



(51) International Patent Classification:

C12N 15/86 (2006.01)

(21) International Application Number:

PCT/GB2017/051023

(22) International Filing Date:

12 April 2017 (12.04.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1606717.5

18 April 2016 (18.04.2016)

GB

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: METHOD FOR PRODUCING RETROVIRAL VECTORS

(57) Abstract: The invention relates to an improved method producing retroviral vectors, the retroviral vectors produced using the method and uses of the vectors.



WO 2017/182774 A1

METHOD FOR PRODUCING RETROVIRAL VECTORS

Field of the Invention

The invention relates to an improved method of producing retroviral vectors, the
5 retroviral vectors produced using the method and uses of the vectors.

Background of the Invention

The development of retroviral-based therapeutics is hindered by the high costs of cGMP
manufacturing, which is a particular concern for applications focused on *in vivo* delivery where
10 both high viral titres and large volumes are typically required. The key cost component of viral
vector manufacturing is the production titre, which is typically several log-orders lower for
retroviral vectors than for non-enveloped vectors such as rAd or rAAV. Some progress has been
made towards the generation of producer cell lines to reduce costs. However, the majority of
effort to increase retroviral production has focused on optimisation and scale-up of transient
15 transfection-based processes, including: the selection of efficient gene transfer reagents, the ratio
of plasmid DNA components, the composition of cell culture media, supplementation with
growth substrates and bioreactor growth conditions (e.g. pH, O₂, lactate, etc). Intriguingly, little
attention has been paid to the optimisation of the mammalian host cell, with nearly all reported
processes relying on commonly available and, readily transfectable, derivatives of HEK 293T
20 cells.

Summary of the Invention

The inventors have surprisingly shown that an increased yield of retroviral vectors is
obtained by decreasing the expression of one or more of fourteen genes. Accordingly, the
25 present invention provides a method of producing a population of retroviral vectors, the method
comprising (1) expressing the retroviral vectors in a culture of human host cells in which the
level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1,
(d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1, (i) CD81, (j) UBE2I, (k) DLG1, (l)
SLFN11, (m) ABCA1 and (n) APOL1 is decreased or abolished and (2) allowing the population
30 of retroviral vectors to accumulate in the culture medium.

The invention also provides:

- a human host cell in which the level of expression and/or transcription of one or more
of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN,
(h) AXIN1, (i) CD81, (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n)
35 APOL1 is decreased;

- a culture of a plurality of human host cells of the invention;
- a retroviral vector produced using a method of the invention;
- a population of retroviral vectors produced using a method of the invention;
- a pharmaceutical composition comprising (a) a retroviral vector of the invention or a population of retroviral vectors of the invention and (b) a pharmaceutically acceptable carrier or diluent;
- a method of delivering a polynucleotide to a cell *in vitro*, comprising administering to the cell a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention, wherein the retroviral vector or retroviral vectors comprise the polynucleotide;
- a method of treating or preventing in a subject a disease which will be benefit from a therapeutic gene, comprising administering to the subject a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene;
- a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention for use in a method of treating or preventing in a subject a disease which will be benefit from a therapeutic gene, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene; and
- a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention for use in gene therapy.

Description of the Figures

Figure 1 is a schematic representation of the lentiviral vector production and titration protocols used during siRNA studies.

Figure 2 shows the effect of non-target siRNA on lentiviral production. LVR2-GFP lentiviral production from 293SF-LVP cells was assessed in 32-replicate wells of a 96-well dish, each well was transfected with a pool of non-target control siRNA under the conditions described in Figure 1 and the Methods. LVR2-GFP titre from each of the 32 replicates was determined as the mean of two independent titration studies. Normalised results from each well is plotted against nominal well number. The overall mean of the 32 wells is represented by the solid horizontal line. The dotted horizontal lines represent the mean $\pm$ two standard deviations of the mean. Normalised results from each well $\pm$ standard error of the mean from the four independent titration studies is plotted against nominal well number. No LVR2-GFP-mediated GFP expression was observed in well number 24.

Figure 3 shows a schematic showing the late phase HIV life cycle. Production of lentiviral vectors through producer cell lines or transient transfection implicates only the late steps of the HIV life cycle. This phase includes: (1) transcription of viral protein mRNA and the viral mRNA, (2) translation of viral protein mRNA to produce viral proteins, (3) initial assembly of viral particles from the viral mRNA and viral proteins, (4) late assembly of the viral particle at the producer cell membrane, (5) budding of the viral particle from the producer cell membrane, (6) maturation of the viral particle and (7) viral particle binding to and infection of a target cell.

Figure 4 shows the siRNA-mediated knockdown of host factors alters lentiviral vector production – Mean Data. The effect of siRNA-mediated knockdown of 108 producer cell factors on LVR2-GFP lentiviral production from 293SF-LVP cells was evaluated under the conditions described in Figure 1 and the Methods. LVR2-GFP titre was determined in four independent siRNA transfection studies each titrated in duplicate. Titre was normalised (100%) to that achieved with non-target control siRNA (solid horizontal line). Symbols represent normalised results $\pm$ standard error of the mean for each target host factor. The dashed and dotted/dashed horizontal lines represent the mean $\pm$ 40% and $\pm$ 30% of non-target control siRNA values respectively. Results are sorted into descending order of the mean values.

Figure 5 shows the siRNA-mediated knockdown of host factors alters lentiviral vector production – Individual Replicate Data. The effect of siRNA-mediated knockdown of 108 producer cell factors on LVR2-GFP lentiviral production from 293SF-LVP cells was evaluated under the conditions described in Figure 1 and the Methods. LVR2-GFP titre was determined in four independent siRNA transfection studies each titrated in duplicate. Titre was normalised (100%) to that achieved with non-target control siRNA (solid horizontal line). Symbols represent normalised mean result for each of the independent target host factor studies. The dashed and dotted/dashed horizontal lines represent the mean $\pm$ 40% and $\pm$ 30% of non-target control siRNA values respectively. Results are sorted into descending order of the mean values.

Figure 6 shows the candidate host factors identified as hits in the siRNA screen and their inferred acting step(s) in the late phase HIV life cycle. The fourteen genes whose siRNA knockdown increased increase lentiviral vector production $\geq 30\%$ compared with treatment with the non-target siRNA pool are indicated on the schematic near the step by which they are presumed to be active in the late steps of the HIV life cycle. Steps as described in Figure 3.

Figure 7 shows the siRNA-mediated knockdown of host factors that increase lentiviral vector production by $\geq 30\%$. Knockdown of 14 host factors was found to increase LVR2-GFP lentiviral production from 293SF-LVP cells 30% under the conditions described in Figure 1 and the Methods. LVR2-GFP titre was determined in four independent siRNA transfection studies

each titrated in duplicate. Titre was normalised (100%) to that achieved with non-target control siRNA (solid horizontal line). Symbols represent normalised results $\pm$ standard error of the mean for each target host factor. The dashed horizontal lines represent the mean $\pm$ 30% of non-target control siRNA values respectively. Results are sorted into descending order of the mean values. Data presented are a subset of the data presented in Figure 4 and Figure 5.

GenBank Entries for the Genes of Interest

The fourteen genes of interest in the invention (referred to herein as (a) to (n)) are known in the art. Their messenger RNA (mRNA) sequences may be found at the following GenBank Accession Numbers.

(a) CD63, gene 967, ENSG00000135404

NM_001257389.1

NM_001780.5

NM_001267698.1

NM_001257390.1

NM_001257391.1

NM_001257392.1

NM_001257400.1

NM_001257401.1

(b) ATP1A1, gene 476, ENSG00000163399

XM_006710655.2

NM_000701.7

NM_001160233.1

NM_001160234.1

(c) LIMK1, gene 3984, ENSG00000106683

NM_002314.3

NM_001204426.1

(d) DDX5, gene 1655, ENSG00000108654

NM_004396.3

XM_005257111.1

XM_006721738.1

XM_011524457.1

XM_011524456.1

(e) UBA7, gene 7318, ENSG00000182179

5 NM_003335.2

XM_011534072.1

XM_011534070.1

XM_011534071.1

XM_005265430.2

10 XM_006713321.2

XM_011534073.1

(f) DUSP1, gene 1843, ENSG00000120129

NM_004417.3

15

(g) SPN, gene 6693, ENSG00000197471

NM_001030288.2

NM_003123.

20 (h) AXIN1, gene 8312, ENSG00000103126

XM_011522687.1

XM_011522685.1

XM_011522684.1

NM_181050.2

25 NM_003502.3

XM_011522683.1

XM_011522682.1

XM_011522688.1

30 (i) CD81, gene 975, ENSG00000110651

NM_001297649.1

NM_004356.3

XM_011520492.1

(j) UBE2I, gene 7329, ENSG00000103275

NM_194260.2

NM_194261.2

XM_011522645.1

5 NM_194259.2

NM_003345.4

XM_005255540.2

XM_011522644.1

XM_005255541.3

10

(k) DLG1, gene 1739, ENSG00000075711

NM_001290983.1

NM_001204386.1

NM_001204386.1

15 NM_004087.2

NM_001204388.1

NM_001204387.1

XM_011512501.1

XM_011512495.1

20 XM_011512497.1

XM_011512506.1

XM_011512504.1

XM_011512505.1

XM_011512503.1

25 XM_011512502.1

XM_011512500.1

XM_011512499.1

XM_005269289.2

XM_011512493.1

30 XM_011512494.1

XM_011512496.1

XM_011512507.1

XM_005269301.3

XM_011512498.1

35 XM_011512508.1

XR_246039.2

XR_924108.1

XM_011512509.1

5 (l) SLFN11, gene 91607, ENSG00000172716

NM_001104590.1

NM_152270.3

NM_001104589.1

NM_001104588.1

10 NM_001104587.1

XM_005258068.2

XM_011525465.1

XM_006722171.2

15 (m) ABCA1, gene 19, ENSG00000165029

NM_005502.3

XM_011518342.1

XM_005251773.1

XM_011518339.1

20 XM_011518341.1

XM_005251776.1

XM_011518340.1

XM_011518343.1

XM_011518344.1

25

(n) APOL1, gene 8542, ENSG00000100342

NM_145343.2

NM_001136540.1

NM_003661.3

30 NM_001136541.1

XM_011530478.1

XM_005261796.2

Detailed Description of the Invention

It is to be understood that different applications of the disclosed products and methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

5 In addition as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a cell” includes two or more such cells, or reference to “a vector” includes two or more such vectors and the like.

All publications, patents and patent applications cited herein, whether supra or infra, are
10 hereby incorporated by reference in their entirety.

Method of producing a population of retroviral vectors

The invention concerns producing a population of retroviral vectors. The number of vectors in a population is discussed in more detail below. The population typically contains one
15 type of retroviral vector. There may be some minor variation between the vectors in the population. The population may comprise two or more different types of retroviral vectors.

Retroviral vectors and their productions are known in the art (Maetzig, Viruses. 2011 Jun;3(6):677-713. doi: 10.3390/v3060677. Epub 2011 Jun 3). The retroviral vectors may be replication-competent. The retroviral vectors are typically replication-defective or replication-
20 deficient. Replication-defective vectors typically have the coding regions for the genes necessary for additional rounds of virion replication and packaging deleted and/or replaced with other genes. These vectors are capable of entering their target cells and delivering their viral payload, but then fail to continue the typical lytic pathway that leads to cell lysis and death. Methods for creating replication-defective viral vectors are known in the art.

25 The method may concern producing any type of retroviral vector. The retroviral vectors produced using the invention are preferably derived from:

- Human Immunodeficiency Virus (HIV-1);
- Human Immunodeficiency Virus (HIV-2);
- Simian Immunodeficiency Virus (SIV);
- 30 - Foamy virus;
- Bovine Immunodeficiency Virus (BIV);
- Feline Immunodeficiency Virus (FIV);
- Equine Infectious Anemia Virus (EIAV);
- Murine Leukemia Virus (MLV);
- 35 - Bovine Leukemia Virus (BLV);

- Rous Sarcoma Virus (RSV);
- Spleen Necrosis Virus (SNV); or
- Mouse Mammary Tumor Virus (MMTV).

The retroviral vectors are modified versions of these viruses that are suitable for use as vectors. For instance, they may be replication-defective or replication-deficient as described above or comprise envelope proteins as discussed below. The vectors are not the viruses. Where the retroviral vector is a modified version of an HIV, it may be non-replicating and lack one or more or all of the HIV proteins Tat, Nef, Vif, Vpr and Vpu.

The retroviral vectors produced using the invention are more preferably derived from HIV-1, SIV, FIV, EIAV or MLV.

The retroviral vectors produced using the invention are preferably lentiviral vectors. Preferred lentiviral vectors are derived from HIV-1, HIV-2, SIV, BIV, FIV or EIAV.

Retroviruses enter cells by taking advantage of virus envelope protein/host cell receptor interactions. The retroviruses produced using the invention preferably comprise one or more of the following envelope proteins:

- Ecotropic MLV envelope;
- Amphotropic MLV envelope;
- Xenotropic MLV envelope;
- Vesicular Stomatitis Virus glycoprotein VSV-G;
- Simian Endogenous Retrovirus envelope RD114;
- Gibbon Ape Leukemia Virus envelope GALV;
- Measles Virus F and H proteins;
- Sendai virus F and HN proteins;
- Influenza virus HA and NA proteins;
- Baculovirus GP64 protein;
- Human Immunodeficiency Virus p120 envelope;
- Ebola virus envelope;
- Rabies virus envelope G protein; and
- Mokola virus envelope G protein.

The retroviral vectors produced using the invention typically comprise a non-native envelope protein. Thus, the envelope protein is not natively present in the virus from which the retroviral vector is derived. The envelope protein(s) comprised in the retroviral vectors may only be non-native envelope protein(s). The retroviral vectors may not comprise any native envelope protein. Where the retroviral vector is derived from an HIV, it may not comprise the Human Immunodeficiency Virus p120 envelope.

The retroviral vectors produced using the invention more preferably comprise one or more of the following envelope proteins:

- VSV-G;
- Measles Virus F and H proteins;
- 5 - Sendai virus F and HN proteins;
- Influenza virus HA and NA proteins; and
- Baculovirus GP64 protein.

The VSV-G envelope protein is particularly preferred. In some embodiments, the only envelope protein comprised in the retroviral vector may be a VSV-G envelope protein. Preferred
10 retroviral vectors are replication-defective or replication-deficient and comprise one or more the above preferred envelope proteins, most preferably VSV-G. Such preferred retroviral vectors typically do not comprise any native envelope protein.

The method of the invention is improved in the sense that it provides an increased yield of retroviral vectors. In other words, the population produced using the invention is larger than
15 the population produced in the absence of the one or more changes to genes (a) to (n) defined above. The invention therefore provides an improved method of producing a population of retroviral vectors. The invention provides a method of producing an increased amount of retroviral vectors or a larger population of retroviral vectors.

The amount of retroviral vectors produced in accordance with the invention may be
20 increased by any amount. The amount may be increased by at least 30%, 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%. The level of expression or transcription may be increased by at least a factor of 2, such as at least a factor of 3, at least a factor of 5 or at least a factor of 10. Genes (a) to (i) defined above were identified on the basis of an increase in retroviral vector yield of at least 40%. Genes (j) to (n)
25 were identified on the basis of an increase in retroviral vector yield of between 30% and 39%. Amounts may be increased further using combinations of one or more if (a) to (n) as set out below.

An increased amount is measured in comparison with a control culture of human host cells in which the expression and/or transcription of one or more of (a) to (n) has not been altered.
30 The host cells in the control culture are typically the same cells as in the culture used in the invention. The number of retroviral vectors may be measured as disclosed in the Examples.

The method of the invention typically produces between about 1×10^6 and about 1×10^7 retroviral vectors per ml of culture medium. The method of the invention preferably further comprises purifying and concentrating the retroviral vectors produced using the invention. This
35 can be done using any of the methods known in the art, such as using a sucrose cushion,

centrifugation, ultra-centrifugation, tangential flow filtration or anion exchange chromatography. Following purification and concentration, the method of the invention typically produces between about 1×10^8 and about 1×10^9 retroviral vectors per ml of medium.

The method of the invention is also improved in the sense that it may provide a population of retroviral vectors with a decreased particle:infectivity (P:I) ratio. In other words, a greater proportion of the retroviral vectors in the population produced using the invention are infective compared with the population produced in the absence of the changes to one or more of genes (a) to (n) defined above. The invention therefore provides method of producing a population of retroviral vectors with a decreased particle:infectivity (P:I) ratio.

The P:I ratio of the population produced in accordance with the invention may be decreased by any amount. The P:I ratio may be decreased by at least 10%, at least 20%, at least 30%, 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%. The P:I ratio of the population may be at least 5000, such as at least 4000, at least 3000, at least 2000, at least 1000, at least 900, at least 500, at least 100 or at least 10. The P:I ratio of the population may be 5000 or lower, such as 4000 or lower, 3000 or lower, 2000 or lower, 1000 or lower, 900 or lower, 500 or lower, 100 or lower or 10 or lower.

The particle:infectivity (P:I) ratio of a retroviral vector is typically calculated by dividing the particle titre by the number of transduction units in an identical volume of virus. The retroviral vector particle titre (VP/mL) is typically determined using real-time reverse transcriptase-PCR. Virus RNA may be purified using a QIAamp viral RNA mini-kit (QIAGEN, Strasse, Germany), and reverse transcribed using reverse transcriptase (Life Technologies). TaqMan quantitative PCR (Life Technologies) using an ABI PRISM 7700 Sequence Detector System (Life Technologies) and using primers amplifying a portion of the WPRE sequence (forward primer: 5'-ggctgttgggcactgacaa-3' (SEQ ID: 1), reverse primer: 5'-ccaaggaaaggacgatgatttc-3' (SEQ ID NO: 2), probe: FAM-5'-cgacaacaccacgga-3' (SEQ ID NO: 3)-TAMRA) may be used to determine the number of viral RNA copies. *In vitro* transcribed WPRE RNA molecules may be used as quantitative standards. The number of viral particles may be determined by assuming that each particle contained two copies of viral RNA.

Transduction units (TU/mL) are typically determined by transducing 293T/17 or Freestyle 293F cells with serial dilutions of a retroviral vector and quantification of WPRE containing provirus DNA by TaqMan quantitative PCR system (Life Technologies) using primers amplifying a portion of the WPRE sequence (forward primer: 5'-ggctgttgggcactgacaa-3' (SEQ ID NO: 4), reverse primer: 5'-ccaaggaaaggacgatgatttc-3' (SEQ ID NO: 5), probe: FAM-5'-cgacaacaccacgga-3' (SEQ ID NO: 6)-TAMRA) in an ABI PRISM 7700 Sequence Detector

System (Life Technologies). Plasmid DNA molecules containing WPRE sequences may be used as quantitative standards.

After retroviral vector production, but before purification and concentration, a typical preparation contains between 1×10^6 and 1×10^7 TU/mL. A purified and concentrated retrovirus preparation typically has a TU/mL of between 1×10^8 and 2×10^9 TU/mL with a P:I of between 500 and 2000.

Techniques for expressing retroviral vectors are known in the art. Polynucleotide sequences encoding the vectors may be included in one or more plasmids or one or more constructs. The one or more plasmids or the one or more constructs encoding the vectors may be expressed in the host cells. Any number of plasmids or constructs may be used.

Host cells

The method is carried out using a culture of human host cells. The culture of human host cells is *in vitro*. The method may be carried out using any number of cells, such as 2 or more, 5 or more, 10 or more, 100 or more, 1000 or more, 10^4 or more, 10^5 or more, 10^6 or more, 10^7 or more, 10^8 or more, 10^9 or more, or 10^{10} or more cells.

The cells are typically *in vitro*. The culture may be present in a culture flask, culture dish, or in the wells of a flat plate, such as a standard 6, 12, 24, 48, 96, 384 or 1536 well plate. Such plates are commercially available from Corning, Thermo Scientific, VWR, Greiner, Nunc, Starstedt or Falcon. The flask, dish or wells may be modified to facilitate culture of the cells, for instance by including a growth matrix. The flask, dish or wells may be modified to allow, or to prevent, attachment and immobilization of the one or more cells to the flask or wells. The surface(s) of the flask, dish or wells may be coated with Fc receptors, capture antibodies, avidin:biotin, lectins, polymers or any other capture chemicals that bind to the one or more cells and immobilize or capture them.

The culture may be present in an industrial culture vessel or bioreactor for the large-scale production of retroviruses.

Any culture volume may be used from μ l volumes up to several litres, such as 50L or more.

Conditions for culturing cells are known in the art. The cells may be cultured under a range of temperatures and CO₂ concentrations, though typically standard conditions of 37°C, 5-10% CO₂ in medium supplemented with or without serum. Suitable culture media are known in the art.

The human host cells are preferably human embryonic kidney (HEK) cells. Suitable HEK cells include, but are not limited to, HEK 293, HEK 293-6E, HEK 293A, HEK 293E, HEK

293F, HEK 293FT, HEK 293FTM, HEK 293S, HEK 293SF, HEK 293SG, HEK 293SGGD, HEK 293T, HEK 293T14, HEK 293T/17 and HEK 293TDMBR. Alternatively, other human cells, (such as HeLa cells) or non-human cells, such as CHO cells, may be used.

5 *Genes (a) to (n)*

The method of the invention uses human host cells in which the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1, (i) CD81, (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n) APOL1 is decreased or abolished. The level of expression of one or more of (a) to (n) may be decreased or abolished. The level of transcription of one or more of (a) to (n) may be decreased or abolished. The level of expression and level of transcription of one or more of (a) to (n) may be decreased or abolished.

