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(54) **Genetically stable oncolytic RNA virus, method of manufacturing and use thereof**

Genetisch stabiles onkolytisches RNA-Virus, Verfahren zur Herstellung und Verwendung davon

Virus d'ARN oncolytique génétiquement stable, son procédé de fabrication et utilisation associée

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(56) References cited:
EP-A1- 1 537 872

- **FERDAT A K: "MECHANISM OF IMMUNOMODULATION IN THE ANTI-TUMOUR EFFECT OF THE ECHO-7 ENTEROVIRUS", EKSPERIMENTAL NAA ONKOLOGIA - EXPERIMENTAL ONCOLOGY, KIEV, UU, vol. 11, no. 5, 1 January 1989 (1989-01-01), pages 43-48, XP008054566, ISSN: 0204-3564**
- **CHUA B H ET AL: "Comparison of the complete nucleotide sequences of echovirus 7 strain UMMC and the prototype (Wallace) strain demonstrates significant genetic drift over time", November 2001 (2001-11), JOURNAL OF GENERAL VIROLOGY, VOL. 82, NR. 11, PAGE(S) 2629-2639, XP002717948, ISSN: 0022-1317 * page 2629 ***

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Description

TECHNICAL FIELD

- 5 **[0001]** The invention relates to development of a novel biotechnologically produced anti-cancer preparation, namely to a genetically stable oncolytic RNA virus, a method for manufacturing the oncolytic virus, and use thereof.

BACKGROUND ART

- 10 **[0002]** The ability of viruses to kill cancer cells is known for more than a century [Kelly, E.; Russell, S.J. History of oncolytic viruses: genesis to genetic engineering. Mol. Ther. 2007, 15, pp. 651-659] and there were numerous promising successes in experimental cancer therapy with various viruses, nevertheless their use in clinical practice is hampered by the difficulty to foresee the interaction between the tumour and its host, as well as the virus and response of human immune system to viral antibodies.
- 15 **[0003]** Although the clinical investigations regarding the use of viruses in cancer therapy commenced more than 50 years ago, at present only two viruses are approved for clinical use in cancer therapy. They are adenovirus with deleted E1B 55K gene (Garber, K. China approves world's first oncolytic virus therapy for cancer treatment. J. Natl. Cancer Inst. 2006, 98, pp. 298-300) and unmodified passivized Picornaviridae Enterovirus of Echo type (Eurasian patent 007839; European patent application 03733607), acting as antitumour immunostimulant.
- 20 **[0004]** EP 1537872 discloses the use of an ECHO-7 virus in the active immunotherapy of tumours. Virus was recovered from the intestinal tract, and isolated in a human primary embryonic cell culture. Page 2, paragraph 18 states that nonpathogenic serotypes of virus (in particular ECHO 7), were selected. Virus was cultured in tumour tissue, then in human embryonic fibroblasts, and the process repeated. The virus obtained was demonstrated as being able to improve survival in patients with skin melanomas.
- 25 **[0005]** The development of novel efficient oncolytic viruses is still a topical problem (Han Hsi Wong, Nicholas R. Lemoine, Yaohe Wang, Viruses 2010, 2, pp. 78-106).
- [0006]** In order to increase the potential of virus so selectively infect cancer cells and heighten the oncolytic activity, a number of modified viruses have been disclosed. They are characterised by deletion of specific genes, thus preventing their propagation in normal cells, or integration of additional genes for improving the oncolytic properties.
- 30 **[0007]** However, the limited knowledge concerning the genetical modifications that provide for selectivity and efficiency against the tumour cells, results in modified viruses with lower cytolytic activity, compared to origin, or higher anti-virus response of human immune system (S.Meerani, Yang Yao, Oncolytic viruses in cancer therapy. European Journal of Scientific Research, vol. 40 no.1 (2010), pp.156-171; Han Hsi Wong, Nicholas R. Lemoine, Yaohe Wang, Viruses 2010, 2, 78-106).
- 35 **[0008]** Although viruses are well-established tools for conveying vectors into cell, their use is limited by the high immunogenicity of viruses (Peng, Z. Current status of gendicine in China: recombinant human Ad-p53 agent for treatment of cancers. Hum. Gene. Ther. 2005, 16, 1016-1027).
- [0009]** One of the most serious adverse properties of non-modified ECHO type viruses, including ECHO 7, is their ability to cause infections that may have a fatal result (Wreghitt T.G., Gandy G.M., King A., Sutehall G., Fatal neonatal ECHO 7 virus infection, The Lancet, vol. 324, p.465, 1984). These viruses are known to be responsible for hand, foot and mouth disease in Malaysia (<http://www.vadscorner.com/echovirus7.html>), for myocarditis in leukemic child (Midula M., Marzetti G., Borra G., Sabatino G., Myocarditis associated with ECHO 7 type infection in leukemic child, Acta Paediatrica Volume 65, Issue 4, pp. 649-651, July 1976), aseptic meningitis, paralytic disease and fever (<http://virology-online.com/viruses/Enteroviruses6.htm>).
- 40 **[0010]** Therefore pathogenicity is one of the major limitations that must be overcome in using ECHO 7 type viruses in treating cancer patients.

DISCLOSURE OF THE INVENTION

- 50 **[TECHNICAL PROBLEM]**

[0011] Therefore, the problem to solve was the development a highly efficient, selective oncolytic virus without pathogenicity in normal cells and low immunological response, and possessing high genetic stability.

- 55 **[SOLUTION TO PROBLEM]**

[0012] This problem was surprisingly solved by a targeted modification of a single-strand RNA virus by developing a method that utilized the high mutation potential of single strand RNA virus in combination with a specifically targeted

selection of mutants, providing for fast separation from the pool of mutant species with high and selective oncolytic activity. Many cancer cells are resistant to the virus (the virus can not enter the cell and survive there). By careful selection of cell lines where the virus is modified and by proper pretreatment of the cancer cells it is possible to create a genetically stable and non-pathogenic virus for cancer treatment.

SHORT DESCRIPTION OF THE INVENTION

[0013] We have developed a method for modifying the native ECHO 7 virus, identified by genome sequence SeqNo2, the method comprising initially conducting the virus adaptation in cancer cells, attenuated by an anti-cancer agent such as dacarbazine, passing the modified virus in human embryonal fibroblast culture, propagation in human melanoma cells and passing in human embryonal fibroblast culture, optionally treated by ribavirin, isolation of the virus and purification of the virus. The virus can be isolated and purified by known methods.

[0014] More than one type of cell lines can be used during conducting the virus adaptation.

[0015] The invention is as set out in claims 1-12.

BRIEF DESCRIPTION OF THE APPENDICES CONTAINING THE SEQUENCES (which appendices are part of the description)

[0016]

Appendix 1: Nucleotide sequence of the modified virus(Seq ID No 1)

Appendix 2: Nucleotide sequence of the unmodified (native) virus (Seq ID No 2)

Appendix 3. Nucleotide sequence of the modified virus after propagation for 12 months (Seq ID No 3)

Appendix 4. Comparison of genomes of the modified virus and unmodified (native) virus (Seq ID No 4)

Appendix 5. Comparison of amino acid sequences of the modified virus and unmodified (native) virus (Seq ID No 5).

DETAILED DESCRIPTION OF THE INVENTION

[0017] We have unexpectedly discovered the suitability for this purpose of a known Echo 7 type *Picornaviridae* enterovirus, isolated from a human intestine. The original nucleotide sequence, determined by a standard method, was found to be rather similar to that of Wallace type *Picornaviridae* Enterovirus.

[0018] Checking the oncolytic activity of isolated native enteroviruses in tissue of angiosarcoma demonstrated that neither individual viruses nor their combinations in a dose 3×10^5 TCID₅₀/0.03 ml possessed substantial oncolytic activity with exception of ECHO 7 type that showed more promising activity (Table 1).

