

CLAIMS

1. A live-attenuated yellow fever virus strain adapted to grow on Vero cells from a parent yellow fever virus 17D substrain that is not adapted to grow on Vero cells, wherein said live-attenuated yellow fever virus strain is less neurovirulent than said parent yellow fever virus 17D substrain.
2. The live-attenuated yellow fever virus strain according to claim 1, wherein the parent yellow fever virus strain is a live-attenuated yellow fever virus strain adapted to grow on eggs.
3. The live-attenuated yellow fever virus strain according to claim 1 or 2, wherein the parent yellow fever virus strain is a yellow fever virus 17D-204 substrain.
4. The live-attenuated yellow fever virus strain according to any one of claims 1 to 3, wherein the parent yellow fever virus 17D substrain comprises an RNA sequence of SEQ ID NO. 2.
5. The live-attenuated yellow fever virus strain according to any one of claims 1 to 4, wherein said live-attenuated yellow fever virus strain is less neurovirulent than the parent yellow fever virus 17D-substrain in a mouse lethal dose 50 (MLD₅₀) test.
6. A live-attenuated yellow fever virus strain comprising a nucleic acid comprising:
- i) a mutation in the codon for the amino acid at position 480 of the envelope protein (E) which results in an amino acid change from valine to leucine, or
 - ii) a mutation in the codon for the amino acid at position 65 of the non-structural protein 2A (NS2a) which results in an amino acid change from methionine to valine.
7. The live-attenuated yellow fever virus strain according to claim 6 comprising a nucleic acid comprising:
- i) a mutation in the codon for the amino acid at position 480 of the envelope protein (E) which results in an amino acid change from valine to leucine, and
 - ii) a mutation in the codon for the amino acid at position 65 of the non-structural protein 2A (NS2a) which results in an amino acid change from methionine to valine.
8. The live-attenuated yellow fever virus strain according to claim 6 or 7, wherein the nucleic acid further comprises a mutation in the codon for the amino acid at

position 19 of the non-structural protein 4A (NS4a) which results in a codon change from AAA to AAG.

9. The live-attenuated yellow fever virus strain according to any one of claims 6 to 8, wherein the nucleic acid further comprises a mutation in the codon for the amino acid at position 145 of the envelope protein (E) which results in a codon change from GUA to GUU.

10. A live-attenuated yellow fever virus strain which comprises an envelope protein comprising a mutation at position 480 which results in an amino acid change from valine to leucine.

11. A live-attenuated yellow fever virus strain which comprises an envelope protein comprising a leucine residue at the position within the protein that corresponds to position 480 of SEQ ID NO. 15.

12. The live-attenuated yellow fever virus strain as claimed in claim 11, wherein said envelope protein comprises a sequence at least 90%, 95%, 98% or 100% identical to the sequence of SEQ ID NO. 15

13. A live-attenuated yellow fever virus strain comprising a nucleic acid molecule encoding:

(i) an envelope protein comprising a mutation at position 480 which results in an amino acid change from valine to leucine, and

(ii) a NS2a protein comprising a mutation at position 65 which results in an amino acid change from methionine to valine.

14. The live-attenuated yellow fever virus strain of claim 13, wherein the nucleic acid further comprises a mutation in the codon for the amino acid at position 19 of the non-structural protein 4A (NS4a) which results in a codon change from AAA to AAG.

15. The live-attenuated yellow fever virus strain of claim 13 or 14, wherein the nucleic acid further comprises a mutation in the codon for the amino acid at position 145 of the envelope protein (E) which results in a codon change from GUA to GUU.

16. A live-attenuated yellow fever virus strain comprising a nucleic acid molecule encoding:

(i) an envelope protein which comprises a leucine residue at the position within the protein that corresponds to position 480 of SEQ ID NO. 15; and

(ii) an NS2a protein which comprises a valine residue at the position within the protein that corresponds to position 65 of SEQ ID NO. 16.

17. The live-attenuated yellow fever virus strain as claimed in claim 16, wherein said envelope protein comprises a sequence at least 90%, 95%, 98% or 100% identical to the sequence of SEQ ID NO. 15 and said NS2a protein comprises a sequence at least 90%, 95%, 98% or 100% identical to the sequence of SEQ ID NO. 16.

18. The live-attenuated yellow fever virus strain as claimed in claim 16 or 17, wherein the nucleic acid further comprises a G nucleotide at position within the nucleic acid coding for the non-structural protein 4A (NS4a) that corresponds to position 57 of SEQ ID NO. 17.

19. The live-attenuated yellow fever virus strain according to any one of claims 16 to 18, wherein the nucleic acid further comprises a U nucleotide at position within the nucleic acid coding for the envelope protein (E) that corresponds to position 435 of SEQ ID NO. 18.

20. An immunogenic composition comprising a live-attenuated yellow fever virus strain according to any one of claims 1 to 19 and a pharmaceutically acceptable vehicle.

21. A method for obtaining a live-attenuated yellow fever virus strain adapted to grow on Vero cells, comprising the steps of:

- a) purifying the viral genomic RNA of a parent live-attenuated yellow fever virus strain that is not adapted to grow on Vero cells, and that is optionally adapted to grow on eggs;

- b) transfecting Vero cells with the viral genomic RNA purified in step a), whereby transfected Vero cells are obtained;

- c) growing the transfected Vero cells obtained in step b) in a culture medium, whereby a first yellow fever virus population is obtained and further recovered;

- d) amplifying the recovered first yellow fever virus population obtained at the end of step c) 2 times or more on fresh Vero cells, whereby a second yellow fever virus population is obtained;

- e) cloning the second yellow fever virus population obtained in step d) by two or more successive plaque purifications on Vero cells whereby a plurality of yellow fever virus clones is obtained;

- f) amplifying separately each of the recovered yellow fever virus clones obtained at the end of step e) 2 times or more on fresh Vero cells, whereby a plurality of yellow fever virus strains is obtained; and

- g) selecting from the said plurality of yellow fever virus strains recovered in
5 step f) one or more live-attenuated yellow fever virus strain that is less neurovirulent than the parent live-attenuated yellow fever virus strain, in a mouse lethal dose 50 (MLD₅₀) test.

22. The method according to claim 21, wherein the said culture medium is serum-free, and is optionally free of any human or animal-derived substance.

23. A live-attenuated yellow fever virus strain obtainable by a method
10 according to any one of claims 21 or 22.

24. A live-attenuated yellow fever virus strain according to any one of claims 1 to 19 or claim 23 for use in the preparation of a vaccine.

25. A vaccine comprising a live-attenuated yellow fever virus strain according to any one of claims 1 to 19 or claim 23 for use in preventing an infection by a yellow
15 fever virus.