The level of expression and/or transcription of any number and combination of (a) to (n) may be decreased or abolished. For instance, the level of expression and/or transcription of any of the following may be decreased or abolished (where combinations are contained within {} and commas equal “and”): {a}; {b}; {c}; {d}; {e}; {f}; {g}; {h}; {i}; {j}; {k}; {l}; {m}; {n}; {a,b}; {a,c}; {a,d}; {a,e}; {a,f}; {a,g}; {a,h}; {a,i}; {a,j}; {a,k}; {a,l}; {a,m}; {a,n}; {b,c}; {b,d}; {b,e}; {b,f}; {b,g}; {b,h}; {b,i}; {b,j}; {b,k}; {b,l}; {b,m}; {b,n}; {c,d}; {c,e}; {c,f}; {c,g}; {c,h}; {c,i}; {c,j}; {c,k}; {c,l}; {c,m}; {c,n}; {d,e}; {d,f}; {d,g}; {d,h}; {d,i}; {d,j}; {d,k}; {d,l}; {d,m}; {d,n}; {e,f}; {e,g}; {e,h}; {e,i}; {e,j}; {e,k}; {e,l}; {e,m}; {e,n}; {f,g}; {f,h}; {f,i}; {f,j}; {f,k}; {f,l}; {f,m}; {f,n}; {g,h}; {g,i}; {g,j}; {g,k}; {g,l}; {g,m}; {g,n}; {h,i}; {h,j}; {h,k}; {h,l}; {h,m}; {h,n}; {i,j}; {i,k}; {i,l}; {i,m}; {i,n}; {j,k}; {j,l}; {j,m}; {j,n}; {k,l}; {k,m}; {k,n}; {l,m}; {l,n}; {m,n}; {a,b,c}; {a,b,d}; {a,b,e}; {a,b,f}; {a,b,g}; {a,b,h}; {a,b,i}; {a,b,j}; {a,b,k}; {a,b,l}; {a,b,m}; {a,b,n}; {a,c,d}; {a,c,e}; {a,c,f}; {a,c,g}; {a,c,h}; {a,c,i}; {a,c,j}; {a,c,k}; {a,c,l}; {a,c,m}; {a,c,n}; {a,d,e}; {a,d,f}; {a,d,g}; {a,d,h}; {a,d,i}; {a,d,j}; {a,d,k}; {a,d,l}; {a,d,m}; {a,d,n}; {a,e,f}; {a,e,g}; {a,e,h}; {a,e,i}; {a,e,j}; {a,e,k}; {a,e,l}; {a,e,m}; {a,e,n}; {a,f,g}; {a,f,h}; {a,f,i}; {a,f,j}; {a,f,k}; {a,f,l}; {a,f,m}; {a,f,n}; {a,g,h}; {a,g,i}; {a,g,j}; {a,g,k}; {a,g,l}; {a,g,m}; {a,g,n}; {a,h,i}; {a,h,j}; {a,h,k}; {a,h,l}; {a,h,m}; {a,h,n}; {a,i,j}; {a,i,k}; {a,i,l}; {a,i,m}; {a,i,n}; {a,j,k}; {a,j,l}; {a,j,m}; {a,j,n}; {a,k,l}; {a,k,m}; {a,k,n}; {a,l,m}; {a,l,n}; {a,m,n}; {b,c,d}; {b,c,e}; {b,c,f}; {b,c,g}; {b,c,h}; {b,c,i}; {b,c,j}; {b,c,k}; {b,c,l}; {b,c,m}; {b,c,n}; {b,d,e}; {b,d,f}; {b,d,g}; {b,d,h}; {b,d,i}; {b,d,j}; {b,d,k}; {b,d,l}; {b,d,m}; {b,d,n}; {b,e,f}; {b,e,g}; {b,e,h}; {b,e,i}; {b,e,j}; {b,e,k}; {b,e,l}; {a,b,c,d}; {a,b,c,e}; {a,b,c,f}; {a,b,c,g}; {a,b,c,h}; {a,b,c,i}; {a,b,c,j}; {a,b,c,k}; {a,b,c,l}; {a,b,c,m}; {a,b,c,n}; {a,b,d,e}; {a,b,d,f}; {a,b,d,g}; {a,b,d,h}; {a,b,d,i}; {a,b,d,j}; {a,b,d,k}; {a,b,d,l}; {a,b,d,m}; {a,b,d,n}; {a,b,e,f}; {a,b,e,g}; {a,b,e,h}; {a,b,e,i}; {a,b,e,j}; {a,b,e,k}; {a,b,e,l}; {a,b,e,m};

- {a,b,e,n}; {a,b,f,g}; {a,b,f,h}; {a,b,f,i}; {a,b,f,j}; {a,b,f,k}; {a,b,f,l}; {a,b,f,m}; {a,b,f,n};
 {a,b,g,h}; {a,b,g,i}; {a,b,g,j}; {a,b,g,k}; {a,b,g,l}; {a,b,g,m}; {a,b,g,n}; {a,b,h,i}; {a,b,h,j};
 {a,b,h,k}; {a,b,h,l}; {a,b,h,m}; {a,b,h,n}; {a,b,i,j}; {a,b,i,k}; {a,b,i,l}; {a,b,i,m}; {a,b,i,n};
 {a,b,j,k}; {a,b,j,l}; {a,b,j,m}; {a,b,j,n}; {a,b,k,l}; {a,b,k,m}; {a,b,k,n}; {a,b,l,m}; {a,b,l,n};
 5 {a,b,m,n}; {a,c,d,e}; {a,c,d,f}; {a,c,d,g}; {a,c,d,h}; {a,c,d,i}; {a,c,d,j}; {a,c,d,k}; {a,c,d,l};
 {a,c,d,m}; {a,c,d,n}; {a,c,e,f}; {a,c,e,g}; {a,c,e,h}; {a,c,e,i}; {a,c,e,j}; {a,c,e,k}; {a,c,e,l};
 {a,c,e,m}; {a,c,e,n}; {a,c,f,g}; {a,c,f,h}; {a,c,f,i}; {a,c,f,j}; {a,c,f,k}; {a,c,f,l}; {a,c,f,m};
 {a,c,f,n}; {a,c,g,h}; {a,c,g,i}; {a,c,g,j}; {a,c,g,k}; {a,c,g,l}; {a,c,g,m}; {a,c,g,n}; {a,c,h,i};
 {a,c,h,j}; {a,c,h,k}; {a,c,h,l}; {a,c,h,m}; {a,c,h,n}; {a,c,i,j}; {a,c,i,k}; {a,c,i,l}; {a,c,i,m};
 10 {a,c,i,n}; {a,c,j,k}; {a,c,j,l}; {a,c,j,m}; {a,c,j,n}; {a,c,k,l}; {a,c,k,m}; {a,c,k,n}; {a,c,l,m};
 {a,c,l,n}; {a,c,m,n}; {a,d,e,f}; {a,d,e,g}; {a,d,e,h}; {a,d,e,i}; {a,d,e,j}; {a,d,e,k}; {a,d,e,l};
 {a,d,e,m}; {a,d,e,n}; {a,d,f,g}; {a,d,f,h}; {a,d,f,i}; {a,d,f,j}; {a,d,f,k}; {a,d,f,l}; {a,d,f,m};
 {a,d,f,n}; {a,d,g,h}; {a,d,g,i}; {a,d,g,j}; {a,d,g,k}; {a,d,g,l}; {a,d,g,m}; {a,d,g,n}; {a,d,h,i};
 {a,d,h,j}; {a,d,h,k}; {a,d,h,l}; {a,d,h,m}; {a,d,h,n}; {a,d,i,j}; {a,d,i,k}; {a,d,i,l}; {a,d,i,m};
 15 {a,d,i,n}; {a,d,j,k}; {a,d,j,l}; {a,d,j,m}; {a,d,j,n}; {a,d,k,l}; {a,d,k,m}; {a,d,k,n}; {a,d,l,m};
 {a,d,l,n}; {a,d,m,n}; {a,e,f,g}; {a,e,f,h}; {a,e,f,i}; {a,e,f,j}; {a,e,f,k}; {a,e,f,l}; {a,e,f,m};
 {a,e,f,n}; {a,e,g,h}; {a,e,g,i}; {a,e,g,j}; {a,e,g,k}; {a,e,g,l}; {a,e,g,m}; {a,e,g,n}; {a,e,h,i};
 {a,e,h,j}; {a,e,h,k}; {a,e,h,l}; {a,e,h,m}; {a,e,h,n}; {a,e,i,j}; {a,e,i,k}; {a,e,i,l}; {a,e,i,m};
 {a,e,i,n}; {a,e,j,k}; {a,e,j,l}; {a,e,j,m}; {a,e,j,n}; {a,e,k,l}; {a,e,k,m}; {a,e,k,n}; {a,e,l,m};
 20 {a,e,l,n}; {a,e,m,n}; {a,f,g,h}; {a,f,g,i}; {a,f,g,j}; {a,f,g,k}; {a,f,g,l}; {a,f,g,m}; {a,f,g,n};
 {a,f,h,i}; {a,f,h,j}; {a,f,h,k}; {a,f,h,l}; {a,f,h,m}; {a,f,h,n}; {a,f,i,j}; {a,f,i,k}; {a,f,i,l}; {a,f,i,m};
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 {a,g,i,l}; {a,g,i,m}; {a,g,i,n}; {a,g,j,k}; {a,g,j,l}; {a,g,j,m}; {a,g,j,n}; {a,g,k,l}; {a,g,k,m};
 25 {a,g,k,n}; {a,g,l,m}; {a,g,l,n}; {a,g,m,n}; {a,h,i,j}; {a,h,i,k}; {a,h,i,l}; {a,h,i,m}; {a,h,i,n};
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- {b,d,e,l}; {b,d,e,m}; {b,d,e,n}; {b,d,f,g}; {b,d,f,h}; {b,d,f,i}; {b,d,f,j}; {b,d,f,k}; {b,d,f,l};
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 {b,e,h,i}; {b,e,h,j}; {b,e,h,k}; {b,e,h,l}; {b,e,h,m}; {b,e,h,n}; {b,e,i,j}; {b,e,i,k}; {b,e,i,l};
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 {b,f,l,m}; {b,f,l,n}; {b,f,m,n}; {b,g,h,i}; {b,g,h,j}; {b,g,h,k}; {b,g,h,l}; {b,g,h,m}; {b,g,h,n};
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 15 {b,h,i,m}; {b,h,i,n}; {b,h,j,k}; {b,h,j,l}; {b,h,j,m}; {b,h,j,n}; {b,h,k,l}; {b,h,k,m}; {b,h,k,n};
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 {b,i,k,n}; {b,i,l,m}; {b,i,l,n}; {b,i,m,n}; {b,j,k,l}; {b,j,k,m}; {b,j,k,n}; {b,j,l,m}; {b,j,l,n};
 {b,j,m,n}; {b,k,l,m}; {b,k,l,n}; {b,k,m,n}; {b,l,m,n}; {c,d,e,f}; {c,d,e,g}; {c,d,e,h}; {c,d,e,i};
 {c,d,e,j}; {c,d,e,k}; {c,d,e,l}; {c,d,e,m}; {c,d,e,n}; {c,d,f,g}; {c,d,f,h}; {c,d,f,i}; {c,d,f,j};
 20 {c,d,f,k}; {c,d,f,l}; {c,d,f,m}; {c,d,f,n}; {c,d,g,h}; {c,d,g,i}; {c,d,g,j}; {c,d,g,k}; {c,d,g,l};
 {c,d,g,m}; {c,d,g,n}; {c,d,h,i}; {c,d,h,j}; {c,d,h,k}; {c,d,h,l}; {c,d,h,m}; {c,d,h,n}; {c,d,i,j};
 {c,d,i,k}; {c,d,i,l}; {c,d,i,m}; {c,d,i,n}; {c,d,j,k}; {c,d,j,l}; {c,d,j,m}; {c,d,j,n}; {c,d,k,l};
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 {c,e,f,k}; {c,e,f,l}; {c,e,f,m}; {c,e,f,n}; {c,e,g,h}; {c,e,g,i}; {c,e,g,j}; {c,e,g,k}; {c,e,g,l};
 25 {c,e,g,m}; {c,e,g,n}; {c,e,h,i}; {c,e,h,j}; {c,e,h,k}; {c,e,h,l}; {c,e,h,m}; {c,e,h,n}; {c,e,i,j};
 {c,e,i,k}; {c,e,i,l}; {c,e,i,m}; {c,e,i,n}; {c,e,j,k}; {c,e,j,l}; {c,e,j,m}; {c,e,j,n}; {c,e,k,l};
 {c,e,k,m}; {c,e,k,n}; {c,e,l,m}; {c,e,l,n}; {c,e,m,n}; {c,f,g,h}; {c,f,g,i}; {c,f,g,j}; {c,f,g,k};
 {c,f,g,l}; {c,f,g,m}; {c,f,g,n}; {c,f,h,i}; {c,f,h,j}; {c,f,h,k}; {c,f,h,l}; {c,f,h,m}; {c,f,h,n};
 {c,f,i,j}; {c,f,i,k}; {c,f,i,l}; {c,f,i,m}; {c,f,i,n}; {c,f,j,k}; {c,f,j,l}; {c,f,j,m}; {c,f,j,n}; {c,f,k,l};
 30 {c,f,k,m}; {c,f,k,n}; {c,f,l,m}; {c,f,l,n}; {c,f,m,n}; {c,g,h,i}; {c,g,h,j}; {c,g,h,k}; {c,g,h,l};
 {c,g,h,m}; {c,g,h,n}; {c,g,i,j}; {c,g,i,k}; {c,g,i,l}; {c,g,i,m}; {c,g,i,n}; {c,g,j,k}; {c,g,j,l};
 {c,g,j,m}; {c,g,j,n}; {c,g,k,l}; {c,g,k,m}; {c,g,k,n}; {c,g,l,m}; {c,g,l,n}; {c,g,m,n}; {c,h,i,j};
 {c,h,i,k}; {c,h,i,l}; {c,h,i,m}; {c,h,i,n}; {c,h,j,k}; {c,h,j,l}; {c,h,j,m}; {c,h,j,n}; {c,h,k,l};
 {c,h,k,m}; {c,h,k,n}; {c,h,l,m}; {c,h,l,n}; {c,h,m,n}; {c,i,j,k}; {c,i,j,l}; {c,i,j,m}; {c,i,j,n};
 35 {c,i,k,l}; {c,i,k,m}; {c,i,k,n}; {c,i,l,m}; {c,i,l,n}; {c,i,m,n}; {c,j,k,l}; {c,j,k,m}; {c,j,k,n};

- {c,j,l,m}; {c,j,l,n}; {c,j,m,n}; {c,k,l,m}; {c,k,l,n}; {c,k,m,n}; {c,l,m,n}; {d,e,f,g}; {d,e,f,h};
 {d,e,f,i}; {d,e,f,j}; {d,e,f,k}; {d,e,f,l}; {d,e,f,m}; {d,e,f,n}; {d,e,g,h}; {d,e,g,i}; {d,e,g,j};
 {d,e,g,k}; {d,e,g,l}; {d,e,g,m}; {d,e,g,n}; {d,e,h,i}; {d,e,h,j}; {d,e,h,k}; {d,e,h,l}; {d,e,h,m};
 {d,e,h,n}; {d,e,i,j}; {d,e,i,k}; {d,e,i,l}; {d,e,i,m}; {d,e,i,n}; {d,e,j,k}; {d,e,j,l}; {d,e,j,m};
 5 {d,e,j,n}; {d,e,k,l}; {d,e,k,m}; {d,e,k,n}; {d,e,l,m}; {d,e,l,n}; {d,e,m,n}; {d,f,g,h}; {d,f,g,i};
 {d,f,g,j}; {d,f,g,k}; {d,f,g,l}; {d,f,g,m}; {d,f,g,n}; {d,f,h,i}; {d,f,h,j}; {d,f,h,k}; {d,f,h,l};
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- {a,c,d,l,n}; {a,c,d,m,n}; {a,c,e,f,g}; {a,c,e,f,h}; {a,c,e,f,i}; {a,c,e,f,j}; {a,c,e,f,k}; {a,c,e,f,l};
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 {a,d,h,k,l}; {a,d,h,k,m}; {a,d,h,k,n}; {a,d,h,l,m}; {a,d,h,l,n}; {a,d,h,m,n}; {a,d,i,j,k}; {a,d,i,j,l};
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- {a,e,g,i,n}; {a,e,g,j,k}; {a,e,g,j,l}; {a,e,g,j,m}; {a,e,g,j,n}; {a,e,g,k,l}; {a,e,g,k,m}; {a,e,g,k,n};
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 15 {c,d,e,f,j}; {c,d,e,f,k}; {c,d,e,f,l}; {c,d,e,f,m}; {c,d,e,f,n}; {c,d,e,g,h}; {c,d,e,g,i}; {c,d,e,g,j};
 {c,d,e,g,k}; {c,d,e,g,l}; {c,d,e,g,m}; {c,d,e,g,n}; {c,d,e,h,i}; {c,d,e,h,j}; {c,d,e,h,k}; {c,d,e,h,l};
 {c,d,e,h,m}; {c,d,e,h,n}; {c,d,e,i,j}; {c,d,e,i,k}; {c,d,e,i,l}; {c,d,e,i,m}; {c,d,e,i,n}; {c,d,e,j,k};
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 20 {c,d,f,h,i}; {c,d,f,h,j}; {c,d,f,h,k}; {c,d,f,h,l}; {c,d,f,h,m}; {c,d,f,h,n}; {c,d,f,i,j}; {c,d,f,i,k};
 {c,d,f,i,l}; {c,d,f,i,m}; {c,d,f,i,n}; {c,d,f,j,k}; {c,d,f,j,l}; {c,d,f,j,m}; {c,d,f,j,n}; {c,d,f,k,l};
 {c,d,f,k,m}; {c,d,f,k,n}; {c,d,f,l,m}; {c,d,f,l,n}; {c,d,f,m,n}; {c,d,g,h,i}; {c,d,g,h,j}; {c,d,g,h,k};
 {c,d,g,h,l}; {c,d,g,h,m}; {c,d,g,h,n}; {c,d,g,i,j}; {c,d,g,i,k}; {c,d,g,i,l}; {c,d,g,i,m}; {c,d,g,i,n};
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 25 {c,d,g,l,n}; {c,d,g,m,n}; {c,d,h,i,j}; {c,d,h,i,k}; {c,d,h,i,l}; {c,d,h,i,m}; {c,d,h,i,n}; {c,d,h,j,k};
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 {c,d,j,m,n}; {c,d,k,l,m}; {c,d,k,l,n}; {c,d,k,m,n}; {c,d,l,m,n}; {c,e,f,g,h}; {c,e,f,g,i}; {c,e,f,g,j};
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 {d,f,g,j,k}; {d,f,g,j,l}; {d,f,g,j,m}; {d,f,g,j,n}; {d,f,g,k,l}; {d,f,g,k,m}; {d,f,g,k,n}; {d,f,g,l,m};
 {d,f,g,l,n}; {d,f,g,m,n}; {d,f,h,i,j}; {d,f,h,i,k}; {d,f,h,i,l}; {d,f,h,i,m}; {d,f,h,i,n}; {d,f,h,j,k};
 35 {d,f,h,j,l}; {d,f,h,j,m}; {d,f,h,j,n}; {d,f,h,k,l}; {d,f,h,k,m}; {d,f,h,k,n}; {d,f,h,l,m}; {d,f,h,l,n};

{d,f,h,m,n}; {d,f,i,j,k}; {d,f,i,j,l}; {d,f,i,j,m}; {d,f,i,j,n}; {d,f,i,k,l}; {d,f,i,k,m}; {d,f,i,k,n};
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 10 {a,b,c,d,e,m}; {a,b,c,d,e,n}; {a,b,c,d,f,g}; {a,b,c,d,f,h}; {a,b,c,d,f,i}; {a,b,c,d,f,j}; {a,b,c,d,f,k};
 {a,b,c,d,f,l}; {a,b,c,d,f,m}; {a,b,c,d,f,n}; {a,b,c,d,g,h}; {a,b,c,d,g,i}; {a,b,c,d,g,j}; {a,b,c,d,g,k};
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 15 {a,b,c,d,l,m}; {a,b,c,d,l,n}; {a,b,c,d,m,n}; {a,b,c,e,f,g}; {a,b,c,e,f,h}; {a,b,c,e,f,i}; {a,b,c,e,f,j};
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 35 {a,b,f,l,m,n}; {a,b,g,h,i,j}; {a,b,g,h,i,k}; {a,b,g,h,i,l}; {a,b,g,h,i,m}; {a,b,g,h,i,n}; {a,b,g,h,j,k};