Table 1. Influence of viruses on angiosarcoma tissue culture

| Virus | Number of animals | Number of regressed tumours on Day 4 after infecting | Isolated (surviving) virus | Viral titer on Day 4, TCD ₅₀ /0.1 ml |
|------------------------|-------------------|--|----------------------------|---|
| ECHO 4 | 6 | 0 | ECHO 4 | 10^6 - 10^7 |
| ECHO 7 | 6 | 0 | ECHO 7 | 10^5 - 10^6 |
| Coxsackie B-5 | 6 | 0 | Coxsackie B-5 | 10^7 - 10^8 |
| ECHO 4 + ECHO 7 | 6 | 2 | ECHO 7 | 10^9 |
| ECHO 4 + Coxsackie B-5 | 6 | 1 | ECHO 4, Coxsackie B-5 | 10^3 } 10^8 |
| ECHO 7 + Coxsackie B-5 | 6 | 0 | ECHO 7 | 10^4 } |
| Control | 6 | 0 | | 10^8 |

[0019] The instability of the genome of the RNA single strand viruses is a well-known fact; therefore, such viruses usually are not selected for constructing oncolytic viral agents.

[0020] The modification of the isolated native virus was realised in several consecutive steps.

[0021] The first step takes advantage of the high mutation potential of RNA viruses (on average one mutation on each replication) to develop a cytopathic mutant by replicating the virus in trypsinized monolayer of human embryonic fibroblast

culture in presence of calf serum.

[0022] Cells were incubated for 10 days, carrying out the passage each time when the cells in culture had degenerated for 50%.

[0023] Testing the selected virus in RD cell culture a pronounced cytopathic effect was observed already in 24 hours after infection. The titer of the developed virus, determined by last dilution method was $TCID_{50}=1 \times 10^{-8}$.

[0024] This strain was propagated, isolated and stored at -70°C for further use in producing selective and genetically stable oncolytic strain.

[0025] The virus so obtained was modified, using specially developed method comprising three steps.

1st modification step in a first tumour cell line

[0026] In the first modification step, the virus was propagated in tumour cell lines attenuated by anticancer agent. Human breast adenocarcinoma cell line (MCF-7) was used in this step.

[0027] A monolayer of these cells was treated with dacarbazine DTIC in sub toxic dose ($20 \mu\text{M}$). After treating with dacarbazine, the cells were transferred to fresh culture medium and contacted with the virus, and the propagation continued without adding serum. After 24 hours from contacting with the virus, the cells were removed and the virus was isolated from the media.

[0028] The virus was repeatedly propagated (passaged) in human embryonic fibroblast cell culture and again used for infecting the MCF-7 cell line. This procedure was repeated 10 times. Thus, this modification step comprises alternately propagating the virus in human breast adenocarcinoma cells and human embryonic fibroblast cells.

2nd modification step in a second tumour cell line

[0029] In the next modification step, the virus as described above, was contacted with gastric adenocarcinoma cell culture. A monolayer of these cells was treated with dacarbazine DTIC in sub toxic dose ($20 \mu\text{M}$).

[0030] The monolayer of these cells was infected by the virus, which was isolated after the modification in the first step, and the propagation continued in a culture medium without serum.

[0031] After 24 hours from contacting with the virus, the cells were removed and virus isolated from the media. The virus was repeatedly propagated (passaged) in human embryonic fibroblast cell culture, and thereafter again used for infecting the gastric adenocarcinoma cell line. This procedure was repeated 10 times. Thus, this second modification step comprises alternately propagating the virus in gastric adenocarcinoma cells and in human embryonic fibroblast cells.

[0032] In the first modification step and in the second modification step, the propagation procedure was always finished by propagating the virus last in the human embryonic fibroblast culture.

3rd modification step in human melanoma cells

[0033] The virus produced in the second step was used for infecting human tumours, obtained in surgery.

[0034] Melanoma cancer tissues were obtained in surgery from 23 patients previously treated by chemotherapy.

[0035] Tissues were infected by the modified virus and incubated at 37°C in the absence of carbon dioxide. Before being used for infecting a new tissue material (fresh melanoma tissue from another patient), the modified virus was repeatedly propagated in human embryonic fibroblast culture to titer $7 \lg TCID_{50}/1 \text{ ml}$.

[0036] The modified virus was propagated in human embryonic fibroblast cell culture that was treated by 5 mM ribavirin 7 hours before infection and cultivated for 24 hours. The virus was isolated from the culture, and the procedure of propagating the virus in the fibroblast culture was repeated 10 times.

[0037] Finally, the virus was isolated, purified and propagated in human embryonic fibroblast culture without addition of ribavirin.

[0038] The propagated virus was used for sequence determination. The genome of the modified virus differs from that of frozen unmodified native virus (Echo 7 type Wallace strain from NCBI database) for about 10%, the coat part for about 12%.

[0039] The virus modified by the described method was found to be surprisingly stable. Its genome changed for only 0.7% after continuous passaging for 12 months (propagation for 12 months in human embryonal lung culture MRC 5).

[0040] Especially important is the fact that the modified virus (further on MV) is characterised by exceptionally high cytopathic effect on malignant cells and low cytotoxicity on normal cell lines as well as no toxicity *in vivo* in mice.

[0041] In experiments with cell lines MV was found to be cytotoxic for melanoma cell lines FM9, FM55, FM94 and SK-Mel26, gastric carcinoma cells, human oral squamous cell carcinoma SCC25 cells, human epithelial cell line derived from a lung carcinoma (A549), acute monocyte leukemia THP-1 cells, rhabdomyosarcoma RD cells, human pancreatic adenocarcinoma HPAF-II cells, human breast adenocarcinoma cells (MCF-7) as well as on primary cell cultures of gastric adenocarcinoma GC1 and thyroid cancer line HA007.

[0042] In animal experiments, MV caused regression of murine sarcoma M-1, mice fibrosarcoma MX-17 as well as transplantable tumours - Moloney sarcoma (SM) and KRS-321 sarcoma.

[0043] In a clinical pilot study, a group of 46 melanoma stage I patients no progress of melanoma was observed for 50 months in 43 patients, treated with MV. In the control group, melanoma progressed for 10 of 31 patients undergoing standard therapy.

[0044] In a 50 months study of 44 stage II melanoma patients the progress of melanoma was stopped in 38 patients, compared to control group of 36 patients undergoing standard therapy, where melanoma did not progress in 15 patients, but did progress in 21 patient. No serious adverse effects were observed for patients treated with MV.

INDUSTRIAL APPLICABILITY

[0045] We have developed a novel virus strain (MV) with original genome sequence, stable against genetic drift, possessing cytopathic activity against various types of tumours, characterized by low incidence of adverse effects and low toxicity that can be used with advantage in cancer virotherapy. Thus, we have unexpectedly solved the main obstacle in wider use of RNA viruses in medicine - obtained genetically stable strain that can be used in standardized continuous manufacturing of oncolytic viral preparation. The viral preparation can be used in anticancer therapy against a variety of tumour cells.

EXAMPLES

[0046] The present invention is described in Examples in more detail. However, the invention is not construed as being limited to the examples.

Virus

[0047] The virus modified according to the invention is ECHO-7 virus (Picornaviridae family, Enterovirus genus, ECHO (Enteric Cytopathic Human Orphan) type 7, group IV, positive-sense single stranded RNA virus).

Example 1. The isolation and characterization of the original virus strain

[0048] A known method for isolation (A.C. Rentz, J.E. Libbey, R.S. Fujinami, F.G. Whitby, and C.L. Byington. Investigation of Treatment Failure in Neonatal Echovirus 7 Infection. The Pediatric Infectious Disease Journal, Volume 25, Number 3, March 2006, 259) and propagation in BS-C-1 cell line (CCL 26; ATCC) was used (Libbey JE, McCright IJ, Tsunoda I, et al. Peripheral nerve protein, P0, as a potential receptor for Theiler's murine encephalomyelitis virus. J Neurovirol. 2001;7:97-104. Pevear DC, Tull TM, Seipel ME, et al. Activity of pleconaril against enteroviruses. Antimicrob Agents Chemother. 1999;43:2109-2115). Virus propagation and determination of titer was conducted in concordance with the published method (Zurbriggen A, Fujinami RS. A neutralization-resistant Theiler's virus variant produces an altered disease pattern in the mouse central nervous system. J Virol. 1989;63:1505-1513).

Example 2. Virus modification

[0049] In the first modification step, the virus was propagated in tumour cell lines attenuated by an anticancer agent. Initially, for propagation was used the human breast adenocarcinoma cell culture (MCF-7), cultivated in DME medium (Sigma-Aldrich) with 10% serum (Gibco) and antibiotics (100 IU/ml penicillin, 100 IU/ml streptomycin) at 37 °C under atmosphere, containing 5% CO₂ until developing of the monolayer.

