIKTLKYR SGGMDEKTIA LLVAGLVITV IVIVGAILFV  180
PGEYSLKNAT GLGLIVTSTG ILILLHYYVF STAIGLTSFV IAILVIQVIA YILAVVGLSL  240
CIAACIPMHG PLLISGLSIL ALAQLLGLVY MKFVASNQKT IQPPRKAVEE PLNAFKESKG  300
MMNDE                                                              305

SEQ ID NO: 7            moltype = AA  length = 274
FEATURE                 Location/Qualifiers
source                  1..274
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 7
QLLFNKTKSV EFTFCNDTVV IPCFVTNMEA QNTTEVYVKW KFKGRDIYTF DGALNKSTVP  60
TDFSSAKIEV SQLLKGDASL KMDKSDAVSH TGNYTCEVTE LTREGETIIE LKYRVVSWFS  120
PNENILIVIF PIFAILLFWG QFGIKTLKYR SGGMDEKTIA LLVAGLVITV IVIVGAILFV  180
PGEYSLKNAT GLGLIVTSTG ILILLHYYVF STAIGLTSFV IAILVIQVIA YILAVVGLSL  240
CIAACIPMHG PLLISGLSIL ALAQLLGLVY MKFV                              274

SEQ ID NO: 8            moltype = AA  length = 293
FEATURE                 Location/Qualifiers
source                  1..293
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 8
QLLFNKTKSV EFTFCNDTVV IPCFVTNMEA QNTTEVYVKW KFKGRDIYTF DGALNKSTVP  60
TDFSSAKIEV SQLLKGDASL KMDKSDAVSH TGNYTCEVTE LTREGETIIE LKYRVVSWFS  120
PNENILIVIF PIFAILLFWG QFGIKTLKYR SGGMDEKTIA LLVAGLVITV IVIVGAILFV  180
PGEYSLKNAT GLGLIVTSTG ILILLHYYVF STAIGLTSFV IAILVIQVIA YILAVVGLSL  240
CIAACIPMHG PLLISGLSIL ALAQLLGLVY MKFVASNQKT IQPPRKAVEE PLN         293

SEQ ID NO: 9            moltype = AA  length = 140
FEATURE                 Location/Qualifiers
source                  1..140
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 9
MWPLVAALLL GSACCGSAQL LFNKTKSVEF TFCNDTVVIP CFVTNMEAQN TTEVYVKWKF  60
KGRDIYTFDG ALNKSTVPTD FSSAKIEVSQ LLKGDASLKM DKSDAVSHTG NYTCEVTELT  120
REGETIIELK YRVVSWFSPN                                              140

SEQ ID NO: 10           moltype = AA  length = 122
FEATURE                 Location/Qualifiers
source                  1..122
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 10
QLLFNKTKSV EFTFCNDTVV IPCFVTNMEA QNTTEVYVKW KFKGRDIYTF DGALNKSTVP  60
TDFSSAKIEV SQLLKGDASL KMDKSDAVSH TGNYTCEVTE LTREGETIIE LKYRVVSWFS  120
PN                                                                 122

SEQ ID NO: 11           moltype = AA  length = 365
FEATURE                 Location/Qualifiers
source                  1..365
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 11
MAVMAPRTLL LLLSGALALT QTWAGSHSMR YFFTSVSRPG RGEPRFIAVG YVDDTQFVRF  60
DSDAASQRME PRAPWIEQEG PEYWDQETRN VKAQSQTDRV DLGTLRGYYN QSEAGSHTIQ  120
IMYGCDVGSD GRFLRGYRQD AYDGKDYIAL NEDLRSWTAA DMAAQITKRK WEAAHEAEQL  180
RAYLDGTCVE WLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT  240
WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEL  300
SSQPTIPIVG IIAGLVLLGA VITGAVVAAV MWRRKSSDRK GGSYTQAASS DSAQGSDVSL  360
TACKV                                                              365

SEQ ID NO: 12           moltype = AA  length = 371
```

-continued

```
FEATURE                 Location/Qualifiers
source                  1..371
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 12
MAVMAPRTLL LLLSGALALT QTWAGSHSMR YFFTSVSRPG RGEPRFIAVG YVDDTQFVRF  60
DSDAASQRME PRAPWIEQEG PEYWDQETRN VKAQSQTDRV DLGTLRGYYN QSEAGSHTIQ  120
IMYGCDVGSD GRFLRGYRQD AYDGKDYIAL NEDLRSWTAA DMAAQITKRK WEAAHAAEQQ  180
RAYLEGRCVE WLRRYLENGK ETLQRTDPPK THMTHHPISD HEATLRCWAL GFYPAEITLT  240
WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEL  300
SSQPTIPIVG IIAGLVLLGA VITGAVVAAV MWRRKSSGGE GVKDRKGGSY TQAASSDSAQ  360
GSDVSLTACK V                                                       371

SEQ ID NO: 13           moltype = AA  length = 362
FEATURE                 Location/Qualifiers
source                  1..362
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 13
MLVMAPRTVL LLLSAALALT ETWAGSHSMR YFYTSVSRPG RGEPRFISVG YVDDTQFVRF  60
DSDAASPREE PRAPWIEQEG PEYWDRNTQI YKAQAQTDRE SLRNLRGYYN QSEAGSHTLQ  120
SMYGCDVGPD GRLLRGHDQY AYDGKDYIAL NEDLRSWTAA DTAAQITQRK WEAAREAEQR  180
RAYLEGECVE WLRRYLENGK DKLERADPPK THVTHHPISD HEATLRCWAL GFYPAEITLT  240
WQRDGEDQTQ DTELVETRPA GDRTFQKWAA VVVPSGEEQR YTCHVQHEGL PKPLTLRWEP  300
SSQSTVPIVG IVAGLAVLAV VVIGAVVAAV MCRRKSSGGK GGSYSQAACS DSAQGSDVSL  360
TA                                                                 362

SEQ ID NO: 14           moltype = AA  length = 366
FEATURE                 Location/Qualifiers
source                  1..366
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 14
MRVMAPRALL LLLSGGLALT ETWACSHSMR YFDTAVSRPG RGEPRFISVG YVDDTQFVRF  60
DSDAASPRGE PRAPWVEQEG PEYWDRETQK YKRQAQADRV SLRNLRGYYN QSEDGSHTLQ  120
RMSGCDLGPD GRLLRGYDQS AYDGKDYIAL NEDLRSWTAA DTAAQITQRK LEAARAAEQL  180
RAYLEGTCVE WLRRYLENGK ETLQRAEPPK THVTHHPLSD HEATLRCWAL GFYPAEITLT  240
WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGQEQR YTCHMQHEGL QEPLTLSWEP  300
SSQPTIPIMG IVAGLAVLVV LAVLGAVVTA MMCRRKSSGG KGGSCSQAAC SNSAQGSDES  360
LITCKA                                                             366

SEQ ID NO: 15           moltype = AA  length = 326
FEATURE                 Location/Qualifiers
source                  1..326
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 15
MRVMAPRALL LLLSGGLALT ETWACSHSMR YFDTAVSRPG RGEPRFISVG YVDDTQFVRF  60
DSDAASPRGE PRAPWVEQEG PEYWDRETQK YKRQAQADRV SLRNLRGYYN QSEDGSHTLQ  120
RMSGCDLGPD GRLLRGYDQS AYDGKDYIAL NEHLRSCTAA DTAAQITQRK LEAARAAEQL  180
RAYLEGTCVE WLRRYLENGK ETLQRAEPPK THVTHHPLSD HEATLRCWAL GFYPAEITLT  240
WQRDGEDQTQ DTELVETRPA GDGTFQKWAA VVVPSGQEQR YTCHMQHEGL QEPLTLRWGG  300
KGGSCSQAAC SNSAQGSDES LITCKA                                       326

SEQ ID NO: 16           moltype = AA  length = 119
FEATURE                 Location/Qualifiers
source                  1..119
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 16
MSRSVALAVL ALLSLSGLEA IQRTPKIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLL  60
KNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM   119

SEQ ID NO: 17           moltype = DNA  length = 23
FEATURE                 Location/Qualifiers
source                  1..23
                        mol_type = other DNA
                        organism = unidentified
SEQUENCE: 17
tccataaagt aggaaacact aca                                          23

SEQ ID NO: 18           moltype = DNA  length = 106
FEATURE                 Location/Qualifiers
source                  1..106
                        mol_type = other DNA
                        organism = unidentified
```

-continued

```
SEQUENCE: 18
tccataaagt aggaaacact acacgattcc ataaagtagg aaacactaca accggttcca  60
taaagtagga aacactacat cactccataa agtaggaaac actaca                 106

SEQ ID NO: 19          moltype = DNA  length = 553
FEATURE                Location/Qualifiers
source                 1..553
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 19
cgcgagttaa taattaccag cgcgggccaa ataaataatc cgcgaggggc aggtgacgtt  60
tgcccagcgc gcgctggtaa ttattaacct cgcgaatatt gattcgaggc cgcgattgcc  120
gcaatcgcga ggggcaggtg acctttgccc agcgcgcgtt cgccccgccc cggacggtat  180
cgataagctt aggagcttgg gctgcaggtc gagggcactg ggaggatgtt gagtaagatg  240
gaaaactact gatgaccctt gcagagacag agtattagga catgtttgaa cagggccggg  300
gcgatcagca ggtagctcta gaggatcccc gtctgtctgc acatttcgta gagcgagtgt  360
tccgatactc taatctccct aggcaaggtt catatttgtg taggttactt attctccttt  420
tgttgactaa gtcaataatc agaatcagca ggtttggagt cagcttggca gggatcagca  480
gcctgggttg gaaggagggg gtataaaagc cccttcacca ggagaagccg tcacacagat  540
ccacaagctc ctg                                                     553

SEQ ID NO: 20          moltype = DNA  length = 347
FEATURE                Location/Qualifiers
source                 1..347
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 20
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta  60
agtggtactc tcccagagac tgtctgactc acgccacccc ctccaccttg gacacaggac  120
gctgtggttt ctgagccagg tacaatgact cctttcggta agtgcagtgg aagctgtaca  180
ctgcccaggc aaagcgtccg ggcagcgtag gcgggcgact cagatcccag ccagtggact  240
tagcccctgt ttgctcctcc gataactggg gtgaccttgg ttaatattca ccagcagcct  300
cccccgttgc ccctctggat ccactgctta aatacggacg aggacag                347

SEQ ID NO: 21          moltype = DNA  length = 504
FEATURE                Location/Qualifiers
source                 1..504
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 21
aatcaacctc tggattacaa aatttgtgaa agattgactg gtattcttaa ctatgttgct  60
ccttttacgc tatgtggata cgctgcttta atgcctttgt atcatgctat tgcttcccgt  120
atggctttca ttttctcctc cttgtataaa tcctggttgc tgtctcttta tgaggagttg  180
tggcccgttg tcaggcaacg tggcgtggtg tgcactgtgt ttgctgacgc aacccccact  240
ggttggggca ttgccaccac ctgtcagctc ctttccggga ctttcgcttt ccccctccct  300
attgccacgg cggaactcat cgccgcctgc cttgcccgct gctggacagg ggctcggctg  360
ttgggcactg acaattccgt ggtgttgtcg gggaaatcat cgtcctttcc ttggctgctc  420
gcctgtgttg ccacctggat tctgcgcggg acgtccttct gctacgtccc ttcggccctc  480
aatccagcgg accttccttc ccgc                                         504

SEQ ID NO: 22          moltype =   length =
SEQUENCE: 22
000

SEQ ID NO: 23          moltype = DNA  length = 235
FEATURE                Location/Qualifiers
source                 1..235
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 23
tggaagggct aattcactcc caacgaagac aagatctgct ttttgcttgt actgggtctc  60
tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta  120
agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact  180
ctggtaacta gagatccctc agaccctttt agtcagtgtg gaaaatctct agcag       235

SEQ ID NO: 24          moltype = DNA  length = 182
FEATURE                Location/Qualifiers
source                 1..182
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 24
gggtctctct ggttagacca gatctgagcc tgggagctct ctggctaact agggaaccca  60
ctgcttaagc ctcaataaag cttgccttga gtgcttcaag tagtgtgtgc ccgtctgttg  120
tgtgactctg gtaactagag atccctcaga ccctttagt cagtgtggaa aatctctagc  180
ag                                                                 182
```

-continued

```
SEQ ID NO: 25          moltype = DNA  length = 235
FEATURE                Location/Qualifiers
source                 1..235
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 25
tggaagggct aattcactcc caacgaagac aagatctgct ttttgcttgt actgggtctc  60
tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta  120
agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact  180
ctggtaacta gagatccctc agaccctttt agtcagtgtg gaaaatctct agcag       235

SEQ ID NO: 26          moltype = DNA  length = 154
FEATURE                Location/Qualifiers
source                 1..154
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 26
tggcgcccga acagggactt gaaagcgaaa gggaaaccag aggagctctc tcgacgcagg  60
actcggcttg ctgaagcgcg cacggcaaga ggcgaggggc ggcgactggt gagtacgcca  120
aaaattttga ctagcggagg ctagaaggag agag                              154

SEQ ID NO: 27          moltype = DNA  length = 151
FEATURE                Location/Qualifiers
source                 1..151
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 27
tggcgcccga acagggacct gaaagcgaaa gggaaaccag agctctctcg acgcaggact  60
cggcttgctg aagcgcgcac ggcaagaggc gaggggcggc gactggtgag tacgccaaaa  120
attttgacta gcggaggcta gaaggagaga g                                 151

SEQ ID NO: 28          moltype = DNA  length = 365
FEATURE                Location/Qualifiers
source                 1..365
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 28
atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgcgatgg gaaaaaattc  60
ggttaaggcc agggggaaag aaaaaatata aattaaaaca tatagtatgg gcaagcaggg  120
agctagaacg attcgcagtt aatcctggcc tgttagaaac atcagaaggc tgtagacaaa  180
tactgggaca gctacaacca tcccttcaga caggatcaga agaacttaga tcattatata  240
atacagtagc aaccctctat tgtgtgcatc aaaggataga gataaaagac accaaggaag  300
ctttagacaa gatagaggaa gagcaaaaca aaagtaagac caccgcacag caagcggccg  360
ctgat                                                              365

SEQ ID NO: 29          moltype = DNA  length = 241
FEATURE                Location/Qualifiers
source                 1..241
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 29
ggagctttgt tccttgggtt cttgggagca gcaggaagca ctatgggcgc agcgtcaatg  60
acgctgacgg tacaggccag acaattattg tctggtatag tgcagcagca gaacaatttg  120
ctgagggcta ttgaggcgca acagcatctg ttgcaactca cagtctgggg catcaagcag  180
ctccaggcaa gaatcctggc tgtggaaaga tacctaaagg atcaacagct cctggggatt  240
t                                                                  241

SEQ ID NO: 30          moltype = DNA  length = 241
FEATURE                Location/Qualifiers
source                 1..241
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 30
ggagctttgt tccttgggtt cttgggagca gcaggaagca ctatgggcgc agcctcaatg  60
acgctgacgg tacaggccag acaattattg tctggtatag tgcagcagca gaacaatttg  120
ctgagggcta ttgaggcgca acagcatctg ttgcaactca cagtctgggg catcaagcag  180
ctccaggcaa gaatcctggc tgtggaaaga tacctaaagg atcaacagct cctggggatt  240
t                                                                  241

SEQ ID NO: 31          moltype = DNA  length = 124
FEATURE                Location/Qualifiers
source                 1..124
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 31
aacttttaaa agaaaagggg ggattggggg gtacagtgca ggggaaagaa tagtagacat  60
```

-continued