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 {a,b,g,i,k,l}; {a,b,g,i,k,m}; {a,b,g,i,k,n}; {a,b,g,i,l,m}; {a,b,g,i,l,n}; {a,b,g,i,m,n}; {a,b,g,j,k,l};
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 10 {a,b,j,l,m,n}; {a,b,k,l,m,n}; {a,c,d,e,f,g}; {a,c,d,e,f,h}; {a,c,d,e,f,i}; {a,c,d,e,f,j}; {a,c,d,e,f,k};
 {a,c,d,e,f,l}; {a,c,d,e,f,m}; {a,c,d,e,f,n}; {a,c,d,e,g,h}; {a,c,d,e,g,i}; {a,c,d,e,g,j}; {a,c,d,e,g,k};
 {a,c,d,e,g,l}; {a,c,d,e,g,m}; {a,c,d,e,g,n}; {a,c,d,e,h,i}; {a,c,d,e,h,j}; {a,c,d,e,h,k}; {a,c,d,e,h,l};
 {a,c,d,e,h,m}; {a,c,d,e,h,n}; {a,c,d,e,i,j}; {a,c,d,e,i,k}; {a,c,d,e,i,l}; {a,c,d,e,i,m}; {a,c,d,e,i,n};
 {a,c,d,e,j,k}; {a,c,d,e,j,l}; {a,c,d,e,j,m}; {a,c,d,e,j,n}; {a,c,d,e,k,l}; {a,c,d,e,k,m}; {a,c,d,e,k,n};
 15 {a,c,d,e,l,m}; {a,c,d,e,l,n}; {a,c,d,e,m,n}; {a,c,d,f,g,h}; {a,c,d,f,g,i}; {a,c,d,f,g,j}; {a,c,d,f,g,k};
 {a,c,d,f,g,l}; {a,c,d,f,g,m}; {a,c,d,f,g,n}; {a,c,d,f,h,i}; {a,c,d,f,h,j}; {a,c,d,f,h,k}; {a,c,d,f,h,l};
 {a,c,d,f,h,m}; {a,c,d,f,h,n}; {a,c,d,f,i,j}; {a,c,d,f,i,k}; {a,c,d,f,i,l}; {a,c,d,f,i,m}; {a,c,d,f,i,n};
 {a,c,d,f,j,k}; {a,c,d,f,j,l}; {a,c,d,f,j,m}; {a,c,d,f,j,n}; {a,c,d,f,k,l}; {a,c,d,f,k,m}; {a,c,d,f,k,n};
 {a,c,d,f,l,m}; {a,c,d,f,l,n}; {a,c,d,f,m,n}; {a,c,d,g,h,i}; {a,c,d,g,h,j}; {a,c,d,g,h,k}; {a,c,d,g,h,l};
 20 {a,c,d,g,h,m}; {a,c,d,g,h,n}; {a,c,d,g,i,j}; {a,c,d,g,i,k}; {a,c,d,g,i,l}; {a,c,d,g,i,m}; {a,c,d,g,i,n};
 {a,c,d,g,j,k}; {a,c,d,g,j,l}; {a,c,d,g,j,m}; {a,c,d,g,j,n}; {a,c,d,g,k,l}; {a,c,d,g,k,m}; {a,c,d,g,k,n};
 {a,c,d,g,l,m}; {a,c,d,g,l,n}; {a,c,d,g,m,n}; {a,c,d,h,i,j}; {a,c,d,h,i,k}; {a,c,d,h,i,l}; {a,c,d,h,i,m};
 {a,c,d,h,i,n}; {a,c,d,h,j,k}; {a,c,d,h,j,l}; {a,c,d,h,j,m}; {a,c,d,h,j,n}; {a,c,d,h,k,l}; {a,c,d,h,k,m};
 {a,c,d,h,k,n}; {a,c,d,h,l,m}; {a,c,d,h,l,n}; {a,c,d,h,m,n}; {a,c,d,i,j,k}; {a,c,d,i,j,l}; {a,c,d,i,j,m};
 25 {a,c,d,i,j,n}; {a,c,d,i,k,l}; {a,c,d,i,k,m}; {a,c,d,i,k,n}; {a,c,d,i,l,m}; {a,c,d,i,l,n}; {a,c,d,i,m,n};
 {a,c,d,j,k,l}; {a,c,d,j,k,m}; {a,c,d,j,k,n}; {a,c,d,j,l,m}; {a,c,d,j,l,n}; {a,c,d,j,m,n}; {a,c,d,k,l,m};
 {a,c,d,k,l,n}; {a,c,d,k,m,n}; {a,c,d,l,m,n}; {a,c,e,f,g,h}; {a,c,e,f,g,i}; {a,c,e,f,g,j}; {a,c,e,f,g,k};
 {a,c,e,f,g,l}; {a,c,e,f,g,m}; {a,c,e,f,g,n}; {a,c,e,f,h,i}; {a,c,e,f,h,j}; {a,c,e,f,h,k}; {a,c,e,f,h,l};
 {a,c,e,f,h,m}; {a,c,e,f,h,n}; {a,c,e,f,i,j}; {a,c,e,f,i,k}; {a,c,e,f,i,l}; {a,c,e,f,i,m}; {a,c,e,f,i,n};
 30 {a,c,e,f,j,k}; {a,c,e,f,j,l}; {a,c,e,f,j,m}; {a,c,e,f,j,n}; {a,c,e,f,k,l}; {a,c,e,f,k,m}; {a,c,e,f,k,n};
 {a,c,e,f,l,m}; {a,c,e,f,l,n}; {a,c,e,f,m,n}; {a,c,e,g,h,i}; {a,c,e,g,h,j}; {a,c,e,g,h,k}; {a,c,e,g,h,l};
 {a,c,e,g,h,m}; {a,c,e,g,h,n}; {a,c,e,g,i,j}; {a,c,e,g,i,k}; {a,c,e,g,i,l}; {a,c,e,g,i,m}; {a,c,e,g,i,n};
 {a,c,e,g,j,k}; {a,c,e,g,j,l}; {a,c,e,g,j,m}; {a,c,e,g,j,n}; {a,c,e,g,k,l}; {a,c,e,g,k,m}; {a,c,e,g,k,n};
 {a,c,e,g,l,m}; {a,c,e,g,l,n}; {a,c,e,g,m,n}; {a,c,e,h,i,j}; {a,c,e,h,i,k}; {a,c,e,h,i,l}; {a,c,e,h,i,m};
 35 {a,c,e,h,i,n}; {a,c,e,h,j,k}; {a,c,e,h,j,l}; {a,c,e,h,j,m}; {a,c,e,h,j,n}; {a,c,e,h,k,l}; {a,c,e,h,k,m};

- {a,c,e,h,k,n}; {a,c,e,h,l,m}; {a,c,e,h,l,n}; {a,c,e,h,m,n}; {a,c,e,i,j,k}; {a,c,e,i,j,l}; {a,c,e,i,j,m};
 {a,c,e,i,j,n}; {a,c,e,i,k,l}; {a,c,e,i,k,m}; {a,c,e,i,k,n}; {a,c,e,i,l,m}; {a,c,e,i,l,n}; {a,c,e,i,m,n};
 {a,c,e,j,k,l}; {a,c,e,j,k,m}; {a,c,e,j,k,n}; {a,c,e,j,l,m}; {a,c,e,j,l,n}; {a,c,e,j,m,n}; {a,c,e,k,l,m};
 {a,c,e,k,l,n}; {a,c,e,k,m,n}; {a,c,e,l,m,n}; {a,c,f,g,h,i}; {a,c,f,g,h,j}; {a,c,f,g,h,k}; {a,c,f,g,h,l};
 5 {a,c,f,g,h,m}; {a,c,f,g,h,n}; {a,c,f,g,i,j}; {a,c,f,g,i,k}; {a,c,f,g,i,l}; {a,c,f,g,i,m}; {a,c,f,g,i,n};
 {a,c,f,g,j,k}; {a,c,f,g,j,l}; {a,c,f,g,j,m}; {a,c,f,g,j,n}; {a,c,f,g,k,l}; {a,c,f,g,k,m}; {a,c,f,g,k,n};
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 {a,c,f,h,k,n}; {a,c,f,h,l,m}; {a,c,f,h,l,n}; {a,c,f,h,m,n}; {a,c,f,i,j,k}; {a,c,f,i,j,l}; {a,c,f,i,j,m};
 10 {a,c,f,i,j,n}; {a,c,f,i,k,l}; {a,c,f,i,k,m}; {a,c,f,i,k,n}; {a,c,f,i,l,m}; {a,c,f,i,l,n}; {a,c,f,i,m,n};
 {a,c,f,j,k,l}; {a,c,f,j,k,m}; {a,c,f,j,k,n}; {a,c,f,j,l,m}; {a,c,f,j,l,n}; {a,c,f,j,m,n}; {a,c,f,k,l,m};
 {a,c,f,k,l,n}; {a,c,f,k,m,n}; {a,c,f,l,m,n}; {a,c,g,h,i,j}; {a,c,g,h,i,k}; {a,c,g,h,i,l}; {a,c,g,h,i,m};
 {a,c,g,h,i,n}; {a,c,g,h,j,k}; {a,c,g,h,j,l}; {a,c,g,h,j,m}; {a,c,g,h,j,n}; {a,c,g,h,k,l}; {a,c,g,h,k,m};
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 15 {a,c,g,i,j,n}; {a,c,g,i,k,l}; {a,c,g,i,k,m}; {a,c,g,i,k,n}; {a,c,g,i,l,m}; {a,c,g,i,l,n}; {a,c,g,i,m,n};
 {a,c,g,j,k,l}; {a,c,g,j,k,m}; {a,c,g,j,k,n}; {a,c,g,j,l,m}; {a,c,g,j,l,n}; {a,c,g,j,m,n}; {a,c,g,k,l,m};
 {a,c,g,k,l,n}; {a,c,g,k,m,n}; {a,c,g,l,m,n}; {a,c,h,i,j,k}; {a,c,h,i,j,l}; {a,c,h,i,j,m}; {a,c,h,i,j,n};
 {a,c,h,i,k,l}; {a,c,h,i,k,m}; {a,c,h,i,k,n}; {a,c,h,i,l,m}; {a,c,h,i,l,n}; {a,c,h,i,m,n}; {a,c,h,j,k,l};
 {a,c,h,j,k,m}; {a,c,h,j,k,n}; {a,c,h,j,l,m}; {a,c,h,j,l,n}; {a,c,h,j,m,n}; {a,c,h,k,l,m}; {a,c,h,k,l,n};
 20 {a,c,h,k,m,n}; {a,c,h,l,m,n}; {a,c,i,j,k,l}; {a,c,i,j,k,m}; {a,c,i,j,k,n}; {a,c,i,j,l,m}; {a,c,i,j,l,n};
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 {a,d,e,f,g,l}; {a,d,e,f,g,m}; {a,d,e,f,g,n}; {a,d,e,f,h,i}; {a,d,e,f,h,j}; {a,d,e,f,h,k}; {a,d,e,f,h,l};
 {a,d,e,f,h,m}; {a,d,e,f,h,n}; {a,d,e,f,i,j}; {a,d,e,f,i,k}; {a,d,e,f,i,l}; {a,d,e,f,i,m}; {a,d,e,f,i,n};
 25 {a,d,e,f,j,k}; {a,d,e,f,j,l}; {a,d,e,f,j,m}; {a,d,e,f,j,n}; {a,d,e,f,k,l}; {a,d,e,f,k,m}; {a,d,e,f,k,n};
 {a,d,e,f,l,m}; {a,d,e,f,l,n}; {a,d,e,f,m,n}; {a,d,e,g,h,i}; {a,d,e,g,h,j}; {a,d,e,g,h,k}; {a,d,e,g,h,l};
 {a,d,e,g,h,m}; {a,d,e,g,h,n}; {a,d,e,g,i,j}; {a,d,e,g,i,k}; {a,d,e,g,i,l}; {a,d,e,g,i,m}; {a,d,e,g,i,n};
 {a,d,e,g,j,k}; {a,d,e,g,j,l}; {a,d,e,g,j,m}; {a,d,e,g,j,n}; {a,d,e,g,k,l}; {a,d,e,g,k,m}; {a,d,e,g,k,n};
 {a,d,e,g,l,m}; {a,d,e,g,l,n}; {a,d,e,g,m,n}; {a,d,e,h,i,j}; {a,d,e,h,i,k}; {a,d,e,h,i,l}; {a,d,e,h,i,m};
 30 {a,d,e,h,i,n}; {a,d,e,h,j,k}; {a,d,e,h,j,l}; {a,d,e,h,j,m}; {a,d,e,h,j,n}; {a,d,e,h,k,l}; {a,d,e,h,k,m};
 {a,d,e,h,k,n}; {a,d,e,h,l,m}; {a,d,e,h,l,n}; {a,d,e,h,m,n}; {a,d,e,i,j,k}; {a,d,e,i,j,l}; {a,d,e,i,j,m};
 {a,d,e,i,j,n}; {a,d,e,i,k,l}; {a,d,e,i,k,m}; {a,d,e,i,k,n}; {a,d,e,i,l,m}; {a,d,e,i,l,n}; {a,d,e,i,m,n};
 {a,d,e,j,k,l}; {a,d,e,j,k,m}; {a,d,e,j,k,n}; {a,d,e,j,l,m}; {a,d,e,j,l,n}; {a,d,e,j,m,n}; {a,d,e,k,l,m};
 {a,d,e,k,l,n}; {a,d,e,k,m,n}; {a,d,e,l,m,n}; {a,d,f,g,h,i}; {a,d,f,g,h,j}; {a,d,f,g,h,k}; {a,d,f,g,h,l};
 35 {a,d,f,g,h,m}; {a,d,f,g,h,n}; {a,d,f,g,i,j}; {a,d,f,g,i,k}; {a,d,f,g,i,l}; {a,d,f,g,i,m}; {a,d,f,g,i,n};

- {a,d,f,g,j,k}; {a,d,f,g,j,l}; {a,d,f,g,j,m}; {a,d,f,g,j,n}; {a,d,f,g,k,l}; {a,d,f,g,k,m}; {a,d,f,g,k,n};
 {a,d,f,g,l,m}; {a,d,f,g,l,n}; {a,d,f,g,m,n}; {a,d,f,h,i,j}; {a,d,f,h,i,k}; {a,d,f,h,i,l}; {a,d,f,h,i,m};
 {a,d,f,h,i,n}; {a,d,f,h,j,k}; {a,d,f,h,j,l}; {a,d,f,h,j,m}; {a,d,f,h,j,n}; {a,d,f,h,k,l}; {a,d,f,h,k,m};
 {a,d,f,h,k,n}; {a,d,f,h,l,m}; {a,d,f,h,l,n}; {a,d,f,h,m,n}; {a,d,f,i,j,k}; {a,d,f,i,j,l}; {a,d,f,i,j,m};
 5 {a,d,f,i,j,n}; {a,d,f,i,k,l}; {a,d,f,i,k,m}; {a,d,f,i,k,n}; {a,d,f,i,l,m}; {a,d,f,i,l,n}; {a,d,f,i,m,n};
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 {a,d,g,h,k,n}; {a,d,g,h,l,m}; {a,d,g,h,l,n}; {a,d,g,h,m,n}; {a,d,g,i,j,k}; {a,d,g,i,j,l}; {a,d,g,i,j,m};
 10 {a,d,g,i,j,n}; {a,d,g,i,k,l}; {a,d,g,i,k,m}; {a,d,g,i,k,n}; {a,d,g,i,l,m}; {a,d,g,i,l,n}; {a,d,g,i,m,n};
 {a,d,g,j,k,l}; {a,d,g,j,k,m}; {a,d,g,j,k,n}; {a,d,g,j,l,m}; {a,d,g,j,l,n}; {a,d,g,j,m,n}; {a,d,g,k,l,m};
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 {a,d,h,j,k,m}; {a,d,h,j,k,n}; {a,d,h,j,l,m}; {a,d,h,j,l,n}; {a,d,h,j,m,n}; {a,d,h,k,l,m}; {a,d,h,k,l,n};
 15 {a,d,h,k,m,n}; {a,d,h,l,m,n}; {a,d,i,j,k,l}; {a,d,i,j,k,m}; {a,d,i,j,k,n}; {a,d,i,j,l,m}; {a,d,i,j,l,n};
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 {a,e,f,g,h,m}; {a,e,f,g,h,n}; {a,e,f,g,i,j}; {a,e,f,g,i,k}; {a,e,f,g,i,l}; {a,e,f,g,i,m}; {a,e,f,g,i,n};
 {a,e,f,g,j,k}; {a,e,f,g,j,l}; {a,e,f,g,j,m}; {a,e,f,g,j,n}; {a,e,f,g,k,l}; {a,e,f,g,k,m}; {a,e,f,g,k,n};
 20 {a,e,f,g,l,m}; {a,e,f,g,l,n}; {a,e,f,g,m,n}; {a,e,f,h,i,j}; {a,e,f,h,i,k}; {a,e,f,h,i,l}; {a,e,f,h,i,m};
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 {a,e,g,j,k,l}; {a,e,g,j,k,m}; {a,e,g,j,k,n}; {a,e,g,j,l,m}; {a,e,g,j,l,n}; {a,e,g,j,m,n}; {a,e,g,k,l,m};
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 35 {a,e,j,k,m,n}; {a,e,j,l,m,n}; {a,e,k,l,m,n}; {a,f,g,h,i,j}; {a,f,g,h,i,k}; {a,f,g,h,i,l}; {a,f,g,h,i,m};

- {a,f,g,h,i,n}; {a,f,g,h,j,k}; {a,f,g,h,j,l}; {a,f,g,h,j,m}; {a,f,g,h,j,n}; {a,f,g,h,k,l}; {a,f,g,h,k,m};
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 {b,c,d,e,h,l}; {b,c,d,e,h,m}; {b,c,d,e,h,n}; {b,c,d,e,i,j}; {b,c,d,e,i,k}; {b,c,d,e,i,l}; {b,c,d,e,i,m};
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 {b,c,f,g,i,n}; {b,c,f,g,j,k}; {b,c,f,g,j,l}; {b,c,f,g,j,m}; {b,c,f,g,j,n}; {b,c,f,g,k,l}; {b,c,f,g,k,m};
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 20 {b,c,f,k,l,m}; {b,c,f,k,l,n}; {b,c,f,k,m,n}; {b,c,f,l,m,n}; {b,c,g,h,i,j}; {b,c,g,h,i,k}; {b,c,g,h,i,l};
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 35 {b,d,e,g,h,l}; {b,d,e,g,h,m}; {b,d,e,g,h,n}; {b,d,e,g,i,j}; {b,d,e,g,i,k}; {b,d,e,g,i,l}; {b,d,e,g,i,m};

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 {b,d,f,g,h,l}; {b,d,f,g,h,m}; {b,d,f,g,h,n}; {b,d,f,g,i,j}; {b,d,f,g,i,k}; {b,d,f,g,i,l}; {b,d,f,g,i,m};
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 {b,d,f,i,m,n}; {b,d,f,j,k,l}; {b,d,f,j,k,m}; {b,d,f,j,k,n}; {b,d,f,j,l,m}; {b,d,f,j,l,n}; {b,d,f,j,m,n};
 15 {b,d,f,k,l,m}; {b,d,f,k,l,n}; {b,d,f,k,m,n}; {b,d,f,l,m,n}; {b,d,g,h,i,j}; {b,d,g,h,i,k}; {b,d,g,h,i,l};
 {b,d,g,h,i,m}; {b,d,g,h,i,n}; {b,d,g,h,j,k}; {b,d,g,h,j,l}; {b,d,g,h,j,m}; {b,d,g,h,j,n}; {b,d,g,h,k,l};
 {b,d,g,h,k,m}; {b,d,g,h,k,n}; {b,d,g,h,l,m}; {b,d,g,h,l,n}; {b,d,g,h,m,n}; {b,d,g,i,j,k};
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 {b,d,h,j,m,n}; {b,d,h,k,l,m}; {b,d,h,k,l,n}; {b,d,h,k,m,n}; {b,d,h,l,m,n}; {b,d,i,j,k,l};
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 {c,d,e,g,h,m}; {c,d,e,g,h,n}; {c,d,e,g,i,j}; {c,d,e,g,i,k}; {c,d,e,g,i,l}; {c,d,e,g,i,m}; {c,d,e,g,i,n};
 {c,d,e,g,j,k}; {c,d,e,g,j,l}; {c,d,e,g,j,m}; {c,d,e,g,j,n}; {c,d,e,g,k,l}; {c,d,e,g,k,m}; {c,d,e,g,k,n};
 {c,d,e,g,l,m}; {c,d,e,g,l,n}; {c,d,e,g,m,n}; {c,d,e,h,i,j}; {c,d,e,h,i,k}; {c,d,e,h,i,l}; {c,d,e,h,i,m};
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- {c,d,e,h,k,n}; {c,d,e,h,l,m}; {c,d,e,h,l,n}; {c,d,e,h,m,n}; {c,d,e,i,j,k}; {c,d,e,i,j,l}; {c,d,e,i,j,m};
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 {c,e,f,g,h,m}; {c,e,f,g,h,n}; {c,e,f,g,i,j}; {c,e,f,g,i,k}; {c,e,f,g,i,l}; {c,e,f,g,i,m}; {c,e,f,g,i,n};
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 35 {e,g,h,i,j,n}; {e,g,h,i,k,l}; {e,g,h,i,k,m}; {e,g,h,i,k,n}; {e,g,h,i,l,m}; {e,g,h,i,l,n}; {e,g,h,i,m,n};

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 $\{a,c,d,f,j,l,n\}$; $\{a,c,d,f,j,m,n\}$; $\{a,c,d,f,k,l,m\}$; $\{a,c,d,f,k,l,n\}$; $\{a,c,d,f,k,m,n\}$; $\{a,c,d,f,l,m,n\}$;
35 $\{a,c,d,g,h,i,j\}$; $\{a,c,d,g,h,i,k\}$; $\{a,c,d,g,h,i,l\}$; $\{a,c,d,g,h,i,m\}$; $\{a,c,d,g,h,i,n\}$; $\{a,c,d,g,h,j,k\}$;

- {a,c,d,g,h,j,l}; {a,c,d,g,h,j,m}; {a,c,d,g,h,j,n}; {a,c,d,g,h,k,l}; {a,c,d,g,h,k,m}; {a,c,d,g,h,k,n};
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- {a,c,f,g,i,k,m}; {a,c,f,g,i,k,n}; {a,c,f,g,i,l,m}; {a,c,f,g,i,l,n}; {a,c,f,g,i,m,n}; {a,c,f,g,j,k,l};
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 {a,d,e,f,g,h,k}; {a,d,e,f,g,h,l}; {a,d,e,f,g,h,m}; {a,d,e,f,g,h,n}; {a,d,e,f,g,i,j}; {a,d,e,f,g,i,k};
 20 {a,d,e,f,g,i,l}; {a,d,e,f,g,i,m}; {a,d,e,f,g,i,n}; {a,d,e,f,g,j,k}; {a,d,e,f,g,j,l}; {a,d,e,f,g,j,m};
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 25 {a,d,e,f,i,j,m}; {a,d,e,f,i,j,n}; {a,d,e,f,i,k,l}; {a,d,e,f,i,k,m}; {a,d,e,f,i,k,n}; {a,d,e,f,i,l,m};
 {a,d,e,f,i,l,n}; {a,d,e,f,i,m,n}; {a,d,e,f,j,k,l}; {a,d,e,f,j,k,m}; {a,d,e,f,j,k,n}; {a,d,e,f,j,l,m};
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 {a,d,e,g,i,j,n}; {a,d,e,g,i,k,l}; {a,d,e,g,i,k,m}; {a,d,e,g,i,k,n}; {a,d,e,g,i,l,m}; {a,d,e,g,i,l,n};
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 {a,d,f,j,l,m,n}; {a,d,f,k,l,m,n}; {a,d,g,h,i,j,k}; {a,d,g,h,i,j,l}; {a,d,g,h,i,j,m}; {a,d,g,h,i,j,n};
 {a,d,g,h,i,k,l}; {a,d,g,h,i,k,m}; {a,d,g,h,i,k,n}; {a,d,g,h,i,l,m}; {a,d,g,h,i,l,n}; {a,d,g,h,i,m,n};
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 30 {b,c,e,f,h,j,k}; {b,c,e,f,h,j,l}; {b,c,e,f,h,j,m}; {b,c,e,f,h,j,n}; {b,c,e,f,h,k,l}; {b,c,e,f,h,k,m};
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- {b,g,h,i,k,l,m}; {b,g,h,i,k,l,n}; {b,g,h,i,k,m,n}; {b,g,h,i,l,m,n}; {b,g,h,j,k,l,m}; {b,g,h,j,k,l,n};
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 {a,c,d,e,f,h,j,m}; {a,c,d,e,f,h,j,n}; {a,c,d,e,f,h,k,l}; {a,c,d,e,f,h,k,m}; {a,c,d,e,f,h,k,n};
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- {a,c,e,g,h,i,j,l}; {a,c,e,g,h,i,j,m}; {a,c,e,g,h,i,j,n}; {a,c,e,g,h,i,k,l}; {a,c,e,g,h,i,k,m};
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 {a,c,e,g,i,k,l,m}; {a,c,e,g,i,k,l,n}; {a,c,e,g,i,k,m,n}; {a,c,e,g,i,l,m,n}; {a,c,e,g,j,k,l,m};
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 {a,d,e,f,g,i,k,n}; {a,d,e,f,g,i,l,m}; {a,d,e,f,g,i,l,n}; {a,d,e,f,g,i,m,n}; {a,d,e,f,g,j,k,l};
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- {a,d,e,f,h,i,j,l}; {a,d,e,f,h,i,j,m}; {a,d,e,f,h,i,j,n}; {a,d,e,f,h,i,k,l}; {a,d,e,f,h,i,k,m};
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- {a,e,f,g,h,i,j,l}; {a,e,f,g,h,i,j,m}; {a,e,f,g,h,i,j,n}; {a,e,f,g,h,i,k,l}; {a,e,f,g,h,i,k,m};
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 {a,b,c,e,f,g,k,l,n}; {a,b,c,e,f,g,k,m,n}; {a,b,c,e,f,g,l,m,n}; {a,b,c,e,f,h,i,j,k}; {a,b,c,e,f,h,i,j,l};
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 30 {a,b,c,e,f,i,k,l,n}; {a,b,c,e,f,i,k,m,n}; {a,b,c,e,f,i,l,m,n}; {a,b,c,e,f,j,k,l,m}; {a,b,c,e,f,j,k,l,n};
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- {a,b,c,e,g,i,j,k,n}; {a,b,c,e,g,i,j,l,m}; {a,b,c,e,g,i,j,l,n}; {a,b,c,e,g,i,j,m,n}; {a,b,c,e,g,i,k,l,m};
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- {a,b,d,e,f,i,j,k,n}; {a,b,d,e,f,i,j,l,m}; {a,b,d,e,f,i,j,l,n}; {a,b,d,e,f,i,j,m,n}; {a,b,d,e,f,i,k,l,m};
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 35 {a,b,c,d,e,g,h,i,j,k}; {a,b,c,d,e,g,h,i,j,l}; {a,b,c,d,e,g,h,i,j,m}; {a,b,c,d,e,g,h,i,j,n};