[0050] The obtained monolayer of these cells was treated with dacarbazine DTIC in sub toxic dose (20 µM). After treating with dacarbazine cells were transferred to fresh culture medium without added serum, the cells contacted with virus and the propagation continued.

[0051] After 24 hours from contacting with the virus the cells were removed and virus isolated from the media. The virus was repeatedly propagated in human embryonal fibroblast cell culture and again used for infecting the MCF-7 cell line. This procedure was repeated 10 times.

[0052] In the next, second step, the virus as described above, was contacted with gastric adenocarcinoma cell culture. The cell culture for propagation was cultivated in DME medium (Sigma-Aldrich) with 10% serum (Gibco) and antibiotics (100 IU/ml penicillin, 100 IU/ml streptomycin) at 37 °C under atmosphere, containing 5% CO₂ until developing of the monolayer.

[0053] The obtained monolayer of these cells was treated with dacarbazine DTIC in sub toxic dose (20 µM). After treating with dacarbazine cells were transferred to fresh culture medium without added serum, the cells contacted with virus and the propagation continued.

[0054] After 24 hours from contacting with the virus the cells were removed and virus isolated from the media. The virus was repeatedly propagated in human embryonal fibroblast cell culture and again used for infecting the gastric adenocarcinoma cell line. This procedure was repeated 10 times.

[0055] In the third step, the virus produced in the second step was used for infecting human tumours, obtained in surgery. Melanoma cancer tissues were obtained in surgery from 23 patients previously treated by chemotherapy.

[0056] The tumour cells were separated from fat cells, necrotic tissue and blood, kept at 0 °C for 24 hours, fragmented and as approximately 0.1 cm³ large tissue pieces immersed in Eagle medium (4 ml of medium for 10 mg of tissue), infected with the prepared virus and incubated in the absence of carbon dioxide at 37 °C.

[0057] The medium was replaced by a fresh portion every day until the destruction of tumour, determined morphologically and visually by the oxidation level of medium.

[0058] The virus titer was determined every day in tumor tissue free medium sample. The reproduction rate of virus was determined from the virus titer at the conclusion of an experiment in comparison with that on Day 0. Such modification of virus was performed in tissues obtained from 23 patients.

[0059] Before being used for infecting a new tissue material, the modified virus was each time repeatedly propagated in human embryonal fibroblast culture to titer 7 lg TCID₅₀/1-ml.

[0060] The modified virus was propagated in human embryonal fibroblast cell culture that was treated by 5 mM ribavirin 7 hours before infection and cultivated for 24 hours. Virus was isolated from culture medium, and the procedure repeated 10 times.

[0061] Finally, the virus was isolated, purified and propagated in human embryonal fibroblast culture without addition of ribavirin.

[0062] The propagated virus sample was used for determination of genome sequence, anticancer activity and replicative stability by passaging it for 12 months in human embryonal fibroblast culture with repeated determination of genome sequence (Appendix 1).

Example 3. Determination of virus genome sequence

[0063] The isolation, amplification and sequencing of the isolated, modified and cultivated virus genome were performed according to the known method [Chua BH, McMinn PC, Lam SK, Chua KB. Comparison of the complete nucleotide sequences of echovirus 7 strains UMMC and the prototype (Wallace) strain demonstrates significant genetic drift over time. J Gen Virol. 2001 Nov; 82(Pt 11): 2629-39].

[0064] For this purpose, 96 enteroviruses with complete genome sequence were selected from the NCBI Gene bank. The complete genome sequences for these viruses were downloaded and compared by Vector NTI program.

[0065] Based on the results of comparing the most conservative regions of virus genomes were determined and 13 degenerated oligonucleotide pairs selected in these regions, covering the length of the potential enteroviruses genome. After the synthesis of the first 13 fragments, another 13 nucleotide pairs were produced. These oligonucleotide pairs were virus-specific and designed so as to produce overlaying fragments. After the building of the full genome sequence the virus genome was repeatedly sequenced with the virus-specific primers.

Example 3.1. The genome sequence of the unmodified (native) virus

[0066] The sequence of the native virus was produced from 26 separate overlapping PCR fragments, synthesized from the primers listed in Table 2.

Table 2. Primers used to sequence the complete genome of viruses.

| Primer | Sequence (5'- 3') | Length (bp) | Position | Target region |
|--------|---------------------------|-------------|-----------|---------------|
| Eo7-1F | TTAAACAGCCTGTGGGTTG | 20 | 1-20 | 5'UTR |
| Eo7-1R | GAAACACGGACACCCAAAGTAG | 22 | 545-566 | 5'UTR |
| Eo7-2F | CCATGGGACGCTTCAATACT | 20 | 391-410 | 5'UTR |
| Eo7-2R | GCACAGTCTTTTGTGTCGA | 20 | 758-777 | VP4 |
| Eo7-3F | CGACTACTTTGGGTGTCCGTGTTTC | 25 | 542-566 | 5' UTR |
| Eo7-3R | TCDGGRAAYTTCCACCACCACCC | 23 | 1178-1200 | VP2 |
| Eo7-4F | CGACAGGGTGAGATCCCTAA | 20 | 979-998 | VP2 |
| Eo7-4R | TTTACCCTTCGTGAGGTTTC | 20 | 1381-1400 | VP2 |

EP 2 826 856 B9

(continued)

| Primer | Sequence (5'- 3') | Length (bp) | Position | Target region |
|---------|-----------------------------|-------------|-----------|---------------|
| Eo7-5F | GCATCYAARTTYCAYCARGG | 20 | 1289-1308 | VP2 |
| Eo7-5R | CACATKGGKGAATSGTGAC | 20 | 1676-1695 | VP2 |
| Eo7-6F | GTGGATCAACTTGCGCACTA | 20 | 1513-1532 | VP2 |
| Eo7-6R | AAATTGTGGCATAGCCGAAG | 20 | 1797-1816 | VP3 |
| Eo7-7F | GTCACSATTGCMCCMATGTG | 20 | 1676-1695 | VP2 |
| Eo7-7R | CTTNATRCTYCCTGACCAGTGTG | 23 | 2055-2077 | VP3 |
| Eo7-8F | AAGCATGGACGCATATCACA | 20 | 1921-1940 | VP3 |
| Eo7-8R | GATATGGGTTCCCACATTGC | 20 | 2174-2194 | VP3 |
| Eo7-9F | CACACTGGTCAGGRAGYATNAAG | 23 | 2055-2077 | VP3 |
| Eo7-10F | CAAGTGTGTCGTCCTGTGCT | 20 | 2350-2369 | VP3 |
| Eo7-9R | CCTATTGGCGCTGTCTTGAT | 20 | 2694-2713 | VP1 |
| Eo7-11F | ACCAAAGATCAAGACAGCGC | 20 | 2687-2706 | VP1 |
| Eo7-11R | TTGGCACCCACACTCTGATA | 20 | 3178-3197 | VP1 |
| Eo7-12F | ACCAGTCCGGTGCTGTTTAC | 20 | 3336-3355 | VP1-2A |
| Eo7-12R | TCCCAYACACARTTYTGCCAGTC | 23 | 3401-3423 | 2A |
| Eo7-13F | CARAAYTGTGTGTGGGAAGACTA | 23 | 3407-3429 | 2A |
| Eo7-13R | CCCTGYTCCATKGCTTCATCYTCYARC | 27 | 3748-3774 | 2A-2B |
| Eo7-14F | TTACCCAGTCACCTTCGAGG | 20 | 3535-3554 | 2A |
| Eo7-14R | TGTTTTTCCTTCACTTCCGG | 20 | 4181-4200 | 2C |
| Eo7-15F | GTTRGARGATGATGCNATGGARCARGG | 27 | 3748-3774 | 2A-2B |
| Eo7-15R | TCAATACGGYRTTTSWCTTGAA | 23 | 4409-4431 | 2C |
| Eo7-16F | CCTYTRTAYGCVGCGYGARGC | 20 | 4343-4362 | 2C |
| Eo7-17F | TTCAAGWSCAAAYRCCGTATTGA | 23 | 4409-4431 | 2C |
| Eo7-16R | AAYTGAATGGCCTTHCCACACAC | 23 | 4922-4944 | 2C |
| Eo7-18F | CTDGTGTGTGGRAAGGCTATNCA | 23 | 4919-4941 | 2C |
| Eo7-18R | TATGCTCCYTGRAARCTGCAAA | 23 | 5309-5330 | 3A-3B |
| Eo7-19F | CAAGCCCTAACCACGTTTGT | 20 | 5252-5271 | 3A |
| Eo7-19R | ACCCGTAGTCAGTCACCTGG | 20 | 5740-5759 | 3C |
| Eo7-20F | TTTGCAGGMTTYCARGGWGCATA | 23 | 5309-5330 | 3A-3B |
| Eo7-20R | GCTCTWGTGGGRAAGTTRTACAT | 23 | 5723-5745 | 3C |
| Eo7-21F | GTGTTGGATGCCAAGGAACT | 20 | 5555-5574 | 3C |
| Eo7-21R | ATGGGCTCCGATCTGATGTC | 20 | 6203-6222 | 3D |
| Eo7-22F | TTCCCCACWAGRGACGGCCARTGYGG | 26 | 5907-5832 | 3C |
| Eo7-22R | CTCCAAAABASRTCYGGGTCTCA | 23 | 6572-6594 | 3D |
| Eo7-23F | TGAAGGAATGCATGGACAAA | 20 | 6360-6379 | 3D |
| Eo7-23R | ATGGGTATTGCTCATCTGCC | 20 | 7078-7097 | 3D |
| Eo7-24F | TGYGACCCRGAYSTVTTTTGGAG | 23 | 6572-6594 | 3D |
| Eo7-24R | TCRTGDATDTCYTTTCATGGGCA | 22 | 7116-7137 | 3D |