```
aatagcaaca gacatacaaa ctaaagaatt acaaaaacaa attacaaaaa ttcaaaattt  120
tatc                                                               124

SEQ ID NO: 32          moltype = DNA  length = 146
FEATURE                Location/Qualifiers
source                 1..146
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 32
tcttcagacc tggaggagga gatatgaggg acaattggag aagtgaatta tataaatata  60
aagtagtaaa aattgaacca ttaggagtag cacccaccaa ggcaaagaga agagtggtgc  120
agagagaaaa aagagcagtg ggaata                                       146

SEQ ID NO: 33          moltype = DNA  length = 468
FEATURE                Location/Qualifiers
source                 1..468
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 33
gggttgctct ggaaaactca tttgcaccac tgctgtgcct tggaatgcta gttggagtaa  60
taaatctctg gaacagattt ggaatcacac gacctggatg gagtgggaca gagaaattaa  120
caattacaca agcttaatac actccttaat tgaagaatcg caaaaccagc aagaaaagaa  180
tgaacaagaa ttattggaat tagataaatg ggcaagtttg tggaattggt ttaacataac  240
aaattggctg tggtatataa aattattcat aatgatagta ggaggcttgg taggtttaag  300
aatagttttt gctgtacttt ctatagtgaa tagagttagg cagggatatt caccattatc  360
gtttcagacc cacctcccaa ccccgagggg acccgacagg cccgaaggaa tagaagaaga  420
aggtggagag agagacagag acagatccat tcgattagtg aacggatc               468

SEQ ID NO: 34          moltype = DNA  length = 902
FEATURE                Location/Qualifiers
source                 1..902
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 34
tggcgcccga acagggacct gaaagcgaaa gggaaaccag agctctctcg acgcaggact  60
cggcttgctg aagcgcgcac ggcaagaggc gaggggcggc gactggtgag tacgccaaaa  120
attttgacta gcggaggcta gaaggagaga gatgggtgcg agagcgtcag tattaagcgg  180
gggagaatta gatcgcgatg ggaaaaaatt cggttaaggc caggggggaaa gaaaaaatat  240
aaattaaaac atatagtatg ggcaagcagg gagctagaac gattcgcagt taatcctggc  300
ctgttagaaa catcagaagg ctgtagacaa atactgggac agctacaacc atcccttcag  360
acaggatcag aagaacttag atcattatat aatacagtag caaccctcta ttgtgtgcat  420
caaaggatag agataaaaga caccaaggaa gctttagaca agatagagga agagcaaaac  480
aaaagtaaga ccaccgcaca gcaagcggcc gctgatcttc agacctggag gaggagatat  540
gagggacaat tggagaagtg aattatataa atataaagta gtaaaaattg aaccattagg  600
agtagcaccc accaaggcaa agagaagagt ggtgcagaga gaaaaaagag cagtgggaat  660
aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcctcaat  720
gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt  780
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca  840
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcctggggat  900
tt                                                                 902

SEQ ID NO: 35          moltype = DNA  length = 1510
FEATURE                Location/Qualifiers
source                 1..1510
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 35
tggcgcccga acagggacct gaaagcgaaa gggaaaccag agctctctcg acgcaggact  60
cggcttgctg aagcgcgcac ggcaagaggc gaggggcggc gactggtgag tacgccaaaa  120
attttgacta gcggaggcta gaaggagaga gatgggtgcg agagcgtcag tattaagcgg  180
gggagaatta gatcgcgatg ggaaaaaatt cggttaaggc caggggggaaa gaaaaaatat  240
aaattaaaac atatagtatg ggcaagcagg gagctagaac gattcgcagt taatcctggc  300
ctgttagaaa catcagaagg ctgtagacaa atactgggac agctacaacc atcccttcag  360
acaggatcag aagaacttag atcattatat aatacagtag caaccctcta ttgtgtgcat  420
caaaggatag agataaaaga caccaaggaa gctttagaca agatagagga agagcaaaac  480
aaaagtaaga ccaccgcaca gcaagcggcc gctgatcttc agacctggag gaggagatat  540
gagggacaat tggagaagtg aattatataa atataaagta gtaaaaattg aaccattagg  600
agtagcaccc accaaggcaa agagaagagt ggtgcagaga gaaaaaagag cagtgggaat  660
aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcctcaat  720
gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt  780
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca  840
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcctggggat  900
ttggggttgc tctggaaaac tcatttgcac cactgctgtg ccttggaatg ctagttggag  960
taataaatct ctggaacaga tttggaatca cacgacctgg atggagtggg acagagaaat  1020
taacaattac acaagcttaa tacactcctt aattgaagaa tcgcaaaacc agcaagaaaa  1080
gaatgaacaa gaattattgg aattagataa atgggcaagt ttgtggaatt ggtttaacat  1140
```

-continued

```
aacaaattgg ctgtggtata taaaattatt cataatgata gtaggaggct tggtaggttt  1200
aagaatagtt tttgctgtac tttctatagt gaatagagtt aggcagggat attcaccatt  1260
atcgtttcag acccacctcc caaccccgag gggacccgac aggcccgaag gaatagaaga  1320
agaaggtgga gagagagaca gagacagatc cattcgatta gtgaacggat ctcgacggta  1380
tcggttaact tttaaaagaa aagggggat tgggggtac agtgcagggg aaagaatagt  1440
agacataata gcaacagaca tacaaactaa agaattacaa aaacaaatta caaaaattca  1500
aaattttatc                                                         1510

SEQ ID NO: 36          moltype = DNA  length = 7216
FEATURE                Location/Qualifiers
source                 1..7216
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 36
gggtctctct ggttagacca gatctgagcc tgggagctct ctggctaact agggaaccca  60
ctgcttaagc ctcaataaag cttgccttga gtgcttcaag tagtgtgtgc ccgtctgttg  120
tgtgactctg gtaactagag atccctcaga cccttttagt cagtgtggaa aatctctagc  180
agtggcgccc gaacagggac ctgaaagcga aagggaaacc agagctctct cgacgcagga  240
ctcggcttgc tgaagcgcgc acggcaagag gcgaggggcg gcgactggtg agtacgccaa  300
aaattttgac tagcggaggc tagaaggaga gagatgggtg cgagagcgtc agtattaagc  360
gggggagaat tagatcgcga tgggaaaaaa ttcggttaag gccaggggga aagaaaaaat  420
ataaattaaa acatatagta tgggcaagca gggagctaga acgattcgca gttaatcctg  480
gcctgttaga aacatcagaa ggctgtagac aaatactggg acagctacaa ccatcccttc  540
agacaggatc agaagaactt agatcattat ataatacagt agcaaccctc tattgtgtgc  600
atcaaaggat agagataaaa gacaccaagg aagctttaga caagatagag gaagagcaaa  660
acaaaagtaa gaccaccgca cagcaagcgg ccgctgatct tcagacctgg aggaggagat  720
atgagggaca attggagaag tgaattatat aaatataaag tagtaaaaat tgaaccatta  780
ggagtagcac ccaccaaggc aaagagaaga gtggtgcaga gagaaaaaag agcagtggga  840
ataggagctt tgttccttgg gttcttggga gcagcaggaa gcactatggg cgcagcctca  900
atgacgctga cggtacaggc cagacaatta ttgtctggta tagtgcagca gcagaacaat  960
ttgctgaggg ctattgaggc gcaacagcat ctgttgcaac tcacagtctg gggcatcaag  1020
cagctccagg caagaatcct ggctgtggaa agatacctaa aggatcaaca gctcctgggg  1080
atttggggtt gctctggaaa actcatttgc accactgctg tgccttggaa tgctagttgg  1140
agtaataaat ctctggaaca gatttggaat cacacgacct ggatggagtg ggacagagaa  1200
attaacaatt acacaagctt aatacactcc ttaattgaag aatcgcaaaa ccagcaagaa  1260
aagaatgaac aagaattatt ggaattagat aaatgggcaa gtttgtggaa ttggtttaac  1320
ataacaaatt ggctgtggta tataaaatta ttcataatga tagtaggagg cttggtaggt  1380
ttaagaatag tttttgctgt actttctata gtgaatagag ttaggcaggg atattcacca  1440
ttatcgtttc agacccacct cccaaccccg aggggacccg acaggcccga aggaatagaa  1500
gaagaaggtg gagagagaga cagagacaga tccattcgat tagtgaacgg atctcgacgg  1560
tatcggttaa cttttaaaag aaaagggggg attgggggt acagtgcagg ggaaagaata  1620
gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt  1680
caaaatttta tcgatcacga gactagcctc gagcacgcga gttaataatt accagcgcgg  1740
gccaaataaa taatccgcga ggggcaggtg acgtttgccc agcgcgcgct ggtaattatt  1800
aacctcgcga atattgattc gaggccgcga ttgccgcaat cgcgaggggc aggtgacctt  1860
tgcccagcgc gcgttcgccc cgccccggac ggtatcgata agcttaggag cttgggctgc  1920
aggtcgaggg cactgggagg atgttgagta agatggaaaa ctactgatga cccttgcaga  1980
gacagagtat taggacatgt ttgaacaggg gccgggcgat cagcaggtag ctctagagga  2040
tccccgtctg tctgcacatt tcgtagagcg agtgttccga tactctaatc tccctaggca  2100
aggttcatat ttgtgtaggt tacttattct ccttttgttg actaagtcaa taatcagaat  2160
cagcaggttt ggagtcagct tggcagggat cagcagcctg ggttggaagg agggggtata  2220
aaagcccctt caccaggaga agccgtcaca cagatccaca agctcctggc tagcgtacgc  2280
caccatgagc gactccgtga tcctgcggag catcaagaag tttggcgagg agaacgacgg  2340
cttcgagtct gataagagct acaacaatga taagaagtcc agactgcagg acgagaagaa  2400
gggcgatggc gtgcgggtgg gcttctttca gctgttccgg ttcagcagca gcaccgacat  2460
ctggctgatg tttgtgggct ccctgtgcgc cttcctgcac ggaatcgcac agccaggcgt  2520
gctgctgatc tttggcacca tgacagacgt gttcatcgac tatgatgtgg agctgcagga  2580
gctgcagatc cctggcaagg cctgcgtgaa caataccatc gtgtggacaa atagctccct  2640
gaaccagaat atgaccaacg gcacaagatg tggcctgctg aatatcgagt ctgagatgat  2700
caagtttgcc agctactatg caggaatcgc agtggccgtg ctgatcaccg gctacatcca  2760
gatctgcttc tgggtcatcg cagcagcaag gcagatccag aagatgagaa agttctattt  2820
tcggagaatc atgcggatgg agatcggctg gtttgactgt aactctgtgg gcgagctgaa  2880
tacaagattc agcgacgaca tcaacaagat caatgacgcc atcgccgatc agatggccct  2940
gtttatccag cggatgacca gcacaatctg tggcttcctg ctgggcttct ttagaggctg  3000
gaagctgacc ctggtcatca tctctgtgag cccactgatc ggaatcggag cagcaacaat  3060
cggcctgtcc gtgtctaagt tcaccgacta cgagctgaag gcatatgcaa aggcaggagt  3120
ggtggcagat gaagtgatca gcagcatgag gaccgtggca gcctttggcg gagagaagag  3180
ggaggtggag cggtacgaga agaacctggt gttcgcccag cggtggggca tcagaaaggg  3240
catcgtgatg ggcttcttta caggcttcgt gtggtgcctg atcttcctgt gctacgccct  3300
ggccttttgg tatggctcca ccctggtgct ggacgaggga gagtataccc ctggcacact  3360
ggtgcagatc ttcctgagcg tgatcgtggg cgccctgaac ctgggaaatg cctccccatg  3420
cctggaggcc tttgccacag gcagggcagc agccacctcc atcttcgaga caatcgaccg  3480
caagcccatc atcgactgta tgtctgagga tggctacaag ctggacagga tcaagggcga  3540
gatcgagttt cacaatgtga ccttccacta tcccagccgc cctgaggtga agatcctgaa  3600
cgatctgaat atggtcatca agccaggaga gatgacagcc ctggtgggac cctctggagc  3660
aggcaagagc accgccctgc agctgatcca gcggttttac gacccttgcg agggaatggt  3720
```

-continued

```
gaccgtggac ggacacgaca tcaggtccct gaacatccag tggctgcgcg atcagatcgg  3780
catcgtggag caggagccag tgctgttctc taccacaatc gccgagaata tcagatacgg  3840
ccgcgaggat gccacaatgg aggacatcgt gcaggccgcc aaggaggcca acgcctataa  3900
cttcatcatg gatctgcccc agcagttcga caccctggtg ggagagggag gaggacagat  3960
gtccggaggc cagaagcaga gagtggccat cgccagagcc ctgatccgca accctaagat  4020
cctgctgctg gatatggcca caagcgccct ggacaatgag tccgaggcca tggtgcagga  4080
ggtgctgagc aagatccagc acggccacac catcatctct gtggcacacc ggctgagcac  4140
agtgagagca gccgacacca tcatcggctt tgagcacgga acagcagtgg agaggggaac  4200
ccacgaggag ctgctggaga ggaagggcgt gtacttcacc ctggtgacac tgcagagcca  4260
gggcaaccag gccctgaatg aggaggacat caaggatgcc acagaggacg atatgctggc  4320
ccggaccttc tccagaggct cttatcagga ttctctgcgg gccagcatca ggcagcgcag  4380
caagtcccag ctgagctacc tggtgcacga gccacctctg gccgtggtgg accacaagtc  4440
cacctatgag gaggatagaa aggacaagga catccccgtg caggaggagg tggagcctgc  4500
cccagtgagg cgcatcctga agttttccgc cccagagtgg ccctacatgc tggtgggatc  4560
tgtgggagca gcagtgaacg gcaccgtgac accactgtat gccttcctgt tttcccagat  4620
cctgggcacc ttctctatcc ccgacaagga ggagcagagg tcccagatca atggcgtgtg  4680
cctgctgttt gtggccatgg gctgcgtgag cctgtttaca cagttcctgc agggctacgc  4740
cttcgccaag agcggcgagc tgctgaccaa gcggctgaga aagttcggct ttcgcgcaat  4800
gctgggacag gacatcgcat ggtttgacga tctgcggaac agcccaggcg ccctgaccac  4860
aagactggcc acagatgcct ctcaggtgca gggagcagca ggcagccaga tcggcatgat  4920
cgtgaactcc ttcaccaatg tgacagtggc catgatcatc gccttctctt ttagctggaa  4980
gctgtctctg gtcatcctgt gcttcttccc ctttctggcc ctgagcggag caacccagac  5040
aaggatgctg accggcttcg cctccagaga caagcaggcc ctggagatgg tgggccagat  5100
cacaaacgag gccctgagca atatcaggac cgtggcagga atcggcaagg agcggcggtt  5160
catcgaggcc ctggagacag agctggagaa gcctttcaag accgccatcc agaaggccaa  5220
catctacggc ttctgctttg ccttcgccca gtgtatcatg ttcatcgcca actccgcctc  5280
ttacagatat ggcggctacc tgatcagcaa tgagggcctg cacttttcct acgtgttcag  5340
agtgatcagc gccgtggtgc tgtctgccac agccctggga agggccttct cctacacccc  5400
atcttatgcc aaggccaaga tctctgccgc caggttcttt cagctgctgg accgccagcc  5460
acccatcagc gtgtacaaca cagccggcga gaagtgggat aatttccagg gcaagatcga  5520
ctttgtggat tgcaagttca cctatcctag caggccagac tcccaggtgc tgaacggcct  5580
gagcgtgtcc atctctccag gccagacact ggcctttgtg ggctcctctg gctgtggcaa  5640
gagcacctcc atccagctgc tggagaggtt ctacgacccc gatcagggca aagtgatgat  5700
cgacggccac gattctaaga aggtgaacgt gcagtttctg cgctccaata tcggcatcgt  5760
gtctcaggag cctgtgctgt tcgcctgcag catcatggat aacatcaagt acggcgacaa  5820
tacaaaggag atcccaatgg agagagtgat cgcagcagca aagcaggcac agctgcacga  5880
tttcgtgatg tccctgcccg agaagtatga gacaaacgtg ggcagccagg gctcccagct  5940
gtctaggggc gagaagcaga ggatcgcaat cgccagggcc atcgtgcgcg atcccaagat  6000
cctgctgctg gacgaggcca ccagcgccct ggatacagag tccgagaaga ccgtgcaggt  6060
ggccctggac aaggcccggg agggaagaac atgtatcgtg atcgcccacc gcctgagcac  6120
catccagaat gccgacatca tcgccgtgat ggcccagggc gtggtcatcg agaagggcac  6180
ccacgaggag ctgatggccc agaagggcgc ctactataag ctggtgacca caggcagccc  6240
tatctcctga tgagtcgact cgacaatcaa cctctggatt acaaaatttg tgaaagattg  6300
actggtattc ttaactatgt tgctcctttt acgctatgtg gatacgctgc tttaatgcct  6360
ttgtatcatg ctattgcttc ccgtatggct ttcattttct cctccttgta taaatcctgg  6420
ttgctgtctc tttatgagga gttgtggccc gttgtcaggc aacgtggcgt ggtgtgcact  6480
gtgtttgctg acgcaacccc cactggttgg ggcattgcca ccacctgtca gctcctttcc  6540
gggactttcg ctttcccccct ccctattgcc acggcggaac tcatcgccgc ctgccttgcc  6600
cgctgctgga caggggctcg gctgttgggc actgacaatt ccgtggtgtt gtcggggaaa  6660
tcatcgtcct ttccttggct gctcgcctgt gttgccacct ggattctgcg cgggacgtcc  6720
ttctgctacg tcccttcggc cctcaatcca gcggaccttc cttcccgcgg cctgctgccg  6780
gctctagata atccataaag taggaaacac tacacgattc cataaagtag gaaacactac  6840
aaccggttcc ataaagtagg aaacactaca tcactccata aagtaggaaa cactacaccc  6900
gggtcgagct cggtaccttt aagaccaatg acttacaagg cagctgtaga tcttagccac  6960
tttttaaaag aaaagggggg actggaaggg ctaattcact cccaacgaag acaagatctg  7020
ctttttgctt gtactgggtc tctctggtta gaccagatct gagcctggga gctctctggc  7080
taactaggga acccactgct taagcctcaa taaagcttgc cttgagtgct tcaagtagtg  7140
tgtgcccgtc tgttgtgtga ctctggtaac tagagatccc tcagaccctt ttagtcagtg  7200
tggaaaatct ctagca                                                  7216