- $\{a,b,c,d,e,g,h,i,k,l\}$; $\{a,b,c,d,e,g,h,i,k,m\}$; $\{a,b,c,d,e,g,h,i,k,n\}$; $\{a,b,c,d,e,g,h,i,l,m\}$;
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30 $\{a,b,c,d,g,h,i,k,m,n\}$; $\{a,b,c,d,g,h,i,l,m,n\}$; $\{a,b,c,d,g,h,j,k,l,m\}$; $\{a,b,c,d,g,h,j,k,l,n\}$;
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35 {a,b,f,g,h,i,k,l,m,n}; {a,b,f,g,h,j,k,l,m,n}; {a,b,f,g,i,j,k,l,m,n}; {a,b,f,h,i,j,k,l,m,n};

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35 {b,d,e,g,h,i,j,k,m,n}; {b,d,e,g,h,i,j,l,m,n}; {b,d,e,g,h,i,k,l,m,n}; {b,d,e,g,h,i,k,l,m,n};

- $\{b,d,e,g,i,j,k,l,m,n\}$; $\{b,d,e,h,i,j,k,l,m,n\}$; $\{b,d,f,g,h,i,j,k,l,m\}$; $\{b,d,f,g,h,i,j,k,l,n\}$;
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15 $\{c,d,e,h,i,j,k,l,m,n\}$; $\{c,d,f,g,h,i,j,k,l,m\}$; $\{c,d,f,g,h,i,j,k,l,n\}$; $\{c,d,f,g,h,i,j,k,m,n\}$;
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30 $\{a,b,c,d,e,f,g,i,l,m,n\}$; $\{a,b,c,d,e,f,g,j,k,l,m\}$; $\{a,b,c,d,e,f,g,j,k,l,n\}$; $\{a,b,c,d,e,f,g,j,k,m,n\}$;
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35 $\{a,b,c,d,e,f,h,k,l,m,n\}$; $\{a,b,c,d,e,f,i,j,k,l,m\}$; $\{a,b,c,d,e,f,i,j,k,l,n\}$; $\{a,b,c,d,e,f,i,j,k,m,n\}$;

[illegible]

- {a,b,d,e,g,h,i,j,k,l,m}; {a,b,d,e,g,h,i,j,k,l,n}; {a,b,d,e,g,h,i,j,k,m,n}; {a,b,d,e,g,h,i,j,l,m,n};
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 35 {b,c,d,f,h,i,j,k,l,m,n}; {b,c,d,g,h,i,j,k,l,m,n}; {b,c,e,f,g,h,i,j,k,l,m}; {b,c,e,f,g,h,i,j,k,l,n};

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 15 {a,b,c,d,e,f,h,i,k,l,m,n}; {a,b,c,d,e,f,h,j,k,l,m,n}; {a,b,c,d,e,f,i,j,k,l,m,n}; {a,b,c,d,e,g,h,i,j,k,l,m};
 {a,b,c,d,e,g,h,i,j,k,l,n}; {a,b,c,d,e,g,h,i,j,k,m,n}; {a,b,c,d,e,g,h,i,j,l,m,n}; {a,b,c,d,e,g,h,i,k,l,m,n};
 {a,b,c,d,e,g,h,j,k,l,m,n}; {a,b,c,d,e,g,i,j,k,l,m,n}; {a,b,c,d,e,h,i,j,k,l,m,n}; {a,b,c,d,f,g,h,i,j,k,l,m};
 {a,b,c,d,f,g,h,i,j,k,l,n}; {a,b,c,d,f,g,h,i,j,k,m,n}; {a,b,c,d,f,g,h,i,j,l,m,n}; {a,b,c,d,f,g,h,i,k,l,m,n};
 {a,b,c,d,f,g,h,j,k,l,m,n}; {a,b,c,d,f,g,i,j,k,l,m,n}; {a,b,c,d,f,h,i,j,k,l,m,n}; {a,b,c,d,g,h,i,j,k,l,m,n};
 20 {a,b,c,e,f,g,h,i,j,k,l,m}; {a,b,c,e,f,g,h,i,j,k,l,n}; {a,b,c,e,f,g,h,i,j,k,m,n}; {a,b,c,e,f,g,h,i,j,l,m,n};
 {a,b,c,e,f,g,h,i,k,l,m,n}; {a,b,c,e,f,g,h,j,k,l,m,n}; {a,b,c,e,f,g,i,j,k,l,m,n}; {a,b,c,e,f,h,i,j,k,l,m,n};
 {a,b,c,e,g,h,i,j,k,l,m,n}; {a,b,c,f,g,h,i,j,k,l,m,n}; {a,b,d,e,f,g,h,i,j,k,l,m}; {a,b,d,e,f,g,h,i,j,k,l,n};
 {a,b,d,e,f,g,h,i,j,k,m,n}; {a,b,d,e,f,g,h,i,j,l,m,n}; {a,b,d,e,f,g,h,i,k,l,m,n}; {a,b,d,e,f,g,h,j,k,l,m,n};
 {a,b,d,e,f,g,i,j,k,l,m,n}; {a,b,d,e,f,h,i,j,k,l,m,n}; {a,b,d,e,g,h,i,j,k,l,m,n}; {a,b,d,f,g,h,i,j,k,l,m,n};
 25 {a,b,e,f,g,h,i,j,k,l,m,n}; {a,c,d,e,f,g,h,i,j,k,l,m}; {a,c,d,e,f,g,h,i,j,k,l,n}; {a,c,d,e,f,g,h,i,j,k,m,n};
 {a,c,d,e,f,g,h,i,j,l,m,n}; {a,c,d,e,f,g,h,i,k,l,m,n}; {a,c,d,e,f,g,h,j,k,l,m,n}; {a,c,d,e,f,g,i,j,k,l,m,n};
 {a,c,d,e,f,h,i,j,k,l,m,n}; {a,c,d,e,g,h,i,j,k,l,m,n}; {a,c,d,f,g,h,i,j,k,l,m,n}; {a,c,e,f,g,h,i,j,k,l,m,n};
 {a,d,e,f,g,h,i,j,k,l,m,n}; {b,c,d,e,f,g,h,i,j,k,l,m}; {b,c,d,e,f,g,h,i,j,k,l,n}; {b,c,d,e,f,g,h,i,j,k,m,n};
 {b,c,d,e,f,g,h,i,j,l,m,n}; {b,c,d,e,f,g,h,i,k,l,m,n}; {b,c,d,e,f,g,h,j,k,l,m,n}; {b,c,d,e,f,g,i,j,k,l,m,n};
 30 {b,c,d,e,f,h,i,j,k,l,m,n}; {b,c,d,e,g,h,i,j,k,l,m,n}; {b,c,d,f,g,h,i,j,k,l,m,n}; {b,c,e,f,g,h,i,j,k,l,m,n};
 {b,d,e,f,g,h,i,j,k,l,m,n}; {c,d,e,f,g,h,i,j,k,l,m,n}; {a,b,c,d,e,f,g,h,i,j,k,l,m};
 {a,b,c,d,e,f,g,h,i,j,k,l,n}; {a,b,c,d,e,f,g,h,i,j,k,m,n}; {a,b,c,d,e,f,g,h,i,j,l,m,n};
 {a,b,c,d,e,f,g,h,i,k,l,m,n}; {a,b,c,d,e,f,g,h,j,k,l,m,n}; {a,b,c,d,e,f,g,i,j,k,l,m,n};
 {a,b,c,d,e,f,h,i,j,k,l,m,n}; {a,b,c,d,e,g,h,i,j,k,l,m,n}; {a,b,c,d,f,g,h,i,j,k,l,m,n};

{a,b,c,e,f,g,h,i,j,k,l,m,n}; {a,b,d,e,f,g,h,i,j,k,l,m,n}; {a,c,d,e,f,g,h,i,j,k,l,m,n};
 {b,c,d,e,f,g,h,i,j,k,l,m,n}; or {a,b,c,d,e,f,g,h,i,j,k,l,m,n}.

The level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1 and (i) CD81 is preferably

- 5 decreased or abolished. The level of expression and/or transcription of any number and combination of (a) to (i) may be decreased. For instance, the level of expression and/or transcription of any of the following may be decreased or abolished (where combinations are contained within {} and commas equal “and”): {a}; {b}; {c}; {d}; {e}; {f}; {g}; {h}; {i}; {a,b}; {a,c}; {a,d}; {a,e}; {a,f}; {a,g}; {a,h}; {a,i}; {b,c}; {b,d}; {b,e}; {b,f}; {b,g}; {b,h}; {b,i};
- 10 {c,d}; {c,e}; {c,f}; {c,g}; {c,h}; {c,i}; {d,e}; {d,f}; {d,g}; {d,h}; {d,i}; {e,f}; {e,g}; {e,h}; {e,i}; {f,g}; {f,h}; {f,i}; {g,h}; {g,i}; {h,i}; {a,b,c}; {a,b,d}; {a,b,e}; {a,b,f}; {a,b,g}; {a,b,h}; {a,b,i}; {a,c,d}; {a,c,e}; {a,c,f}; {a,c,g}; {a,c,h}; {a,c,i}; {a,d,e}; {a,d,f}; {a,d,g}; {a,d,h}; {a,d,i}; {a,e,f}; {a,e,g}; {a,e,h}; {a,e,i}; {a,f,g}; {a,f,h}; {a,f,i}; {a,g,h}; {a,g,i}; {a,h,i}; {b,c,d}; {b,c,e}; {b,c,f}; {b,c,g}; {b,c,h}; {b,c,i}; {b,d,e}; {b,d,f}; {b,d,g}; {b,d,h}; {b,d,i}; {b,e,f};
- 15 {b,e,g}; {b,e,h}; {b,e,i}; {b,f,g}; {b,f,h}; {b,f,i}; {b,g,h}; {b,g,i}; {b,h,i}; {c,d,e}; {c,d,f}; {c,d,g}; {c,d,h}; {c,d,i}; {c,e,f}; {c,e,g}; {c,e,h}; {c,e,i}; {c,f,g}; {c,f,h}; {c,f,i}; {c,g,h}; {c,g,i}; {c,h,i}; {d,e,f}; {d,e,g}; {d,e,h}; {d,e,i}; {d,f,g}; {d,f,h}; {d,f,i}; {d,g,h}; {d,g,i}; {d,h,i}; {e,f,g}; {e,f,h}; {e,f,i}; {e,g,h}; {e,g,i}; {e,h,i}; {f,g,h}; {f,g,i}; {f,h,i}; {g,h,i}; {a,b,c,d}; {a,b,c,e}; {a,b,c,f}; {a,b,c,g}; {a,b,c,h}; {a,b,c,i}; {a,b,d,e}; {a,b,d,f}; {a,b,d,g};
- 20 {a,b,d,h}; {a,b,d,i}; {a,b,e,f}; {a,b,e,g}; {a,b,e,h}; {a,b,e,i}; {a,b,f,g}; {a,b,f,h}; {a,b,f,i}; {a,b,g,h}; {a,b,g,i}; {a,b,h,i}; {a,c,d,e}; {a,c,d,f}; {a,c,d,g}; {a,c,d,h}; {a,c,d,i}; {a,c,e,f}; {a,c,e,g}; {a,c,e,h}; {a,c,e,i}; {a,c,f,g}; {a,c,f,h}; {a,c,f,i}; {a,c,g,h}; {a,c,g,i}; {a,c,h,i}; {a,d,e,f}; {a,d,e,g}; {a,d,e,h}; {a,d,e,i}; {a,d,f,g}; {a,d,f,h}; {a,d,f,i}; {a,d,g,h}; {a,d,g,i}; {a,d,h,i}; {a,e,f,g}; {a,e,f,h}; {a,e,f,i}; {a,e,g,h}; {a,e,g,i}; {a,e,h,i}; {a,f,g,h}; {a,f,g,i}; {a,f,h,i};
- 25 {a,g,h,i}; {b,c,d,e}; {b,c,d,f}; {b,c,d,g}; {b,c,d,h}; {b,c,d,i}; {b,c,e,f}; {b,c,e,g}; {b,c,e,h}; {b,c,e,i}; {b,c,f,g}; {b,c,f,h}; {b,c,f,i}; {b,c,g,h}; {b,c,g,i}; {b,c,h,i}; {b,d,e,f}; {b,d,e,g}; {b,d,e,h}; {b,d,e,i}; {b,d,f,g}; {b,d,f,h}; {b,d,f,i}; {b,d,g,h}; {b,d,g,i}; {b,d,h,i}; {b,e,f,g}; {b,e,f,h}; {b,e,f,i}; {b,e,g,h}; {b,e,g,i}; {b,e,h,i}; {b,f,g,h}; {b,f,g,i}; {b,f,h,i}; {b,g,h,i}; {c,d,e,f}; {c,d,e,g}; {c,d,e,h}; {c,d,e,i}; {c,d,f,g}; {c,d,f,h}; {c,d,f,i}; {c,d,g,h}; {c,d,g,i};
- 30 {c,d,h,i}; {c,e,f,g}; {c,e,f,h}; {c,e,f,i}; {c,e,g,h}; {c,e,g,i}; {c,e,h,i}; {c,f,g,h}; {c,f,g,i}; {c,f,h,i}; {c,g,h,i}; {d,e,f,g}; {d,e,f,h}; {d,e,f,i}; {d,e,g,h}; {d,e,g,i}; {d,e,h,i}; {d,f,g,h}; {d,f,g,i}; {d,f,h,i}; {d,g,h,i}; {e,f,g,h}; {e,f,g,i}; {e,f,h,i}; {e,g,h,i}; {f,g,h,i}; {a,b,c,d,e}; {a,b,c,d,f}; {a,b,c,d,g}; {a,b,c,d,h}; {a,b,c,d,i}; {a,b,c,e,f}; {a,b,c,e,g}; {a,b,c,e,h}; {a,b,c,e,i}; {a,b,c,f,g}; {a,b,c,f,h}; {a,b,c,f,i}; {a,b,c,g,h}; {a,b,c,g,i}; {a,b,c,h,i}; {a,b,d,e,f}; {a,b,d,e,g}; {a,b,d,e,h};
- 35 {a,b,d,e,i}; {a,b,d,f,g}; {a,b,d,f,h}; {a,b,d,f,i}; {a,b,d,g,h}; {a,b,d,g,i}; {a,b,d,h,i}; {a,b,e,f,g};

- {a,b,e,f,h}; {a,b,e,f,i}; {a,b,e,g,h}; {a,b,e,g,i}; {a,b,e,h,i}; {a,b,f,g,h}; {a,b,f,g,i}; {a,b,f,h,i};
 {a,b,g,h,i}; {a,c,d,e,f}; {a,c,d,e,g}; {a,c,d,e,h}; {a,c,d,e,i}; {a,c,d,f,g}; {a,c,d,f,h}; {a,c,d,f,i};
 {a,c,d,g,h}; {a,c,d,g,i}; {a,c,d,h,i}; {a,c,e,f,g}; {a,c,e,f,h}; {a,c,e,f,i}; {a,c,e,g,h}; {a,c,e,g,i};
 {a,c,e,h,i}; {a,c,f,g,h}; {a,c,f,g,i}; {a,c,f,h,i}; {a,c,g,h,i}; {a,d,e,f,g}; {a,d,e,f,h}; {a,d,e,f,i};
 5 {a,d,e,g,h}; {a,d,e,g,i}; {a,d,e,h,i}; {a,d,f,g,h}; {a,d,f,g,i}; {a,d,f,h,i}; {a,d,g,h,i}; {a,e,f,g,h};
 {a,e,f,g,i}; {a,e,f,h,i}; {a,e,g,h,i}; {a,f,g,h,i}; {b,c,d,e,f}; {b,c,d,e,g}; {b,c,d,e,h}; {b,c,d,e,i};
 {b,c,d,f,g}; {b,c,d,f,h}; {b,c,d,f,i}; {b,c,d,g,h}; {b,c,d,g,i}; {b,c,d,h,i}; {b,c,e,f,g}; {b,c,e,f,h};
 {b,c,e,f,i}; {b,c,e,g,h}; {b,c,e,g,i}; {b,c,e,h,i}; {b,c,f,g,h}; {b,c,f,g,i}; {b,c,f,h,i}; {b,c,g,h,i};
 {b,d,e,f,g}; {b,d,e,f,h}; {b,d,e,f,i}; {b,d,e,g,h}; {b,d,e,g,i}; {b,d,e,h,i}; {b,d,f,g,h}; {b,d,f,g,i};
 10 {b,d,f,h,i}; {b,d,g,h,i}; {b,e,f,g,h}; {b,e,f,g,i}; {b,e,f,h,i}; {b,e,g,h,i}; {b,f,g,h,i}; {c,d,e,f,g};
 {c,d,e,f,h}; {c,d,e,f,i}; {c,d,e,g,h}; {c,d,e,g,i}; {c,d,e,h,i}; {c,d,f,g,h}; {c,d,f,g,i}; {c,d,f,h,i};
 {c,d,g,h,i}; {c,e,f,g,h}; {c,e,f,g,i}; {c,e,f,h,i}; {c,e,g,h,i}; {c,f,g,h,i}; {d,e,f,g,h}; {d,e,f,g,i};
 {d,e,f,h,i}; {d,e,g,h,i}; {d,f,g,h,i}; {e,f,g,h,i}; {a,b,c,d,e,f}; {a,b,c,d,e,g}; {a,b,c,d,e,h};
 {a,b,c,d,e,i}; {a,b,c,d,f,g}; {a,b,c,d,f,h}; {a,b,c,d,f,i}; {a,b,c,d,g,h}; {a,b,c,d,g,i}; {a,b,c,d,h,i};
 15 {a,b,c,e,f,g}; {a,b,c,e,f,h}; {a,b,c,e,f,i}; {a,b,c,e,g,h}; {a,b,c,e,g,i}; {a,b,c,e,h,i}; {a,b,c,f,g,h};
 {a,b,c,f,g,i}; {a,b,c,f,h,i}; {a,b,c,g,h,i}; {a,b,d,e,f,g}; {a,b,d,e,f,h}; {a,b,d,e,f,i}; {a,b,d,e,g,h};
 {a,b,d,e,g,i}; {a,b,d,e,h,i}; {a,b,d,f,g,h}; {a,b,d,f,g,i}; {a,b,d,f,h,i}; {a,b,d,g,h,i}; {a,b,e,f,g,h};
 {a,b,e,f,g,i}; {a,b,e,f,h,i}; {a,b,e,g,h,i}; {a,b,f,g,h,i}; {a,c,d,e,f,g}; {a,c,d,e,f,h}; {a,c,d,e,f,i};
 {a,c,d,e,g,h}; {a,c,d,e,g,i}; {a,c,d,e,h,i}; {a,c,d,f,g,h}; {a,c,d,f,g,i}; {a,c,d,f,h,i}; {a,c,d,g,h,i};
 20 {a,c,e,f,g,h}; {a,c,e,f,g,i}; {a,c,e,f,h,i}; {a,c,e,g,h,i}; {a,c,f,g,h,i}; {a,d,e,f,g,h}; {a,d,e,f,g,i};
 {a,d,e,f,h,i}; {a,d,e,g,h,i}; {a,d,f,g,h,i}; {a,e,f,g,h,i}; {b,c,d,e,f,g}; {b,c,d,e,f,h}; {b,c,d,e,f,i};
 {b,c,d,e,g,h}; {b,c,d,e,g,i}; {b,c,d,e,h,i}; {b,c,d,f,g,h}; {b,c,d,f,g,i}; {b,c,d,f,h,i}; {b,c,d,g,h,i};
 {b,c,e,f,g,h}; {b,c,e,f,g,i}; {b,c,e,f,h,i}; {b,c,e,g,h,i}; {b,c,f,g,h,i}; {b,d,e,f,g,h}; {b,d,e,f,g,i};
 {b,d,e,f,h,i}; {b,d,e,g,h,i}; {b,d,f,g,h,i}; {b,e,f,g,h,i}; {c,d,e,f,g,h}; {c,d,e,f,g,i}; {c,d,e,f,h,i};
 25 {c,d,e,g,h,i}; {c,d,f,g,h,i}; {c,e,f,g,h,i}; {d,e,f,g,h,i}; {a,b,c,d,e,f,g}; {a,b,c,d,e,f,h};
 {a,b,c,d,e,f,i}; {a,b,c,d,e,g,h}; {a,b,c,d,e,g,i}; {a,b,c,d,e,h,i}; {a,b,c,d,f,g,h}; {a,b,c,d,f,g,i};
 {a,b,c,d,f,h,i}; {a,b,c,d,g,h,i}; {a,b,c,e,f,g,h}; {a,b,c,e,f,g,i}; {a,b,c,e,f,h,i}; {a,b,c,e,g,h,i};
 {a,b,c,f,g,h,i}; {a,b,d,e,f,g,h}; {a,b,d,e,f,g,i}; {a,b,d,e,f,h,i}; {a,b,d,e,g,h,i}; {a,b,d,f,g,h,i};
 {a,b,e,f,g,h,i}; {a,c,d,e,f,g,h}; {a,c,d,e,f,g,i}; {a,c,d,e,f,h,i}; {a,c,d,e,g,h,i}; {a,c,d,f,g,h,i};
 30 {a,c,e,f,g,h,i}; {a,d,e,f,g,h,i}; {b,c,d,e,f,g,h}; {b,c,d,e,f,g,i}; {b,c,d,e,f,h,i}; {b,c,d,e,g,h,i};
 {b,c,d,f,g,h,i}; {b,c,e,f,g,h,i}; {b,d,e,f,g,h,i}; {c,d,e,f,g,h,i}; {a,b,c,d,e,f,g,h}; {a,b,c,d,e,f,g,i};
 {a,b,c,d,e,f,h,i}; {a,b,c,d,e,g,h,i}; {a,b,c,d,f,g,h,i}; {a,b,c,e,f,g,h,i}; {a,b,d,e,f,g,h,i};
 {a,c,d,e,f,g,h,i}; {b,c,d,e,f,g,h,i}; or {a,b,c,d,e,f,g,h,i}.

