CLAIMS

1. A method for producing infectious influenza viruses, wherein said method comprises the steps consisting of :

a) transfecting cells with a set of expression vectors to generate a seed of infectious influenza virus,

b) infecting CHO cells with said seed of infectious influenza virus.

2. The method according to claim 1, wherein the cells at step a) are cells of primate origin, a mixture of cells of primate origin and CHO cells or consist of CHO cells.

3. The method according to claim 3, wherein the cells of primate origin are Vero cells.

4. The method according to any of claims 1 to 3, wherein said CHO cells are CHO-K1 cells.

5. The method according to any one of claims 1 to 4, wherein said set of expression vectors comprises:

- expression vectors allowing the expression of mRNAs encoding at least influenza PB1, PB2, PA and NP proteins, and

- expression vectors allowing the expression of at least influenza PB1, PB2, PA, NP, M, NS, HA and NA vRNAs, or the corresponding cRNAs,

it being understood that expression of said set of expression vectors allows (i) the formation of the ribonucleoprotein complex (RNP) containing the influenza vRNA(s), and (ii) the generation of infectious influenza viruses in said transfected cells.

6. The method according to claim 5, wherein:

(i) said expression vectors allowing the expression of mRNAs encoding influenza PB1, PB2, PA and NP proteins comprise four different uni directional plasmids, each plasmid containing a cDNA complementary to a mRNA encoding one of the four distinct proteins selected from PB1, PB2, PA and NP influenza proteins under the control of a promoter that binds to RNA polymerase II, and

(ii) said expression vectors allowing the expression of influenza PB1, PB2, PA, NP, M, NS, HA and NA vRNAs, or the corresponding cRNAs, comprise eight different uni

directional plasmids, each plasmid containing a cDNA complementary to one of the eight distinct vRNAs selected from said PB1, PB2, PA, NP, M, NS, HA and NA influenza vRNAs, or to the corresponding cRNAs, under the control of a promoter that binds to RNA polymerase I.

7. The method according to claim 6, wherein said each plasmid containing a cDNA complementary to one of said influenza PB1, PB2, PA, NP, M, NS, HA and NA vRNAs, or the corresponding cRNAs, under the control of a promoter that binds to RNA polymerase I has been obtained by cloning said cDNA sequence into a vector comprising, in the 5' to 3' sense :

a) a promoter that binds to RNA polymerase I, or a T7 RNA polymerase;

b) a recombination cassette comprising, in the 5' to 3' sense:

- an inverted complementary recognition sequence for a first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;
- a restriction site for a second restriction enzyme which has its cutting site inside of its recognition sequence;
- a restriction site for a third restriction enzyme which has its cutting site inside of its recognition sequence; and
- a recognition sequence for said first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;

wherein said second and third restriction enzymes are different; and

c) a terminator sequence;

it being understood that :

- when the promoter binds to RNA polymerase I, said terminator sequence is hepatitis delta ribozyme sequence; and
- when the promoter binds to T7 RNA polymerase, said terminator sequence is T7 polymerase terminator sequence.

8. The method according to claim 7, wherein said vector comprises sequence SEQ ID NO: 1.

9. The method according to any of claims 1 to 6, wherein said set of expression vectors comprises eight different bidirectional plasmids, each plasmid containing a cDNA complementary to one of the eight distinct vRNAs selected from said PB1, PB2, PA, NP, M, NS, HA and NA influenza vRNAs under the control of two

promoters, wherein said first promoter binds to polymerase I and said second promoter binds to polymerase II,

it being understood that expression of said set of expression vectors allows (i) the formation of the ribonucleoprotein complex (RNP) containing the influenza vRNA(s), and (ii) the generation of infectious influenza viruses in said transfected cells.

10. The method according to any one of claims 1 to 9, wherein the infectious influenza viruses produced are type A or type B influenza viruses.

11. The method according to any one of claims 1 to 10, wherein the infectious influenza viruses produced are reassortant infectious influenza viruses, the genetic material of which results from the combination of the genetic material of at least two donor viruses.

12. The method according to claim 11, wherein one of the donor virus is A/Puerto Rico/8/34 (H1N1) (A/PR/8/34), B/Lee/40 or B/Panama/45/90.

13. The method according to any of claims 1 to 12, wherein the infectious influenza viruses produced are chimeric viruses.

14. The method according to claim 13, wherein said chimeric virus contains a chimeric influenza HA and/or NA vRNAs.

15. The method according to claim 14, wherein the chimeric influenza HA vRNA and/or NA vRNAs comprise one or more domains of a HA vRNA or one or more domains of a NA vRNA from a seasonal or pandemic virus and one or more domains of a HA vRNA or one or more domains of a NA vRNA from another donor virus, wherein at least one domain of the HA vRNA from said seasonal or pandemic influenza virus is complementary to the region of a mRNA encoding the antigenic ectodomain of HA, such as HA1 and/or HA2 from said seasonal or pandemic influenza virus, and at least one domain of the NA vRNA from said seasonal or pandemic influenza virus is complementary to the region of a mRNA encoding the antigenic ectodomain of NA from said seasonal or pandemic influenza virus.