SEQ ID NO: 37          moltype = AA  length = 1251
FEATURE                Location/Qualifiers
source                 1..1251
                       mol_type = protein
                       organism = unidentified
SEQUENCE: 37
MSTERDSETT FDEDSQPNDE VVPYSDDETE DELDDQGSAV EPEQNRVNRE AEENREPFRK  60
ECTWQVKAND RKYHEQPHFM NTKFLCIKES KYANNAIKTY KYNAFTFIPM NLFEQFKRAA  120
NLYFLALLIL QAVPQISTLA WYTTLVPLLV VLGVTAIKDL VDDVARHKMD KEINNRTCEV  180
IKDGRFKVAK WKEIQVGDVI RLKKNDFVPA DILLLSSSEP NSLCYVETAE LDGETNLKFK  240
MSLEITDQYL QREDTLATFD GFIECEEPNN RLDKFTGTLF WRNTSFPLDA DKILLRGCVI  300
RNTDFCHGLV IFAGADTKIM KNSGKTRFKR TKIDYLMNYM VYTIFVVLIL LSAGLAIGHA  360
YWEAQVGNSS WYLYDGEDDT PSYRGFLIFW GYIIVLNTMV PISLYVSVEV IRLGQSHFIN  420
WDLQMYYAEK DTPAKARTTT LNEQLGQIHY IFSDKTGTLT QNIMTFKKCC INGQIYGDHR  480
DASQHNHNKI EQVDFSWNTY ADGKLAFYDH YLIEQIQSGK EPEVRQFFFL LAVCHTVMVD  540
RTDGQLNYQA ASPDEGALVN AARNFGFAFL ARTQNTITIS ELGTERTYNV LAILDFNSDR  600
```

-continued

```
KRMSIIVRTP EGNIKLYCKG ADTVIYERLH RMNPTKQETQ DALDIFANET LRTLCLCYKE  660
IEEKEFTEWN KKFMAASVAS TNRDEALDKV YEEIEKDLIL LGATAIEDKL QDGVPETISK  720
LAKADIKIWV LTGDKKETAE NIGFACELLT EDTTICYGED INSLLHARME NQRNRGGVYA  780
KFAPPVQESF FPPGGNRALI ITGSWLNEIL LEKKTKRNKI LKLKFPRTEE ERRMRTQSKR  840
RLEAKKEQRQ KNFVDLACEC SAVICCRVTP KQKAMVVDLV KRYKKAITLA IGDGANDVNM  900
IKTAHIGVGI SGQEGMQAVM SSDYSFAQFR YLQRLLLVHG RWSYIRMCKF LRYFFYKNFA  960
FTLVHFWYSF FNGYSAQTAY EDWFITLYNV LYTSLPVLLM GLLDQDVSDK LSLRFPGLYI  1020
VGQRDLLFNY KRFFVSLLHG VLTSMILFFI PLGAYLQTVG QDGEAPSDYQ SFAVTIASAL  1080
VITVNFQIGL DTSYWTFVNA FSIFGSIALY FGIMFDFHSA GIHVLFPSAF QFTGTASNAL  1140
RQPYIWLTII LAVAVCLLPV VAIRFLSMTI WPSESDKIQK HRKRLKAEEQ WQRRQQVFRR  1200
GVSTRRSAYA FSHQRGYADL ISSGRSIRKK RSPLDAIVAD GTAEYRRTGD S           1251

SEQ ID NO: 38          moltype = AA  length = 3756
FEATURE                Location/Qualifiers
source                 1..3756
                       mol_type = protein
                       organism = unidentified
SEQUENCE: 38
ATGAGTACAG AAAGAGACTC AGAAACGACA TTTGACGAGG ATTCTCAGCC TAATGACGAA  60
GTGGTTCCCT ACAGTGATGA TGAAACAGAA GATGAACTTG ATGACCAGGG GTCTGCTGTT  120
GAACCAGAAC AAAACCGAGT CAACAGGGAA GCAGAGGAGA ACCGGGAGCC ATTCAGAAAA  180
GAATGTACAT GGCAAGTCAA AGCAAACGAT CGCAAGTACC ACGAACAACC TCACTTTATG  240
AACACAAAAT TCTTGTGTAT TAAGGAGAGT AAATATGCGA ATAATGCAAT TAAAACATAC  300
AAGTACAACG CATTTACCTT TATACCAATG AATCTGTTTG AGCAGTTTAA GAGAGCAGCC  360
AATTTATATT TCCTGGCTCT TCTTATCTTA CAGGCAGTTC CTCAAATCTC TACCCTGGCT  420
TGGTACACCA CACTAGTGCC CCTGCTTGTG GTGCTGGGCG TCACTGCAAT CAAAGACCTG  480
GTGGACGATG TGGCTCGCCA TAAAATGGAT AAGGAAATCA ACAATAGGAC GTGTGAAGTC  540
ATTAAGGATG GCAGGTTCAA AGTTGCTAAG TGGAAAGAAA TTCAAGTTGG AGACGTCATT  600
CGTCTGAAAA AAAATGATTT TGTTCCAGCT GACATTCTCC TGCTGTCTAG CTCTGAGCCT  660
AACAGCCTCT GCTATGTGGA AACAGCAGAA CTGGATGGAG AAACCAATTT AAAATTTAAG  720
ATGTCACTTG AAATCACAGA CCAGTACCTC CAAAGAGAAG ATACATTGGC TACATTTGAT  780
GGTTTTATTG AATGTGAAGA ACCCAATAAC AGACTAGATA AGTTTACAGG AACACTATTT  840
TGGAGAAACA CAAGTTTTCC TTTGGATGCT GATAAAATTT TGTTACGTGG CTGTGTAATT  900
AGGAACACCG ATTTCTGCCA CGGCTTAGTC ATTTTTGCAG GTGCTGACAC TAAAATAATG  960
AAGAATAGTG GGAAAACCAG ATTTAAAAGA ACTAAAATTG ATTACTTGAT GAACTACATG  1020
GTTTACACGA TCTTTGTTGT TCTTATTCTG CTTTCTGCTG GTCTTGCCAT CGGCCATGCT  1080
TATTGGGAAG CACAGGTGGG CAATTCCTCT TGGTACCTCT ATGATGGAGA AGACGATACA  1140
CCCTCCTACC GTGGATTCCT CATTTTCTGG GGCTATATCA TTGTTCTCAA CACCATGGTA  1200
CCCATCTCTC TCTATGTCAG CGTGGAAGTG ATTCGTCTTG GACAGAGTCA CTTCATCAAC  1260
TGGGACCTGC AAATGTACTA TGCTGAGAAG GACACACCCG CAAAAGCTAG AACCACCACA  1320
CTCAATGAAC AGCTCGGGCA GATCCATTAT ATCTTCTCTG ATAAGACGGG GACACTCACA  1380
CAAAATATCA TGACCTTTAA AAAGTGCTGT ATCAACGGGC AGATATATGG GGACCATCGG  1440
GATGCCTCTC AACACAACCA CAACAAAATA GAGCAAGTTG ATTTTAGCTG GAATACATAT  1500
GCTGATGGGA AGCTTGCATT TTATGACCAC TATCTTATTG AGCAAATCCA GTCAGGGAAA  1560
GAGCCAGAAG TACGACAGTT CTTCTTCTTG CTCGCAGTTT GCCACACAGT CATGGTGGAT  1620
AGGACTGATG GTCAGCTCAA CTACCAGGCA GCCTCTCCCG ATGAAGGTGC CCTGGTAAAC  1680
GCTGCCAGGA ACTTTGGCTT TGCCTTCCTC GCCAGGACCC AGAACACCAT CACCATCAGT  1740
GAACTGGGCA CTGAAAGGAC TTACAATGTT CTTGCCATTT TGGACTTCAA CAGTGACCGG  1800
AAGCGAATGT CTATCATTGT AAGAACCCCA GAAGGCAATA TCAAGCTTTA CTGTAAAGGT  1860
GCTGACACTG TTATTTATGA ACGGTTACAT CGAATGAATC CTACTAAGCA AGAAACACAG  1920
GATGCCCTGG ATATCTTTGC AAATGAAACT CTTAGAACCC TATGCCTTTG CTACAAGGAA  1980
ATTGAAGAAA AAGAATTTAC AGAATGGAAT AAAAAGTTTA TGGCTGCCAG TGTGGCCTCC  2040
ACCAACCGGG ACGAAGCTCT GGATAAAGTA TATGAGGAGA TTGAAAAAGA CTTAATTCTC  2100
CTGGGAGCTA CAGCTATTGA AGACAAGCTA CAGGATGGAG TTCCAGAAAC CATTTCAAAA  2160
CTTGCAAAAG CTGACATTAA GATCTGGGTG CTTACTGGAG ACAAAAAGGA AACTGCTGAA  2220
AATATAGGAT TTGCTTGTGA ACTTCTGACT GAAGACACCA CCATCTGCTA TGGGGAGGAT  2280
ATTAATTCTC TTCTTCATGC AAGGATGGAA AACCAGAGGA ATAGAGGTGG CGTCTACGCA  2340
AAGTTTGCAC CTCCTGTGCA GGAATCTTTT TTTCCACCCG GTGGAAACCG TGCCTTAATC  2400
ATCACTGGTT CTTGGTTGAA TGAAATTCTT CTCGAGAAAA AGACCAAGAG AAATAAGATT  2460
CTGAAGCTGA AGTTCCCAAG AACAGAAGAA GAAAGACGGA TGCGGACCCA AAGTAAAAGG  2520
AGGCTAGAAG CTAAGAAAGA GCAGCGGCAG AAAAACTTTG TGGACCTGGC CTGCGAGTGC  2580
AGCGCAGTCA TCTGCTGCCG CGTCACCCCC AAGCAGAAGG CCATGGTGGT GGACCTGGTG  2640
AAGAGGTACA AGAAAGCCAT CACGCTGGCC ATCGGAGATG GGGCCAATGA CGTGAACATG  2700
ATCAAAACTG CCCACATTGG CGTTGGAATA AGTGGACAAG AAGGAATGCA AGCTGTCATG  2760
TCGAGTGACT ATTCCTTTGC TCAGTTCCGA TATCTGCAGA GGCTACTGCT GGTGCATGGC  2820
CGATGGTCTT ACATAAGGAT GTGCAAGTTC CTACGATACT TCTTTTACAA AAACTTTGCC  2880
TTTACTTTGG TTCATTTCTG GTACTCCTTC TTCAATGGCT ACTCTGCGCA GACTGCATAC  2940
GAGGATTGGT TCATCACCCT CTACAACGTG CTGTACACCA GCCTGCCCGT GCTCCTCATG  3000
GGGCTGCTCG ACCAGGATGT GAGTGACAAA CTGAGCCTCC GATTCCCTGG GTTATACATA  3060
GTGGGACAAA GAGACTTACT ATTCAACTAT AAGAGATTCT TTGTAAGCTT GTTGCATGGG  3120
GTCCTAACAT CGATGATCCT CTTCTTCATA CCTCTTGGAG CTTATCTGCA AACCGTAGGG  3180
CAGGATGGAG AGGCACCTTC CGACTACCAG TCTTTTGCCG TCACCATTGC CTCTGCTCTT  3240
GTAATAACAG TCAATTTCCA GATTGGCTTG GATACTTCTT ATTGGACTTT TGTGAATGCT  3300
TTTTCAATTT TTGGAAGCAT TGCACTTTAT TTTGGCATCA TGTTTGACTT TCATAGTGCT  3360
GGAATACATG TTCTCTTTCC ATCTGCATTT CAATTTACAG GCACAGCTTC AAACGCTCTG  3420
AGACAGCCAT ACATTTGGTT AACTATCATC CTGGCTGTTG CTGTGTGCTT ACTACCCGTC  3480
```

-continued

```
GTTGCCATTC GATTCCTGTC AATGACCATC TGGCCATCAG AAAGTGATAA GATCCAGAAG  3540
CATCGCAAGC GGTTGAAGGC GGAGGAGCAG TGGCAGCGAC GGCAGCAGGT GTTCCGCCGG  3600
GGCGTGTCAA CGCGGCGCTC GGCCTACGCC TTCTCGCACC AGCGGGGCTA CGCGGACCTC  3660
ATCTCCTCCG GGCGCAGCAT CCGCAAGAAG CGCTCGCCGC TTGATGCCAT CGTGGCGGAT  3720
GGCACCGCGG AGTACAGGCG CACCGGGGAC AGCTGA                            3756