The level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1 and (d) DDX5 is decreased, such as {a}; {b}; {c}; {d}; {a,b}; {a,c}; {a,d}; {b,c}; {b,d}; {c,d}; {a,b,c}; {a,b,d}; {a,c,d}; {b,c,d}; or {a,b,c,d}.

The level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1 and
5 (c) LIMK1 is decreased, such as {a} {b} {c} {a,b} {a,c} {b,c}; or {a,b,c}.

The level of expression and/or transcription of CD63 is preferably decreased.

The level of expression and/or transcription of one or more of (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n) APOL1 is decreased, such as {j}; {k}; {l}; {m}; {n}; {j,k}; {j,l}; {j,m}; {j,n}; {k,l}; {k,m}; {k,n}; {l,m}; {l,n}; {m,n}; {j,k,l}; {j,k,m}; {j,k,n}; {j,l,m}; {j,l,n};
10 {j,m,n}; {k,l,m}; {k,l,n}; {k,m,n}; {l,m,n}; {j,k,l,m}; {j,k,l,n}; {j,k,m,n}; {j,l,m,n}; {k,l,m,n};
or {j,k,l,m,n}.

The level of expression and/or transcription of UBE2I is preferably decreased.

Decreased or abolished expression and/or transcription

15 The level of expression and/or transcription of one or more of (a) to (n) may be decreased by any amount. For instance, the level of expression and/or transcription may be decreased by at least 10%, at least 20%, at least 30% at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95%. The expression and/or transcription may be abolished (i.e. the expression or transcription is decreased by 100%).

20 The level of transcription can be determined by measuring the amount of mRNA encoded by the gene. The amount of mRNA can be measured using quantitative reverse transcription polymerase chain reaction (qRT-PCR), such as real time qRT-PCR, northern blotting or microarrays.

The level of expression can be determined by measuring the amount of protein encoded
25 by the gene. The amount of the protein can be measured using immunohistochemistry, western blotting, mass spectrometry or fluorescence-activated cell sorting (FACS).

The level of expression and/or transcription of one or more of (a) to (n) may be decreased in any way. The method of the invention may comprise (1) decreasing in a culture of human host cells the level of expression and/or transcription of one or more of (a) to (n); (2) expressing
30 the retroviral vectors in the culture provided in (1); and (3) allowing the population of retroviral vectors to accumulate in the culture medium.

The level of expression and/or transcription of the one or more genes in (a) to (n) is decreased using histone modification, antisense oligonucleotides, ribozymes, micro RNA (miRNA), small interfering RNA (siRNA), small hairpin RNA (shRNA), DNA-directed RNA
35 interference (ddRNAi), clustered regularly interspaced short palindromic repeats (CRISPR)

interference (CRISPRi), transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs) and meganucleases.

siRNA and shRNA

RNA interference (RNAi) technology for knocking down protein expression is well known in the art and standard methods can be employed to knock down expression of any one of (a) to (n). RNAi involves the use of double-stranded RNA, such small interfering RNA (siRNA) or small hairpin RNA (shRNA), which can bind to the mRNA and inhibit protein expression.

Accordingly, the level of expression of any one of (a) to (n) may be decreased or abolished using an oligonucleotide which specifically hybridises to a part of the mRNA of any one of (a) to (n). The human host cells may be contacted with or administered an oligonucleotide which specifically hybridises to a part of the mRNA of any one of (a) to (n). Combinations of different oligonucleotides each of which specifically hybridises to a part of the mRNA of any one of (a) to (n) may be used/administered. Combinations of (a) to (n) are listed above. The mRNA sequences for (a) to (n) may be found at the GenBank entries listed above.

Oligonucleotides are short nucleotide polymers which typically have 50 or fewer nucleotides, such as 40 or fewer, 30 or fewer, 22 or fewer, 21 or fewer, 20 or fewer, 10 or fewer or 5 or fewer nucleotides. The oligonucleotides used in the invention are preferably 19 to 25 nucleotides in length, more preferably 21 or 22 nucleotides in length. The nucleotides can be naturally occurring or artificial. A nucleotide typically contains a nucleobase, a sugar and at least one linking group, such as a phosphate, 2'O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate group. The nucleobase is typically heterocyclic. Nucleobases include, but are not limited to, purines and pyrimidines and more specifically adenine (A), guanine (G), thymine (T), uracil (U) and cytosine (C). The sugar is typically a pentose sugar. Nucleotide sugars include, but are not limited to, ribose and deoxyribose. The nucleotide is typically a ribonucleotide or deoxyribonucleotide. The nucleotide typically contains a monophosphate, diphosphate or triphosphate. Phosphates may be attached on the 5' or 3' side of a nucleotide.

Nucleotides include, but are not limited to, adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), guanosine monophosphate (GMP), guanosine diphosphate (GDP), guanosine triphosphate (GTP), thymidine monophosphate (TMP), thymidine diphosphate (TDP), thymidine triphosphate (TTP), uridine monophosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP), cytidine monophosphate (CMP), cytidine diphosphate (CDP), cytidine triphosphate (CTP), 5-methylcytidine monophosphate, 5-methylcytidine diphosphate, 5-methylcytidine triphosphate, 5-hydroxymethylcytidine

monophosphate, 5-hydroxymethylcytidine diphosphate, 5-hydroxymethylcytidine triphosphate, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), deoxyadenosine monophosphate (dAMP), deoxyadenosine diphosphate (dADP), deoxyadenosine triphosphate (dATP), deoxyguanosine monophosphate (dGMP),

5 deoxyguanosine diphosphate (dGDP), deoxyguanosine triphosphate (dGTP), deoxythymidine monophosphate (dTMP), deoxythymidine diphosphate (dTDP), deoxythymidine triphosphate (dTTP), deoxyuridine monophosphate (dUMP), deoxyuridine diphosphate (dUDP), deoxyuridine triphosphate (dUTP), deoxycytidine monophosphate (dCMP), deoxycytidine diphosphate (dCDP) and deoxycytidine triphosphate (dCTP), 5-methyl-2'-deoxycytidine monophosphate, 5-
10 methyl-2'-deoxycytidine diphosphate, 5-methyl-2'-deoxycytidine triphosphate, 5-hydroxymethyl-2'-deoxycytidine monophosphate, 5-hydroxymethyl-2'-deoxycytidine diphosphate and 5-hydroxymethyl-2'-deoxycytidine triphosphate. The nucleotides are preferably selected from AMP, TMP, GMP, UMP, dAMP, dTMP, dGMP or dCMP.

The nucleotides may contain additional modifications. In particular, suitable modified
15 nucleotides include, but are not limited to, 2' amino pyrimidines (such as 2'-amino cytidine and 2'-amino uridine), 2'-hydroxyl purines (such as 2'-fluoro pyrimidines (such as 2'-fluorocytidine and 2'-fluoro uridine), hydroxyl pyrimidines (such as 5'- α -P-borano uridine), 2'-O-methyl nucleotides (such as 2'-O-methyl adenosine, 2'-O-methyl guanosine, 2'-O-methyl cytidine and 2'-O-methyl uridine), 4'-thio pyrimidines (such as 4'-thio uridine and 4'-thio cytidine) and
20 nucleotides have modifications of the nucleobase (such as 5-pentynyl-2'-deoxy uridine, 5-(3-aminopropyl)-uridine and 1,6-diaminohexyl-N-5-carbamoylmethyl uridine).

One or more nucleotides can be oxidized or methylated. The nucleotides may be attached to each other in any manner. The nucleotides may be linked by phosphate, 2'-O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate or phosphorothioate linkages. The
25 nucleotides are typically attached by their sugar and phosphate groups as in nucleic acids.

The oligonucleotide can be a nucleic acid, such as deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). The oligonucleotide may be any synthetic nucleic acid known in the art, such as peptide nucleic acid (PNA), glycerol nucleic acid (GNA), threose nucleic acid (TNA), locked nucleic acid (LNA), morpholino nucleic acid or other synthetic polymers with
30 nucleotide side chains.

An oligonucleotide preferably specifically hybridises to a part of hybridises to a part of the mRNA of any one of (a) to (n), hereafter called the target sequence. The length of the target sequence typically corresponds to the length of the oligonucleotide. For instance, a 21 or 22 nucleotide oligonucleotide typically specifically hybridises to a 21 or 22 nucleotide target
35 sequence. The target sequence may therefore be any of the lengths discussed above with

reference to the length of the oligonucleotide. The target sequence is typically consecutive nucleotides within the target mRNA.

An oligonucleotide “*specifically hybridises*” to a target sequence when it hybridises with preferential or high affinity to the target sequence but does not substantially hybridise, does not
 5 hybridise or hybridises with only low affinity to other sequences.

An oligonucleotide “*specifically hybridises*” if it hybridises to the target sequence with a melting temperature (T_m) that is at least 2 °C, such as at least 3 °C, at least 4 °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C or at least 10 °C, greater than its T_m for other sequences. More preferably, the oligonucleotide hybridises to the target sequence with a T_m that
 10 is at least 2 °C, such as at least 3 °C, at least 4 °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C, at least 10 °C, at least 20 °C, at least 30 °C or at least 40 °C, greater than its T_m for other nucleic acids. Preferably, the portion hybridises to the target sequence with a T_m that is at least 2 °C, such as at least 3 °C, at least 4 °C, at least 5 °C, at least 6 °C, at least 7 °C, at least 8 °C, at least 9 °C, at least 10 °C, at least 20 °C, at least 30 °C or at least 40 °C, greater than
 15 its T_m for a sequence which differs from the target sequence by one or more nucleotides, such as by 1, 2, 3, 4 or 5 or more nucleotides. The portion typically hybridises to the target sequence with a T_m of at least 90 °C, such as at least 92 °C or at least 95 °C. T_m can be measured experimentally using known techniques, including the use of DNA microarrays, or can be calculated using publicly available T_m calculators, such as those available over the internet.

Conditions that permit the hybridisation are well-known in the art (for example,
 20 Sambrook et al., 2001, Molecular Cloning: a laboratory manual, 3rd edition, Cold Spring Harbour Laboratory Press; and Current Protocols in Molecular Biology, Chapter 2, Ausubel et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995)). Hybridisation can be carried out under low stringency conditions, for example in the presence of a buffered solution of
 25 30 to 35% formamide, 1 M NaCl and 1 % SDS (sodium dodecyl sulfate) at 37 °C followed by a 20 wash in from 1X (0.1650 M Na⁺) to 2X (0.33 M Na⁺) SSC (standard sodium citrate) at 50 °C. Hybridisation can be carried out under moderate stringency conditions, for example in the presence of a buffer solution of 40 to 45% formamide, 1 M NaCl, and 1 % SDS at 37 °C, followed by a wash in from 0.5X (0.0825 M Na⁺) to 1X (0.1650 M Na⁺) SSC at 55 °C.
 30 Hybridisation can be carried out under high stringency conditions, for example in the presence of a buffered solution of 50% formamide, 1 M NaCl, 1% SDS at 37 °C, followed by a wash in 0.1X (0.0165 M Na⁺) SSC at 60 °C.

The oligonucleotide may comprise a sequence which is substantially complementary to the target sequence. Typically, the oligonucleotides are 100% complementary. However, lower
 35 levels of complementarity may also be acceptable, such as 95%, 90%, 85% and even 80%.

Complementarity below 100% is acceptable as long as the oligonucleotides specifically hybridise to the target sequence. An oligonucleotide may therefore have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatches across a region of 5, 10, 15, 20, 21, 22, 30, 40 or 50 nucleotides.

The oligonucleotide can be a nucleic acid, such as any of those discussed above. The
5 oligonucleotide is preferably RNA.

The oligonucleotide may be single stranded. The oligonucleotide may be double stranded. The oligonucleotide may comprise a hairpin.

Oligonucleotides may be synthesised using standard techniques known in the art. Alternatively, oligonucleotides may be purchased.

10 Oligonucleotides may be administered to the human host cells in any of the forms discussed below with reference to the Cas9 polynucleotides.

CRISPRi

The level of expression and/or transcription of one or more of (a) to (n) may be altered
15 using clustered regularly interspaced short palindromic repeats (CRISPR) interference (CRISPRi). This involves targeting a catalytically inactive CRISPR associated protein 9 (Cas9) to a position in any one or (a) to (n) in the cell. The may be position proximal to the transcription start site (TSS) of the gene(s).

A catalytically inactive Cas9 may be used. The Cas9 may be derived from any source.
20 The Cas9 is preferably derived from *Streptococcus pyogenes* or from *Staphylococcus aureus*. The Cas9 may be derived from *Corynebacter diphtheria*, *Eubacterium ventriosum*, *Streptococcus pasteurianus*, *Lactobacillus farciminis*, *Sphaerochaeta globus*, *Azospirillum*, *Gluconacetobacter diazotrophicus*, *Neisseria cinerea*, *Roseburia intestinalis*, *Parvibaculum lavamentivorans*, *Nitratifractor salsuginis*, *Campylobacter lari* or *Streptococcus thermophiles*.

25 The Cas9 is catalytically inactive. This means that the Cas9 lacks the endonuclease activity needed to make a double strand break in DNA. This means that it is incapable of mutating genes, but it can still regulate them (in accordance with the invention). The endonuclease activity of the Cas9 can be measured using routine techniques, such as Surveyor or T7 endonuclease assays, high resolution melt analysis (HRMA) or *in vitro* cleavage of naked
30 DNA. Catalytically inactive Cas9s are also known in the art.

For instance, the Cas9 from *Streptococcus pyogenes* is made inactive via the substitutions D10A and H840A (i.e. replacement of aspartic acid at position 10 with alanine and replacement of histidine at position 840 with alanine).

The cells may be contacted with the protein form of the catalytically inactive Cas9.
35 Alternatively or in addition, the catalytically inactive Cas9 may be expressed in the cells. This

involves contacting the cells with a polynucleotide which encodes the catalytically inactive Cas9. A polynucleotide, such as a nucleic acid, is a polymer comprising two or more nucleotides. The nucleotides may be any of those discussed above. The polynucleotide is typically a nucleic acid, such as deoxyribonucleic acid (DNA) or a ribonucleic acid (RNA). The polynucleotide may be any synthetic nucleic acid known in the art and discussed above.

The Cas9 is targeted to a position in any one of (a) to (n), such as proximal to the transcription start site (TSS). The TSS of the gene can be identified using standard techniques, such as rapid amplification of cDNA ends (5' RACE) or derivatives, such as the high throughput cap analysis of gene expression (CAGE) pioneered by the Riken FANTOM5 consortium. The sgRNA base pairs with the DNA to provide specificity, but the Cas9 protein-sgRNA complex will bind to a larger region, including sequence specific contacts of the protein with the PAM sequence (NGG) immediately downstream of the sgRNA.

The Cas9 is preferably targeted to a position that is less than 300 nucleotides from the TSS, such as less than 250 nucleotides, less than 200 nucleotides, less than 150 nucleotides, less than 100 nucleotides, less than 50 nucleotides, less than 30 nucleotides or less than 10 nucleotides from the TSS. The Cas9 is preferably targeted to the TSS itself, immediately upstream of the TSS or immediately downstream of the TSS. The Cas9 is preferably targeted to a non-protein coding region of the gene.

The Cas9 is preferably targeted using a synthetic-guide RNA (sgRNA) or a CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) pair. The method may therefore comprise contacting the cell with a synthetic-guide RNA (sgRNA) or a CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) pair.

The method may comprise expressing the sgRNA or crRNA and tracrRNA in the cell. The method may therefore comprise contacting the cell with a polynucleotide which encodes the sgRNA or crRNA and tracrRNA. The polynucleotide may be any of those discussed above. As discussed in more detail below, this polynucleotide may be part of the same expression vector which comprises a polynucleotide sequence encoding the Cas9.

The sgRNA, crRNA or tracrRNA preferably comprises a RNA oligonucleotide which specifically hybridises to a target sequence on the gene. The target sequence is designed such that the Cas9 is targeted to a position in the gene, such as proximal to the TSS as described above. RNA oligonucleotides are discussed above.

Since the RNA oligonucleotide are intended to specifically hybridise to the target gene, it may correspond to a continuous section of the mRNA of gene (which is complementary to the gene itself). The mRNAs of (a) to (n) can be found in the GenBank entries listed above. The RNA oligonucleotides may be 50 or fewer continuous ribonucleotides, such as 40 or fewer, 30 or

fewer, 22 or fewer, 21 or fewer, 20 or fewer, 10 or fewer or 5 or fewer nucleotides, from any of the mRNAs listed in these entries. The oligonucleotide used in the invention is preferably 20 to 25 nucleotides in length, more preferably 21 or 22 nucleotides in length.

The oligonucleotide may comprise a sequence which is substantially complementary to the target (gene) sequence. Typically, the oligonucleotides are 100% complementary. However, lower levels of complementarity may also be acceptable, such as 95%, 90%, 85% and even 80%. Complementarity below 100% is acceptable as long as the oligonucleotides specifically hybridise to the target sequence. An oligonucleotide may therefore have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatches across a region of 5, 10, 15, 20, 21, 22, 30, 40 or 50 nucleotides.

“Complementarity” refers to the ability of the oligonucleotide to form hydrogen bond(s) with the gene sequence by either traditional Watson-Crick base pairing or other non-traditional types.

Cas9 can be delivered to the cells prior to the sgRNA or crRNA and tracrRNA to give time for Cas to be expressed. The Cas9 might be administered 1-12 hours (preferably around 2-6 hours) prior to the administration of the sgRNA or crRNA and tracrRNA. Alternatively, Cas9 and the sgRNA or crRNA and tracrRNA and guide RNA can be administered together. Advantageously, a second booster dose of the sgRNA or crRNA and tracrRNA can be administered 1-12 hours (preferably around 2-6 hours) after the initial administration of Cas 9 and the sgRNA or crRNA and tracrRNA. Additional administrations of Cas9 and/or the sgRNA or crRNA and tracrRNA might be useful to achieve the most efficient levels of expression alteration

The Cas9 and the sgRNA or crRNA and tracrRNA may administered to the cells in any appropriate way. The Cas9 and the sgRNA or crRNA and tracrRNA may be delivered in the form of a polynucleotide sequence. The polynucleotide sequence may be cloned into any suitable expression vector. In an expression vector, the polynucleotide sequence encoding a construct is typically operably linked to a control sequence, such a promoter, which is capable of providing for the expression of the coding sequence by the brain cell. Such expression vectors can be used to express the polynucleotide in the human host cells. The expression vector may also comprise a polynucleotide sequence encoding the Cas9 and a polynucleotide sequence encoding the sgRNA or crRNA and tracrRNA.

The term “*operably linked*” refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence “*operably linked*” to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. Multiple copies of the same or different polynucleotide may be introduced into the vector.

The term “control sequence” is intended to include promoters, enhancers, internal ribosomal entry sites (IRES), and other expression control elements (e.g. transcription termination signals, such as polyadenylation signals and poly-U sequences). Such control sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Control sequences include those that direct constitutive expression of a nucleotide sequence in many types of brain cell and those that direct expression of the nucleotide sequence only in certain brain cells. A non-limiting example of a suitable neuron-specific promoter includes the neurofilament promoter; Byrne and Ruddle, 1989. Proc. Natl. Acad. Sci. USA 86: 5473-5477.

Control sequences may also direct expression in a temporal-dependent manner, such as in a cell-cycle dependent or developmental stage-dependent manner, which may or may not also be tissue or cell-type specific. In some embodiments, a vector comprises one or more pol III promoters (e.g. 1, 2, 3, 4, 5, or more pol III promoters), one or more pol II promoters (e.g. 1, 2, 3, 4, 5, or more pol II promoters), one or more pol I promoters (e.g. 1, 2, 3, 4, 5, or more pol I promoters), or combinations thereof. Examples of pol III promoters include, but are not limited to, U6 and H1 promoters. Examples of pol II promoters include, but are not limited to, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, e.g., Boshart et al, Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β -actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 α promoter. Also encompassed by the term “control sequence” are enhancer elements, such as WPRE; CMV enhancers; the R-U5' segment in LTR of HTLV-I (Mol. Cell. Biol., Vol. 8(1), p. 466-472, 1988); SV40 enhancer; and the intron sequence between exons 2 and 3 of rabbit β -globin (Proc. Natl. Acad. Sci. USA., Vol. 78(3), p. 1527-31, 1981). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression desired, etc. With regards to control sequences, mention is made of U.S. patent application 10/491,026. With regards to promoters, mention is made of PCT publication WO 2011/028929 and U.S. application 12/511,940.

Conventional viral and non-viral based gene transfer methods can be used to introduce the polynucleotide, sgRNA or crRNA and tracrRNA into the cells. Non-viral vector delivery systems include DNA plasmids, RNA, naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a liposome. Methods of non-viral delivery of nucleic acids include lipofection, electroporation, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and

4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Felgner, WO 91/17424; WO 91/16024. The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

Conventional viral based expression systems could include retroviral, lentiviral, adenoviral, adeno-associated (AAV), herpes simplex virus (HSV) and Sendai viral vectors for gene transfer. Methods for producing and purifying such vectors are known in the art.

The Cas9 and the sgRNA or crRNA and tracrRNA or any expression vector containing them may be delivered using nanoparticle delivery systems. Such delivery systems include, but are not limited to, lipid-based systems, liposomes, micelles, microvesicles, exosomes, and gene gun. With regard to nanoparticles that can deliver RNA, see, e.g., Alabi et al., Proc Natl Acad Sci U S A. 2013 Aug 6;110(32):12881-6; Zhang et al., Adv Mater. 2013 Sep 6;25(33):4641-5; Jiang et al., Nano Lett. 2013 Mar 13;13(3):1059-64; Karagiannis et al., ACS Nano. 2012 Oct 23;6(10):8484-7; Whitehead et al., ACS Nano. 2012 Aug 28;6(8):6922-9 and Lee et al., Nat Nanotechnol. 2012 Jun 3;7(6):389-93. Lipid Nanoparticles, Spherical Nucleic Acid (SNA™) constructs, nanoplexes and other nanoparticles (particularly gold nanoparticles) are also contemplated as a means for delivery of Cas9 and optionally the the sgRNA or crRNA and tracrRNA or any expression vector.

