

**CLAIMS**

1. A method for generating reassortant influenza viruses comprising the steps of:
  - (i) contacting a culture host with a parent influenza virus strain comprising a first hemagglutinin (HA) gene and a first neuraminidase (NA) gene;
  - 5 (ii) introducing into the culture host one or more expression construct(s) comprising one or more influenza genes, wherein said influenza genes comprise a second HA gene or a second NA gene;
  - (iii) culturing the culture host in order to produce reassortant viruses; and
  - (iv) selecting for reassortant viruses that comprise the second HA gene or the second NA
  - 10 gene.
2. The method of claim 1, further comprising step (v) isolating a reassortant influenza virus comprising the second HA gene or the second NA gene.
3. The method of claim 2, wherein the isolated reassortant influenza virus is a 7:1 reassortant influenza virus.
- 15 4. The method of claim 1, wherein the one or more influenza genes comprise a second HA gene and a second NA gene, and step (iv) comprises selecting for reassortant viruses that comprise the second HA gene and/or the second NA gene.
5. The method of claim 4, further comprising step (v) isolating a reassortant influenza virus comprising (i) the second HA gene; and/or (ii) the second NA gene.
- 20 6. The method of claim 4, wherein the isolated reassortant influenza virus is a 6:2 reassortant influenza virus.
7. The method of claim 4, wherein the one or more influenza genes comprise a second PB1, PB2, PA, M, NS or NP, and step (iv) comprises selecting for reassortant viruses that comprise the second PB1, PB2, PA, M, NS or NP gene.
- 25 8. The method of claim 7, further comprising the step (v) of isolating a reassortant influenza virus comprising (i) the second HA gene; and/or (ii) the second NA gene; and (iii) the second PB1, PB2, PA, M, NS or NP gene.
9. The method of claim 8, wherein the isolated reassortant influenza virus is a 5:3 reassortant influenza virus.
- 30 10. The method of any preceding claim, wherein the one or more expression construct(s) comprise no more than seven, six, five, four or three influenza genes.
11. The method of any of claims 1-6, wherein the one or more expression construct(s) comprise no more than two influenza genes.

12. The method of any preceding claim, wherein the one or more expression construct(s) is one or more plasmid(s), one or more linear DNA molecule(s), or one or more RNA molecule(s).
13. The method of claim 12, wherein the one or expression construct(s) comprises a synthetic nucleic acid molecule, for example a synthetic DNA molecule or a synthetic RNA molecule.
- 5 14. The method of any preceding claim, wherein the one or more expression construct(s) are bi-directional expression construct(s), wherein at least one gene is located between an upstream pol II promoter and a downstream non-endogenous pol I promoter.
15. The method of any preceding claim, wherein the one or more expression construct(s) are introduced into the culture host at the same time as the culture host is contacted with the parent  
10 influenza virus strain.
16. The method of any one of claims 1-14, wherein the one or more expression construct(s) are introduced into the culture host before or after the culture host is contacted with the parent influenza virus strain.
17. The method of any preceding claim, comprising a step of separating reassortant influenza  
15 viruses from the culture host prior to step (iv).
18. The method of any preceding claim, wherein step (iv) comprises negative selection against the first HA.
19. The method of claim 18, wherein the negative selection comprises contacting the culture host or the reassortant influenza viruses that have been separated from the culture host with one or more  
20 antibodies which are specific for the first HA.
20. The method of claim 18 or 19, wherein the negative selection comprises contacting the culture host with an inhibitory agent that reduces or prevents the transcription and/or translation of the first HA.
21. The method of any preceding claim, wherein step (iv) comprises negative selection against the  
25 first NA.
22. The method of claim 21, wherein the negative selection comprises contacting the culture host or the reassortant influenza viruses that have been separated from the culture host with one or more antibodies which are specific for the first NA.
23. The method of claim 21 or 22, wherein the negative selection comprises contacting the culture  
30 host with an inhibitory agent that reduces or prevents the transcription and/or translation of the first NA.
24. The method of any preceding claim, wherein step (iv) comprises positive selection for the second HA or the second NA.

25. The method of claim 24, wherein the positive selection comprises contacting the culture host or the reassortant influenza viruses that have been separated from the culture host with one or more antibodies which are specific for the second HA or the second NA.
26. The method of any of claims 17-25, wherein step (iv) comprises three or fewer selection steps.
- 5 27. The method of any claims 2-3, 5-6 or 8-26, wherein the isolation of the reassortant virus comprises a limited dilution step and/or a plaque isolation step.
28. The method of any of claims 2-3, 5-6 or 8-27, further comprising the step (vi) of purifying the virus obtained in step (v).
- 10 29. The method of any preceding claim, wherein the second HA gene comprises one or more modifications relative to the wild-type influenza virus from which the second HA gene is derived.
30. The method of claim 29, wherein the modification of the second HA gene is the deletion of a polybasic cleavage site.
- 15 31. The method of any preceding claim, wherein the reassortant influenza virus produced or isolated is less virulent than the wild-type influenza virus from which the second HA gene or second NA gene is derived.
32. The method of any preceding claim, wherein the culture host is an embryonated avian egg.
33. The method of any of claims 1-31, wherein the culture host is a mammalian cell or an avian cell.
34. The method of claim 33, wherein the culture host is an MDCK, Vero, PerC6, CEF or EB66 cell.
- 20 35. The method of claim 34, wherein the cell grows adherently.
36. The method of claim 34, wherein the cell grows in suspension.
37. The method of claim 36, wherein the MDCK cell is of the cell line MDCK 33016 (DSM ACC2219).
38. The method of any preceding claim, wherein the reassortant influenza virus is an influenza A virus or an influenza B virus.
- 25 39. A population of reassortant influenza viruses produced by the method of any one of claims 1, 4, 7, 10-26 or 29-32.
40. An isolated reassortant influenza virus produced by the method of any one of claims 2-3, 5-6 or 8-38.
- 30 41. A method of preparing a vaccine, comprising the steps of (a) preparing a reassortant influenza virus by the method of any of claims 1-38, and (b) preparing a vaccine from the reassortant influenza virus.
42. A vaccine produced by the method of claim 41.