SEQ ID NO: 39          moltype = AA  length = 1321
FEATURE                Location/Qualifiers
source                 1..1321
                       mol_type = protein
                       organism = unidentified
SEQUENCE: 39
MSDSVILRSI KKFGEENDGF ESDKSYNNDK KSRLQDEKKG DGVRVGFFQL FRFSSSTDIW  60
LMFVGSLCAF LHGIAQPGVL LIFGTMTDVF IDYDVELQEL QIPGKACVNN TIVWTNSSLN  120
QNMTNGTRCG LLNIESEMIK FASYYAGIAV AVLITGYIQI CFWVIAAARQ IQKMRKFYFR  180
RIMRMEIGWF DCNSVGELNT RFSDDINKIN DAIADQMALF IQRMTSTICG FLLGFFRGWK  240
LTLVIISVSP LIGIGAATIG LSVSKFTDYE LKAYAKAGVV ADEVISSMRT VAAFGGEKRE  300
VERYEKNLVF AQRWGIRKGI VMGFFTGFVW CLIFLCYALA FWYGSTLVLD EGEYTPGTLV  360
QIFLSVIVGA LNLGNASPCL EAFATGRAAA TSIFETIDRK PIIDCMSEDG YKLDRIKGEI  420
EFHNVTFHYP SRPEVKILND LNMVIKPGEM TALVGPSGAG KSTALQLIQR FYDPCEGMVT  480
VDGHDIRSLN IQWLRDQIGI VEQEPVLFST TIAENIRYGR EDATMEDIVQ AAKEANAYNF  540
IMDLPQQFDT LVGEGGGQMS GGQKQRVAIA RALIRNPKIL LLDMATSALD NESEAMVQEV  600
LSKIQHGHTI ISVAHRLSTV RAADTIIGFE HGTAVERGTH EELLERKGVY FTLVTLQSQG  660
NQALNEEDIK DATEDDMLAR TFSRGSYQDS LRASIRQRSK SQLSYLVHEP PLAVVDHKST  720
YEEDRKDKDI PVQEEVEPAP VRRILKFSAP EWPYMLVGSV GAAVNGTVTP LYAFLFSQIL  780
GTFSIPDKEE QRSQINGVCL LFVAMGCVSL FTQFLQGYAF AKSGELLTKR LRKFGFRAML  840
GQDIAWFDDL RNSPGALTTR LATDASQVQG AAGSQIGMIV NSFTNVTVAM IIAFSFSWKL  900
SLVILCFFPF LALSGATQTR MLTGFASRDK QALEMVGQIT NEALSNIRTV AGIGKERRFI  960
EALETELEKP FKTAIQKANI YGFCFAFAQC IMFIANSASY RYGGYLISNE GLHFSYVFRV  1020
ISAVVLSATA LGRAFSYTPS YAKAKISAAR FFQLLDRQPP ISVYNTAGEK WDNFQGKIDF  1080
VDCKFTYPSR PDSQVLNGLS VSISPGQTLA FVGSSGCGKS TSIQLLERFY DPDQGKVMID  1140
GHDSKKVNVQ FLRSNIGIVS QEPVLFACSI MDNIKYGDNT KEIPMERVIA AAKQAQLHDF  1200
VMSLPEKYET NVGSQGSQLS RGEKQRIAIA RAIVRDPKIL LLDEATSALD TESEKTVQVA  1260
LDKAREGRTC IVIAHRLSTI QNADIIAVMA QGVVIEKGTH EELMAQKGAY YKLVTTGSPI  1320
S                                                                  1321

SEQ ID NO: 40          moltype = DNA  length = 3966
FEATURE                Location/Qualifiers
source                 1..3966
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 40
atgtctgact cagtaattct tcgaagtata aagaaatttg gagaggagaa tgatggtttt  60
gagtcagata aatcatataa taatgataag aaatcaaggt tacaagatga gaagaaaggt  120
gatggcgtta gagttggctt ctttcaattg tttcggtttt cttcatcaac tgacatttgg  180
ctgatgtttg tgggaagttt gtgtgcattt ctccatggaa tagcccagcc aggcgtgcta  240
ctcatttttg gcacaatgac agatgttttt attgactacg acgttgagtt acaagaactc  300
cagattccag gaaaagcatg tgtgaataac accattgtat ggactaacag ttccctcaac  360
cagaacatga caaatggaac acgttgtggg ttgctgaaca tcgagagcga aatgatcaaa  420
tttgccagtt actatgctgg aattgctgtc gcagtactta tcacaggata tattcaaata  480
tgcttttggg tcattgccgc agctcgtcag atacagaaaa tgagaaaatt ttactttagg  540
agaataatga gaatggaaat agggtggttt gactgcaatt cagtggggga gctgaataca  600
agattctctg atgatattaa taaaatcaat gatgccatag ctgaccaaat ggccctttc  660
attcagcgca tgacctcgac catctgtggt ttcctgttgg gatttttcag gggttggaaa  720
ctgaccttgg ttattatttc tgtcagccct ctcattggga ttggagcagc caccattggt  780
ctgagtgtgt ccaagtttac ggactatgag ctgaaggcct atgccaaagc aggggtggtg  840
gctgatgaag tcatttcatc aatgagaaca gtggctgctt ttggtggtga gaaaagagag  900
gttgaaaggt atgagaaaaa tcttgtgttc gcccagcgtt ggggaattag aaaaggaata  960
gtgatgggat tctttactgg attcgtgtgg tgtctcatct ttttgtgtta tgcactggcc  1020
ttctggtacg gctccacact tgtcctggat gaaggagaat atacaccagg aacccttgtc  1080
cagattttcc tcagtgtcat agtaggagct ttaaatcttg gcaatgcctc tccttgtttg  1140
gaagcctttg caactggacg tgcagcagcc accagcattt ttgagacaat agacaggaaa  1200
cccatcattg actgcatgtc agaagatggt tacaagttgg atcgaatcaa gggtgaaatt  1260
gagttccata atgtgacctt ccattatcct tccagaccag aggtgaagat tctaaatgac  1320
ctcaacatgg tcattaaacc aggggaaatg acagctctgg taggacccag tggagctgga  1380
aaaagtacag cactgcaact cattcagcga ttctatgacc cctgtgaagg aatggtgacc  1440
gtggatggcc atgacattcg ctctcttaac attcagtggc ttagagatca gattgggata  1500
gtggagcaag agccagttct gttctctacc accattgcag aaaatattcg ctatggcaga  1560
gaagatgcaa caatggaaga catagtccaa gctgccaagg aggccaatgc ctacaacttc  1620
atcatggacc tgccacagca atttgacacc cttgttggag aaggaggagg ccagatgagt  1680
ggtggccaga aacaaagggt agctatcgcc agagccctca tccgaaatcc caagattctg  1740
cttttggaca tggccacctc agctctggac aatgagagtg aagccatggt gcaagaagtg  1800
ctgagtaaga ttcagcatgg gcacacaatc atttcagttg ctcatcgctt gtctacggtc  1860
agagctgcag ataccatcat tggttttgaa catggcactg cagtggaaag agggacccat  1920
gaagaattac tggaaaggaa aggtgtttac ttcactctag tgactttgca aagccaggga  1980
aatcaagctc ttaatgaaga ggacataaag gatgcaactg aagatgacat gcttgcgagg  2040
```

-continued

```
acctttagca gagggagcta ccaggatagt ttaagggctt ccatccggca acgctccaag  2100
tctcagcttt cttacctggt gcacgaacct ccattagctg ttgtagatca taagtctacc  2160
tatgaagaag atagaaagga caaggacatt cctgtgcagg aagaagttga acctgcccca  2220
gttaggagga ttctgaaatt cagtgctcca gaatggccct acatgctggt agggtctgtg  2280
ggtgcagctg tgaacgggac agtcacaccc ttgtatgcct ttttattcag ccagattctt  2340
gggacttttt caattcctga taaagaggaa caaaggtcac agatcaatgg tgtgtgccta  2400
ctttttgtag caatgggctg tgtatctctt ttcacccaat ttctacaggg atatgccttt  2460
gctaaatctg gggagctcct aacaaaaagg ctacgtaaat ttggtttcag ggcaatgctg  2520
gggcaagata ttgcctggtt tgatgacctc agaaatagcc ctggagcatt gacaacaaga  2580
cttgctacag atgcttccca agttcaaggg gctgccggct ctcagatcgg gatgatagtc  2640
aattccttca ctaacgtcac tgtggccatg atcattgcct tctcctttag ctggaagctg  2700
agcctggtca tcttgtgctt cttccccttc ttggctttat caggagccac acagaccagg  2760
atgttgacag gatttgcctc tcgagataag caggccctgg agatggtggg acagattaca  2820
aatgaagccc tcagtaacat ccgcactgtt gctggaattg gaaaggagag gcggttcatt  2880
gaagcacttg agactgagct ggagaagccc ttcaagacag ccattcagaa agccaatatt  2940
tacggattct gctttgcctt tgcccagtgc atcatgttta ttgcgaactc tgcttcctac  3000
agatatggag gttacttaat ctccaatgag gggctccatt tcagctatgt gttcagggtg  3060
atctctgcag ttgtactgag tgcaacagct cttggaagag ccttctctta caccccaagt  3120
tatgcaaaag ctaaaatatc agctgcacgc ttttttcaac tgctggaccg acaaccccca  3180
atcagtgtat acaatactgc aggtgaaaaa tgggacaact tccaggggaa gattgatttt  3240
gttgattgta aatttacata tccttctcga cctgactcgc aagttctgaa tggtctctca  3300
gtgtcgatta gtccagggca gacactggcg tttgttggga gcagtggatg tggcaaaagc  3360
actagcattc agctgttgga acgtttctat gatcctgatc aagggaaggt gatgatagat  3420
ggtcatgaca gcaaaaaagt aaatgtccag ttcctccgct caaacattgg aattgtttcc  3480
caggaaccag tgttgtttgc ctgtagcata atggacaata tcaagtatgg agacaacacc  3540
aaagaaattc ccatggaaag agtcatagca gctgcaaaac aggctcagct gcatgatttt  3600
gtcatgtcac tcccagagaa atatgaaact aacgttgggt cccaggggtc tcaactctct  3660
agaggggaga aacaacgcat tgctattgct cgggccattg tacgagatcc taaaatcttg  3720
ctactagatg aagccacttc tgccttagac acagaaagtg aaaagacggt gcaggttgct  3780
ctagacaaag ccagagaggg tcggacctgc attgtcattg cccatcgctt gtccaccatc  3840
cagaacgcgg atatcattgc tgtcatggca cagggggtgg tgattgaaaa ggggacccat  3900
gaagaactga tggcccaaaa aggagcctac tacaaactag tcaccactgg atccccccatc  3960
agttga                                                             3966
```

SEQ ID NO: 41 moltype = DNA length = 3966
FEATURE Location/Qualifiers
source 1..3966
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 41

```
atgagcgact ccgtgatcct gcggagcatc aagaagtttg gcgaggagaa cgacggcttc  60
gagtctgata agagctacaa caatgataag aagtccagac tgcaggacga gaagaagggc  120
gatggcgtgc gggtgggctt ctttcagctg ttccggttca gcagcagcac cgacatctgg  180
ctgatgtttg tgggctccct gtgcgccttc ctgcacggaa tcgcacagcc aggcgtgctg  240
ctgatctttg gcaccatgac agacgtgttc atcgactatg atgtggagct gcaggagctg  300
cagatccctg gcaaggcctg cgtgaacaat accatcgtgt ggacaaatag ctccctgaac  360
cagaatatga ccaacggcac aagatgtggc ctgctgaata tcgagtctga gatgatcaag  420
tttgccagct actatgcagg aatcgcagtg gccgtgctga tcaccggcta catccagatc  480
tgcttctggg tcatcgcagc agcaaggcag atccagaaga tgagaaagtt ctattttcgg  540
agaatcatgc ggatggagat cggctggttt gactgtaact ctgtgggcga gctgaataca  600
agattcagcg acgacatcaa caagatcaat gacgccatcg ccgatcagat ggccctgttt  660
atccagcgga tgaccagcac aatctgtggc ttcctgctgg gcttctttag aggctggaag  720
ctgaccctgg tcatcatctc tgtgagccca ctgatcggaa tcggagcagc aacaatcggc  780
ctgtccgtgt ctaagttcac cgactacgag ctgaaggcat atgcaaaggc aggagtggtg  840
gcagatgaag tgatcagcag catgaggacc gtggcagcct ttggcggaga gaagagggag  900
gtggagcggt acgagaagaa cctggtgttc gcccagcggt ggggcatcag aaagggcatc  960
gtgatgggct tctttacagg cttcgtgtgg tgcctgatct tcctgtgcta cgccctggcc  1020
ttttggtatg gctccaccct ggtgctggac gagggagagt ataccccctgg cacactggtg  1080
cagatcttcc tgagcgtgat cgtgggcgcc ctgaacctgg gaaatgcctc cccatgcctg  1140
gaggcctttg ccacaggcag ggcagcagcc acctccatct tcgagacaat cgaccgcaag  1200
cccatcatcg actgtatgtc tgaggatggc tacaagctgg acaggatcaa gggcgagatc  1260
gagtttcaca atgtgacctt ccactatccc agccgccctg aggtgaagat cctgaacgat  1320
ctgaatatgg tcatcaagcc aggagagatg acagccctgg tgggaccctc tggagcaggc  1380
aagagcaccg ccctgcagct gatccagcgg ttttacgacc cttgcgaggg aatggtgacc  1440
gtggacggac acgacatcag gtccctgaac atccagtggc tgcgcgatca gatcggcatc  1500
gtggagcagg agccagtgct gttctctacc acaatcgccg agaatatcag atacggccgc  1560
gaggatgcca caatggagga catcgtgcag gccgccaagg aggccaacgc ctataacttc  1620
atcatggatc tgccccagca gttcgacacc ctggtgggag agggaggagg acagatgtcc  1680
ggaggccaga agcagagagt ggccatcgcc agagccctga tccgcaaccc taagatcctg  1740
ctgctggata tggccacaag cgccctggac aatgagtccg aggccatggt gcaggaggtg  1800
ctgagcaaga tccagcacgg ccacaccatc atctctgtgg cacaccggct gagcacagtg  1860
agagcagccg acaccatcat cggctttgag cacggaacag cagtggagag gggaacccac  1920
gaggagctgc tggagaggaa gggcgtgtac ttcaccctgg tgacactgca gagccagggc  1980
aaccaggccc tgaatgagga ggacatcaag gatgccacag aggacgatat gctggcccgg  2040
accttctcca gaggctctta tcaggattct ctgcgggcca gcatcaggca gcgcagcaag  2100
tcccagctga gctacctggt gcacgagcca cctctggccg tggtggacca caagtccacc  2160
```

-continued