Other methods of altering gene expression and/or transcription

ZFN and TALEN-based methods for gene editing are described in Govindan and Ramalingam, J Cell Physiol, 9999:1-13 (2016); Gaj et al., Trends in Biotechnology, 31(7): 397-405 (2013); Sander and Young, Nature Biotechnology, 32: 347-355 (2014); Hu et al., Cell Chem Biol, 23(1):57-73 (2016). HDR and NHEJ are described in Pardo et al, Cell Mol Life Sci, 66(6):1039-1056 (2009) and Cox et al., Nature Medicine, 21(2): 121-131 (2015).

ZFNs may be used to introduce one or more site-specific double-stranded breaks (DSBs) in one or more of (a) to (n). TALEN may be used to repair the DSB in such a way as to modify the sequence and/or expression of the gene in which the DSB occurs.

Meganucleases are "molecular DNA scissors" that can be used to replace, eliminate or modify sequences in a highly targeted way.

Host cells and cultures of the invention

The invention also provides a human host cell in which the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1, (i) CD81, (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n) APOL1 is decreased. Any of the embodiments discussed above with reference to the methods of the invention, including the type of cell and the combinations of (a) to (n), equally apply to the cell of the invention. The level of expression and/or transcription of one or more of (a) to (n) can be decreased using any of the methods discussed above.

The cell of the invention may be isolated, substantially isolated, purified or substantially purified. The cell is isolated or purified if it is completely free of any other components, such as culture medium, other cells of the invention or other cell types. The cell is substantially isolated if it is mixed with carriers or diluents, such as culture medium, which will not interfere with its intended use. Alternatively, the cell of the invention may be present in a growth matrix or immobilized on a surface as discussed above.

Cells of the invention may be isolated using a variety of techniques including antibody-based techniques. Cells may be isolated using negative and positive selection techniques based on the binding of monoclonal antibodies to those surface markers which are present on the cell. Hence, the cells may be separated using any antibody-based technique, including fluorescent activated cell sorting (FACS) and magnetic bead separation.

The invention also provides a culture of a plurality of human host cells of the invention. The culture may be in any of the forms discussed above with reference to the method of the invention. The culture may comprise any number of cells of the invention. The culture may comprises at least about 1×10^6 , at least about 2×10^6 , at least about 2.5×10^6 , at least about 5×10^6 , at least about 1×10^7 , at least about 2×10^7 , at least about 5×10^7 , at least about 1×10^8 or at least about 2×10^8 cells of the invention. In some instances, the population may comprise at least about 1×10^7 , at least about 1×10^8 , at least about 1×10^9 , at least about 1×10^{10} , at least about 1×10^{11} or at about least 1×10^{12} cells of the invention or even more.

The host cell and culture of the invention may be used in the method of the invention.

Vectors and populations of the invention

The invention also provides a retroviral vector produced using a method of the invention. Any of the embodiments discussed above with reference to the method of the invention, such as the type of vector, equally apply to the vector of the invention. The vector preferably further comprises a therapeutic gene or a gene editing tool as indicated above, such siRNA, shRNA or

any of the components of CRISPRi. This is discussed in more detail below with reference to the medical uses of the invention.

The invention also provides a population of retroviral vectors produced using a method of the invention. Any of the embodiments discussed above with reference to the method of the invention, such as the type of vectors, equally apply to the population of the invention. The population may comprise any number of vectors of the invention, such as at least about 1×10^6 , least about 1×10^7 , at least about 1×10^8 , at least about 1×10^9 , at least about 1×10^{10} , at least about 1×10^{11} or at about least 1×10^{12} vectors of the invention or even more. The population may have any of the P:I ratios discussed above with reference to the method of the invention.

The vectors in the population preferably further comprise a therapeutic gene or gene editing tool. This is discussed in more detail below with reference to the non-medical and medical uses of the invention.

The vector and population of the invention may be any form. They may be used for any pharmaceutical or non-pharmaceutical use.

Pharmaceutical compositions of the invention

The invention also provides a pharmaceutical composition comprising (a) a vector of the invention or population of the invention and (b) a pharmaceutically acceptable carrier or diluent. The carrier or diluent may be any of those discussed above with reference to the CRISPRi vectors which may be used in the invention.

The carrier(s) or diluent(s) present in the pharmaceutical composition must be “acceptable” in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. Typically, carriers for injection, and the final formulation, are sterile and pyrogen free. Preferably, the carrier or diluent is water. A pharmaceutically acceptable carrier or diluent may comprise as one of its components thioglycerol or thioanisole.

Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances and the like, may be present in the excipient or vehicle. These excipients, vehicles and auxiliary substances are generally pharmaceutical agents that do not induce an immune response in the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, polyethyleneglycol, hyaluronic acid, glycerol, thioglycerol and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of

pharmaceutically acceptable excipients, vehicles and auxiliary substances is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

The active agents are typically present at 0.1% to 50% by weight in the pharmaceutical composition, more preferably at 0.1% to 5% by weight. They may be present at less than 0.1% by weight in the pharmaceutical composition.

The pharmaceutically acceptable carrier or diluent is typically present at 50% to 99.9% by weight in the pharmaceutical composition, more preferably at 95% to 99.9% by weight. The pharmaceutically acceptable carrier or diluents may be present at more than 99.9% by weight in the pharmaceutical composition.

Pharmaceutical compositions include, but are not limited to pharmaceutically acceptable solutions, lyophilisates, suspensions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable compositions. Such pharmaceutical compositions may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. A lyophilisate may comprise one or more of trehalose, thioglycerol and thioanisole. In one embodiment of a pharmaceutical composition for parenteral administration, the active ingredient is provided in dry form (e.g., a lyophilisate, powder or granules) for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted pharmaceutical composition.

The pharmaceutical composition may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable compositions may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides.

Other parenterally-administrable pharmaceutical compositions which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Pharmaceutical compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

For example, solid oral forms may contain, together with the active substance, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents;

e.g. starches, gum arabic, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical compositions. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tableting, sugar-coating, or film-coating processes.

Liquid dispersions for oral administration may be syrups, emulsions or suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with the active substance, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for intravenous administration or infusion may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1% to 2%.

Oral compositions include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release compositions or powders and contain 10% to 95% of active ingredient, preferably 25% to 70%. Where the pharmaceutical composition is lyophilised, the lyophilised material may be reconstituted prior to administration, e.g. a suspension. Reconstitution is preferably effected in buffer.

Capsules, tablets and pills for oral administration to an individual may be provided with an enteric coating comprising, for example, Eudragit "S", Eudragit "L", cellulose acetate, cellulose acetate phthalate or hydroxypropylmethyl cellulose.

Polynucleotides may be present in combination with cationic lipids, polymers or targeting systems.

Uptake of polynucleotide or oligonucleotide constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents.

Examples of these agents include cationic agents, for example, calcium phosphate and DEAE-

Dextran and lipofectants, for example, lipofectamine and transfectam. The dosage of the polynucleotide or oligonucleotide to be administered can be altered.

Alternatively, the active agent may be encapsulated, adsorbed to, or associated with, particulate carriers. Suitable particulate carriers include those derived from polymethyl methacrylate polymers, as well as PLG microparticles derived from poly(lactides) and poly(lactide-co-glycolides). See, e.g., Jeffery et al. (1993) Pharm. Res. 10:362-368. Other particulate systems and polymers can also be used, for example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules. The composition will depend upon factors such as the nature of the active agent and the method of delivery. The pharmaceutical composition may be administered in a variety of dosage forms. It may be administered orally (e.g. as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules), topically, parenterally, subcutaneously, by inhalation, intravenously, intramuscularly, intralymphatically (such as to lymph nodes in the groin), intrasternally, transdermally, intradermally, epidermally, sublingually, intranasally, buccally or by infusion techniques. The administration may be intratonsillar. The administration may be as suppositories. The administration may be made by iontophoresis. Preferably, the administration is intradermal, epidermal or transdermal. The administration may be made by a patch, such as a microtine patch. Administration is discussed in more detail below.

A physician will be able to determine the required route and means of administration for each particular individual.

The pharmaceutical compositions of the invention are preferably provided sealed in a container. The pharmaceutical compositions are typically provided in unit dose form, for example single dose form. They may alternatively be provided in multi-dose form. Where the pharmaceutical composition is a pharmaceutically acceptable solution, the solution may be provided in an ampoule, sealed vial, syringe, cartridge, flexible bag or glass bottle. Where the pharmaceutical composition is a lyophilisate, it is preferably provided in a sealed vial.

The pharmaceutical compositions of the invention will comprise a suitable concentration of each agent to be effective without causing adverse reaction. Where the pharmaceutical composition is for example a lyophilisate, the relevant concentration will be that of each vector following reconstitution. Typically, the concentration of each agent in the pharmaceutical composition when in solution will be in the range of 0.03 to 200 nmol/ml. The concentration of each agent may be more preferably in the range of 0.3 to 200 nmol/ml, 3 to 180 nmol/ml, 5 to 160 nmol/ml, 10 to 150 nmol/ml, 50 to 200 nmol/ml or 30 to 120 nmol/ml, for example about 100 nmol/ml. The pharmaceutical composition should have a purity of greater than 95% or 98% or a purity of at least 99%.

In an embodiment where the invention involves combined therapy, the other therapeutic agents or adjuvants may be administered separately, simultaneously or sequentially. They may be administered in the same or different pharmaceutical compositions. A pharmaceutical composition may therefore be prepared which comprises an agent of the invention and also one or more other therapeutic agents or adjuvants. A pharmaceutical composition of the invention may alternatively be used simultaneously, sequentially or separately with one or more other therapeutic compositions as part of a combined treatment.

In vitro methods of the invention

The invention also provides a method of delivering a polynucleotide to a cell *in vitro*, comprising administering to the cell a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention, wherein the retroviral vector or retroviral vectors comprise the polynucleotide. The polynucleotide may be a gene or a therapeutic gene.

The method may comprise delivering any number of polynucleotides, such as 1, 2, 3, 4, 5, 10, 20 or more polynucleotides. The polynucleotide(s) may be any polynucleotide (s) of interest.

Polynucleotides are discussed above. The polynucleotide is typically operably linked to a control sequence which is capable of providing for the expression of the polynucleotide by the host cell. Control sequences are discussed above.

The cell may be any cell type. Suitable *in vitro* culture conditions are discussed above with reference to the method of the invention.

The vectors are being used a polynucleotide delivery system. The vector, population or pharmaceutical composition of the invention may be administered in any of the forms discussed above.

Therapeutic methods of the invention

The invention also provides a method of treating or preventing in a subject a disease which will be benefit from a therapeutic gene, comprising administering to the subject a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene.

The invention also provides a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention for use in a method of

treating or preventing in a subject a disease which will benefit from a therapeutic gene, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene.

The invention also provides use of a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention in
5 manufacture of a medicament for treating or preventing in a subject a disease which will benefit from a therapeutic gene, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene.

The invention also provides a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention for use in gene
10 therapy.

The invention also provides use of a retroviral vector of the invention, a population of retroviral vectors of the invention or a pharmaceutical composition of the invention in the manufacture of a medicament for use in gene therapy.

A disease will benefit from a therapeutic gene if the administration of the gene treats or
15 prevents one or more symptoms of the disease. The disease may be associated with the therapeutic gene in the sense that an alteration in the function of the naturally-occurring version of the gene is associated with the disease. For instance, the function of the gene may be altered in a patient having the disease when compared with a patient of the same sex and of approximately the same age without the disease.

The disease may be associated with an increased function of the gene. The function of
20 the gene may be increased by any amount. For instance, the function may be decreased by at least 10%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% or at least 100% compared with the level of the function in a normal brain cell. The function may be increased by at least a factor of 2 compared with the function in
25 a normal brain cell, such as at least a factor of 3, at least a factor of 10, at least a factor of 300, at least a factor of 500, at least a factor of 1000 or more.

The disease may be associated with a decreased function of the gene. The function of the gene may be decreased by any amount. For instance, the function may be decreased by at least 10%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least
30 90% or at least 95% compared with the level of the function in a normal brain cell. The disease may have a complete loss of gene function (i.e. the function is decreased 100% compared with a normal brain cell). Gene function may be measured in any of the ways discussed below. The gene function may be the expression of the gene. The disease may be associated with an alteration (increase or decrease) in the expression of the gene (an increase or a decrease in the
35 expression of the gene). This is discussed in more detail below.

The gene may be associated with the disease because of a polymorphism or mutation in the gene. The gene may comprise a missense mutation. Missense mutations change the amino acid sequence of the encoded protein and thus can reduce the function of the protein or abolish it altogether.

5 The gene may comprise a nonsense mutation. This leads to decay of mRNA and thus a reduction in protein expression.

 The gene may comprise a frameshift mutation. The frameshift mutation may be a deletion frameshift mutation or an insertion frameshift mutation. Both types of mutation can decrease the function of the gene or abolish it altogether. Some frameshift mutations can also
10 introduce a pre-mature stop codon and lead to loss of protein expression.

 The gene may comprise a deletion inframe mutation. This mutation may also decrease the function of the encoded protein or abolish it altogether.

 The mutations discussed above are preferably homozygous.

 Mutations in mRNA may be identified using RNA sequencing including next-generation
15 sequencing. Mutations in the gene may be identified using DNA sequencing including next-generation sequencing. This may also be done using Southern blotting, measuring copy-number variation and investigating promoter methylation.

 It will be clear from the above that mutations may affect the level expression of the gene, its stability or its ability to function. The disease may be associated with an increased amount or
20 a decreased amount of the protein encoded by the gene. The disease may comprise an increased amount or a decreased amount of the protein encoded by the gene compared with the amount in a normal brain cell. The amount of the protein may be increased or decreased by any amount and in particular the % amounts discussed above in relation to gene function.

 The amount of the protein can be measured as discussed above.

25 The disease may be associated with a protein encoded by the gene with an increased function or a decreased function. The disease may be associated with a protein encoded by the disease with an increased function or a decreased function compared with the protein in a normal brain cell. The function of the protein may be increased or decreased by any amount and in particular the % amounts discussed above in relation to gene function. Any function of the
30 protein may be altered. The function of the protein can be measured using standard assays depending on its function. The disease may be associated with misfolding and/or aggregation of the protein.

 It will be clear from the above that mutations may affect the amount of the gene's mRNA. The disease may be associated with an increased amount or a decreased amount of the
35 gene's mRNA. The disease may comprise an increased amount or a decreased amount of the

gene's mRNA compared with a normal brain cell. The amount of the mRNA may be increased or decreased by any amount and in particular the % amounts discussed above in relation to gene function.

The amount of mRNA can be measured as described above.

5 The administration of the therapeutic gene in accordance with the invention corrects the altered function of the gene in the subject. For instance, a mutation of the gene that results in decreased expression of the gene may be corrected by administration of the therapeutic gene in accordance with the invention.

Any disease may be treated in accordance with the invention. The disease may be
10 cancer. The cancer may be colorectal cancer, prostate cancer, ovarian cancer, lung cancer, central nervous system (CNS) cancer, breast cancer, pancreatic cancer, large intestine cancer or kidney cancer.

The disease may be a respiratory, cardiovascular, gastroenterological, skin,
musculoskeletal, neurological, ophthalmological, genitourinary, immune system or inflammatory
15 disease.

Respiratory diseases include, but are not limited to asthma, respiratory allergy, pneumonia, bronchitis, rhinitis, sinusitis, tracheitis, pharyngitis, croup and otitis.

Cardiovascular diseases include, but are not limited to angina pectoris, ischemic myocardial infarction, arrhythmia, post-myocardial infarction pain, myocarditis, heart failure and
20 hypertension.

Gastroenterological diseases include, but are not limited to stomach ulcers, gastritis, liver cirrhosis, pathological states of the oesophagus, gallstones, pancreatitis, constipation, diarrhea, hemorrhoids and fistulae or inflammation of the rectum.

Skin diseases include, but are not limited to psoriasis, neurodermatitis, dermatitis and
25 atopic dermatitis.

Muscular-skeletal diseases include, but are not limited to back pain, lumbago, fractures, pulled muscle, torn ligament or tendon, disc prolapse, ischiatitis, osteoporosis, Perthes disease, osteoarthritis, gout, muscle cramp and diseases affecting the integrity of joints, such as age-related disintegration of joints.

30 Neurological diseases include, but are not limited to neurodegenerative diseases, multiple sclerosis, dementia, neuralgias, and stroke. The neurodegenerative disease is preferably Parkinson's disease (PD), Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), Alzheimer's disease (AD), Pick's disease, frontotemporal dementia, frontotemporal dementia and parkinsonism linked to chromosome 17

(FTDP-17), cortico-basal degeneration, progressive supranuclear palsy, Huntington's disease or amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease and Charcot disease).

Ophthalmological diseases include, but are not limited to glaucoma, retinopathy, retinal macula degeneration, age-related macula degeneration (AMD), eye infections, and retinal detachment.

Genito-urinary diseases include, but are not limited to male genital conditions selected from prostatism, impotence, infertility, testicular disease, female conditions selected from pre/postmenstrual pains, fibroids, endometriosis, infertility, myoma, fibromyoma, inflammatory pelvic conditions, diseases of the ovaries, oviduct(s) or cervix and menopause.

Immune system or inflammatory diseases include, but are not limited to, rheumatoid arthritis, chronic obstructive pulmonary disease, asthma, angina pectoris, osteo-arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, psoriasis, multiple sclerosis, systemic lupus erythematosus, arteriosclerosis, pathogenic infection, injury or inflammation of the skin, inflammation of internal organs, colitis, gastroenteritis, pneumonia, wound infections, tuberculosis, influenza, sinusitis, chest infections, bronchitis, allergies such as hay fever, and hemorrhoids

Any subject may be treated in accordance with the invention. The subject is typically human. However, the subject can be another animal or mammal, such as a research animal, such as a rat, a mouse, a rabbit or a guinea pig, a commercially farmed animal, such as a horse, a cow, a sheep or a pig, or a pet, such as a cat, a dog or a hamster.

The subject may be asymptomatic. A prophylactically effective amount of the vector, population or pharmaceutical composition is administered to such a subject. A prophylactically effective amount is an amount which prevents the onset of one or more, preferably all of, symptoms of the one or more diseases.

Alternatively, the subject may be in need thereof. That is, the subject may exhibit one or more symptoms of the disease. A therapeutically effective amount of the vector, population or pharmaceutical composition is administered to such a subject. A therapeutically effective amount is an amount which is effective to ameliorate one or more of, preferably all of, the symptoms of the disease.

The the vector, population or pharmaceutical composition may be administered to the subject in any appropriate way. In the invention, the vector, population or pharmaceutical composition may be administered in a variety of dosage forms. Thus, it can be administered orally, for example as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules. It may also be administered by enteral or parenteral routes such as via buccal, anal, pulmonary, intravenous, intra-arterial, intramuscular, intraperitoneal, intraarticular,

topical or other appropriate administration routes. A physician will be able to determine the required route of administration for each particular subject.

The the vector, population or pharmaceutical composition may be in any of the forms discussed above with reference to the pharmaceutical composition of the invention.

5 Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859 and 5,589,466. The nucleic acid molecule can be introduced directly into the recipient subject, such as by standard intramuscular or intradermal injection; transdermal particle delivery; inhalation; topically, or by oral, intranasal or mucosal modes of administration. The molecule alternatively can be introduced ex vivo into cells that have been removed from a subject. For
10 example, the vector, population or pharmaceutical composition may be introduced into APCs of an individual ex vivo. Cells containing the nucleic acid molecule of interest are re-introduced into the subject such that an immune response can be mounted against the peptide encoded by the nucleic acid molecule. The nucleic acid molecules used in such immunization are generally referred to herein as “nucleic acid vaccines.”

15 The dose may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the subject to be treated; the route of administration; and the required regimen. Again, a physician will be able to determine the required route of administration and dosage for any particular subject. A typical daily dose is from about 0.1 to 50 mg per kg of body weight, according to the activity of the specific inhibitor,
20 the age, weight and conditions of the subject to be treated and the frequency and route of administration. The dose may be provided as a single dose or may be provided as multiple doses, for example taken at regular intervals, for example 2, 3 or 4 doses administered hourly. Preferably, dosage levels of inhibitors are from 5 mg to 2 g.

 Typically vectors are administered in the range of 1 pg to 1 mg, preferably to 1 pg to 10
25 μg nucleic acid for particle mediated delivery and 10 μg to 1 mg for other routes.

 The vector, population or pharmaceutical composition is preferably administered in combination with another therapy

 The inhibitor may be used in combination with one or more other therapies intended to treat the same subject. By a combination is meant that the therapies may be administered
30 simultaneously, in a combined or separate form, to the subject. The therapies may be administered separately or sequentially to a subject as part of the same therapeutic regimen. For example, the vector, population or pharmaceutical composition may be used in combination with another therapy intended to treat the disease. The other therapy may be a general therapy aimed at treating or improving the condition of the subject. For example, treatment with methotrexate,
35 glucocorticoids, salicylates, nonsteroidal anti-inflammatory drugs (NSAIDs), analgesics, other

DMARDs, aminosaliclates, corticosteroids, and/or immunomodulatory agents (e.g., 6-mercaptopurine and azathioprine) may be combined with the inhibitor. The other therapy may be a specific treatment directed at the disease. Such treatments are known in the art.

5 **Example**

Introduction

Gene transfer vectors have been made from various members of the *Retroviridae*. Such vectors are widely used in cell biology and gene and cell therapy. The production of retroviral
10 vectors in general and lentiviral vectors in particular is a time-consuming and expensive operation. The production of such vectors typically occurs in mammalian host cells (preferably human cells, more preferably human HEK293 derived cells) into which the genetic components necessary for viral production are introduced by permanent or transient transfection/transduction. We sought to maximise the yields of viral vector production by reducing/eliminating the
15 expression of mammalian host cell proteins that reduce viral productivity.

Gene transfer vectors derived from the lentivirus HIV are a widely used example of a retroviral gene transfer vector. Reviews of HIV replication typically identify four host cell proteins – sometimes termed canonical host restriction factors, which inhibit HIV replication. These host factors are APOBEC3G, BST2 (Tetherin), SAMHD1, and TRIM5 α (Harris et al 2012
20 PMID: 23043100, Malim and Bieniasz 2012 PMID: 22553496, Santa-Marta et al 2013 PMID: 24167505, Strebel 2013 PMID: 24246762, Jia et al 2015 PMID: 25939065, Simon et al 2015 PMID: 25988886).

