

CLAIMS

1. A method of producing a retroviral vector pseudotyped with hemagglutinin-neuraminidase (HN) and fusion (F) proteins from a respiratory paramyxovirus, and which comprises a promoter and a transgene, wherein said method comprises the use of codon-optimised gag-pol genes.
2. The method of claim 1, wherein the retroviral vector is a lentiviral vector.
3. ~~The method of claim 2,~~ optionally wherein the lentiviral vector is selected from the group consisting of a Simian immunodeficiency virus (SIV) vector, a Human immunodeficiency virus (HIV) vector, a Feline immunodeficiency virus (FIV) vector, an Equine infectious anaemia virus (EIAV) vector, and a Visna/maedi virus vector.
4. ~~The method of claim 2 or 3,~~ wherein the lentiviral vector is an SIV vector.
5. The method of any one of the preceding claims, wherein
(i) the codon-optimised gag-pol genes are SIV gag-pol genes; and/or
6. ~~The method of any one of the preceding claims, wherein~~ (ii) the codon-optimised gag-pol genes comprise or consist of a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO: 1.
7. ~~The method of claim 6,~~ optionally wherein the codon-optimised gag-pol genes comprise or consist of the nucleic acid sequence of SEQ ID NO: 1; and/or
8. ~~The method of any one of the preceding claims, wherein~~ (iii) the codon-optimised gag-pol genes are comprised in a plasmid that comprises or consists of a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO: 5.
9. ~~The method of claim 8,~~ optionally wherein the codon-optimised gag-pol genes are comprised in a plasmid that comprises or consists of the nucleic acid sequence of SEQ ID NO: 5.

105. The method of any one of the preceding claims, wherein the respiratory paramyxovirus is a Sendai virus.

116. The method of any one of the preceding claims, wherein the titre of retroviral vector produced is:

a) equivalent to the titre of retroviral vector produced by a corresponding method which does not use codon-optimised gal-pol genes; or

b) increased compared with the titre of retroviral vector produced by a corresponding method which does not use codon-optimised gal-pol genes.

c) ~~12.~~ The method of claim 11, optionally wherein the titre of retroviral vector is at least 2-fold, or at least 2.5-fold greater than the titre of retroviral vector produced by a corresponding method which does not use codon-optimised gal-pol genes.

137. The method of any one of the preceding claims, wherein the promoter is selected the group consisting of a cytomegalovirus (CMV) promoter, elongation factor 1a (EF1a) promoter, and a hybrid human CMV enhancer/EF1a (hCEF) promoter.

148. The method of any one of the preceding claims, wherein the vector comprises a hybrid human CMV enhancer/EF1a (hCEF) promoter.

159. The method of any one of the preceding claims, wherein the transgene is selected from:

a) a secreted therapeutic protein, optionally Alpha-1 Antitrypsin (A1AT), Factor VIII, Surfactant Protein B (SFTPB), Factor VII, Factor IX, Factor X, Factor XI, von Willebrand Factor, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and a monoclonal antibody against an infectious agent; or

b) *CFTR*, *ABCA3*, *DNAH5*, *DNAH11*, *DNAI1*, and *DNAI2*.

1610. The method of any one of the preceding claims, wherein the transgene encodes:

a) *CFTR*;

b) A1AT; or

c) FVIII.

1711. The method of any one of the preceding claims, wherein:

- a) the promoter is a hCEF promoter and the transgene encodes CFTR;
- b) the promoter is a hCEF promoter and the transgene encodes A1AT; or
- c) the promoter is a hCEF or CMV promoter and the transgene encodes FVIII.

5

1812. The method of any one of the preceding claims, said method comprising the following steps:

- a) growing cells in suspension;
- b) transfecting the cells with one or more plasmids;
- c) adding a nuclease;
- d) harvesting the lentivirus;
- e) adding trypsin; and
- f) purification.

10

1913. The method according to claim 1812, wherein the one or more plasmids comprise or consist of:

15

- a) a vector genome plasmid, preferably selected from selected from pGM830 and pGM326;
- b) a co-gagpol plasmid, preferably pGM691;
- c) a Rev plasmid, preferably pGM299;
- d) a fusion (F) protein plasmid, preferably pGM301; and
- e) a hemagglutinin-neuraminidase (HN) plasmid, preferably pGM303; and

20

20. — The method according to claim 19, preferably wherein the ratio of vector genome plasmid: co-gagpol plasmid: Rev plasmid: F plasmid: HN plasmid is 20:9:6:6:6.

25

2114. The method according to any one of claims 18 to 20 claim 12 or 13, wherein (i) steps (a)-(f) are carried out sequentially; and/or

30

22. — The method according to any one of claims 18 to 21, wherein (ii) the cells are HEK293T or 293T/17 cells; and/or

23. — The method according to any one of claims 18 to 22, wherein (iii) the addition of the nuclease is at the pre-harvest stage; and/or

24. ~~The method according to any one of claims 18 to 23, wherein(iv) the addition of trypsin is at the post-harvest stage-; and/or~~
- 5 25. ~~The method according to any one of claims 18 to 24, wherein(v) the purification step comprises a chromatography step-; and/or~~
26. ~~The method according to any one of the claims 19 to 24, wherein(vi) the vector genome plasmid is modified to reduce the number of retroviral ORFs.~~
- 10 ~~27~~15. A nucleic acid comprising codon-optimised gag-pol genes, said nucleic acid having at least 80% sequence identity to SEQ ID NO: 1-, and
28. ~~The~~wherein preferably the nucleic acid ~~of claim 27 which comprises or consists of the~~ nucleic acid sequence of SEQ ID NO: 1.
- 15 ~~29~~16. A plasmid comprising a nucleic acid as defined in claim ~~27 or 28~~15, wherein optionally:
- a) the plasmid comprises or consists of a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO: 5; or
- b) the plasmid comprises or consists of the nucleic acid sequence of SEQ ID NO: 5.
- 20 ~~30~~17. A host cell comprising a nucleic acid as defined in claim ~~27 or 28~~15, and/or a plasmid as defined in claim ~~29~~16.
- 25 ~~31~~18. A retroviral vector pseudotyped with hemagglutinin-neuraminidase (HN) and fusion (F) proteins from a respiratory paramyxovirus which is obtainable by a method as defined in any one of claims 1 to 14.
19. A retroviral vector pseudotyped with hemagglutinin-neuraminidase (HN) and fusion (F) proteins from a respiratory paramyxovirus which is obtainable by a method as defined in any
- 30 one of claims 1 to ~~26~~14 for use in a method of treating a disease.
- ~~32. A method of treating a disease comprising administering~~20. A retrovirus for use according to claim 19 wherein the disease to be treated is a lung disease, preferably cystic fibrosis.

- 5 21. Use of a retroviral vector pseudotyped with hemagglutinin-neuraminidase (HN) and fusion (F) proteins from a respiratory paramyxovirus which is obtainable by a method as defined in any one of claims 1 to 26, to a subject in need thereof¹⁴ for the manufacture of a medicament for the treatment of disease, wherein the disease to be treated is a lung disease, preferably cystic fibrosis.
33. The method of treatment according to claim 32, wherein the disease to be treated is a lung disease, preferably cystic fibrosis.