```
tatgaggagg atagaaagga caaggacatc cccgtgcagg aggaggtgga gcctgcccca  2220
gtgaggcgca tcctgaagtt ttccgcccca gagtggccct acatgctggt gggatctgtg  2280
ggagcagcag tgaacggcac cgtgacacca ctgtatgcct tcctgttttc ccagatcctg  2340
ggcaccttct ctatccccga caaggaggag cagaggtccc agatcaatgg cgtgtgcctg  2400
ctgtttgtgg ccatgggctg cgtgagcctg tttacacagt tcctgcaggg ctacgccttc  2460
gccaagagcg gcgagctgct gaccaagcgg ctgagaaagt tcggctttcg cgcaatgctg  2520
ggacaggaca tcgcatggtt tgacgatctg cggaacagcc caggcgccct gaccacaaga  2580
ctggccacag atgcctctca ggtgcaggga gcagcaggca gccagatcgg catgatcgtg  2640
aactccttca ccaatgtgac agtggccatg atcatcgcct tctcttttag ctggaagctg  2700
tctctggtca tcctgtgctt cttccccttt ctggccctga gcggagcaac ccagacaagg  2760
atgctgaccg gcttcgcctc cagagacaag caggccctgg agatggtggg ccagatcaca  2820
aacgaggccc tgagcaatat caggaccgtg gcaggaatcg gcaaggagcg gcggttcatc  2880
gaggccctgg agacagagct ggagaagcct ttcaagaccg ccatccagaa ggccaacatc  2940
tacggcttct gctttgcctt cgcccagtgt atcatgttca tcgccaactc cgcctcttac  3000
agatatggcg gctacctgat cagcaatgag ggcctgcact tttcctacgt gttcagagtg  3060
atcagcgccg tggtgctgtc tgccacagcc ctgggaaggg ccttctccta caccccatct  3120
tatgccaagg ccaagatctc tgccgccagg ttctttcagc tgctggaccg ccagccaccc  3180
atcagcgtgt acaacacagc cggcgagaag tgggataatt tccagggcaa gatcgacttt  3240
gtggattgca agttcaccta tcctagcagg ccagactccc aggtgctgaa cggcctgagc  3300
gtgtccatct ctccaggcca gacactggcc tttgtgggct cctctggctg tggcaagagc  3360
acctccatcc agctgctgga gaggttctac gaccccgatc agggcaaagt gatgatcgac  3420
ggccacgatt ctaagaaggt gaacgtgcag tttctgcgct ccaatatcgg catcgtgtct  3480
caggagcctg tgctgttcgc ctgcagcatc atggataaca tcaagtacgg cgacaataca  3540
aaggagatcc caatggagag agtgatcgca gcagcaaagc aggcacagct gcacgatttc  3600
gtgatgtccc tgcccgagaa gtatgagaca aacgtgggca gccagggctc ccagctgtct  3660
aggggcgaga agcagaggat cgcaatcgcc agggccatcg tgcgcgatcc caagatcctg  3720
ctgctggacg aggccaccag cgccctggat acagagtccg agaagaccgt gcaggtggcc  3780
ctggacaagg cccgggaggg aagaacatgt atcgtgatcg cccaccgcct gagcaccatc  3840
cagaatgccg acatcatcgc cgtgatggcc cagggcgtgg tcatcgagaa gggcacccac  3900
gaggagctga tggcccagaa gggcgcctac tataagctgg tgaccacagg cagccctatc  3960
tcctga                                                             3966
```

```
SEQ ID NO: 42          moltype = DNA  length = 3966
FEATURE                Location/Qualifiers
source                 1..3966
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 42
atgagcgatt ctgtgatcct gcggagcatc aagaagttcg gcgaggaaaa cgacggcttc  60
gagagcgaca agagctacaa caacgacaag aagtcccggc tgcaggacga gaagaaaggc  120
gacggtgtca gagtgggctt cttccagctg ttccggttca gcagcagcac cgacatctgg  180
ctgatgttcg tgggcagcct gtgcgccttt ctgcacggaa ttgctcagcc tggcgtgctg  240
ctgatcttcg gcaccatgac cgacgtgttc atcgactacg acgtggaact gcaagagctg  300
cagatccctg gcaaggcctg cgtgaacaac accatcgtgt ggaccaacag cagcctgaac  360
cagaacatga ccaacggcac cagatgcggc ctgctgaata tcgagtccga gatgatcaag  420
ttcgccagct actacgccgg aatcgccgtg gccgttctga tcaccggcta catccagatc  480
tgcttttggg ttatcgccgc tgccagacag atccagaaga tgcggaagtt ctacttccgg  540
cggatcatgc ggatggaaat cggatggttc gactgcaaca gcgtgggcga gctgaacacc  600
agattcagcg acgacatcaa caagatcaac gacgccattg ccgaccagat ggccctgttc  660
atccagcgga tgacctctac catctgcggc tttctgctgg gctttttcag aggctggaag  720
ctgaccctgg tcatcatcag cgtgtcccca ctgatcggaa tcggagccgc cacaatcggc  780
ctgagcgtgt ccaagttcac cgattacgag ctgaaggcct acgccaaagc tggcgtggtg  840
gccgatgaag tgatcagctc catgagaacc gtggccgcct ttggcggcga gaagagagag  900
gtggaacgct acgagaaaaa cctggtgttc gcccagagat ggggcatcag aaagggcatc  960
gtgatgggat tcttcaccgg cttcgtgtgg tgcctgatct tcctgtgtta cgccctggcc  1020
ttttggtacg gcagcaccct ggttctggat gagggcgagt ataccccctgg aactctggtg  1080
cagatctttc tgagcgtgat cgtgggcgcc ctgaacctgg gaaatgcctc tccatgcctg  1140
gaagcctttg ccacaggcag agccgccgct accagcatct tcgagacaat cgacagaaag  1200
cccatcatcg actgcatgag cgaggacggc tacaagctgg acagaatcaa gggcgagatc  1260
gagttccaca acgtgacctt tcactacccc agcagacccg aagtgaagat cctgaacgac  1320
ctgaacatgg tcatcaagcc cggcgagatg acagccctcg ttggaccttc tggcgccgga  1380
aaatctacag ccctgcagct gatccagcgg ttctacgatc cttgcgaagg catggtcacc  1440
gtggacggcc acgacatcag atctctgaac atccagtggc tgcgggacca gatcggcatc  1500
gtggaacaag agcctgtgct gttcagcacc acaatcgccg agaacatcag atacggccgc  1560
gaggatgcca ccatggaaga tattgtgcag gccgctaaag aggccaacgc ctacaacttc  1620
atcatggacc tgcctcagca gttcgacacc ctcgtcggag aaggcggagg acaaatgtct  1680
ggcggccaga aacagagagt ggccattgct agagccctga tcagaaaccc caagatcctg  1740
ctgctggaca tggccacaag cgccctggat aatgagagcg aggccatggt gcaagaggtg  1800
ctgagcaaga tccagcacgg ccacaccatc atcagtgtgg cccacagact gagcaccgtg  1860
cgggctgccg ataccatcat cggatttgag cacggcaccg ccgtggaaag aggcacacac  1920
gaggaactgc tggaacggaa gggcgtgtac ttcaccctcg tgacactgca gagccagggc  1980
aatcaggccc tgaacgaaga ggacatcaag gacgccaccg aggacgacat gctggccaga  2040
acctttagca gaggcagcta ccaggacagc ctgcgggcct ctatcagaca gagaagcaag  2100
agccagctga gctacctggt gcacgaacct ccactggctg tggtggatca caagagcacc  2160
tacgaggaag atcggaagga caaggacatc cccgtgcaag aagaagtgga acccgctcct  2220
gtgcggcgga tcctgaaatt ttctgcccct gagtggccct acatgctcgt gggatctgtg  2280
```

-continued

```
ggagccgccg tgaatggaac agtgacccct ctgtacgcct tcctgttctc ccagatcctg  2340
ggcaccttca gcatccccga caaagaggaa cagcggagcc agatcaatgg cgtgtgcctg  2400
ctgtttgtgg ctatgggctg cgtgtccctg tttacccagt tcctgcaggg atacgccttc  2460
gccaagagcg gagagctgct gaccaagcgg ctgagaaagt tcggcttcag agccatgctc  2520
ggccaggata tcgcttggtt tgacgacctg agaaacagcc ctggcgctct gacaaccaga  2580
ctggccacag atgcttctca ggtgcaaggc gctgccggct ctcagatcgg aatgatcgtg  2640
aacagcttca ccaatgtgac cgtggccatg atcattgcct ttagcttcag ctggaaactg  2700
agcctcgtga tcctgtgctt cttcccattt ctggccctgt ccggcgctac ccagaccaga  2760
atgcttacag gcttcgccag cagagacaag caggccctgg aaatggtcgg acagatcacc  2820
aacgaggccc tgagcaacat cagaacagtg gccggcatcg gcaaagagcg gcggtttatt  2880
gaggctctgg aaaccgagct ggaaaaaccc ttcaagaccg ccatccagaa ggccaatatc  2940
tacggcttct gcttcgcctt cgctcagtgc atcatgttta tcgccaacag cgccagctat  3000
agatacggcg gctacctgat cagcaatgag ggcctgcact tcagctacgt gttccgcgtg  3060
atctctgccg tggtgctgtc tgccacagct ctgggcagag cattcagcta cacacccagc  3120
tacgccaagg ccaagattag cgccgccaga ttctttcagc tgctggatag acagcctcct  3180
atctccgtgt acaacaccgc tggcgagaag tgggacaact tccagggcaa gatcgacttc  3240
gtggattgca agttcacata cccctctcgg cccgacagcc aggtgctgaa tggactgtcc  3300
gtgtctatca gccccggaca gacactggcc tttgtgggaa gctctggctg cggcaaaagc  3360
accagcatcc agctgctcga gagattctac gaccccgacc agggcaaagt gatgatcgac  3420
ggacacgaca gcaagaaagt gaacgtgcag ttcctgagat ccaacatcgg gatcgtgtct  3480
caagaacccg tgctgtttgc ctgctccatc atggataaca ttaagtacgg cgacaacacc  3540
aaagaaatcc ccatggaaag agtgatcgct gccgccaaac aggcccagct gcacgatttc  3600
gtgatgagcc tgcctgagaa gtacgagaca aacgtgggct ctcagggcag ccagctgtct  3660
agaggcgaaa agcagcggat cgctatcgcc agagccatcg tgcgggatcc taagattctg  3720
ctcctggatg aagccaccag cgcactggac accgagtccg aaaagacagt gcaagtggcc  3780
ctggacaaag ccagagaggg cagaacctgt atcgtgatcg cccaccggct gagcacaatc  3840
cagaacgccg atattatcgc cgtgatggcc cagggcgtcg tgatcgaaaa gggaacccac  3900
gaagaactga tggctcagaa gggcgcctac tacaagctcg tgacaacagg cagccctatc  3960
tcctga                                                             3966
```

```
SEQ ID NO: 43          moltype = AA  length = 1286
FEATURE                Location/Qualifiers
source                 1..1286
                       mol_type = protein
                       organism = unidentified
SEQUENCE: 43
MDLEAAKNGT AWRPTSAEGD FELGISSKQK RKKTKTVKMI GVLTLFRYSD WQDKLFMSLG  60
TIMAIAHGSG LPLMMIVFGE MTDKFVDTAG NFSFPVNFSL SLLNPGKILE EEMTRYAYYY  120
SGLGAGVLVA AYIQVSFWTL AAGRQIRKIR QKFFHAILRQ EIGWFDINDT TELNTRLTDD  180
ISKISEGIGD KVGMFFQAVA TFFAGFIVGF IRGWKLTLVI MAISPILGLS AAVWAKILSA  240
FSDKELAAYA KAGAVAEEAL GAIRTVIAFG GQNKELERYQ KHLENAKEIG IKKAISANIS  300
MGIAFLLIYA SYALAFWYGS TLVISKEYTI GNAMTVFFSI LIGAFSVGQA APCIDAFANA  360
RGAAYVIFDI IDNNPKIDSF SERGHKPDSI KGNLEFNDVH FSYPSRANVK ILKGLNLKVQ  420
SGQTVALVGS SGCGKSTTVQ LIQRLYDPDE GTINIDGQDI RNFNVNYLRE IIGVVSQEPV  480
LFSTTIAENI CYGRGNVTMD EIKKAVKEAN AYEFIMKLPQ KFDTLVGERG AQLSGGQKQR  540
IAIARALVRN PKILLLDEAT SALDTESEAE VQAALDKARE GRTTIVIAHR LSTVRNADVI  600
AGFEDGVIVE QGSHSELMKK EGVYFKLVNM QTSGSQIQSE EFELNDEKAA TRMAPNGWKS  660
RLFRHSTQKN LKNSQMCQKS LDVETDGLEA NVPPVSFLKV LKLNKTEWPY FVVGTVCAIA  720
NGGLQPAFSV IFSEIIAIFG PGDDAVKQQK CNIFSLIFLF LGIISFFTFF LQGFTFGKAG  780
EILTRRLRSM AFKAMLRQDM SWFDDHKNST GALSTRLATD AAQVQGATGT RLALIAQNIA  840
NLGTGIIISF IYGWQLTLLL LAVVPIIAVS GIVEMKLLAG NAKRDKKELE AAGKIATEAI  900
ENIRTVVSLT QERKFESMYV EKLYGPYRNS VQKAHIYGIT FSISQAFMYF SYAGCFRFGA  960
YLIVNGHMRF RDVILVFSAI VFGAVALGHA SSFAPDYAKA KLSAAHLFML FERQPLIDSY  1020
SEEGLKPDKF EGNITFNEVV FNYPTRANVP VLQGLSLEVK KGQTLALVGS SGCGKSTVVQ  1080
LLERFYDPLA GTVFVDFGFQ LLDGQEAKKL NVQWLRAQLG IVSQEPILFD CSIAENIAYG  1140
DNSRVVSQDE IVSAAKAANI HPFIETLPHK YETRVGDKGT QLSGGQKQRI AIARALIRQP  1200
QILLLDEATS ALDTESEKVV QEALDKAREG RTCIVIAHRL STIQNADLIV VFQNGRVKEH  1260
GTHQQLLAQK GIYFSMVSVQ AGTQNL                                       1286
```

```
SEQ ID NO: 44          moltype = DNA  length = 3861
FEATURE                Location/Qualifiers
source                 1..3861
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 44
atggatcttg aggcggcaaa gaacggaaca gcctggcgcc ccacgagcgc ggagggcgac  60
tttgaactgg gcatcagcag caaacaaaaa aggaaaaaaa cgaagacagt gaaaatgatt  120
ggagtattaa cattgtttcg atactccgat tggcaggata aattgtttat gtcgctgggt  180
accatcatgg ccatagctca cggatcaggt ctccccctca tgatgatagt atttggagag  240
atgactgaca aatttgttga tactgcagga aacttctcct ttccagtgaa cttttccttg  300
tcgctgctaa atccaggcaa aattctggaa gaagaaatga ctagatatgc atattactac  360
tcaggattgg gtgctggagt tcttgttgct gcctatatac aagtttcatt ttggactttg  420
gcagctggtc gacagatcag gaaaattagg cagaagtttt ttcatgctat tctacgacag  480
gaaataggat ggtttgacat caacgacacc actgaactca atacgcggct aacagatgac  540
atctccaaaa tcagtgaagg aattggtgac aaggttggaa tgttctttca agcagtagcc  600
acgtttttg caggattcat agtgggattc atcagaggat ggaagctcac ccttgtgata  660
```

-continued