(continued)

| Primer | Sequence (5'- 3') | Length (bp) | Position | Target region |
|-----------------------------------|---------------------------------|-------------|------------------|---------------|
| Eo7-25F | CCTGGACGAATGTGACCTTT | 20 | 7041-7060 | 3D |
| Eo7-25R | CCCTACCGCACTTTTATCCA | 20 | 7384-7403 | 3'UTR |
| Eo7-26F | ATCCAYGARTCHATYAGRTGGAC | 23 | 7130-7152 | 3D |
| Eo7-26R | CCGCACCGAATGCGGAGAATTTAC | 24 | 7404-7427 | 3'UTR |
| UTR - untranslated region. | | | | |

[0067] The 5'-terminal and the 3'-terminal sequences were obtained, using 5'-RACE and 3'-RACE methods, correspondingly.

[0068] As a result, the full genome sequence of the unmodified virus was found to consist of 7434 nucleotides, excluding the poly A sequence (Appendix 2; Seq ID No 2).

[0069] The untranslatable 5'-terminal (5'NTR) contains 742 nucleotides, followed by coding part starting with start codon (AUG) at position 743, containing codons for 2196 amino acids and ending with stop codon (UAA) at position 7331 (Appendix 2). The untranslatable 3'-terminal (3'NTR) of this strain contains 100 nucleotides, followed by poly A sequence.

Example 3.2. The sequence of the modified virus (MV)

[0070] The sequence of the starting virus was produced from 26 separate overlapping PCR fragments, synthesized using the primers listed in Table 2.

[0071] The 5'-terminal and the 3'-terminal sequences were obtained, using 5'-RACE and 3'-RACE methods, correspondingly.

[0072] As a result, the full genome sequence of the modified virus was found to consist of 7427 nucleotides, excluding the poly A sequence (Appendix 1; Seq ID No 1).

[0073] The untranslatable 5'-terminal (5'NTR) of this strain contains 742 nucleotides, followed by the coding sequence. The coding part that contains information about the virus polyprotein, begins with the start codon (AUG) at position 743, contains codons for 2194 amino acids and ends with stop codon (UAA) at position 7325 (Appendix 1). The untranslatable 3'-terminal (3'NTR) of this strain contains 100 nucleotides, followed by poly A sequence.

Example 3.3. The genome sequence of the modified virus after propagation for 12 months

[0074] The sequence of the modified virus was produced from 26 separate overlapping PCR fragments, synthesized the primers listed in Table 2.

[0075] The 5'-terminal and the 3'-terminal sequences of this strain were obtained, using 5'-RACE and 3'-RACE methods, correspondingly.

[0076] As a result, the full genome sequence of the modified virus was found to consist of 7427 nucleotides, excluding the poly a sequence (Appendix 3). The untranslatable 5'-terminal (5'NTR) contains 742 nucleotides, followed by coding part, starting with start codon (AUG) at position 743, containing codons for 2194 amino acids and ending with stop codon (UAA) at position 7325 (Appendix 3). The untranslatable 3'-terminal (3'NTR) of this strain contains 100 nucleotides, followed by poly A sequence.

Example 3.4. Comparison of genomes of modified virus (MV) and native strain

[0077] Comparison of genomes of modified virus (MV) and starting strain is provided in Appendix 4.

[0078] The difference in nucleotide sequence, calculated by programme Vector NTI is substantial, 10% for the complete genome and 12% for the part coding the virus coat proteins. The amino acid sequences for the modified and starting strains are listed in Appendix 5.

Example 3.5. The genome sequence of the modified virus after propagation for 12 months

[0079] The changes in the sequence of modified virus (MV) genome after continuous passaging for 12 months did not exceed 0.7% of the initial sequence.

[0080] All found changes were one nucleotide replacements, partially the mute mutations (without change of amino

acid). If the amino acid was changed, its position was in the genome polymorphic part, evidently without relevant influence on virus activity.

Example 4. Virus passaging

[0081] Virus MV was passaged by known methods and propagated for 12 months in human embryonal lung culture MRC 5 (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia - Laboratorio Centro Substrati Cellulari, Catalogue No. BS CL 68 (origin: American Type Culture centre Collection, Rockville, Md, USA), free of bacteria, viruses, fungi or mycoplasmas, and later stored frozen at -70 °C.

Example 5. Determination of anti-cancer activity of the modified virus (MV)

[0082] In experiments with cell lines, MV was found to be cytotoxic for melanoma cell lines FM9, FM55, FM94 and SK-Mel26, gastric carcinoma cells, human oral squamous cell carcinoma SCC25 cells, human epithelial cell line derived from a lung carcinoma (A549), acute monocytic leukemia THP-1 cells, rhabdomyosarcoma RD cells, human pancreatic adenocarcinoma HPAF-II cells, human breast adenocarcinoma cells (MCF-7) as well as on primary cell cultures of gastric adenocarcinoma GC1 and thyroid cancer line HA007.

[0083] Thus, for example, MV injections for 3 days caused reducing of sarcoma M-1 mass in 55% (in 11 of 22) of animals, compared with 6% (in 1 of 18) spontaneous regression in the control group.

[0084] Transplanting sarcoma KRS-321 on Day 5 after the injecting MV in a dose 15×10^6 TCID₅₀ on Wistar rats in 44% of animals (11/25) the regression of tumour was observed, while in the control group there were no cases of regression.

[0085] Testing the anti-cancer activity of the virus sample after the 12 months passaging on the same cancer cell lines and transplanted tumours no statistically significant difference from the original MV was observed.

[0086] Neither MV nor the virus passaged for 12 months caused any toxic reactions in intact mice.

Example 6. Anti-cancer activity of modified virus in treating patients

[0087] Treating of melanoma patients by the modified virus (MV) was conducted according to the following scheme: therapy was commenced 2-3 weeks after the excision of the tumour by intramuscular administration of 2 ml of solution with titer 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml for 3 days consecutively with supporting injections at monthly intervals according to the same 3 day schedule. After the fourth month, the virus preparation was administered once monthly for the next 8 months. In the next 2 years the supporting therapy was continued with the same dose, gradually increasing the interval between administrations to 6, 8 and 12 weeks.

[0088] In a clinical pilot study, a group of 46 melanoma stage I patients no progress of melanoma was observed for 50 months in 43 patients, treated with MV. In the control group, melanoma progressed for 10 of 31 patients undergoing standard therapy.

[0089] In a 50 months study of 44 stage II melanoma patients the progress of melanoma was stopped in 38 patients, compared to control group of 36 patients undergoing standard therapy, where melanoma did not progress in 15 patients, but did progress in 21 patients.

