CLAIMS

1. A method for the production of a recombinant lentiviral vector, comprising:
- culturing, in suspension in a serum-free medium, ~~mammalian~~ HEK293T cells transfected with at least one plasmid adapted for the production of a lentiviral vector, the culture being carried out in a volume of at least 5 L;
- harvesting the produced recombinant lentiviral vector from the culture medium.

~~2. The method according to claim 1, wherein the mammalian cells are HEK293T cells.~~

~~3~~2. The method according to claim 1 ~~or 2~~, wherein the harvesting step consists of a single lentivirus harvest.

~~4~~3. The method according to claim ~~3~~2, wherein the transfection is a transient transfection and the single harvest is implemented between 48 and 72 hours post-transfection.

~~5~~4. The method according to any one of claims 1 to 3, comprising a transfection step wherein the cells are transfected with a mixture of polyethylenimine (PEI) and plasmids.

~~6~~5. The method according to claim ~~5~~4, wherein the PEI is a 20 – 25 kD linear PEI.

~~7~~6. The method according to claims ~~5~~ 4 or ~~6~~5, wherein transfection is carried out with a total DNA amount of at least 1.5 µg/10^6 cells.

~~8~~7. The method according to any one of claims ~~5~~ 4 to ~~7~~6, wherein the PEI and the plasmids are mixed before transfection according to a N/P ratio of less than 10, wherein N/P refers to the number of nitrogen atoms in the PEI per oligonuclotide phosphate.

~~9~~8. The method according to claim ~~8~~7, wherein the N/P ratio is of around 6.

~~10~~9. The method according to any one of claims ~~5~~ 4 to ~~9~~8, wherein the contact time between PEI and the plasmids before addition to the cell culture is comprised between 5 and 30 minutes, the contact time being in particular of around 10 minutes.

~~11~~10. The method according to any one of claims 1 to ~~10~~9, wherein sodium butyrate is added to the cell culture 24 hours after transfection of the cells without changing the medium.

~~12~~11. The method according to claim ~~11~~10, wherein sodium butyrate is added to the cell culture at a final concentration in the culture comprised between 2 mM and 12 mM, in particular between 2 mM and 10 mM, more particularly at a final concentration of 5 mM.

~~13~~12. The method according to any one of claims 1 to ~~12~~11, wherein the cells are transfected with four plasmids including a plasmid encoding the envelope proteins (Env plasmid), a plasmid encoding the lentiviral GagPol proteins (Gag-Pol plasmid), a plasmid encoding the lentiviral Rev protein (Rev plasmid) and a plasmid comprising a transgene of interest (TOI) between a lentiviral 3'-LTR and a lentiviral 5'LTR (TOI plasmid).

~~14~~13. The method according to any one of claims 1 to ~~13~~12, wherein the culture is implemented in a volume of at least 50 L.

~~15. The method according to any one of claims 1 to 14, wherein at least 10^7 infectious genomes /mL are produced.~~

~~16~~14. The method according to any one of claims 1 to ~~15~~13, wherein:
~~- the cells are 293T cells;~~
- transfection of the cells is carried out with a mixture of PEI and the required plasmid(s);
- sodium butyrate is added 24 hours post-transfection without changing the medium of the culture; and
- a single harvest of produced lentiviral vectors is carried-out.

~~17~~15. A cell culture device, wherein said culture device contains a volume of at least 5 L of a serum-free culture medium comprising ~~mammalian~~ HEK 293T cells transfected with at least one plasmid adapted for the production of a lentiviral vector, said cells growing in suspension in said culture device.

~~18. A cell culture device according to claim 17, wherein the cells are HEK 293T cells.~~

19. A method for optimizing the production of a lentiviral vector by a mammalian cell grown in suspension in a serum-free medium, transfected with plasmids required for said production, comprising adding sodium butyrate 24 hours post-transfection to a cell culture without changing the medium of the culture.

20. The method according to claim 19, wherein sodium butyrate is added at a final concentration of 5 mM.