Here, we identify other host cell gene products that also reduce HIV replication and/or the production of lentiviral and/or retroviral gene transfer vectors.

25

Methods Overview

Cells

The HEK293 derived cell lines 293SF-PacLV #29-6-14 (termed hereafter 293SF-LVP) and 293SF-CymR-rtTA2s-M2 (termed hereafter 293SF-Titr) (Broussou et al 2008 PMID:
30 18180776) were maintained in tissue culture plates and Erlenmeyer flasks on an orbital rotating platform (orbit 0.75 inch) at 185 rpm (ThermoFisher Scientific, Paisley, UK) in a humidified incubator at 8% CO₂ in Freestyle 293 Expression Media (ThermoFisher Scientific). Cells were passaged twice a week by diluting existing cultures to 3.5x10⁵ cells per mL.

35 *LVR2-GFP Lentiviral Vector Production*

The addition of 1µg/mL doxycycline (Sigma-Aldrich, Gillingham, UK) and 10µg/mL cumate (Sigma-Aldrich) to cultures of 293SF-LVP cells induces the production of LVR2-GFP a third generation self-inactivating HIV-based lentiviral vector pseudotyped with VSV-G, that contains the Green Fluorescence Protein (GFP) cDNA under the transcriptional control of a Tet-Off promoter (Broussou et al, Vigna et al 2002 PMID: 11863414).

LVR2-GFP Lentiviral Vector Titration

In cells harbouring the rtTA2s-M2 transactivator protein (Vigna et al), and in the presence of 1µg/mL doxycycline the Tet-Off promoter found in LVR2-GFP provirus is transcriptionally active. To quantify the amount of LVR2-GFP produced in experimental samples, 203SF-Titr cells (which express the rtTA2s-M2 transactivator protein) were transduced and the number of GFP positive cells was determined.

siRNA Transfection

siRNA molecules were selected that targeted human protein encoding mRNAs predicted to be expressed in 293SF-LVP proteins. siRNA molecules (Dharmacon ON-TARGET Plus siRNA) to a given mRNA were provided by the manufacturer (GE Healthcare Dharmacon) as a pool of four individual siRNA molecules. siRNA molecule pools were introduced into 293SF-LVP cells 3 days prior to the initiation of LVR2-GFP production using Lipofectamine 2000 Transfection Reagent as described by the manufacturer.

High-Throughput Screening Methods

Introduction of siRNA Molecules Into 293SF-LVP Cells (Figure 1A)

Individual wells of 96-well tissue culture plates (ThermoFisher Scientific) were seeded with $2 \times 10^4 \pm 10\%$ 293SF-LVP cells in Freestyle 293 Expression Media. Approximately 10 minutes later, 20µl of Freestyle 293 Expression Media containing 500nM experimental siRNA pool (GE Healthcare Dharmacon) and 0.5µL Lipofectamine 2000 Transfection Reagent (ThermoFisher Scientific) were added. Cells were cultured on an agitating platform at 185 rpm in a humidified incubator at 8% CO₂.

Induction of LVR2-GFP Production (Figure 1B)

LVR2-GFP production was initiated by addition to each well of 293SF-LVP cells of 10µL of Freestyle 293 Expression Media that contained 10µg/mL doxycycline and 100µg/mL cumate, for a final concentration of approximately 1µg/mL doxycycline and 10µg/mL cumate.

LVR2-GFP Lentiviral Vector Harvesting (Figure 1C)

Three days after induction of LVR2-GFP production, the supernatant of each well of 293SF-LVP cells was clarified by filtration using a 96-well 0.2 µm filter plate (ThermoFisher Scientific) as described by the manufacturer.

5

LVR2-GFP Lentiviral Vector Titration (Figure 1D & 1E)

Individual wells of 96-well black-sided, clear/flat-bottomed tissue culture plates (PerkinElmer, Llantrisant, UK) were seeded with $5 \times 10^3 \pm 10\%$ 293SF-Titr cells in 80µL Freestyle 293 Expression Media. Approximately 10 minutes later, 30 or 45µL of 0.2µm filtered tissue culture media containing LVR2-GFP lentiviral particles was added. Three days later, the number of GFP positive 293SF-Titr cells in each well was determined using an Operetta High Content Imaging System (PerkinElmer) and Harmony high content imaging and analysis software with the PhenoLOGIC plug-in (PerkinElmer).

10

15 Controls, Quantitation & Replicates

Typically high-throughput siRNA experiment utilised the 32 central wells of each 96-well tissue culture plate. The remaining wells (the two external rows and columns in a standard 12x8 well 96-well plate) were filled with 200µL Dulbecco's Phosphate-Buffered Saline (ThermoFisher Scientific) to minimise variation in evaporation in the central experimental 32 wells.

20

Typically, on each siRNA transfection plate (Figure 1A & Figure 1B), a single experimental replicate was used. The number of LVR2-GFP lentiviral particles produced under each experimental condition was typically determined twice – once by using 15µL and once by using 30µL of the harvested tissue culture supernatant (Figure 1C). LVR2-GFP production under each experimental condition was normalised to an in-plate sample that was treated with a non-target control siRNA pool designed and tested by the manufacturer for minimal targeting of human, mouse or rat genes and recommended for establishing baseline cellular responses in RNAi studies. Each experimental condition was evaluated on at least four independent siRNA transfection plates.

25

30

Other controls used included: (1) non-target control siRNA pool, (2) a transfection efficiency control that targeted the GFP cDNA in the LVR2-GFP viral mRNA, (3) a cell viability control that targeted the PLK1 mRNA - inhibiting cell division and stimulating apoptosis (Spänkuch-Schmitt et al 2002 PMID: 12488480), (4) a lentivirus production knockdown control that targeted the TSG101 mRNA - arresting HIV-1 budding at a late stage. This later target has

35

previously been used as a positive control for inhibition of HIV replication in similar screens (Nguyen et al., 2006 PMID: 16352537, Zhou et al., 2008 PMID: 18976975, Wen et al., 2014 PMID: 25187981).

5 Results

siRNA Screen – Discriminatory Power

Using the conditions described above, an siRNA transfection study was performed in which 32 replicate wells were transfected with non-target control siRNA. LVR2-GFP lentiviral vector productivity was assessed in two independent titration studies. Data and presented in
10 Figure 2.

Identification of mammalian host cell factors that might inhibit lentiviral production

To identify potential host cell factors inhibiting lentiviral production, a search for publications was conducted on MEDLINE using the PubMed interface, as well as the Google
15 Scholar database, using the following keywords “HIV restriction factor”, “restrict HIV” and “HIV siRNA”. All papers obtained up to December 2015 were surveyed to specifically identify genes involved in the late phase of the HIV life cycle, as categorised in Figure 3 as these are hypothesised to potentially affect lentiviral vector production. The reference lists from the resulting studies were also consulted to identify any published studies missed by the database
20 search. Additional database enquiries were run using the name of possible candidate genes and the keywords “HIV”, “restriction factor” or “siRNA” to find further articles confirming findings. PubMed’s Gene database’s profile for each gene also provided supplementary studies that were investigated.

A selection of the 93 candidate host cell factors that may inhibit lentiviral production
25 restriction factors were selected for screening – their knockdown was anticipated to increase lentiviral production. A further 11 host cell factors were also selected for investigation whose knockdown was anticipated to decrease lentiviral production. The four canonical restriction factor, APOBEC3G, BST2, SAMHD1 and TRIM5 were also included as controls. The setup of each siRNA transfection plate included 24 candidate siRNA pools plus eight selected siRNA
30 reference to help control plate to plate variability. These eight include the four controls described in the methods (GFP, Non-target, PLK1 & TSG101) plus BST2, CHMP5, UBE2I & VTA1. Each siRNA transfection plate was repeated in quadruplicate with each being titrated in duplicate as described in the methods.

Each result was normalised to the non-target control value for each plate and then the
35 average of all the plates was determined. Figure 4 and Table 1 show the results for the whole

screen. In Figure 5, the spread of all individual data points from each siRNA transfection plate is shown.

Table 1 Ranked average results of the siRNA screen for all genes tested.

Rank	Gene	Screen Result	Rank	Gene	Screen Result	Rank	Gene	Screen Result
1	CD63	206.68	37	AGO2	91.75	73	MX2	56.85
2	ATP1A1	165.40	38	UBP1	89.13	74	DDX24	55.55
3	UBA7	162.40	39	EIF3E	88.57	75	EIF3F	55.25
4	DDX5	158.77	40	SUMO2	87.83	76	MAP3K5	54.59
5	DUSP1	158.31	41	TRIM22	86.70	77	COMM D1	52.30
6	LIMK1	156.69	42	UBE2L6	86.60	78	DNAJB1	51.88
7	SPN	145.30	43	CD9	83.18	80	HSP90AB1	51.63
8	AXIN1	144.04	44	APOBEC3B	82.45	81	CDKN1A	49.55
9	CD81	143.33	45	APOBEC3A	82.18	82	USP18	48.40
10	DLG1	139.14	46	CD3G	81.53	83	KAT5	48.05
11	SLFN11	137.56	47	TRIM32	81.33	84	PRKAA1	46.98
12	ABCA1	137.20	48	APOBEC3G	81.16	85	TRIM11	45.95
13	UBE2I	135.07	49	DNAJB6	81.03	86	HDAC2	45.48
14	APOL1	130.44	50	ROCK2	80.05	87	RNF115	44.86
15	CD53	129.32	51	HEXIM1	79.53	88	ISG15	44.00
16	DDX6	129.18	52	PRKACA	77.78	89	DDX58	43.91
17	SIRT1	123.99	53	SAMHD1	74.72	90	IFI16	43.25
18	APOBEC3H	121.68	54	DHX30	73.78	91	BANP	39.25
19	39508.0	119.88	55	GBP5	71.89	92	GFP	38.79
20	UBASH3A	118.54	56	HDAC1	71.58	93	TRIM26	37.30
21	SUMO1	113.79	57	CD82	70.05	94	LSM1	35.68
22	DROSHA	108.28	58	ZC3H12A	69.78	95	HSPA8	34.90
23	MOV10	106.91	59	TSG101	69.48	96	TSPO	32.45
24	EIF2AK2	106.74	60	DNAJC5	68.55	97	ADAR	32.28
25	HSPA1A	106.34	61	RNASL	68.45	98	TCF4	29.18
26	BST2	105.59	62	DNAJA1	68.15	99	APOBEC3C	29.00
27	CHMP5	103.27	63	TRIM5	67.67	100	TRIM21	28.73
28	RSAD2	100.10	64	MB21D1	67.40	101	IFITM3	23.10
29	HSPA4	100.00	65	RUVBL2	67.39	102	DICER1	20.00
30	Non-Target	100.00	66	HSPA1B	65.13	103	APOBEC3F	19.60
31	PRMT6	99.53	67	TARBP2	64.38	104	TRIM28	19.25

32	CTNNB1	98.39	68	SETDB1	62.90	105	HERC5	18.60
33	CNP	97.67	69	IFITM2	62.63	106	PLK1	17.91
34	EZR	97.67	70	IFITM1	62.20	107	PRKRA	16.13
35	TSPAN7	97.39	71	SP3	58.55			
36	CD151	96.26	72	VTG1	57.91			

Legend: Screen result is the mean normalised value of the titre obtained following knock-down with the indicated siRNA when the non-target control siRNA result set at 100%.

5 Discussion

Knockdown of nine host factors resulted an increase of $\geq 40\%$ in lentiviral vector production compared with treatment with the non-target siRNA pool. These were: CD63 (206.68%), ATP1A1 (165.40%), UBA7 (162.40%), DDX5 (158.77%), DUSP1 (158.31%), LIMK1 (156.69%), SPN (145.30%), AXIN1 (144.04%), CD81 (143.33%).

10 The step these hits are presumed to act in the late stage of the HIV life cycle shown in Figure 6.

Knockdown of a further five host factors resulted in an increase of $\geq 30\% \leq 40\%$ in lentiviral vector production compared with treatment with the non-target siRNA pool. These were: DLG1 (139.14%), SLFN11 (137.56%), ABCA1 (137.20%), UBE2I (135.07%), APOL1 (130.44%).

15 The increase achieved with just these 14 siRNAs is plotted in Figure 7.

CLAIMS

1. A method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1, (i) CD81, (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n) APOL1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium.
2. A method according to claim 1, wherein the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1 and (i) CD81 is decreased.
3. A method according to claim 1, wherein the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1 and (d) DDX5 is decreased.
4. A method according to claim 1, wherein the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1 and (c) LIMK1 is decreased.
5. A method according to claim 1, wherein the level of expression and/or transcription of CD63 is decreased.
6. A method according to claim 1, wherein the level of expression and/or transcription of one or more of (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n) APOL1 is decreased.
7. A method according to claim 1, wherein the level of expression and/or transcription of UBE2I is decreased.
8. A method according to any one of the preceding claims, wherein the human host cells are human embryonic kidney (HEK) cells.
9. A method according to any one of the preceding claims, wherein the retroviral vectors are derived from Human Immunodeficiency Virus-1 (HIV-1), Human Immunodeficiency Virus-2 (HIV-2), Simian Immunodeficiency Virus (SIV), Foamy virus, Bovine Immunodeficiency Virus (BIV), Feline Immunodeficiency Virus (FIV), Equine Infectious Anemia Virus (EIAV), Murine

Leukemia Virus (MLV), Bovine Leukemia Virus (BLV), Rous Sarcoma Virus (RSV), Spleen Necrosis Virus (SNV) or Mouse Mammary Tumor Virus (MMTV).

10. A method according to any one of the preceding claims, wherein the retroviral vectors comprise one or more of the following envelope proteins (a) Ecotropic MLV envelope, (b) Amphotropic MLV envelope, (c) Xenotropic MLV envelope, (d) Vesicular Stomatitis Virus glycoprotein VSV-G, (e) Simian Endogenous Retrovirus envelope RD114, (f) Gibbon Ape Leukemia Virus envelope GALV, (g) Measles Virus F and H proteins, (h) Sendai virus F and HN proteins (i) Influenza virus HA and NA proteins, (j) Baculovirus GP64 protein, (k) Human Immunodeficiency Virus p120 envelope, (l) Ebola virus envelope, (m) Rabies virus envelope G protein and (n) Mokola virus envelope G protein.

11. A method according to any one of claims 1 to 9, wherein the retroviral vector does not comprise a native envelope protein.

12. A method according to any one of the preceding claims, wherein the retroviral vector comprises one or more of the following envelope proteins: Vesicular Stomatitis Virus glycoprotein VSV-G, Sendai virus F and HN proteins, Influenza virus HA and NA proteins, and Baculovirus GP64 protein.

13. A method according to claim 12, wherein the retroviral vector comprises the envelope protein Vesicular Stomatitis Virus glycoprotein VSV-G.

14. A method according to any one of the preceding claims, wherein the level of expression and/or transcription of the one or more genes in (a) to (n) is decreased using one or more of small interfering RNA (siRNA), small hairpin RNA (shRNA), clustered regularly interspaced short palindromic repeats (CRISPR) interference (CRISPRi), transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs) and meganucleases.

15. A method according to any one of the preceding claims, wherein the method further comprises purifying and concentrating the population of retroviral vectors.

16. A method according to any one of the preceding claims, wherein the retroviral vectors are lentiviral vectors.

17. A method according to any one of the preceding claims, wherein the retroviral vectors are replication-defective or replication-deficient.
18. A human host cell in which the level of expression and/or transcription of one or more of (a) CD63, (b) ATP1A1, (c) LIMK1, (d) DDX5, (e) UBA7, (f) DUSP1, (g) SPN, (h) AXIN1, (i) CD81, (j) UBE2I, (k) DLG1, (l) SLFN11, (m) ABCA1 and (n) APOL1 is decreased.
19. A human host cell according to claim 18, wherein the level of expression and/or transcription of the genes defined in any one of claims 2 to 7 is decreased.
20. A culture of a plurality of human host cells according to claim 18 or 19.
21. A retroviral vector produced using a method according to any one of claims 1 to 17.
22. A retroviral vector according to claim 21, wherein the vector further comprises a therapeutic gene.
23. A population of retroviral vectors produced using a method according to any one of claims 1 to 17.
24. A population according claim 23, wherein the retroviral vectors comprise a therapeutic gene.
25. A pharmaceutical composition comprising (a) a retroviral vector according to claim 21 or 22 or a population of retroviral vectors according to claim 23 or 24 and (b) a pharmaceutically acceptable carrier or diluent.
26. A method of delivering a polynucleotide to a cell *in vitro*, comprising administering to the cell a retroviral vector according to claim 21 or 22, a population of retroviral vectors according to claim 23 or 24 or a pharmaceutical composition according to claim 25, wherein the retroviral vector or retroviral vectors comprise the polynucleotide.
27. A method of treating or preventing in a subject a disease which will benefit from a therapeutic gene, comprising administering to the subject a retroviral vector according to claim 21 or 22, a population of retroviral vectors according to claim 23 or 24 or a pharmaceutical

composition according to claim 25, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene.

28. A method according to claim 27, wherein the retroviral vector, the population of retroviral vectors or the pharmaceutical composition is administered in combination with another therapy.

29. A retroviral vector according to claim 21 or 22, a population of retroviral vectors according to claim 23 or 24 or a pharmaceutical composition according to claim 25 for use in a method of treating or preventing in a subject a disease which will be benefit from a therapeutic gene, wherein the retroviral vector or retroviral vectors comprise the therapeutic gene.

30. A retroviral vector according to claim 21 or 22, a population of retroviral vectors according to claim 23 or 24 or a pharmaceutical composition according to claim 25 for use in gene therapy.