[0090] The efficiency of treatment is characterized by the following examples:

Case 1. Female, age 76, *Melanoma cutis dorsii*

Op. 11.09.2009. *Excisio tu cutis dorsii*

pT4b N0 M0

SN biopsy was not performed

Ex consilio: follow-up

Op. 07.04.2010. *LAE axillaris sin.*

Mts lln axillaris sin

Ex consilio: *Roferon*

Roferon 6 ml 3x per week from 24.06.2010 till 30.08.2010.

The treatment was discontinued due to the side effects.

From October 2010 the therapy with virus preparation in 2 ml dose with titer 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml was commenced. The treatment was well tolerated, and no progression of the disease was documented until 01.02.2012.

Case 2. Female, age 42, *Melanoma cutis dorsii*

Op. 25.05.2008. *Excisio tu cutis dorsii*

pT4a N0 M0, *Clark V*, *Breslow* 9 mm

SN biopsy was not performed

Virus preparation (2 ml with titer 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml was administered from 27.06.2008 till 27.06.2011.

21.01.2011. US examination: recurrence in the scar

Op. 02.02.2011. *Excisio*. Histological examination: granuloma.

Virus preparation (2 ml with titer 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml was continued till 27.06.2011.

During the observation period (till December 2011) no evidence of the disease progression was documented.

Case 3. Female, age 57, *Melanoma cutis dorsi*

Op. 19.08.2007. *Excisio tu cutis dorsi*

P T3b N0 M0

SN biopsy was not performed

Recommendations: follow-up

Op. 10.12.2009. *LAE colli dx*. Histological examination: *mts l/n colli dx* Progression of the disease - US examination on 22.02.2010: *mts l/n colli* 22.02.2010. *Ex consilio*: no surgery was recommended due to bulky disease Virus preparation (2 ml with titer 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml was administered from 22.02.2010 and still is in progress.

Last visit at clinic on 22.11.2011 - the disease has stabilized.

Case 4. Female, age 58, *Melanoma cutis dorsi*

Op. April 2004. *Excisio tu cutis dorsi*, *LAE axillaris sin.*

pT4b, N2c, M0 (*Breslow* 15 mm)

Reexcisio January 2006, September 2006 (local recurrence)

Therapy with IFN from October 2006 till May 2007.

Reexcisio cum dermoplasticum February 2007, May 2007, September 2007. Virus preparation (2 ml with titer 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml was administered from February 2008 till April 2011.

Visceral metastasis February 2011.

Exitus letalis October 2011.

Dose form and administration

[0091] The viral preparation for therapeutic treatment can be in the form of injectable aqueous solution containing the modified virus having the stable genome sequence as explained above, for example in the titer of 2×10^6 TCID₅₀/ml - 2×10^8 TCID₅₀/ml. The solution carrying the virus can be any physiologically acceptable sterile solution, especially sodium chloride solution. The preparation is stored and transported in frozen condition and defrozen at room temperature before the use. The preparation can be in vials or other container units in volumes that correspond a single dose injected at a time to the patient.

[0092] The preparation can be administered by injecting it intramuscularly (*i.m.*) to the patient after the excision of the tumour in question, when the wound has healed. The dosage can be 2 ml of the above-mentioned solution at a time. The intramuscular administration by injection is repeated according to the planned therapy schedule

Appendix 1: **Seq ID No 1**

Sequence of the modified virus

[0093]

| | | | | | | |
|----|------|------------|------------|------------|-------------|-------------|
| | 0 | UUAAAACAGC | CUGUGGGUUG | UUCCCACCCA | CAGGGCCCAC | UGGGCGCUAG |
| | 50 | CACACUGGUA | UCACGGUACC | CUUGUGCGCC | UGUUUUUAUCU | UCCCCUCCCC |
| | 100 | ACUGUAACUU | AGAAGAAUGA | CAUAAACGGU | CAACAGAUAG | CUCAGUACAC |
| 5 | 150 | CAACUGAGCC | CCGACCAAGC | ACUUCUGUUA | CCCCGGACCG | AGUAACAAUA |
| | 200 | GGCUGCUCGC | GCGGCUGAAG | GUGAAAACGU | UCGUUACCCG | GCCAAUUACU |
| | 250 | UCGAGAAACC | UAGUACCACC | AUGAAGGUUG | CGCAGCGUUU | CGCUCCGCAC |
| | 300 | AACCCCAGUG | UAGAUCAAGU | CGAUGAGUCA | CCGCACUCCC | CACGGGCGAC |
| 10 | 350 | CGUGGCGGUG | GCUGCGCUGG | CGGCCUGCCU | AUGGGGCAAC | CCAUGGGACG |
| | 400 | CUUCAAUACU | GACAUGGUGC | GAAGAGUCUA | UUGAGCUAAU | UGGUAGUCCU |
| | 450 | CCGGCCCCUG | AAUGCGGCUA | AUCCUAACUG | CGGGGCAAGU | GCCCACAAAC |
| | 500 | CAGUGGGUGG | CUUGUCGUAA | CGGGUAACCC | UGCAGCGGAA | CCGACUACUU |
| | 550 | UGGGUGUCCG | UGUUUCCUUU | UAUUCUUAUU | CUGGCUGCUU | AUGGUGACAA |
| 15 | 600 | UUGAGAGAUU | GUURCCAUAU | AGCUAUUGGA | UUGGCCRUCU | GGUGAGUAAC |
| | 650 | AGAGCAAUCA | UAUUCCUCUU | UGUUGGAUUU | AUACCACUUG | AUUCCACUAG |
| | 700 | UUACAACACU | CUGCUACAUA | UUAUUUGCUU | AAAUACAAGA | AGAUGGGAGC |
| | 750 | ACAAGUAUCG | ACACAAAAGA | CUGGUGCACA | CGAGACCSGU | UUGAGCGCUA |
| 20 | 800 | ACGGACACUC | UAUCAUUCAC | UAUACCAACA | UCAACUACUA | CAAAGAUGCA |
| | 850 | GCAUCCAACU | CAGCCAACAG | GCAGGAUUUC | ACCCAGGAUC | CAGGUAAGUU |
| | 900 | CACUGAACCG | GUCAAGGAUA | UCAUGAUCAA | AUCGAUGCCC | GCCCUAAACU |
| | 950 | CACCGUCCGC | GGAGGAGUGC | GGGUACAGCG | ACAGGGUGAG | AUCCCUAACG |
| 25 | 1000 | CUCGGCAACU | CAACCAUUAC | CACUCAAGAA | AGUGCAAACG | UAGUUGUUGG |
| | 1050 | CUAUGGCAGG | UGGCCAGAGU | ACUUGAAAGA | UGAAGAAGCU | ACUGCGGAAG |
| | 1100 | AUCAGCCAAC | ACAACCCGAU | GUAGCCACRU | GCAGGUUCUA | CACGUUGGAA |
| | 1150 | UCCGUCCAGU | GGGAGAAAAA | UAGCGCUGGA | UGGUGGUGGA | AGUUCCCCGA |
| | 1200 | AGCACUUAAG | GACAUGGGCC | UCUUUGGUCA | GAACAUGCUU | UACCACUAUC |
| 30 | 1250 | UCGGUAGAGC | AGGCUACACU | AUACAUGUGC | AGUGCAACGC | AUCCAAAUUU |
| | 1300 | CAUCAGGGCU | GUCUACUUGU | UGUCUGUGUA | CCUGAAGCUG | AGAUGGGGUG |
| | 1350 | UUCCCAGACG | GACAAAGAGG | UUGCUGCGAU | GAACCUCACG | AAGGGUGAAA |
| | 1400 | CGGCGCACAA | GUUUGAACCA | ACCAAAACCA | CAGGCGGCCA | CACAGUGCAA |
| 35 | 1450 | UCCAUAGUGU | GCAACGCGGG | UAUGGGCAUC | GGCGUGGGGA | ACCUCACCAU |
| | 1500 | CUACCCUCAC | CAGUGGAUCA | ACUUGCGCAC | UAAUAACUGC | GCUACAAUUG |
| | 1550 | UGAUGCCGUA | UAUAAAUUCA | GUACCCAUGG | AUAACAUGUU | UAGGCACUAC |
| | 1600 | AAUUUCACGC | UAAUGGUGAU | CCCAUUUGCA | CCCCUGGAUU | ACAAUGCCCA |
| | 1650 | AGCAUCUGAG | UACGUACCUG | UAACUGUCAC | AAUAGCCCCA | AUGUGUGCAG |
| 40 | 1700 | AAUACAAUGG | UUUAAGGCUG | GCUUACCAGC | AAGGGCUGCC | AGUGCUAAAU |
| | 1750 | ACACCGGGAA | GCAAUCAGUU | UAUGACAUCG | GAUGAUUUUUC | AAUCCCCUUC |
| | 1800 | GGCUAUGCCA | CAAUUUGAUG | UGACUCCGCA | CAUGGACAUC | CCAGGUGAAG |
| | 1850 | UGCACAACCU | CAUGGAGAUU | GCAGAAGUUG | AUUCGGUGGU | ACCUGUUAAC |
| 45 | 1900 | AACACUGCGG | CCAAUCUGCA | AAGCAUGGAC | GCAUAUCACA | UAGAGGUGAA |
| | 1950 | CRCAGGAAAU | CACCAAGGUG | AAAAGAUAAU | CGCUUUCCAG | AUACAACCCG |
| | 2000 | GGCUGGAUUC | AGUGUUUAAG | AGAACACUGC | UAGGUGAAGU | GCUCAAUUUAU |
| | 2050 | UACGCGCACU | GGUCAGGGAG | CAUUAAGCUA | ACAUUCACAU | UUUGUGGUUC |
| 50 | 2100 | AGCAAUGGCC | ACGGGCAAGC | UACUCUUAGC | AUACUCCCCA | CCUGGCGCCG |
| | 2150 | AUGUACCGGC | UAGCAGAAAG | CAGGCAUGA | UGGGAACCCA | UAUCAUCUGG |
| | 2200 | GACUUAGGGC | UGCAAUCCAG | UUGCGUUCUA | UGUAUUCCAU | GGAUCAGUCA |
| | 2250 | GACACAUUAU | CGCCUAGUGC | AACAGGAUGA | GUACACCAGC | GCCGGCAAUG |
| | 2300 | UCACCUUCUG | GUAUCAGACA | GGUAUAGUGG | UUCCACCCGG | CACACCCAAC |
| 55 | 2350 | AAGUGUGUCG | UCCUGUGCUU | UGUGUCAGCG | UGUAAUGACU | UCUCCGUGCG |
| | 2400 | CAUGCUGCGU | GACACACCAU | UCAUCGGCCA | AACAACACUG | CUACAAGGUG |