```
atggccatca gccctattct aggactctct gcagccgttt gggcaaagat actctcggca  720
tttagtgaca aagaactagc tgcttatgca aaagcaggcg ccgtggcaga agaggctctg  780
ggggccatca ggactgtgat agctttcggg ggccagaaca aagagctgga aaggtatcag  840
aaacatttag aaaatgccaa agagattgga attaaaaaag ctatttcagc aaacatttcc  900
atgggtattg ccttcctgtt aatatatgca tcatatgcac tggccttctg gtatggatcc  960
actctagtca tatcaaaaga atatactatt ggaaatgcaa tgacagtttt tttttcaatc  1020
ctaattggag ctttcagtgt tggccaggct gccccatgta ttgatgcttt tgccaatgca  1080
agaggagcag catatgtgat ctttgatatt attgataata atcctaaaat tgacagtttt  1140
tcagagagag gacacaaacc agacagcatc aaagggaatt tggagttcaa tgatgttcac  1200
ttttcttacc cttctcgagc taacgtcaag atcttgaagg gcctcaacct gaaggtgcag  1260
agtgggcaga cggtggccct ggttggaagt agtggctgtg ggaagagcac aacggtccag  1320
ctgatacaga ggctctatga ccctgatgag ggcacaatta acattgatgg gcaggatatt  1380
aggaacttta atgtaaacta tctgagggaa atcattggtg tggtgagtca ggagccggtg  1440
ctgttttcca ccacaattgc tgaaaatatt tgttatggcc gtggaaatgt aaccatggat  1500
gagataaaga aagctgtcaa agaggccaac gcctatgagt ttatcatgaa attaccacag  1560
aaatttgaca ccctggttgg agagagaggg gcccagctga gtggtgggca gaagcagagg  1620
atcgccattg cacgtgccct ggttcgcaac cccaagatcc ttctgctgga tgaggccacg  1680
tcagcattgg acacagaaag tgaagctgag gtacaggcag ctctggataa ggccagagaa  1740
ggccggacca ccattgtgat agcacaccga ctgtctacgg tccgaaatgc agatgtcatc  1800
gctgggtttg aggatggagt aattgtggag caaggaagcc acagcgaact gatgaagaag  1860
gaaggggtgt acttcaaact tgtcaacatg cagacatcag gaagccagat ccagtcagaa  1920
gaatttgaac taaatgatga aaaggctgcc actagaatgg ccccaaatgg ctggaaatct  1980
cgcctattta ggcattctac tcagaaaaac cttaaaaatt cacaaatgtg tcagaagagc  2040
cttgatgtgg aaaccgatgg acttgaagca aatgtgccac cagtgtcctt tctgaaggtc  2100
ctgaaactga ataaaacaga atggccctac tttgtcgtgg gaacagtatg tgccattgcc  2160
aatgggggc ttcagccggc attttcagtc atattctcag agatcatagc gatttttgga  2220
ccaggcgatg atgcagtgaa gcagcagaag tgcaacatat tctctttgat tttcttattt  2280
ctgggaatta tttctttttt tactttcttc cttcagggtt tcacgtttgg gaaagctggc  2340
gagatcctca ccagaagact gcggtcaatg gcttttaaag caatgctaag acaggacatg  2400
agctggtttg atgaccataa aaacagtact ggtgcacttt ctacaagact tgccacagat  2460
gctgcccaag tccaaggagc cacaggaacc aggttggctt taattgcaca gaatatagct  2520
aaccttggaa ctggtattat catatcattt atctacggtt ggcagttaac cctattgcta  2580
ttagcagttg ttccaattat tgctgtgtca ggaattgttg aaatgaaatt gttggctgga  2640
aatgccaaaa gagataaaaa agaactggaa gctgctggaa agattgcaac agaggcaata  2700
gaaaatatta ggacagttgt gtctttgacc caggaaagaa aatttgaatc aatgtatgtt  2760
gaaaaattgt atggacctta caggaattct gtgcagaagg cacacatcta tggaattact  2820
tttagtatct cacaagcatt tatgtatttt tcctatgccg gttgttttcg atttggtgca  2880
tatctcattg tgaatggaca tatgcgcttc agagatgtta ttctggtgtt ttctgcaatt  2940
gtatttggtg cagtggctct aggacatgcc agttcatttg ctccagacta tgctaaagct  3000
aagctgtctg cagcccactt attcatgctg tttgaaagac aacctctgat tgacagctac  3060
agtgaagagg ggctgaagcc tgataaattt gaaggaaata taacatttaa tgaagtcgtg  3120
ttcaactatc ccacccgagc aaacgtgcca gtgcttcagg ggctgagcct ggaggtgaag  3180
aaaggccaga cactagccct ggtgggcagc agtggctgtg ggaagagcac ggtggtccag  3240
ctcctggagc ggttctacga ccccttggcg gggacagtgt ttgtggactt tggttttcag  3300
cttctcgatg gtcaagaagc aaagaaactc aatgtccagt ggctcagagc tcaactcgga  3360
atcgtgtctc aggagcctat cctatttgac tgcagcattg ccgagaatat tgcctatgga  3420
gacaacagcc gggttgtatc acaggatgaa attgtgagtg cagccaaagc tgccaacata  3480
catcctttca tcgagacgtt accccacaaa tatgaaacaa gagtgggaga taaggggact  3540
cagctctcag gaggtcaaaa acagaggatt gctattgccc gagccctcat cagacaacct  3600
caaatcctcc tgttggatga agctacatca gctctggata ctgaaagtga aaaggttgtc  3660
caagaagccc tggacaaagc cagagaaggc cgcacctgca ttgtgattgc tcaccgcctg  3720
tccaccatcc agaatgcaga cttaatagtg gtgtttcaga atgggagagt caaggagcat  3780
ggcacgcatc agcagctgct ggcacagaaa ggcatctatt tttcaatggt cagtgtccag  3840
gctgggacac agaacttatg a                                            3861
```

```
SEQ ID NO: 45          moltype = AA  length = 1190
FEATURE                Location/Qualifiers
source                 1..1190
                       mol_type = protein
                       organism = unidentified
SEQUENCE: 45
MPVRGDRGFP PRRELSGWLR APGMEELIWE QYTVTLQKDS KRGFGIAVSG GRDNPHFENG  60
ETSIVISDVL PGGPADGLLQ ENDRVVMVNG TPMEDVLHSF AVQQLRKSGK VAAIVVKRPR  120
KVQVAALQAS PPLDQDDRAF EVMDEFDGRS FRSGYSERSR LNSHGGRSRS WEDSPERGRP  180
HERARSRERD LSRDRSRGRS LERGLDQDHA RTRDRSRGRS LERGLDHDFG PSRDRDRDRS  240
RGRSIDQDYE RAYHRAYDPD YERAYSPEYR RGARHDARSR GPRSRSREHP HSRSPSPEPR  300
GRPGPIGVLL MKSRANEEYG LRLGSQIFVK EMTRTGLATK DGNLHEGDII LKINGTVTEN  360
MSLTDARKLI EKSRGKLQLV VLRDSQQTLI NIPSLNDSDS EIEDISEIES NRSFSPEERR  420
HQYSDYDYHS SSEKLKERPS SREDTPSRLS RMGATPTPFK STGDIAGTVV PETNKEPRYQ  480
EDPPAPQPKA APRTFLRPSP EDEAIYGPNT KMVRFKKGDS VGLRLAGGND VGIFVAGIQE  540
GTSAEQEGLQ EGDQILKVNT QDFRGLVRED AVLYLLEIPK GEMVTILAQS RADVYRDILA  600
CGRGDSFFIR SHFECEKETP QSLAFTRGEV FRVVDTLYDG KLGNWLAVRI GNELEKGLIP  660
NKSRAEQMAS VQNAQRDNAG DRADFWRMRG QRSGVKKNLR KSREDLTAVV SVSTKFPAYE  720
RVLLREAGFK RPVVLFGPIA DIAMEKLANE LPDWFQTAKT EPKDAGSEKS TGVVRLNTVR  780
QIIEQDKHAL LDVTPKAVDL LNYTQWFPIV IFFNPDSRQG VKTMRQRLNP TSNKSSRKLF  840
DQANKLKKTC AHLFTATINL NSANDSWFGS LKDTIQHQQG EAVWVSEGKM EGMDDDPEDR  900
```

-continued

```
MSYLTAMGAD YLSCDSRLIS DFEDTDGEGG AYTDNELDEP AEEPLVSSIT RSSEPVQHEE  960
SIRKPSPEPR AQMRRAASSD QLRDNSPPPA FKPEPPKAKT QNKEESYDFS KSYEYKSNPS  1020
AVAGNETPGA STKGYPPPVA AKPTFGRSIL KPSTPIPPQE GEEVGESSEE QDNAPKSVLG  1080
KVKIFEKMDH KARLQRMQEL QEAQNARIEI AQKHPDIYAV PIKTHKPDPG TPQHTSSRPP  1140
EPQKAPSRPY QDTRGSYGSD AEEEEYRQQL SEHSKRGYYG QSARYRDTEL             1190

SEQ ID NO: 46          moltype = DNA  length = 3573
FEATURE                Location/Qualifiers
source                 1..3573
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 46
atgccggtgc gaggagaccg cgggtttcca ccccggcggg agctgtcagg ttggctccgc  60
gccccaggca tggaagagct gatatgggaa cagtacactg tgaccctaca aaaggattcc  120
aaaagaggat ttggaattgc agtgtccgga ggcagagaca acccccactt tgaaaatgga  180
gaaacgtcaa ttgtcatttc tgatgtgctc ccgggtgggc ctgctgatgg gctgctccaa  240
gaaaatgaca gagtggtcat ggtcaatggc acccccatgg aggatgtgct tcattcgttt  300
gcagttcagc agctcagaaa aagtgggaag gtcgctgcta ttgtggtcaa gaggccccgg  360
aaggtccagg tggccgcact tcaggccagc cctcccctgg atcaggatga ccgggctttt  420
gaggtgatgg acgagtttga tggcagaagt ttccggagtg gctacagcga gaggagccgg  480
ctgaacagcc atgggggcg cagccgcagc tgggaggaca gcccggaaag ggggcgtccc  540
catgagcggg cccggagccg ggagcgggac ctcagccggg accggagccg tggccggagc  600
ctggagcggg gcctggacca agaccatgcg cgcacccgag accgcagccg tggccggagc  660
ctggagcggg gcctggacca cgactttggg ccatcccggg accgggaccg tgaccgcagc  720
cgcggccgga gcattgacca ggactacgag cgagcctatc accgggccta cgacccagac  780
tacgagcggg cctacagccc ggagtacagg cgcggggccc gccacgatgc ccgctctcgg  840
ggaccccgaa gccgcagccg cgagcacccg cactcacgga gccccagccc cgagcctagg  900
gggcggccgg ggcccatcgg ggtcctcctg atgaaaagca gagcgaacga agagtatggt  960
ctccggcttg ggagtcagat cttcgtaaag gaaatgaccc gaacgggtct ggcaactaaa  1020
gatggcaacc ttcacgaagg agacataatt ctcaagatca atgggactgt aactgagaac  1080
atgtctttaa cggatgctcg aaaattgata gaaaagtcaa gaggaaaact acagctagtg  1140
gtgttgagag acagccagca gaccctcatc aacatcccgt cattaaatga cagtgactca  1200
gaaatagaag atatttcaga aatagagtca aaccgatcat tttctccaga ggagagacgt  1260
catcagtatt ctgattatga ttatcattcc tcaagtgaga agctgaagga aaggccaagt  1320
tccagagagg acacgccgag cagattgtcc aggatgggtg cgacacccac tccctttaag  1380
tccacagggg atattgcagg cacagttgtc ccagagacca acaaggaacc cagataccaa  1440
gaggaccccc cagctcctca accaaaagca gccccgagaa cttttcttcg tcctagtcct  1500
gaagatgaag caatatatgg ccctaatacc aaaatggtaa ggttcaagaa gggagacagc  1560
gtgggcctcc ggttggctgg tggcaatgat gtcgggatat ttgttgctgg cattcaagaa  1620
gggacctcgg cggagcagga gggccttcaa gaaggagacc agattctgaa ggtgaacaca  1680
caggatttca gaggattagt gcgggaggat gccgttctct acctgttaga aatccctaaa  1740
ggtgaaatgg tgaccatttt agctcagagc cgagccgatg tgtatagaga catcctggct  1800
tgtggcagag gggattcgtt ttttataaga agccactttg aatgtgagaa ggaaactcca  1860
cagagcctgg ccttcaccag aggggaggtc ttccgagtgg tagacacact gtatgacggc  1920
aagctgggca actggctggc tgtgaggatt gggaacgagt tggagaaagg cttaatcccc  1980
aacaagagca gagctgaaca aatggccagt gttcaaaatg cccagagaga caacgctggg  2040
gaccgggcag atttctggag aatgcgtggc cagaggtctg gggtgaagaa gaacctgagg  2100
aaaagtcggg aagacctcac agctgttgtg tctgtcagca ccaagttccc agcttatgag  2160
agggttttgc tgcgagaagc tggtttcaag agacctgtgg tcttattcgg ccccatagct  2220
gatatagcaa tggaaaaatt ggctaatgag ttacctgact ggtttcaaac tgctaaaacg  2280
gaaccaaaag atgcaggatc tgagaaatcc actggagtgg tccggttaaa taccgtgagg  2340
caaattattg aacaggataa gcatgcacta ctggatgtga ctccgaaagc tgtggacctg  2400
ttgaattaca cccagtggtt cccaattgtg atttttttca acccagactc cagacaaggt  2460
gtcaaaacca tgagacaaag gttaaatcca acgtccaaca aaagttctcg aaagttattt  2520
gatcaagcca acaagcttaa aaaaacgtgt gcacaccttt ttacagctac aatcaaccta  2580
aattcagcca atgatagctg gtttggcagc ttaaaggaca ctattcagca tcagcaagga  2640
gaagcggttt gggtctctga aggaaagatg gaagggatgg atgatgaccc cgaagaccgc  2700
atgtcctact taaccgccat gggcgcggac tatctgagtt gcgacagccg cctcatcagt  2760
gactttgaag acacggacgg tgaaggaggc gcctacactg acaatgagct ggatgagcca  2820
gccgaggagc cgctggtgtc gtccatcacc cgctcctcgg agccggtgca gcacgaggag  2880
agcataagga aacccagccc agagccacga gctcagatga ggagggctgc tagcagcgat  2940
caacttaggg acaatagccc gcccccagca ttcaagccag agccgcccaa ggccaaaacc  3000
cagaacaaag aagaatccta tgacttctcc aaatcctatg aatataagtc aaacccctct  3060
gccgttgctg gtaatgaaac tcctggggca tctaccaaag gttatcctcc tcctgttgca  3120
gcaaaaccta cctttgggcg gtctatactg aagccctcca ctcccatccc tcctcaagag  3180
ggtgaggagg tgggagagag cagtgaggag caagataatg ctcccaaatc agtcctgggc  3240
aaagtcaaaa tatttgagaa gatggatcac aaggccaggt tacagagaat gcaggagctc  3300
caggaagcac agaatgcaag gatcgaaatt gcccagaagc atcctgatat ctatgcagtt  3360
ccaatcaaaa cgcacaagcc agaccctggc acgccccagc acacgagttc cagacccccct  3420
gagccacaga aagctccttc cagaccttat caggatacca gaggaagtta tggcagtgat  3480
gccgaggagg aggagtaccg ccagcagctg tcagaacact ccaagcgcgg ttactatggc  3540
cagtctgccc gataccggga cacagaatta tag                               3573

SEQ ID NO: 47          moltype = AA  length = 486
FEATURE                Location/Qualifiers
source                 1..486
```

-continued

```
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 47
MVMQFQGLEN PIQISPHCSC TPSGFFMEMM SMKPAKGVLT EQVAGPLGQN LEVEPYSQYS  60
NVQFPQVQPQ ISSSSYYSNL GFYPQQPEEW YSPGIYELRR MPAETLYQGE TEVAEMPVTK  120
KPRMGASAGR IKGDELCVVC GDRASGYHYN ALTCEGCKGF FRRSITKNAV YKCKNGGNCV  180
MDMYMRRKCQ ECRLRKCKEM GMLAECMYTG LLTEIQCKSK RLRKNVKQHA DQTVNEDSEG  240
RDLRQVTSTT KSCREKTELT PDQQTLLHFI MDSYNKQRMP QEITNKILKE EFSAEENFLI  300
LTEMATNHVQ VLVEFTKKLP GFQTLDHEDQ IALLKGSAVE AMFLRSAEIF NKKLPSGHSD  360
LLEERIRNSG ISDEYITPMF SFYKSIGELK MTQEEYALLT AIVILSPDRQ YIKDREAVEK  420
LQEPLLDVLQ KLCKIHQPEN PQHFACLLGR LTELRTFNHH HAEMLMSWRV NDHKFTPLLC  480
EIWDVQ                                                             486

