CLAIMS

1. A purified RNA based viral vector composition comprising less than 2% of initial protein contaminants and less than 70 up to 90%, preferably less than 30%, of initial DNA contaminants, compare to the crude RNA based viral vector composition as present in the cell serum free medium, said composition being capable of transducing eukaryotic cells without affecting cell viability.

2. The RNA based viral vector composition of claim 1, wherein the crude RNA based viral vector is one wherein the physical particles/transducing units (PP/TU) is comprised of between 100:1 up to 900:1, preferably between 100:1 up to 600:1, more preferably between 100:1 up to 400:1.

3. The RNA based viral vector of claim 1 or 2, wherein said viral vectors have little to no effect on cell proliferation, viability, and/or the ability of cells, such as stem cells to differentiate or such as primary cells to be reprogrammed into pluripotent cells.

4. A viral DNA construct as contained in a bacterial host as deposited at CNCM Collection under the accession n°CNCM I-4487 or n° CNCM I-4488 or n° CNCM I-4489.

5. The use of the RNA based viral vector of any of claim 1 to 3, to transduce a eukaryotic target cells for transfer of a nucleic acid of interest (transgene) into said cells.

6. A method for preparing modified eukaryotic cells, wherein said cells are transduced without affecting their viability by a purified RNA based viral vector composition according to any of claims 1 to 3.

7 A method for production of RNA based retroviral particles comprising:

(i) transfection of a producer cell, modified to complement deletions in the RNA viral genome upon which the viral vector is based, and culturing the producer cells under suitable conditions to permit the production of viral vector particles, wherein said culturing following transfection is conducted in serum free medium;

(ii) collecting the supernatant containing said RNA based viral vector particles.

8. The method of claim 7, comprising no step of sodium butyrate induction.

9. The method of claim 7 or 8, wherein the supernatant collection is performed by multiple steps comprised between 3 and 6, at specific time intervals.

10. The method of any of claims 7 to 9, wherein the supernatant collection is followed by clarification by centrifugation.

11. The method of any of claims 7 to 10, further comprising a step of tangential ultrafiltration and diafiltration, the ultrafiltration being preferably operated on polysulfone hollow-fiber cartridges.

12. The method of any of claims 7 to 11, further comprising a step of ion-exchange chromatography.

13. A purified RNA based viral vector composition obtainable by the method according to any of claims 7 to 12.

14. A RNA based viral vector composition of claim 13, wherein the removal of DNA contaminants comprises between 70 to 99%, preferably up to 71% of such contaminants presents in the initial serum-free culture medium, and the removal of cellular proteins contaminants comprises between 50 to 99.9%, preferably up to 56% of the cellular proteins contained in the initial serum-free culture medium, and wherein the ratio PP/TU comprises between 100:1 to 900:1, preferably between 100:1 and 600:1.

15. A method of preparing a genetically modified eukaryotic cell comprising contacting an eukaryotic cell with the RNA based viral vector composition of any of claims 1 to 3, 13 or 14.

16. A modified eukaryotic cell wherein said cell is transduced without affecting its viability by a purified RNA based viral vector composition according to any of claims 1 to 3, 13 or 14.

CLAIMS

1. A method for production of RNA based retroviral particles comprising:

(i) transfection of a producer cell, modified to complement deletions in the RNA viral genome upon which the viral vector is based, and culturing the producer cells under suitable conditions to permit the production of viral vector particles, wherein said culturing following transfection is conducted in serum free medium;

(ii) collecting the supernatant containing said RNA based viral vector particles, said supernatant collection being performed by multiple steps comprised between 3 and 6, at specific time intervals;

and further comprising a step of tangential ultrafiltration and diafiltration.

2. The method of claim 1, comprising no step of sodium butyrate induction.

3. The method of any of claims 1 or 2, wherein the supernatant collection is followed by clarification by centrifugation.

4. The method of any of claims 1 to 3, further comprising a step of ion-exchange chromatography.

5. The method of any of claims 1 to 4, wherein the ultrafiltration is operated on polysulfone hollow-fiber cartridges.

6. A purified RNA based viral vector composition obtainable by the method according to any of claims 1 to 5.

7. A RNA based viral vector composition obtained by the method for production of RNA based retroviral particles according to claims 1 to 5, wherein the removal of DNA contaminants comprises between 70 to 99%, preferably up to 71% of such contaminants presents in the initial serum-free culture medium, and the removal of cellular proteins contaminants comprises between 50 to 99.9%, preferably up to 56% of the cellular proteins contained in the initial serum-free culture medium, and wherein the ratio PP/TU comprises between 100:1 to 900:1, preferably between 100:1 and 600:1.

8. A purified RNA based viral vector composition, obtained by the method for production of RNA based retroviral particles according to claims 1 to 5, comprising less than 2% of initial

protein contaminants and less than 70 up to 90%, preferably less than 30%, of initial DNA contaminants, compare to the crude RNA based viral vector composition as present in the cell serum free medium, said composition being capable of transducing eukaryotic cells without affecting cell viability, and wherein the crude RNA based viral vector is one wherein the physical particles/transducing units (PP/TU) is comprised of between 100:1 up to 900:1, preferably between 100:1 up to 600:1, more preferably between 100:1 up to 400:1.

9. The use of the RNA based viral vector of claim 8, to transduce a eukaryotic target cells for transfer of a nucleic acid of interest (transgene) into said cells.

10. An *in vitro* method for preparing modified eukaryotic cells, wherein said cells are transduced without affecting their viability by a purified RNA based viral vector composition according to claim 8.

11. An *in vitro* method of preparing a genetically modified eukaryotic cell comprising contacting an eukaryotic cell with the RNA based viral vector composition of any of claims 6 to 8.

12. A modified eukaryotic cell wherein said cell is transduced *in vitro* without affecting its viability by a purified RNA based viral vector composition according to any of claims 6 to