| | | | | | | |
|----|------|------------|-------------|------------|--------------|-------------|
| | 2450 | AUACGGACGU | GGCCGUCAAC | AAUGCAGUAG | CCAGGGUAGC | UGAUACAAUU |
| | 2500 | GCCAGUGGGC | CCAGCAACUC | CACUAGCAUU | CCUGCACUAA | CCGCAGUUGA |
| | 2550 | GACUGGGCAC | ACAUCACAGG | UAGAGCCUAG | UGAUACAAUG | CAAACACGGC |
| 5 | 2600 | AUGUAAAGAA | CUACCAUUCG | CGAUCUGAAU | CAACAAUAGA | GAACUUCCUU |
| | 2650 | AGCCGGUCGG | CCUGUGUAUA | UAUUGAAGAG | UACUUUACCA | AAGAUCAGA |
| | 2700 | CAGCGCCAAU | AGGUACAUGU | CAUGGACUAA | AAAUGCUAGA | AGGAUGGUGC |
| | 2750 | AAUUGAGGCG | AAAGUUUGAA | CUGUUCACAU | ACAUGC GG UU | UGAUUAUGGAG |
| | 2800 | AUCACAUUUG | UUAUCACUAG | UAGACAACUG | CCUGGGACUA | GCAUCGCGCA |
| 10 | 2850 | AGACAUGCCG | CCACUGACAC | ACCAAAUCAU | GUAUAUACCC | CCUGGUGGUC |
| | 2900 | CAGUACCAA | CAGUGUGACC | GAUUUUGCAU | GGCAAACUUC | GACUAAUCCA |
| | 2950 | AGUAUCUUUU | GGACUGAGGG | CAAUGCCCCC | CCGCGUAUGU | CCAUAACCAU |
| | 3000 | UAUAAGCAUA | GGGAUUGCAU | ACAGCAACUU | UUAUGACGGR | UGGUCGCACU |
| | 3050 | UCUCACAAA | UGGGGUUAUAC | GGCUACAAUG | CAUUAACAA | CAUGGGCAAA |
| 15 | 3100 | UUUAUCGCAC | GCCAUGUGAA | CAAAGACACA | CCGUACCAGA | UGUCCAGUAC |
| | 3150 | GAUUCGUGUG | UACUUUAAAC | CCAAACAUAU | CAGAGUGUGG | GUGCCAAGAC |
| | 3200 | CACCACGUUU | GUGCCCCUAA | AUUAAAUCUA | GUAACGUUAA | CUUUGACCCA |
| | 3250 | ACCAACCUAA | CUGAUUCAAG | AUCAAGUAUA | ACAUUGUGUC | CAGACACUAA |
| 20 | 3300 | CCGUCCGGAA | GUCCGUACAG | CUGGAAAAUU | CGGCCACCAG | UCCGGUGCUG |
| | 3350 | UUUACGUGGG | UAAUUACAGA | AUAGUGAACA | GGCACCUCGC | CACGCACAAC |
| | 3400 | GACUGGCAAA | ACUGUGUGUG | GGAAGACUAC | AACAGAGACC | UCCUUGUGAG |
| | 3450 | CACCACUACA | GCCCAUGGGU | GUGACACUAA | AGCCAGAUUG | CAGUGCACAG |
| | 3500 | CAGGCGUAUA | UUUUUGUGCC | UCAAGGAACA | AACAUUACCC | AGUCACCUUC |
| 25 | 3550 | GAGGGGCCAG | GCUUGGUGGA | AGUUCAGGAG | AGCGAGUACU | ACCCAAAAAG |
| | 3600 | AYAUCAGUCC | CACGUGCUUC | UAGCUGCAGG | AUUUUCUGAA | CCGGGCGAUU |
| | 3650 | GUGGCGGAU | CCUCAGAUUG | CAACACGGCG | UGAUCGGUAA | CGUCACCAUG |
| | 3700 | GGUGGAGAGG | GGGUCGUUGG | GUUUGCCGAC | GUCAGAGACC | UACUGUGGUU |
| | 3750 | AGAGGAUGAU | GCCAUGGAAC | AGGGCGUAAG | AGACUAUGUU | GAACAACUAG |
| 30 | 3800 | GAAAUUCUUU | CGGCUCAGGU | UUCACCAAUC | AAAUUUGUGA | ACAGGUCAAC |
| | 3850 | CUCCUCAAA | AGUCAUUGGU | UGGACAGGAU | UCUAUUCUGG | AAAAAUCCCU |
| | 3900 | UAAGGCUCUA | GUUAAGAUUA | UCUCAGCACU | GGUCRUUGUA | GUGAGAAAU |
| | 3950 | ACGAUGAUUC | CAUAACGGUU | ACCGCCACUC | UAGCUUUAAU | UGGUUGCACC |
| | 4000 | UCUUCUCCGU | GGCGGUGGCU | CAAGCAGAAG | GUGUCACAAU | AUUUAUGGAU |
| 35 | 4050 | ACCCAGGGCC | GAGCGACAAA | ACAAUAGCUG | GCUCAAGAAG | UUUACUGAGA |
| | 4100 | UGACCAACGC | CUGCAAGGGC | AUGGAGUGGA | UAGCCAUAAA | AAUUCAAAAG |
| | 4150 | UUUAUUGAGU | GGCUUAAAGU | CAAGAUUCUG | CCGGAAGUGA | AGGAAAAACA |
| | 4200 | CGAGUUCUC | AACAGGCUAA | AGCAAUUAAC | ACUCCUAGAG | AGCCAGAUUG |
| 40 | 4250 | CAACCAUAGA | GCAGAGUGCA | CCAUCGCAGA | GUGAUCAAGA | GCAACUCUUC |
| | 4300 | UCCAACGUCC | AGUACUUCGC | CCAUAUUAUG | AGAAAGUAUG | CGCCAUUGUA |
| | 4350 | CGCUGCCGAA | GCGAAGAGAG | UGUUCUCACU | UGAGAAGAAA | AUGAGCAACU |
| | 4400 | ACAUACAGUU | CAAGUCCAAA | UGCCGUUAUG | AGCCUGUAUG | CUUACUCCUA |
| | 4450 | CAUGGCAGCC | CAGGGGCCGG | AAAGUCCGUG | GCCACCAACU | UGAUUGGCAG |
| 45 | 4500 | AUCCUCGCA | GAAAAACUCA | ACAGCUCUGU | RUACUCCCUA | CCACCAGACC |
| | 4550 | CCGACCACUU | UGACGGCUAC | AAGCAGCAAG | CGGUCGUGAU | CAUGGAUGAC |
| | 4600 | UUUAUGCCAA | AUCCUGAUGG | AAAAGAUGUC | UCACUAUUUU | GUCAGAUUGU |
| | 4650 | UUCUAGCGUG | GACUUUGUAC | CACCGAUGGC | UGCGCUAGAG | GAAAAAGGAA |
| | 4700 | UCCUAUUUAC | CUCCCCGUUC | GUGUUGGCAU | CAACCAACGC | UGGGUCCAUC |
| 50 | 4750 | AAUGCACCCA | CUGUGUCUGA | CAGCAGAGCG | CUCGCUAGGA | GAUUCCACUU |
| | 4800 | UGACAUGAAC | AUUGAAGUCA | UUUCUAUGUA | CAGUCAAAAC | GGCAAGAUA |
| | 4850 | ACAUGCCCAU | GUCAGUUAUA | ACAUGUGAUG | AAGAGUGUUG | UCCAGUUAAC |
| | 4900 | UUCAAAAGGU | GCUGCCCGUU | GGUGUGUGGA | AAGGCYAUGC | AAUUCAUUGA |
| | 4950 | UAGGAGAACU | CAAGUUAGAU | AUUCGCUGGA | CAUGCUAGUU | ACUGAAAUGU |
| 55 | 5000 | UUAGGGAGUA | UAACCAUAGA | CACAGUGUGG | GAGCCACUCU | UGAAGCUCUG |
| | 5050 | UCCAAGGGC | CACCAGUCUA | CAGAGAGAUC | AAAAUCAGCG | UCGCCCCAGA |