SEQ ID NO: 48           moltype = AA  length = 476
FEATURE                 Location/Qualifiers
source                  1..476
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 48
MGSKMNLIEH SHLPTTDEFS FSENLFGVLT EQVAGPLGQN LEVEPYSQYS NVQFPQVQPQ  60
ISSSSYYSNL GFYPQQPEEW YSPGIYELRR MPAETLYQGE TEVAEMPVTK KPRMGASAGR  120
IKGDELCVVC GDRASGYHYN ALTCEGCKGF FRRSITKNAV YKCKNGGNCV MDMYMRRKCQ  180
ECRLRKCKEM GMLAECMYTG LLTEIQCKSK RLRKNVKQHA DQTVNEDSEG RDLRQVTSTT  240
KSCREKTELT PDQQTLLHFI MDSYNKQRMP QEITNKILKE EFSAEENFLI LTEMATNHVQ  300
VLVEFTKKLP GFQTLDHEDQ IALLKGSAVE AMFLRSAEIF NKKLPSGHSD LLEERIRNSG  360
ISDEYITPMF SFYKSIGELK MTQEEYALLT AIVILSPDRQ YIKDREAVEK LQEPLLDVLQ  420
KLCKIHQPEN PQHFACLLGR LTELRTFNHH HAEMLMSWRV NDHKFTPLLC EIWDVQ      476

SEQ ID NO: 49           moltype = DNA  length = 1461
FEATURE                 Location/Qualifiers
source                  1..1461
                        mol_type = other DNA
                        organism = unidentified
SEQUENCE: 49
atggtaatgc agtttcaggg gttagaaaat ccaattcaaa ttagtcctca ctgcagctgt  60
acgccgtcag gatttttcat ggaaatgatg agtatgaagc ccgcgaaagg tgttttaaca  120
gaacaagtgg caggtcctct gggacagaac ctggaagtgg aaccatactc gcaatacagc  180
aatgttcagt ttccccaagt tcaaccacag atttcctcgt catcctatta ttccaacctg  240
ggtttctacc cccagcagcc tgaagagtgg tactctcctg gaatatatga actcaggcgt  300
atgccagctg agactctcta ccagggagaa actgaggtag cagagatgcc tgtaacaaag  360
aagccccgca tgggcgcgtc agcagggagg atcaaagggg atgagctgtg tgttgtttgt  420
ggagacagag cctctggata ccactataat gcactgacct gtgaggggtg taaaggtttc  480
ttcaggagaa gcattaccaa aaacgctgtg tacaagtgta aaaacggggg caactgtgtg  540
atggatatgt acatgcgaag aaagtgtcaa gagtgtcgac taaggaaatg caaagagatg  600
ggaatgttgg ctgaatgtat gtatacaggc ttgttaactg aaattcagtg taaatctaag  660
cgactgagaa aaaatgtgaa gcagcatgca gatcagaccg tgaatgaaga cagtgaaggt  720
cgtgacttgc gacaagtgac ctcgacaaca aagtcatgca gggagaaaac tgaactcacc  780
ccagatcaac agactcttct acattttatt atggattcat ataacaaaca gaggatgcct  840
caggaaataa caaataaaat tttaaaagaa gaattcagtg cagaagaaaa ttttctcatt  900
ttgacggaaa tggcaaccaa tcatgtacag gttcttgtag aattcacaaa aaagctacca  960
ggatttcaga ctttggacca tgaagaccag attgctttgc tgaaagggtc tgcggttgaa  1020
gctatgttcc ttcgttcagc tgagattttc aataagaaac ttccgtctgg gcattctgac  1080
ctattggaag aaagaattcg aaatagtggt atctctgatg aatatataac acctatgttt  1140
agtttttata aaagtattgg ggaactgaaa atgactcaag aggagtatgc tctgcttaca  1200
gcaattgtta tcctgtctcc agatagacaa tacataaagg atagagaggc agtagagaag  1260
cttcaggagc cacttcttga tgtgctacaa aagttgtgta agattcacca gcctgaaaat  1320
cctcaacact ttgcctgtct cctgggtcgc ctgactgaat tacggacatt caatcatcac  1380
cacgctgaga tgctgatgtc atggagagta aacgaccaca agtttacccc acttctctgt  1440
gaaatctggg acgtgcagtg a                                            1461

SEQ ID NO: 50           moltype = AA  length = 1848
FEATURE                 Location/Qualifiers
source                  1..1848
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 50
MSVGELYSQC TRVWIPDPDE VWRSAELTKD YKEGDKSLQL RLEDETILEY PIDVQRNQLP  60
FLRNPDILVG ENDLTALSYL HEPAVLHNLK VRFLESNHIY TYCGIVLVAI NPYEQLPIYG  120
QDVIYTYSGQ NMGDMDPHIF AVAEEAYKQM ARDEKNQSII VSGESGAGKT VSAKYAMRYF  180
ATVGGSASET NIEEKVLASS PIMEAIGNAK TTRNDNSSRF GKYIQIGFDK RYHIIGANMR  240
TYLLEKSRVV FQADDERNYH IFYQLCAAAG LPEFKELALT SAEDFFYTSQ GGDTSIEGVD  300
DAEDFEKTRQ AFTLLGVKES HQMSIFKIIA SILHLGSVAI QAERDGDSCS ISPQDVYLSN  360
FCRLLGVEHS QMEHWLCHRK LVTTSETYVK TMSLQQVINA RNALAKHIYA QLFGWIVEHI  420
NKALHTSLKQ HSFIGVLDIY GFETFEVNSF EQFCINYANE KLQQQFNSHV FKLEQEEYMK  480
EQIPWTLIDF YDNQPCIDLI EAKLGILDLL DEECKVPKGT DQNWAQKLYD RHSSSQHFQK  540
PRMSNTAFII VHFADKVEYL SDGFLEKNRD TVYEEQINIL KASKFPLVAD LFHDDKDPVP  600
```

-continued

```
ATTPGKGSSS KISVRSARPP MKVSNKEHKK TVGHQFRTSL HLLMETLNAT TPHYVRCIKP  660
NDEKLPFHFD PKRAVQQLRA CGVLETIRIS AAGYPSRWAY HDFFNRYRVL VKKRELANTD  720
KKAICRSVLE NLIKDPDKFQ FGRTKIFFRA GQVAYLEKLR ADKFRTATIM IQKTVRGWLQ  780
KVKYHRLKGA TLTLQRYCRG HLARRLAEHL RRIRAAVVLQ KHYRMQRARQ AYQRVRRAAV  840
VIQAFTRAMF VRRTYRQVLM EHKATTIQKH VRGWMARRHF QRLRDAAIVI QCAFRMLKAR  900
RELKALRIEA RSAEHLKRLN VGMENKVVQL QRKIDEQNKE FKTLSEQLSV TTSTYTMEVE  960
RLKKELVHYQ QSPGEDTSLR LQEEVESLRT ELQRAHSERK ILEDAHSREK DELRKRVADL 1020
EQENALLKDE KEQLNNQILC QSKDEFAQNS VKENLMKKEL EEERSRYQNL VKEYSQLEQR 1080
YDNLRDEMTI IKQTPGHRRN PSNQSSLESD SNYPSISTSE IGDTEDALQQ VEEIGLEKAA 1140
MDMTVFLKLQ KRVRELEQER KKLQVQLEKR EQQDSKKVQA EPPQTDIDLD PNADLAYNSL 1200
KRQELESENK KLKNDLNELR KAVADQATQN NSSHGSPDSY SLLLNQLKLA HEELEVRKEE 1260
VLILRTQIVS ADQRRLAGRN AEPNINARSS WPNSEKHVDQ EDAIEAYHGV CQTNSKTEDW 1320
GYLNEDGELG LAYQGLKQVA RLLEAQLQAQ SLEHEEEVEH LKAQLEALKE EMDKQQQTFC 1380
QTLLLSPEAQ VEFGVQQEIS RLTNENLDLK ELVEKLEKNE RKLKKQLKIY MKKAQDLEAA 1440
QALAQSERKR HELNRQVTVQ RKEKDFQGML EYHKEDEALL IRNLVTDLKP QMLSGTVPCL 1500
PAYILYMCIR HADYTNDDLK VHSLLTSTIN GIKKVLKKHN DDFEMTSFWL SNTCRLLHCL 1560
KQYSGDEGFM TQNTAKQNEH CLKNFDLTEY RQVLSDLSIQ IYQQLIKIAE GVLQPMIVSA 1620
MLENESIQGL SGVKPTGYRK RSSSMADGDN SYCLEAIIRQ MNAFHTVMCD QGLDPEIILQ 1680
VFKQLFYMIN AVTLNNLLLR KDVCSWSTGM QLRYNISQLE EWLRGRNLHQ SGAVQTMEPL 1740
IQAAQLLQLK KKTQEDAEAI CSLCTSLSTQ QIVKILNLYT PLNEFEERVT VAFIRTIQAQ 1800
LQERNDPQQL LLDAKHMFPV LFPFNPSSLT MDSIHIPACL NLEFLNEV              1848

SEQ ID NO: 51          moltype = DNA  length = 5547
FEATURE                Location/Qualifiers
source                 1..5547
                       mol_type = other DNA
                       organism = unidentified
SEQUENCE: 51
atgtcggtgg gcgagctcta cagccagtgc acaagggtct ggatccctga ccctgatgag   60
gtatggcgct cagctgagtt aaccaaggac tacaaagaag gagacaagag cctacagctc  120
agactggagg atgaaacgat tctggaatac ccaattgatg tacaacgcaa ccagctgccc  180
ttcttacgga atccagatat cttggtggga gaaaatgacc tgactgccct tagctatctt  240
catgagcctg cagttttgca taatttgaag gtccgtttcc tggagtccaa ccatatctac  300
acttactgtg gtatcgtact tgttgccatt aatccttatg aacagttgcc aatctatgga  360
caagatgtca tctataccta cagtggccaa aacatgggag acatggaccc ccacatcttt  420
gctgtggcag aagaagccta caagcagatg gccagagatg agaagaatca gtccatcata  480
gtcagtgggg agtctggagc cgggaagacg gtatcagcca agtatgccat gcgctatttc  540
gccaccgttg gtggctcggc cagtgaaacc aacatcgaag agaaggtgct ggcatccagt  600
cccatcatgg aggccattgg aaatgccaag accacccgca atgacaacag cagccgtttt  660
ggcaagtaca tccagattgg ctttgacaaa aggtaccaca tcatcggggc caacatgagg  720
acttacctct tggagaagtc cagagtggtc ttccaggcag atgatgagag gaattaccac  780
atcttttacc agctctgtgc tgctgccggt cttccagaat ttaaagagct tgcactaaca  840
agtgcagagg actttttcta tacatcacag ggaggagaca cttccatcga gggtgtggac  900
gatgctgagg actttgagaa gactcgacaa gccttcacac tcctcggagt gaaagagtcc  960
catcagatga gcatttttaa gataattgct tctatcttgc accttggaag tgtggcgatt 1020
caggctgagc gtgatggtga ttcctgtagt atatcacccc aggatgtata cctaagcaac 1080
ttctgccgac tgctaggggt ggagcacagt cagatggagc actggctgtg tcatcgcaag 1140
ctggtcacca cctcggagac ctacgtcaag accatgtccc tgcagcaggt gatcaatgcg 1200
cgcaacgccc tggcgaagca catctatgcc cagttgttcg gctggattgt ggagcacatc 1260
aacaaggccc tgcacacctc cctcaagcag cactccttca tcggggtcct ggacatctat 1320
gggtttgaga catttgaggt aaacagcttt gagcagttct gtatcaacta tgcaaatgaa 1380
aagctccagc agcagttcaa ctcgcatgtt ttcaaactgg agcaagaaga atacatgaag 1440
gaacagatcc cttggaccct gattgatttt tatgataacc aaccttgtat cgacctcatt 1500
gaagccaagc tgggtatctt ggacctgttg gatgaagaat gtaaggtccc caaaggaact 1560
gaccagaact gggctcagaa gctctatgac cggcactcca gcagccagca cttccagaag 1620
ccccgcatgt ccaacacggc cttcatcatc gtccactttg cagacaaggt ggagtacctc 1680
tctgatggtt ttctggagaa aaacagagac acggtgtatg aagagcagat caatatcctg 1740
aaggccagca agttcccact agtggctgac ttgtttcatg atgacaagga ccctgttcct 1800
gccaccaccc ctgggaaggg gtcatcttcg aagatcagcg tccgttctgc cagacccccc 1860
atgaaagtct ccaacaagga gcacaagaaa accgttggcc accagttccg tacctccctg 1920
catctgctca tggagaccct gaatgccacg acacctcact atgtccgctg catcaagccc 1980
aacgatgaga agctcccctt tcactttgac ccaaagagag cagtgcagca actcagagcc 2040
tgcggggtgt tggagacgat tcgaatcagt gcagctggct acccatccag gtgggcctac 2100
catgactttt tcaaccggta tcgggtgctg gtcaagaaga gagagctcgc caacacagac 2160
aaaaaggcca tctgcaggtc tgtcctggag aacctcatca aggaccccga caagttccag 2220
tttggccgca ccaagatctt ctttcgagca ggccaggtgg cctacctgga gaagctgcgg 2280
gctgacaagt tccggacagc caccatcatg atccagaaaa ctgtccgggg atggctgcag 2340
aaggtgaaat atcacaggct gaagggggct accttaaccc tgcagaggta ctgccgggga 2400
cacctggccc gcaggctggc tgagcacctg cggaggatca gagcggctgt ggtgctccag 2460
aaacattacc gcatgcagag ggcccgccag gcctaccaga gggtccgcag agctgccgtt 2520
gttatccagg ccttcacccg ggccatgttt gtgcggagaa cctaccgcca ggtcctcatg 2580
gagcacaagg ccaccaccat ccagaagcac gtgcggggct ggatggcacg caggcacttc 2640
cagcggctgc gggatgcagc cattgtcatc cagtgtgcct tccggatgct caaggccagg 2700
cgggagctga aggccctcag gattgaggcc cgctcagcag agcatctgaa acgtctcaac 2760
gtgggcatgg agaacaaggt ggtccagctg cagcggaaga tcgatgagca gaacaaagag 2820
ttcaagacac tttcagagca gttgtccgtg accacctcaa catacaccat ggaggtagag 2880
```

-continued