16. The method according to any one of claims 1 to 15, wherein said method is entirely performed in a serum-free medium or in animal component-free conditions.

17. A method of preparing an influenza vaccine composition, which method comprises:

a) producing influenza viruses by a method according to any one of claims 1 to 16;

b) harvesting the infectious influenza viruses after multiplication in CHO cells,

c) purifying the harvested infectious influenza virus,

d) optionally inactivating the purified virus, and

e) mixing the purified virus with a pharmaceutically acceptable carrier.

18. A CHO cell which comprises a set of expression vectors as defined in any one of claims 5 to 9.

19. The CHO cell according to claim 18 which is a CHO-K1 cell.

20. A vector comprising, in the 5' to 3' sense:

- a promoter that binds to RNA polymerase I, or to T7 RNA polymerase;
- a recombination cassette comprising, in the 5' to 3' sense:
  - an inverted complementary recognition sequence for a first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;
  - a restriction site for a second restriction enzyme which has its cutting site inside of its recognition sequence;
  - a restriction site for a third restriction enzyme which has its cutting site inside of its recognition sequence; and
  - a recognition sequence for said first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;

wherein said second and third restriction enzymes are different; and

- a terminator sequence;

it being understood that :

- when the promoter binds to RNA polymerase I, said terminator sequence is hepatitis delta ribozyme sequence,
- when the promoter binds to T7 RNA polymerase, said terminator sequence is T7 polymerase terminator sequence.

21. The vector according to claim 20, wherein said promoter binds to rodent RNA polymerase I or to human RNA polymerase I.

22. The vector according to claim 20 or 21, wherein said first restriction enzyme of the recombination cassette is *BbsI* or *SapI*, and said second and third restriction enzymes of the recombination cassette are selected from the group consisting of *NotI* and *SbfI*.

23. The vector according to any one of claims 20 to 22, wherein said recombination cassette consists of sequence SEQ ID NO:2.

24. The vector according to any one of claims 20 to 23, wherein said vector comprises the sequence SEQ ID NO: 1.

25. A vector according to any one claims 20 to 24 for use in a method for producing reassortant RNA virus by reverse genetic.

CLAIMS

1. A method for producing infectious influenza viruses, wherein said method comprises the steps of:

a) transfecting cells of primate origin, CHO cells, a mixture of cells of CHO cells with another cell line, cells of canine origin, or chicken cells with a set of expression vectors to generate a seed of infectious influenza virus,

b) infecting CHO-K1 cells that do not express Sia2-6Gal receptors with said seed of infectious influenza virus.

2. The method according to claim 1, wherein the cells at step a) are cells of primate origin, a mixture of cells of primate origin and CHO cells or consist of CHO cells.

3. The method according to claim 2, wherein the cells of primate origin are Vero cells.

4. The method according to any of claims 1 to 3, wherein said CHO cells at step a) are CHO-K1 cells.

5. The method according to any one of claims 1 to 4, wherein said set of expression vectors comprises:

- expression vectors allowing the expression of mRNAs encoding at least influenza PB1, PB2, PA and NP proteins, and

- expression vectors allowing the expression of at least influenza PB1, PB2, PA, NP, M, NS, HA and NA vRNAs, or the corresponding cRNAs,

it being understood that expression of said set of expression vectors allows (i) the formation of the ribonucleoprotein complex (RNP) containing the influenza vRNA(s), and (ii) the generation of infectious influenza viruses in said transfected cells.

6. The method according to claim 5, wherein:

(i) said expression vectors allowing the expression of mRNAs encoding influenza PB1, PB2, PA and NP proteins comprise four different uni directional plasmids, each plasmid containing a cDNA complementary to a mRNA encoding one of the four distinct proteins selected from PB1, PB2, PA and NP influenza proteins under the control of a promoter that binds to RNA polymerase II, and

(ii) said expression vectors allowing the expression of influenza PB1, PB2, PA, NP, M, NS, HA and NA vRNAs, or the corresponding cRNAs, comprise eight different uni directional plasmids, each plasmid containing a cDNA complementary to one of the eight distinct vRNAs selected from said PB1, PB2, PA, NP, M, NS, HA and NA influenza vRNAs, or to the corresponding cRNAs, under the control of a promoter that binds to RNA polymerase I.