| | | | | | | |
|----|------|-------------|------------|-------------|-------------|-------------|
| | 5100 | GACACCCCCA | CCACCAGCUA | UUGCUGAUUU | ACUGAAAUCA | GUGGACAGUG |
| | 5150 | AAGCUGUGAG | GGAUACUGC | AAGGAGAGAG | GGUGGCUUGU | GCCAGAGAUC |
| | 5200 | AAUUCUACCC | UACAAAUAGA | GAAGCAUGUG | AGUAGAGCAU | UCAUAUGUUU |
| 5 | 5250 | ACAAGCCCUA | ACCACGUUUG | UUUCAGUUGC | UGGUAAUAAU | UACAUUAUUU |
| | 5300 | ACAAAUUAUU | UGCAGGUUUC | CAAGGCGCCU | ACACAGGGAU | GCCCAACCAG |
| | 5350 | AAACCUAAGG | UGCCCACCCU | GAGACAGGCC | AAAGUACAGG | GCCCAGCGUU |
| | 5400 | UGAGUUCGCU | GUGGCGAUGA | UGAAAAGGAA | CGCCAGUACA | GUAAAAACCG |
| 10 | 5450 | AGUACGGUGA | AUUCACCAUG | CUUGGCAUUU | ACGACAAGUG | GGCGGUGUUA |
| | 5500 | CCGCGCCACG | CCAAGCCUGG | CCCCACCAUC | UUGAUGAAUG | AUCAGGAAGU |
| | 5550 | CGGCGUGUUG | GAUGCCAAGG | AACUAGUUGA | UAAAGAUGGG | ACAAAUCUAG |
| | 5600 | AAUUGACUCU | CCUGAAGCUC | AACCGUAACG | AAAAGUUCAG | AGAUUUUAGG |
| 15 | 5650 | GGGUUUUCUAG | CAAGAGAAGA | GGUUGAAGUG | AAUGAAGCUG | UCCUAGCAAU |
| | 5700 | AAAUACAAGC | AAAUUCCCUA | ACAUGUACAU | ACCAGUGGGC | CAGGUGACUG |
| | 5750 | ACUACGGGUU | UCUGAACCUG | GGAGGGACUC | CCACGAAGAG | AAUGCUC AUG |
| | 5800 | UAUAACUUCC | CAACUAGAGC | AGGUCAGUGU | GGAGGUGUCC | UCAUGUCAAC |
| | 5850 | AGGGAAAGUC | CUGGGAAUAC | AUGUAGGAGG | GAAUGGACAU | CAAGGGUUCU |
| 20 | 5900 | CAGCGGCACU | CCUCAGGCAC | UACUUCAACG | AGGAGCAGGG | UGAAAUAGAA |
| | 5950 | UUCAUUGAGA | GCUCAAAGGA | CGCGGGAUUC | CCUGUGAUCA | ACACUCCAG |
| | 6000 | UAAGACAAAA | UUGGAACCAA | GUGUGUUUCA | CCAGGUGUUC | GAGGGCAACA |
| | 6050 | AGGAACCAGC | GGUCCUUAGA | AAUGGGGACC | CACGACUCAA | AGCCAACUUC |
| 25 | 6100 | GAGGAAGCAA | UCUUCUCCAA | GUACAUUGGC | AAUGUCAACA | CGCAUGUAGA |
| | 6150 | UGAGUACAUG | UUGGAGGCUG | UGGACCAUUA | UGCAGGACAA | CUAGCUACUC |
| | 6200 | UGGACAUCAG | UACGGAGCCC | AUGAAGCUAG | AGGACGCCGU | GUAUGGUACA |
| | 6250 | GAGGGGCUGG | AAGCACUAGA | CCUAACCACC | AGUGCAGGCU | ACCCUUACGU |
| 30 | 6300 | GGCCUUGGGC | AUCAAGAAAA | GAGAUUUUCU | AUCUAAGAAG | ACUAAAGACC |
| | 6350 | UCACUAAGUU | GAAGGAAUGC | AUGGACAAAU | AUGGCCUAAA | UUUGCCAAUG |
| | 6400 | GUAACCUACG | UCAAGAUGA | GUUGAGAUCU | GCUGAGAAGG | UGGCCAAGGG |
| | 6450 | AAA AUCCAGG | CUUAUUGAGG | CUUCUAGUCU | CAAUGACUCA | GUAGCAAUGA |
| | 6500 | GGCAAACAUU | UGGAAAUUUA | UAUAAGACCU | UUCACCUCAA | CCCGGGCAUC |
| 35 | 6550 | GUUACGGGCA | GUGCUGUUGG | GUGUGAUCCA | GAUGUGUUUU | GGAGCAAAGAU |
| | 6600 | CCCUGUUAUG | CUUGAUGGAC | AUCUCAUAGC | UUUUGACU AU | UCAGGCUAUG |
| | 6650 | ACGCUAGCCU | CAGCCCAGUG | UGGUUUGCAU | GUUUGAAACU | UCUCCUAGAG |
| | 6700 | AAACUAGGGU | AUACAAACAA | GGAAACAAAC | UACAUAGAUU | ACCUCUGUAA |
| 40 | 6750 | UUCCCAUCAC | CUGUAUAGAG | ACAAGCACUA | CUUUGUAAGA | GGCGGU AUGC |
| | 6800 | CAUCAGGGUG | UUCAGGCACC | AGCAUAUUUA | AUUCCAUGAU | UAACAACAUC |
| | 6850 | AUAAUCAGGA | CUCUCAUGCU | GAAGGUUU AU | AAAGGCAUUG | AUUUGGACCA |
| | 6900 | AUUCAGAAUG | AUUGCCUAUG | GGGAUGAUGU | GAUUGCUUCC | UAUCCGUGGC |
| 45 | 6950 | CUAUCGAUGC | UUCGCUGUUA | GCUGAAGCAG | GAAAAGAUUA | UGGUUUAAUC |
| | 7000 | AUGACCCCAG | CAGACAAAGG | CGAGUGCUUC | AACGAGGUAA | CCUGGACGAA |
| | 7050 | UGUGACCUUU | CUGAAAAGGU | ACUUUAGGGC | AGAUGAGCAA | UACCCA UUUC |
| | 7100 | UGGUCCAUCC | UGUUAUGCCA | AUGAAGGACA | UCCAUGAGUC | UAUUAGGUGG |
| | 7150 | ACCAAAGAUC | CCAAGAACAC | ACAGGAUCAU | GUGCGCUCGC | UGUGCCUAUU |
| 50 | 7200 | GGCUUGGCAC | AACGGGGAGC | AAGAAUAUGA | GGAGUUUAUU | CGCAAGAUCA |
| | 7250 | GAAGCGUGCC | CGUUGGGCGC | UGC UUGACCC | UACCCGCUUU | UUCAACACUG |
| | 7300 | CGCAGGAAGU | GGCUGGACUC | CUUUUAAAAU | UAGAGCAUAA | UUAGUAAAUC |
| | 7350 | AUAAUUGGCU | UAACCCUACC | GCAUGAACCG | AACUUGAUAA | AAGUGCGGUA |
| 55 | 7400 | GGGGUAAAUU | CUCCGCAUUC | GGUGCGG | | |