```
cggctgaaga aggagctggt gcactaccag cagagcccag gtgaggacac cagcctcagg  2940
ctgcaggagg aggtggagag cctgcgcaca gagctgcaga gggcccactc ggagcgcaag  3000
atcttggagg acgcccacag cagggagaaa gatgagctga ggaagcgagt tgcagacctg  3060
gagcaagaaa atgctctctt gaaagatgag aaagaacagc tcaacaacca aatcctgtgc  3120
cagtctaaag atgaatttgc ccagaactct gtgaaggaaa atctcatgaa gaaagaactg  3180
gaggaggagc gatcccggta ccagaacctt gtgaaggaat attcacagtt ggagcagaga  3240
tacgacaacc ttcgggatga aatgaccatc ataaagcaaa ctccaggtca taggcggaac  3300
ccatcaaacc aaagtagctt agaatctgac tccaattacc cctccatctc cacatctgag  3360
atcggagaca ctgaggatgc cctccagcag gtggaggaaa ttggcctgga gaaggcagcc  3420
atggacatga cggtcttcct gaagctgcag aagagagtac gggagctgga gcaggagagg  3480
aaaaagctgc aagtgcagct ggagaagaga gaacagcagg acagcaagaa agtccaggcg  3540
gaaccaccac agactgacat agatttggac ccgaatgcag atctggccta caatagtctg  3600
aagaggcaag agctggagtc agagaacaaa aagctgaaga atgacctgaa tgagctgagg  3660
aaagccgtgg ccgaccaagc cacgcagaat aactccagcc acggctcccc agatagctac  3720
agcctcctgc tgaaccagct caagctggcc cacgaggagc tcgaggtgcg caaggaggag  3780
gtgctcatcc tcaggaccca gatcgtgagc gccgaccagc ggcgactcgc cggcaggaac  3840
gcggagccga acattaatgc cagatcaagt tggcctaaca gtgaaaagca tgttgaccag  3900
gaggatgcca ttgaggccta tcacggggtc tgccagacaa acagcaagac tgaggattgg  3960
ggatatttaa atgaagatgg agaactcggc ttggcctacc aaggcctaaa gcaagttgcc  4020
aggctgctgg aggctcagct gcaggcccag agcctggagc atgaggagga ggtggagcat  4080
ctcaaggctc agctcgaggc cctgaaggag gagatggaca aacagcagca gaccttctgc  4140
cagacgctac tgctctcccc agaggcccag gtggaattcg gcgttcagca ggaaatatcc  4200
cggctgacca acgagaatct ggaccttaaa gaactggtag aaaagctgga aaagaatgag  4260
aggaagctca aaaagcaact gaagatttac atgaagaaag cccaggacct agaagctgcc  4320
caggcattgg cccagagtga gaggaagcgc catgagctca acaggcaggt cacggtccag  4380
cggaaagaga aggatttcca gggcatgctg gagtaccaca aagaggacga ggccctcctc  4440
atccggaacc tggtgacaga cttgaagccc cagatgctgt cgggcacagt gccctgtctc  4500
cccgcctaca tcctctacat gtgcatccgg cacgcggact acaccaacga cgatctcaag  4560
gtgcactccc tgctgacctc caccatcaac ggcattaaga aagtcctgaa aaagcacaat  4620
gatgactttg agatgacgtc attctggtta tccaacacct gccgccttct tcactgtctg  4680
aagcagtaca gcggggatga gggcttcatg actcagaaca ctgcaaagca gaatgaacac  4740
tgtcttaaga attttgacct caccgaatac cgtcaggtgc tgagtgacct ttccattcag  4800
atctaccagc agctcattaa aattgccgag ggcgtgttac agccgatgat agtttctgcc  4860
atgttggaaa atgagagcat tcagggtcta tctggtgtga agcccaccgg ctaccggaag  4920
cgctcctcca gcatggcaga tggggataac tcatactgcc tggaagctat catccgccag  4980
atgaatgcct ttcatacagt catgtgtgac cagggcttgg accctgagat catcctgcag  5040
gtattcaaac agctcttcta catgatcaac gcagtgactc ttaacaacct gctcttgcgg  5100
aaggacgtct gctcttggag cacaggcatg caactcaggt acaatataag tcagcttgag  5160
gagtggcttc ggggaagaaa ccttcaccag agtggagcag ttcagaccat ggaacctctg  5220
atccaagcag cccagctcct gcaattaaag aagaaaaccc aggaggacgc agaggctatc  5280
tgctccctgt gtacctccct cagcacccag cagattgtca aaattttaaa cctttatact  5340
cccctgaatg aatttgaaga acgggtaaca gtggccttta tacgaacaat ccaggcacaa  5400
ctacaagagc ggaatgaccc tcagcaactg ctattagatg ccaagcacat gtttcctgtt  5460
ttgtttccat ttaatccatc ttctctaacc atggactcaa tccacatccc agcgtgtctc  5520
aatctggaat tcctcaatga agtctga                                      5547

SEQ ID NO: 52          moltype = DNA  length = 18
FEATURE                Location/Qualifiers
source                 1..18
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 52
tactgacgct ctcgcacc                                                18

SEQ ID NO: 53          moltype = DNA  length = 17
FEATURE                Location/Qualifiers
source                 1..17
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 53
tctcgacgca ggactcg                                                 17

SEQ ID NO: 54          moltype = DNA  length = 20
FEATURE                Location/Qualifiers
source                 1..20
                       mol_type = other DNA
                       organism = synthetic construct
misc_feature           1
                       note = FAM linked at 5' terminus
SEQUENCE: 54
atctctctcc ttctagcctc                                              20

SEQ ID NO: 55          moltype = DNA  length = 20
FEATURE                Location/Qualifiers
source                 1..20
                       mol_type = other DNA
```

-continued

```
                        organism = synthetic construct
SEQUENCE: 55
accgattcca gatgattggc                                                20

SEQ ID NO: 56           moltype = DNA  length = 22
FEATURE                 Location/Qualifiers
source                  1..22
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 56
tccatattaa tgcagtgctt gc                                             22

SEQ ID NO: 57           moltype = DNA  length = 20
FEATURE                 Location/Qualifiers
source                  1..20
                        mol_type = other DNA
                        organism = synthetic construct
misc_feature            1
                        note = HEX linked at 5' terminus
SEQUENCE: 57
agaggcctgt cctgcagctc                                                20
```

1. An immune-shielded lentiviral vector comprising a nucleotide sequence encoding a progressive familial intrahepatic cholestasis (PFIC)-associated polypeptide.

2. The lentiviral vector according to claim **1**, wherein the PFIC-associated polypeptide is selected from familial intrahepatic cholestasis type 1 (FIC1), or a fragment thereof; bile salt export pump (BSEP), or a fragment thereof; multiple drug resistance 3 (MDR3), or a fragment thereof; tight junction protein 2 (TJP2), or a fragment thereof; farnesoid X receptor (FXR), or a fragment thereof; and Myosin-Vb (MYO5B), or a fragment thereof.

3. The lentiviral vector according to claim **1** or **2**, wherein the PFIC-associated polypeptide is selected from BSEP, or a fragment thereof; FXR, or a fragment thereof; and MYO5B, or a fragment thereof; preferably wherein the PFIC-associated polypeptide is BSEP, or a fragment thereof.

4. The lentiviral vector according to any preceding claim, wherein the PFIC-associated polypeptide comprises or consists of an amino acid sequence which is at least 70% identical to one of SEQ ID NOs: 37, 39, 43, 45, 47, 48 or 50, or a fragment thereof, preferably wherein the PFIC-associated polypeptide comprises or consists of an amino acid sequence which is at least 70% identical to SEQ ID NO: 39 or a fragment thereof.

5. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to one of SEQ ID NOs: 38, 40, 41, 42, 44, 46, 49 or 51, or a fragment thereof, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to one of SEQ ID NOs: 40-42, or a fragment thereof.

6. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is codon-optimised, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 41 or 42, or a fragment thereof.

7. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is a $CD47^{high}$ lentiviral vector.

8. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is obtained from a $CD47^{high}$ host cell, optionally wherein the host cell is genetically engineered to increase expression of CD47 on the cell surface.

9. The lentiviral vector according to any preceding claim, wherein the lentiviral vector has at least about 2-fold more CD47 on its surface than a lentiviral vector obtained from an unmodified host cell.

10. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is a $MHC\text{-}I^{free}$ lentiviral vector.

11. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is obtained from a $MHC\text{-}I^{free}$ host cell, optionally wherein the host cell is genetically engineered to disrupt expression of MHC-I on the cell surface.

12. The lentiviral vector according to any preceding claim, wherein MHC-I is not detectable on the surface of the lentiviral vector.

13. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is a $CD47^{high}/MHC\text{-}I^{free}$ lentiviral vector.

14. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is obtained from a $CD47^{high}/MHC\text{-}I^{free}$ host cell.

15. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to one or more miRNA target sequences.

16. The lentiviral vector according to claim **15**, wherein the one or more miRNA target sequences suppress transgene expression in one or more cells other than hepatocytes, preferably wherein the one or more miRNA target sequence suppress transgene expression in hematopoietic-lineage cells and/or antigen-presenting cells.

17. The lentiviral vector according to claim **15** or **16**, wherein the one or more miRNA target sequences are selected from miR-181, miR-142, miR-223, and miR-155 target sequences.

18. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to one or more

mir-142 target sequence, two or more mir-142 target sequences, three or more mir-142 target sequences, or four or more mir-142 target sequences.

19. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to four mir-142 target sequences.

20. The lentiviral vector according to any of claims **15-19**, wherein the one or more miRNA target sequences comprise or consist of a nucleotide sequence which is at least 90% identical to SEQ ID NO: 17, preferably wherein the one or more miRNA target sequences comprise or consist of a nucleotide sequence which is at least 90% identical to SEQ ID NO: 18.

21. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a liver-specific promoter, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a hepatocyte-specific promoter.

22. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a transthyretin (TTR) promoter, an alpha-1-antitrypsin (AAT) promoter, a thyroxine-binding globulin (TBG) promoter, a APoE/hAAT promoter, a HCR-hAAT promoter, a LP1 promoter, or a HLP promoter.

23. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a transthyretin (TTR) promoter, preferably wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a Enh1mTTR (ET) promoter.

24. The lentiviral vector according to any preceding claim, wherein the nucleotide sequence encoding a PFIC-associated polypeptide is operably linked to a promoter which comprises or consists of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 19.

25. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is pseudotyped, preferably wherein the lentiviral vector is VSV.G-pseudotyped.

26. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is a self-inactivating (SIN) lentiviral vector, preferably wherein the lentiviral vector comprises self-inactivating (SIN) LTRs which comprise or consist of a nucleotide sequence which is at least 70% identical to SEQ ID NO: 23, or a fragment thereof.

27. The lentiviral vector according to any preceding claim, wherein the lentiviral vector is an integrating lentiviral vector and/or a replication-defective lentiviral vector.

28. The lentiviral vector according to any preceding claim, wherein the lentiviral vector comprises a nucleotide sequence which is at least 70% identical to SEQ ID NO: 36.

29. An isolated cell comprising a lentiviral vector according to any of claims **1-28**.

30. A pharmaceutical composition comprising a lentiviral vector according to any of claims **1-28**, in combination with a pharmaceutically acceptable carrier, diluent or excipient.

31. The lentiviral vector according to any of claims **1-28** or pharmaceutical composition according to claim **30**, for use as a medicament.

32. Use of a lentiviral vector according to any of claims **1-28** or a pharmaceutical composition according to claim **30**, for the manufacture of a medicament.

33. A method comprising administering a therapeutically effective amount of a lentiviral vector according to any of claims **1-28** or a pharmaceutical composition according to claim **30**, to a subject in need thereof.

34. The lentiviral vector according to any of claims **1-28** or pharmaceutical composition according to claim **30**, for use in preventing or treating progressive familial intrahepatic cholestasis (PFIC).

35. Use of a lentiviral vector according to any of claims **1-28** or a pharmaceutical composition according to claim **30**, for the manufacture of a medicament for preventing or treating progressive familial intrahepatic cholestasis (PFIC).

36. A method of preventing or treating progressive familial intrahepatic cholestasis (PFIC), comprising administering a therapeutically effective amount of a lentiviral vector according to any of claims **1-28** or a pharmaceutical composition according to claim **30**, to a subject in need thereof.

37. The lentiviral vector or pharmaceutical composition for use according to claim **34**, the use according to claim **35**, or the method according to claim **36**, wherein the PFIC-associated polypeptide is FIC1, or a fragment thereof, and the PFIC is PFIC type 1 (PFIC-1); the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC type 2 (PFIC-2); the PFIC-associated polypeptide is MDR3, or a fragment thereof, and the PFIC is PFIC type 3 (PFIC-3); the PFIC-associated polypeptide is TJP2, or a fragment thereof, and the PFIC is PFIC type 4 (PFIC-4); the PFIC-associated polypeptide is FXR, or a fragment thereof, and the PFIC is PFIC type 5 (PFIC-5); or the PFIC-associated polypeptide is MYO5B, or a fragment thereof, and the PFIC is PFIC type 6 (PFIC-6).

38. The lentiviral vector or pharmaceutical composition for use according to claim **34** or **37**, the use according to claim **35** or **37**, or the method according to claim **36** or **37**, wherein the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC-2; the PFIC-associated polypeptide is FXR, or a fragment thereof, and the PFIC is PFIC-5; or the PFIC-associated polypeptide is MYO5B, or a fragment thereof, and the PFIC is PFIC-6, preferably wherein the PFIC-associated polypeptide is BSEP, or a fragment thereof, and the PFIC is PFIC-2.

39. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-38**, the use according to any of claims **32**, **35**, **37-38**, or the method according to any of claims **33**, **36-39**, wherein the subject is a human subject.

40. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-39**, the use according to any of claims **32**, **35**, **37-39**, or the method according to any of claims **33**, **36-39**, wherein the subject is a juvenile.

41. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-39**, the use according to any of claims **32**, **35**, **37-39**, or the method according to any of claims **33**, **36-39**, wherein the subject is a paediatric patient, preferably wherein the subject is a neonatal patient or an infantile patient.

42. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-41**, the use according to any of claims **32**, **35**, **37-41**, or the method according to any of claims **33**, **36-41**, wherein said lentiviral vector or said pharmaceutical composition is administered systemically, preferably wherein said lentiviral vector or

said pharmaceutical composition is administered by intravenous injection or intraperitoneal injection.

43. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-41**, the use according to any of claims **32**, **35**, **37-41**, or the method according to any of claims **33**, **36-41**, wherein said lentiviral vector or said pharmaceutical composition is administered locally, preferably wherein said lentiviral vector or said pharmaceutical composition is administered by direct injection, intra-arterial injection, or intraportal injection.

44. The lentiviral vector or pharmaceutical composition for use according to claim **43**, the use according to claim **43**, or the method according to claim **43**, wherein said lentiviral vector or said pharmaceutical composition is administered locally to the liver, preferably wherein said lentiviral vector or said pharmaceutical composition is administered by intrahepatic injection, intrahepatic arterial injection, or intraportal injection.

45. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-44**, the use according to any of claims **32**, **35**, **37-43**, or the method according to any of claims **33**, **36-44**, wherein the lentiviral vector is administered at a dose of at least about 10^8 TU/kg, at least about 10^9 TU/kg, or at least about 10^{10} TU/kg.

46. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-45**, the use according to any of claims **32**, **35**, **37-45**, or the method according to any of claims **33**, **36-45**, wherein the lentiviral vector is administered in a dose of from about 10^8 to about 10^{11} TU/kg, from about 10^8 to about 10^{10} TU/kg, or from about 10^9 to about 10^{10} TU/kg.

47. The lentiviral vector or pharmaceutical composition for use according to any of claims **31**, **34**, **37-46**, the use according to any of claims **32**, **35**, **37-46**, or the method according to any of claims **33**, **36-46**, wherein the lentiviral vector integrates into the genome of liver cells and is maintained as the liver cells duplicate, preferably wherein the lentiviral vector integrates into the genome of hepatocytes and is maintained as the hepatocytes duplicate.

48. The lentiviral vector or pharmaceutical composition for use according to any of claims **34**, **37-47**, the use according to any of claims **35**, **37-47**, or the method according to any of claims **36-47**, wherein serum bile acid levels are reduced and/or normalised.

49. The lentiviral vector or pharmaceutical composition for use according to any of claims **34**, **37-48**, the use according to any of claims **35**, **37-48**, or the method according to any of claims **36-48**, wherein the liver function is improved, preferably wherein the serum level of bilirubin and/or one or more liver enzymes is reduced and/or normalised.

50. The lentiviral vector or pharmaceutical composition for use according to any of claims **34**, **37-49**, the use according to any of claims **35**, **37-49**, or the method according to any of claims **36-49**, wherein the formation of liver fibrosis is slowed and/or reduced.

51. An immune-shielded lentiviral vector for use in a method of therapy, wherein the method comprises administration of the immune-shielded lentiviral vector to a juvenile or paediatric subject.

52. A cell for use in a method of therapy, wherein the cell comprises an immune-shielded lentiviral vector, and wherein the method comprises administration of the cell to a juvenile or paediatric subject.

53. The immune-shielded lentiviral vector or cell for use according to claim **51** or **52**, wherein the subject is a neonatal subject or an infantile subject.

* * * * *