possibility of producing viruses having infectious titers two to three times greater than those obtained in a conventionally used neutral medium.

In an aspect, there is provided a process for producing an lentivirus, optionally pseudotyped, characterized in that the host cells producing said virus are cultivated in a mildly acid medium, the pH of the medium being comprised between 5.8 and 6.2, the pH being more particularly of about 6.

In another aspect, there is provided a kit for applying the process as disclosed herein, comprising a mildly acid culture medium, or a culture medium accompanied by one or several solutions useful for bringing the pH of said medium to a mildly acid value comprised between 5.8 and 6.2, the pH being more particularly of about 6, the kit further comprising: (a) one or several suitable plasmids for producing the optionally pseudotyped lentivirus; and/or (b) suitable cells for producing said lentivirus.

In yet another aspect, there is provided a kit for applying the process as disclosed herein, comprising (i) means for applying said process and (ii) instructions to be followed for applying the process the kit comprising the following means (a) and (b), (a) and (c), (b) and (c) or (a) and (b) and (c):

(a) one or several suitable plasmids for producing the lentivirus, optionally pseudotyped;

(b) suitable cells for producing said lentivirus; and

(c) a mildly acid culture medium, or a culture medium accompanied by one or several solutions useful for bringing the pH of said medium to a mildly acid value comprised between 5.8 and 6.2, the pH being more particularly of about 6.

In a further aspect, there is provided a kit for applying the process as disclosed herein, comprising a culture medium accompanied by one or several solutions useful for bringing the pH of said medium to a mildly acid value comprised between 5.8 and 6.2, the pH being more particularly of about 6.

Therefore the object of the disclosure is the use of mildly acid conditions for producing an enveloped virus. More particularly, the disclosure relates to a process for producing an enveloped lentiviral vector, characterized in that the culture medium used for cultivating the host cells producing said vector is a mildly acid medium.

DETAILED DESCRIPTION OF THE INVENTION

The invention therefore relates to a process for producing enveloped vectors characterized in that said vectors are produced under mildly acid conditions.

The expression "mildly acid condition" refers to the pH of an aqueous solution comprised between 5 and 6.6, in particular between 5.5 and 6.6 or between 5 and 6.2, more particularly between 5.8 and 6.2. The pH will notably be equal to 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1 or 6.2. According to a particular embodiment, the pH is of about 6. The selected pH will also depend on the buffering power of the medium used, which the person skilled in the art will be able to easily determine considering his/her general knowledge.

The person skilled in the art is able to modify the pH of a solution, notably the pH of a cell culture medium. He/she may notably introduce into said solution a solution of an acid, notably of a strong acid such as hydrochloric acid. If need be, a solution of a base, notably of a strong base such as sodium hydroxide, may be used for readjusting the pH to bring it up to the desired value.

Except for mildly acid conditions which are the object of the present invention, the process used for producing enveloped viruses applies processes and materials well known in the field. The person skilled in the art may refer to his/her general knowledge in the production of enveloped viruses, notably illustrated by (Ansorge et al. 2010; Schweizer and Merten 2010; Rodrigues et al. 2011).

Within the scope of the present invention, the term of "virus" designates both a natural virus, as found in nature, and a modified virus, for which the genome includes modifications

CLAIMS

1. A process for producing an entivirus, optionally pseudotyped, characterized in that the host cells producing said virus are cultivated in a mildly acid medium, the pH of the medium being comprised between 5.8 and 6.2, the pH being more particularly of about 6.

2. The process according to claim 1, wherein the lentivirus is pseudotyped with an envelope protein derived from a virus belonging to the family of rhabdoviridae, arenaviridae, togaviridae, filoviridae, retroviridae, coronaviridae, paramyxoviridae, flaviridae, orthomyxoviridae or baculoviridae.

3. The process according to claim 1 or 2, wherein the lentivirus is pseudotyped with an envelope glycoprotein selected from the envelope protein of the vesicular stomatitis virus (VSV-G), rabies virus, Mokola virus, lymphocyte choriomeningitis virus (LCMV), Ross River Virus (RRV), Sindbis virus, Semliki Forest Virus (SFV), Venezuelan equine encephalitis virus, West equine encephalitis virus, Ebola virus, Lassa virus, virus of avian leukosis (ALV), Rous Sarcoma Virus (RSV), „Jaagsiekte sheep retrovirus, different murine leukaemia viruses (MLV), modified Baboon Ape Endogenous Virus (BAEVTR), modified Gibbon Ape Leukaemia Virus (GALVTR), Human T–Lymphtrophic Virus (HTLV–1), human spumous virus, Maedi–Visna Virus (MMV), SaRS–Coronavirus, Sendai virus, human parainfluenza type 3 virus, Nipah virus, measles virus, hepatitis C virus (HCV), influenza virus and Autographa californica multiple nuclear polyhedrosis virus.

4. The process according to claim 3, the lentivirus being pseudotyped with an envelope protein selected from the VSV–G envelope protein or the GALVTR envelope protein.

5. The process according to any one of the preceding claims, the host cell being selected from among a HEK293, HEK293T, HEK293FT, Te671, CEM, NIH–3T3, Mpf or D17 cell.

6. The process according to any one of the preceding claims, the host cell being stably or transiently transfected with one or several plasmids coding for the elements required for producing the lentivirus.

7. The process according to any one of the preceding claims, the lentivirus further contains a transgene of interest introduced into its genome.

8. The process according to any one of the preceding claims, said process comprising the following steps:

- transient transfection of HEK293T cells by means of one or several plasmids coding for the elements required for producing said enveloped vector;
- cultivation of said cells in a suitable medium, for which the pH is of about 6;
- harvesting the enveloped virus in the culture supernatant.

9. The process according to claim 8, the cells being transfected by means of four plasmids: one plasmid bearing an expression cassette comprising a lentiviral *gagpol* gene, one plasmid bearing an expression cassette comprising a lentiviral *rev* gene, one

transfer plasmid comprising an expression cassette of a transgene of interest, comprised between a lentiviral LTR–5' and a LTR–3' and one plasmid bearing an expression cassette of envelope glycoprotein(s).

10. A kit for applying the process according to any one of claims 1 to 5, comprising a mildly acid culture medium, or a culture medium accompanied by one or several solutions useful for bringing the pH of said medium to a mildly acid value comprised between 5.8 and 6.2, the pH being more particularly of about 6, the kit further comprising:

(a) one or several suitable plasmids for producing the lentivirus, optionally pseudotyped; and/or

(b) suitable cells for producing said lentivirus.

11. The kit according to claim 10, further comprising instructions for applying the process for producing a lentivirus, optionally pseudotyped according to any one of claims 1 to 9.

12. A kit for applying the process according to any one of claims 1 to 9, comprising (i) means for applying said process and (ii) instructions to be followed for applying the process, the kit comprising the following means (a) and (b), (a) and (c), (b) and (c) or (a) and (b) and (c):

(a) one or several suitable plasmids for producing the lentivirus, optionally pseudotyped;

(b) suitable cells for producing said lentivirus; and

(c) a mildly acid culture medium, or a culture medium accompanied by one or several solutions useful for bringing the pH of said medium to a mildly acid value comprised between 5.8 and 6.2, the pH being more particularly of about 6.

13. A kit for applying the process according to any one of claims 1 to 9, comprising a culture medium accompanied by one or several solutions useful for bringing the pH of said medium to a mildly acid value comprised between 5.8 and 6.2, the pH being more particularly of about 6.