7. The method according to claim 6, wherein each plasmid containing a cDNA complementary to one of said influenza PB1, PB2, PA, NP, M, NS, HA and NA vRNAs, or the corresponding cRNAs, under the control of a promoter that binds to RNA polymerase I has been obtained by cloning said cDNA sequence into a vector comprising, in the 5' to 3' sense :

a) a promoter that binds to RNA polymerase I, or a T7 RNA polymerase;

b) a recombination cassette comprising, in the 5' to 3' sense:

- an inverted complementary recognition sequence for a first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;
- a restriction site for a second restriction enzyme which has its cutting site inside of its recognition sequence;
- a restriction site for a third restriction enzyme which has its cutting site inside of its recognition sequence; and
- a recognition sequence for said first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;

wherein said second and third restriction enzymes are different; and

c) a terminator sequence;

it being understood that :

- when the promoter binds to RNA polymerase I, said terminator sequence is hepatitis delta ribozyme sequence; and
- when the promoter binds to T7 RNA polymerase, said terminator sequence is T7 polymerase terminator sequence.

8. The method according to claim 7, wherein said vector comprises sequence SEQ ID NO: 1.

9. The method according to any of claims 1 to 6, wherein said set of expression vectors comprises eight different bidirectional plasmids, each plasmid

containing a cDNA complementary to one of the eight distinct vRNAs selected from said PB1, PB2, PA, NP, M, NS, HA and NA influenza vRNAs under the control of two promoters, wherein said first promoter binds to polymerase I and said second promoter binds to polymerase II,

it being understood that expression of said set of expression vectors allows (i) the formation of the ribonucleoprotein complex (RNP) containing the influenza vRNA(s), and (ii) the generation of infectious influenza viruses in said transfected cells.

10. The method according to any one of claims 1 to 9, wherein the infectious influenza viruses produced are type A or type B influenza viruses.

11. The method according to any one of claims 1 to 10, wherein the infectious influenza viruses produced are reassortant infectious influenza viruses, the genetic material of which results from the combination of the genetic material of at least two donor viruses.

12. The method according to claim 11, wherein one of the donor virus is A/Puerto Rico/8/34 (H1N1) (A/PR/8/34), B/Lee/40 or B/Panama/45/90.

13. The method according to any of claims 1 to 12, wherein the infectious influenza viruses produced are chimeric viruses.

14. The method according to claim 13, wherein said chimeric virus contains a chimeric influenza HA and/or NA vRNAs.

15. The method according to claim 14, wherein the chimeric influenza HA vRNA and/or NA vRNAs comprise one or more domains of a HA vRNA or one or more domains of a NA vRNA from a seasonal or pandemic virus and one or more domains of a HA vRNA or one or more domains of a NA vRNA from another donor virus, wherein at least one domain of the HA vRNA from said seasonal or pandemic influenza virus is complementary to the region of a mRNA encoding the antigenic ectodomain of HA, such as HA1 and/or HA2 from said seasonal or pandemic influenza virus, and at least one domain of the NA vRNA from said seasonal or pandemic influenza virus is complementary to the region of a mRNA encoding the antigenic ectodomain of NA from said seasonal or pandemic influenza virus.

16. The method according to any one of claims 1 to 15, wherein said method is entirely performed in a serum-free medium or in animal component-free conditions.

17. A method of preparing an influenza vaccine composition, which method comprises:

a) producing influenza viruses by a method according to any one of claims 1 to 16;

b) harvesting the infectious influenza viruses after multiplication in CHO-K1 cells that do not express Sia2-6Gal receptors,

c) purifying the harvested infectious influenza virus,

d) optionally inactivating the purified virus, and

e) mixing the purified virus with a pharmaceutically acceptable carrier.

18. A CHO-K1 cell which comprises a set of expression vectors as defined in any one of claims 5 to 9.

19. A vector comprising, in the 5' to 3' sense:

- a promoter that binds to RNA polymerase I, or to T7 RNA polymerase;
- a recombination cassette comprising, in the 5' to 3' sense:
  - an inverted complementary recognition sequence for a first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;
  - a restriction site for a second restriction enzyme which has its cutting site inside of its recognition sequence;
  - a restriction site for a third restriction enzyme which has its cutting site inside of its recognition sequence; and
  - a recognition sequence for said first restriction enzyme which has its cutting site outside of its recognition sequence and produces sticky ends;

wherein said second and third restriction enzymes are different; and

- a terminator sequence;

it being understood that :

- when the promoter binds to RNA polymerase I, said terminator sequence is hepatitis delta ribozyme sequence,

- when the promoter binds to T7 RNA polymerase, said terminator sequence is T7 polymerase terminator sequence.

20. The vector according to claim 19, wherein said promoter binds to rodent RNA polymerase I or to human RNA polymerase I.

21. The vector according to claim 19 or 20, wherein said first restriction enzyme of the recombination cassette is *BbsI* or *SapI*, and said second and third restriction enzymes of the recombination cassette are selected from the group consisting of *NotI* and *SbfI*.

22. The vector according to any one of claims 19 to 21, wherein said recombination cassette consists of sequence SEQ ID NO:2.

23. The vector according to any one of claims 19 to 22, wherein said vector comprises the sequence SEQ ID NO: 1.

24. A vector according to any one claims 19 to 23 for use in a method for producing reassortant RNA virus by reverse genetics.