Fig. 1

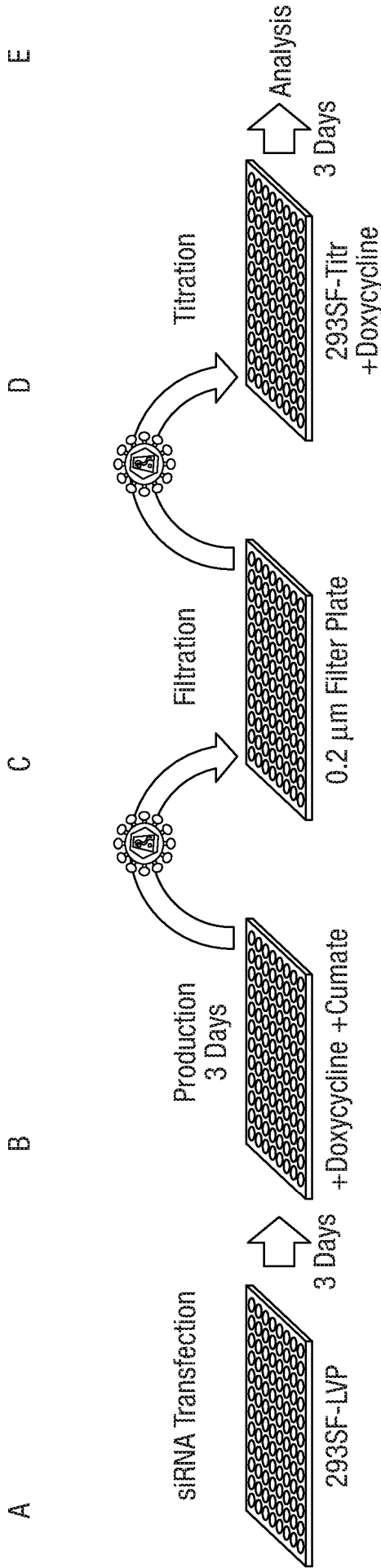


Fig. 2

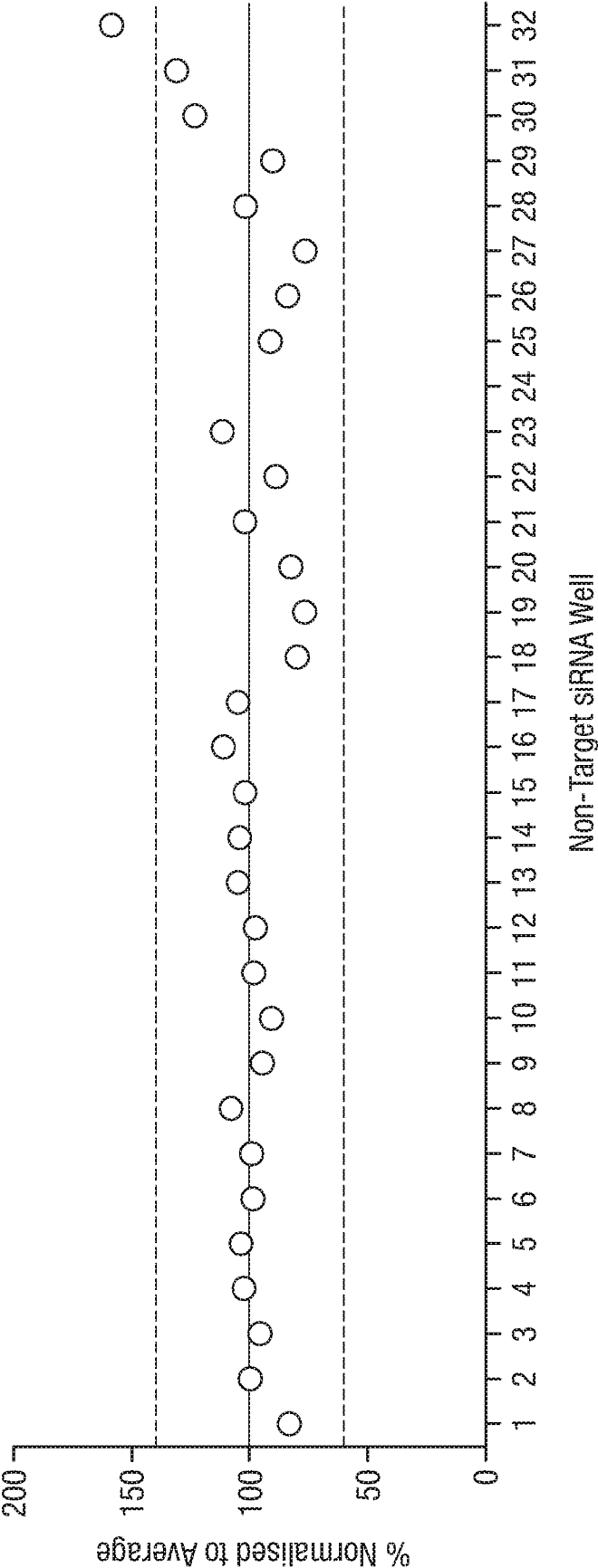


Fig. 3

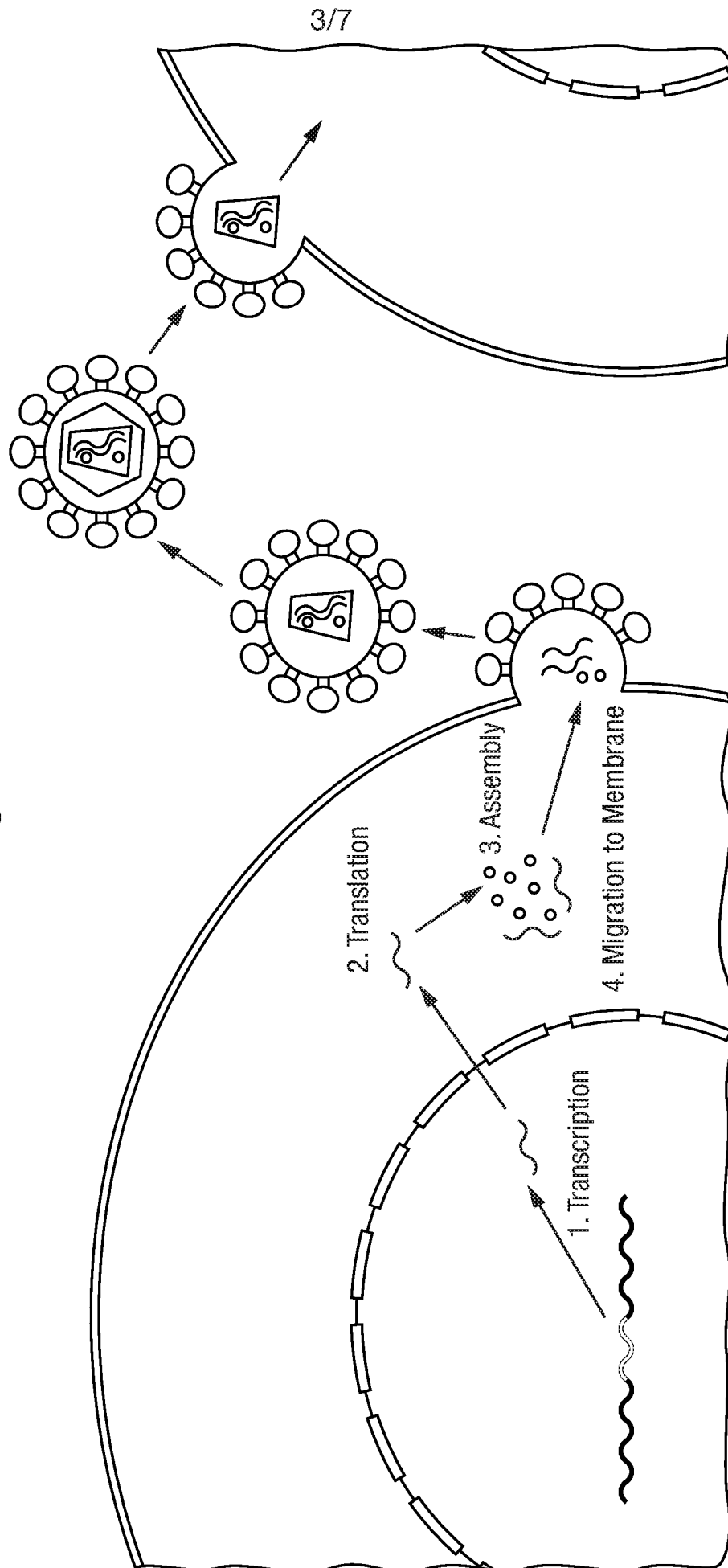
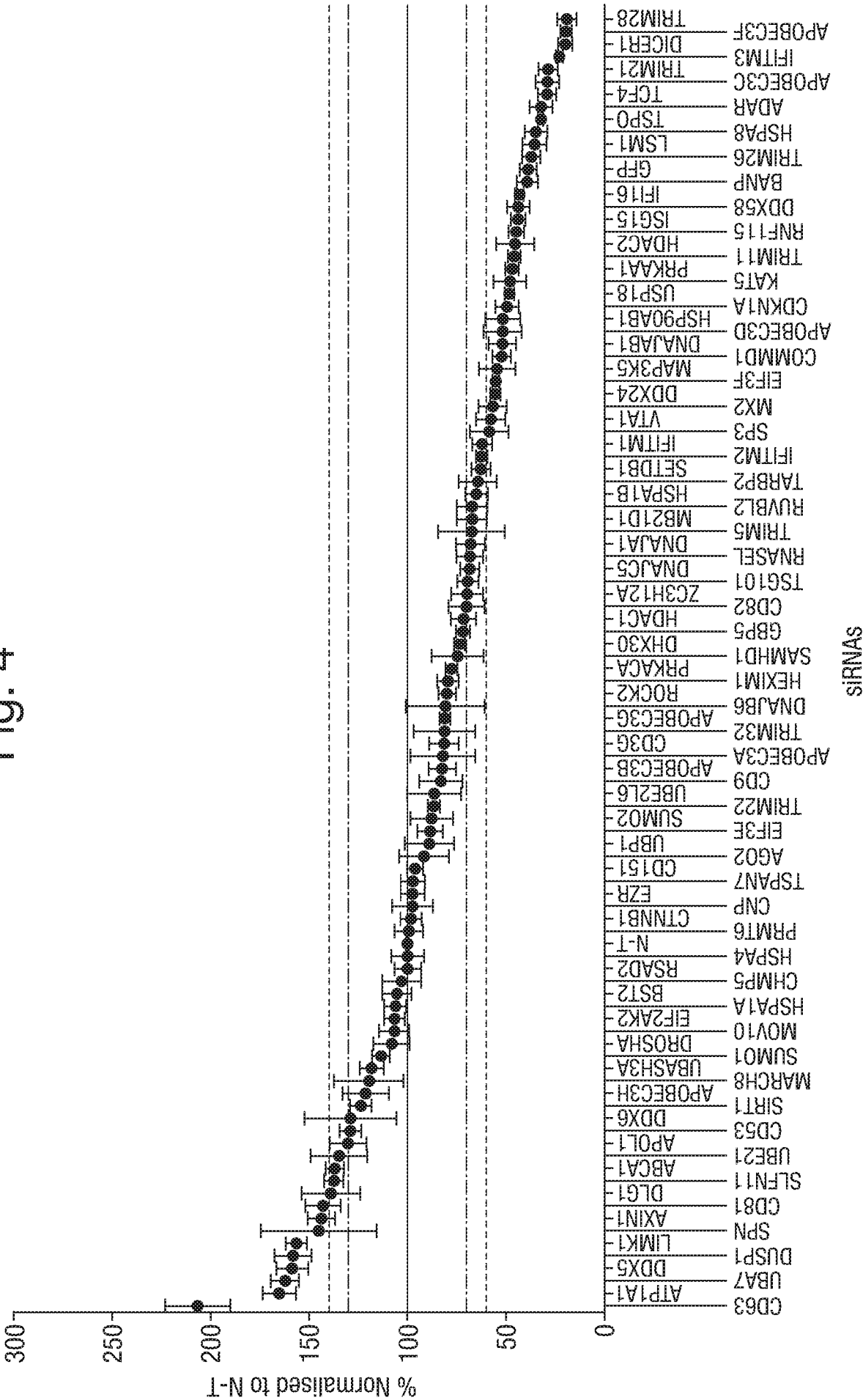


Fig. 4



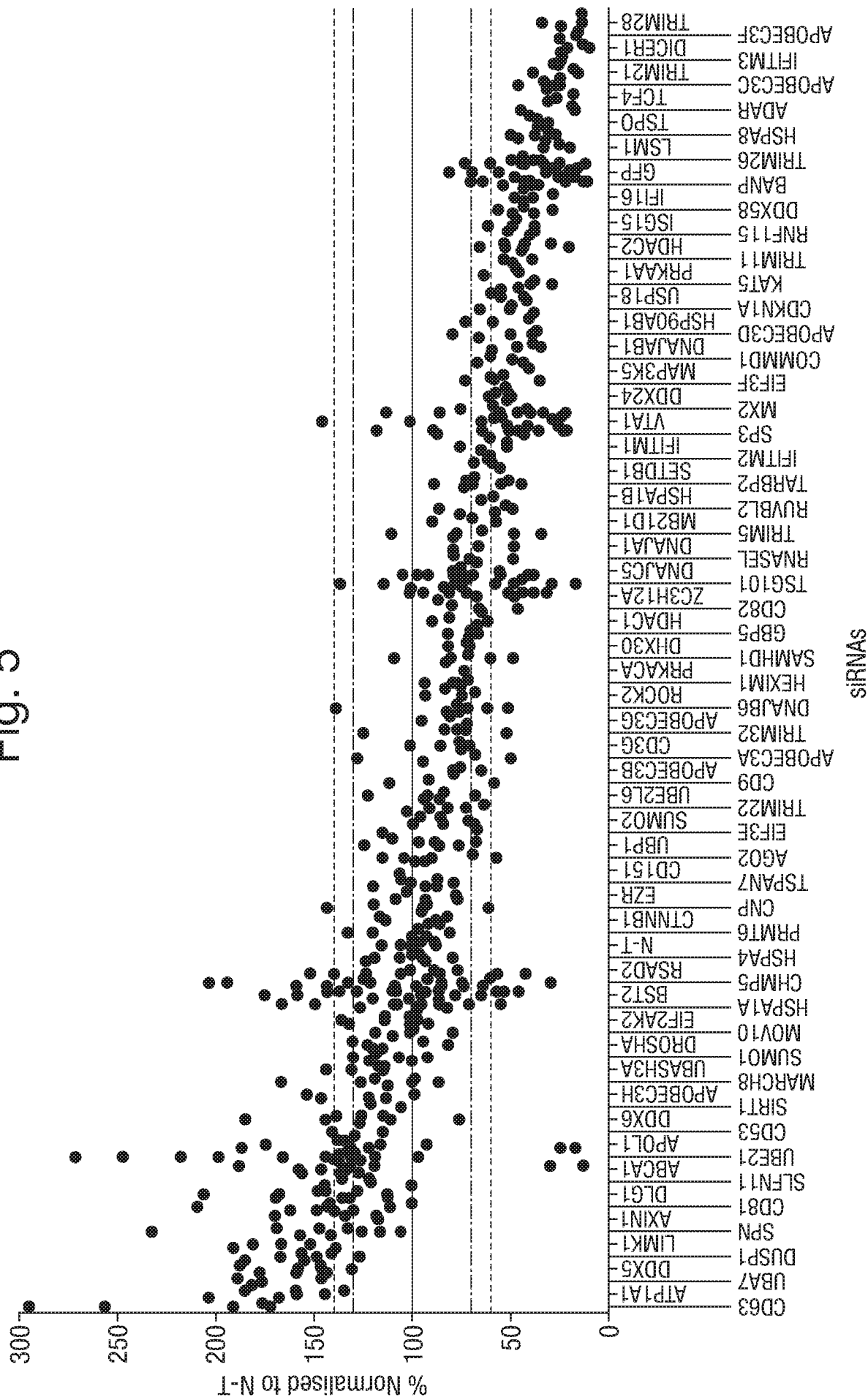
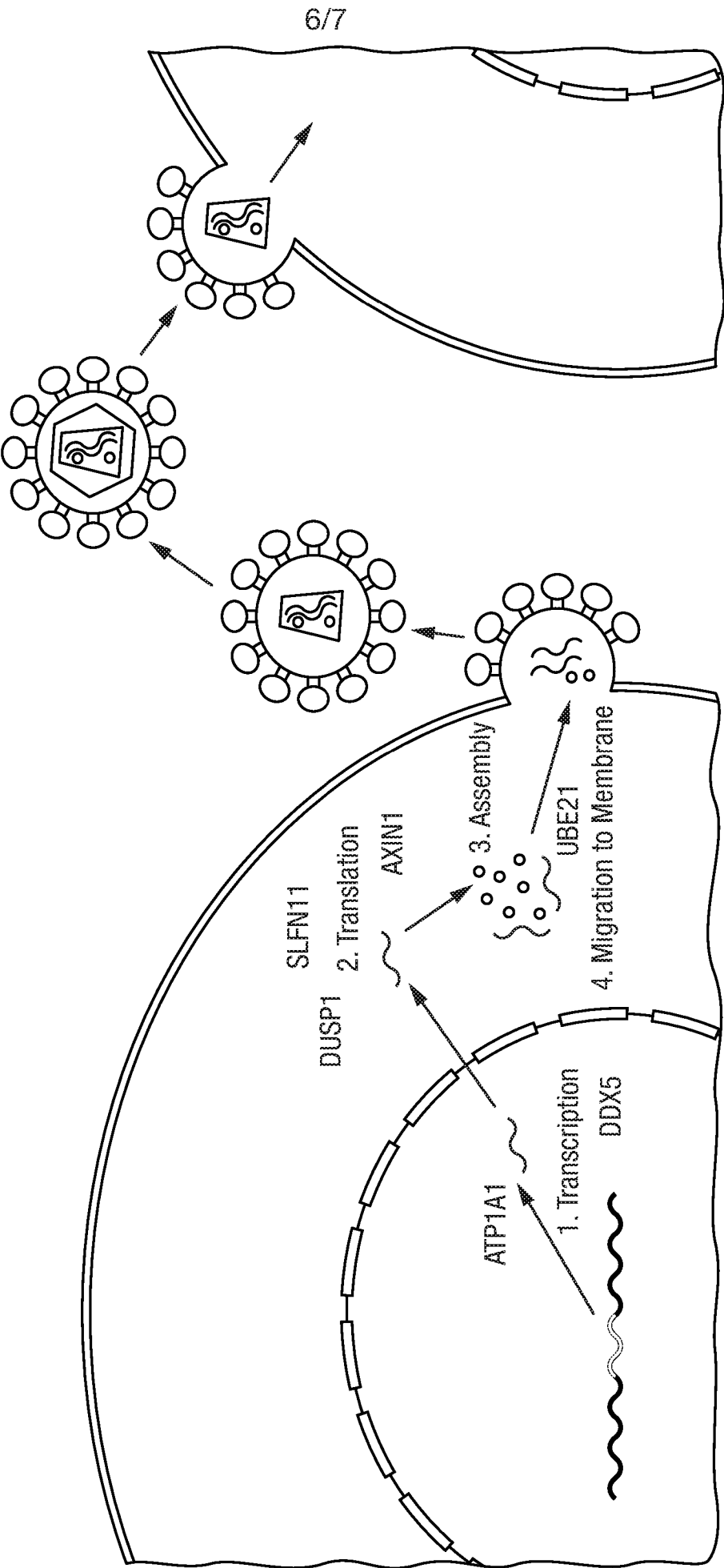
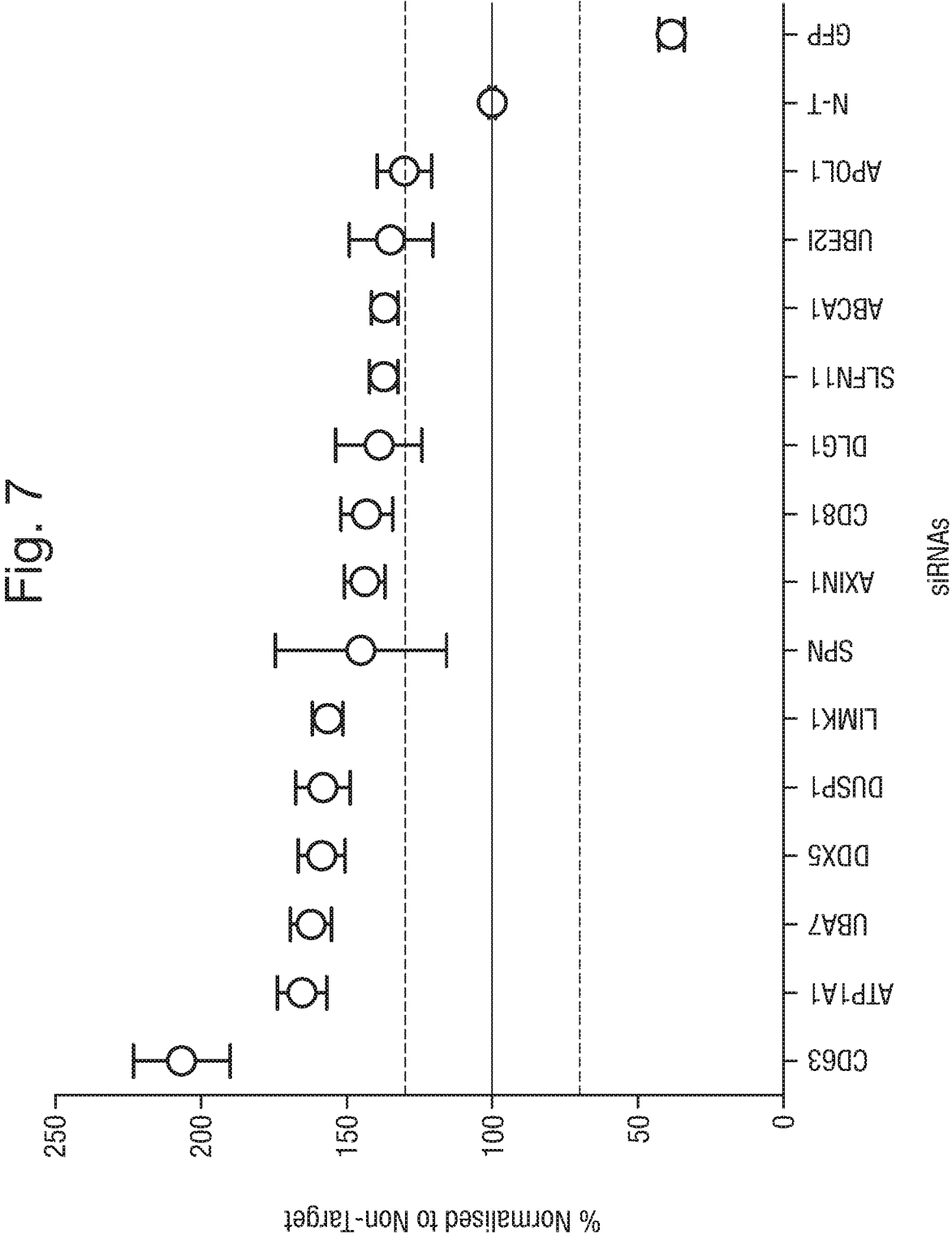


Fig. 6



6/7



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2017/051023

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/86
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/083035 A1 (SPAYD KATIE JANSEN [US] ET AL) 5 April 2012 (2012-04-05)	21,23,25
Y	claims; examples	1-5,8-30
X	----- BROUSSAU S ET AL: "Inducible Packaging Cells for Large-scale Production of Lentiviral Vectors in Serum-free Suspension Culture", MOLECULAR THERAPY, vol. 16, no. 3, 1 March 2008 (2008-03-01), pages 500-507, XP055387760, US ISSN: 1525-0016, DOI: 10.1038/sj.mt.6300383 materials and methods, results, discussion; ----- -/-	21,23,25



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 July 2017

Date of mailing of the international search report

01/09/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Sommer, Birgit

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2017/051023

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

5(completely); 1-4, 8-30(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2017/051023

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CODINA J ET AL: "CD63 interacts with the carboxy terminus of the colonic H ⁺ -K ⁺ -ATPase to increase plasma membrane localization and 86 Rb ⁺ uptake", AMERICAN JOURNAL OF CELL PHYSIOLOGY, vol. 288, 12 January 2005 (2005-01-12), pages C1279-C1286, XP055388273, page C1280, right-hand column, last paragraph; -----	18-20
Y	KREMENTSOV D N ET AL: "Tetraspanins regulate cell-to-cell transmission of HIV-1", RETROVIROLOGY, BIOMED CENTRAL LTD., LONDON, GB, vol. 6, no. 1, 14 July 2009 (2009-07-14), page 64, XP021059343, ISSN: 1742-4690, DOI: 10.1186/1742-4690-6-64 materials and methods; results; figure 1; -----	1-5,8-30
Y,P	GELINAS J-F ET AL: "704. Enhanced Lentiviral Production Through Rational Design of Mammalian Host Cells", MOLECULAR THERAPY, vol. 24, no. Suppl. 1, 1 May 2016 (2016-05-01), page S278, XP055388086, the whole document -----	1-5,8-30
A	WO 2015/194770 A1 (UNIV KOREA RES & BUS FOUND [KR]) 23 December 2015 (2015-12-23) abstract -& EP 3 159 404 A1 (IMMUNOMAX CO LTD [KR]) 26 April 2017 (2017-04-26) -----	1-5,8-30
A	ZHENG Y-H ET AL: "Host restriction factors in retroviral infection: promises in virus-host interaction", RETROVIROLOGY, BIOMED CENTRAL LTD., LONDON, GB, vol. 9, no. 1, 20 December 2012 (2012-12-20), page 112, XP021136969, ISSN: 1742-4690, DOI: 10.1186/1742-4690-9-112 abstract; page 11, left-hand column, paragraph 2 - right-hand column, paragraph 1; -----	1-5,8-30

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2017/051023

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012083035 A1	05-04-2012	US 2012083035 A1	05-04-2012
		US 2013217121 A1	22-08-2013

WO 2015194770 A1	23-12-2015	CN 106661558 A	10-05-2017
		EP 3159404 A1	26-04-2017
		JP 2017518084 A	06-07-2017
		US 2017198025 A1	13-07-2017
		WO 2015194770 A1	23-12-2015

EP 3159404 A1	26-04-2017	CN 106661558 A	10-05-2017
		EP 3159404 A1	26-04-2017
		JP 2017518084 A	06-07-2017
		US 2017198025 A1	13-07-2017
		WO 2015194770 A1	23-12-2015

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 5(completely); 1-4, 8-30(partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of CD63 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

2. claims: 1-4, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of ATP1A1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

3. claims: 1-4, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of LIMK1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

4. claims: 1-3, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of DDX5 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

5. claims: 1, 2, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of UBA7 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

thereto;

6. claims: 1, 2, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of DUSP1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

7. claims: 1, 2, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of SPN is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

8. claims: 1, 2, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of AXIN1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

9. claims: 1, 2, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of CD81 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

10. claims: 7(completely); 1, 6, 8-30(partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of UBE2I is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

related thereto;

11. claims: 1, 6, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of DLG1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

12. claims: 1, 6, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of SLFN11 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

13. claims: 1, 6, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of ABCA1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;

14. claims: 1, 6, 8-30(all partially)

a method of producing a population of retroviral vectors, the method comprising (1) expressing the retroviral vectors in a culture of human host cells in which the level of expression and/or transcription of APOL1 is decreased and (2) allowing the population of retroviral vectors to accumulate in the culture medium as well as subject-matter related thereto;