Appendix 2: Seq ID No 2

Sequence of the unmodified (native) virus

5 [0094]

0 TTAAACAGC CTGTGGGTTG TTCCACCCA CAGGGCCCAC TGGGCGCTAG
 50 CACACTGGTA TCACGGTACC TTTGTGCGCC TGTTTTATAT CCCCCTCCCC
 100 ACTGTAACCT AGAGAAATCA CATAAACGAT CAATAGAAGG CGCAGCACAC
 150 CAGCTGAGTC TTGACCAAGC ACTTCTGTTT CCCC GGACTG AGTATCAATA
 200 GACTGCTCAC GCGGTTGAAG GAGAAAACGT TCGTTACCCG GCCAACTACT
 250 TCGAGAAACC TAGTACCACC ATGAAAAGTTG CGCAGTGTTT CGCTCAGCAC
 300 AACCCAGTG TAGATCAGGT CGATGAGTCA CCGCATTCCC CACGGGCGAC
 350 CGTGGCGGTG GCTGCGTTGG CGGCCTGCCT ATGGGGCAAC CCATGGGACG
 400 CTTCAATACT GACATGGTGC GAAGAGTCTA TTGAGCTAGT TGGTAGTCCT
 450 CCGGCCCTTG AATGCGGCTA ATCCTAACTG CGGAGCAAGT GCCACAAAC
 500 CAGTGGGTAG CTTGTCTGTA CCGGCAACTC TGCAGCGGAA CCGACTACTT
 550 TGGGTGTCG TGTTCCTTT TATTCTTATT CTGGCTGCTT ATGGTGACAA
 600 TTGAGAGATT GTTACCATAT AGCTATTGGA TTGGCCATCC GGTGACTAAC
 650 AGAGCAATTA TATACCTCTT TGTGGATTT ATACCACTTA ATTCCACTAA
 700 TTACAACACT CTGCTACACA TTATTTACTT AAAACCAAGA AGATGGGAGC
 750 ACAAGTATCA ACACAAAAAA CTGGTGACACA TGAGACCSGT TTGAGCGCTA
 800 ACGGAAGCTC CATCATTCAC TACACCAACA TCAATTACTA CAAAGATGCA
 850 GCATCCAAC TACCCAACAG GCAAGACTTC ACCCAAGATC CAGGCAAAAT
 900 CACCGAACCG GTCAAGGATA TCATGATCAA GTCATGCCC GCCCTAAACT
 950 CACCGACCGT GGAGGAGTGT GGGTACAGTG ATAGGGTGAG ATCCATAACG
 1000 CTCGGCAACT CAACCATTAC CACTCAGGAG AGTGCAAAATG TAGTTGTTGG
 1050 CTATGGCGGG TGGCCAGAGT ACTTGAAAGA TGAAGAAGCT ACTGCGGAAG
 1100 ATCAACCAAC ACAACCCGAT GTAGCCACAT GCAGGTTTTA CACGCTGGAA
 1150 TCCGTCCAGT GGGAGAAAAA TTCCGCTGGA TGGTGGTGGA AGTTCCCCGA
 1200 AGCACTTAAG GACATGGGCC TCTTTGGTCA AAACATGCAT TACCCTACC
 1250 TCGGTAGAGC AGGCTACACT ATACACGTGC AGTGCAATGC ATCCAAATTC
 1300 CACCAAGGCT GTCTACTTGT TGTCTGTGTA CCTGAGGCTG AGATGGGGTG
 1350 TTCAAAGTG GACGGTACTG TAAATGAGCA GGAATGACG GAGGGTGAAA
 1400 CGGATATGAA GCTTGAACCC ACCAGAACCA CAGGCGTACG CCGAGTGCAA
 1450 TCCGCAGTGT ACAACGCGGG TATGGGCGTC GGCGTGGGGA ACCTCACCAT
 1500 CTTCCCTCAC CAGTGGATCA ACCTGCGCAC TAACAATGTG GCTACAATTG
 1550 TGATGCCATA CATAAATAGT GTACCCATGG ATAACATGTT TAGGCCTAC
 1600 AACTTCACGC TAATGATGAT CCCATTTGCA CCCCTGGATT ACACCAACCA
 1650 AGCATCTACG TACGTACCTA TAACTGTCAC AATAGCACCA ATGTGTGCTG
 1700 AATACAATGG TTTGAGGCTC GTTACCTCGC AAGGGTTGCC AGTGATGAAC
 1750 ACACCGGGAA GCAATCAGTT CCTGACATCG GATGACTTTC AATCACCTTC
 1800 GGCTATGCCA CAATTTGATG TGAATCCAGA CATGGACATC CCAGGTGAAG
 1850 TGAACAACCT CATGGAGATT GCAGAGGTTG ACTCGGTGGT ACCTGTTAAC
 1900 AACAATGAGG CCAATCTGAA AAGCATGGAC GCATACCGCA TACCGGTGAA
 1950 CRCAGGAAAT CAACAAGGTG AAAAGATATT TGGTTTCCAA ATACAACCCG
 2000 GGCTTGATTC AGTGTTTAAG AGAACACTGC TAGGTGAGAT GCTCAATTAT
 2050 TACACGCACT GGTCAGGGAG CATTAAAGCTA ACATTTATGT TTTGTGGTTC
 2100 AGCAATGGCC ACGGGCAAAT TACTCTTAGC ATACTCACCA CCTGGCGCCG
 2150 ATGTACCGAC TAGCAGAAAG GAGGCAATGC TGGGAACCCA TGTCATCTGG
 2200 GACTTTGGGC TGCAATCCAG TTGTGTTCTG TGTGTTCCAT GGATCAGCCA
 2250 GACACACTAC AGGTTGGTGC AGCAGGATGA GTACACCGGC GCCGGCTATA
 2300 TCACCTGCTG GTACCAACA AGTATAGTGG TTCCACCCGG CACACCCAAA
 2350 AAGTGTGTCA TCCTGTGCTT TGTGTCAGCG TGTAATGATT TCTCCGTGAG
 2400 CATGCTGAGT GACACACCAT TCATCGGCCA AACAGCACTG CTGCAGAGCC
 2450 CTGTGGAAGA AGCTGAAGAG AACGCAGTTG CACGTGTGGC TGACACAATT
 2500 GCCAGTGGGC CCAGCAACTC CGAGAGCGTT CCTGCACTAA CAGCAGTTGA
 2550 GACTGGGCAC ACATCACAGG TAGTGCCCTAG TGACACAATG CAAACAAGGC
 2600 ATGTGAAGAA CTACCATTCG AGATCTGAGT CAACAATAGA GAACTTCCTT
 2650 AGCAGGTCCG CCTGTGTGTA TATTGAAGAG TACTATACCA AACTGAAAC
 2700 CAGACAAAAT TTATACATGT TGCCCACTAT AAATACTAGA TGGATGGTGC
 2750 AATTGAGGAG AAAGTTTGAG ATGTTTACAT ACATGAGGTT TGACATGGAA
 2800 ATCACATTTG TTATCACTAG TAGACAACCTG CATCGAACTA GCATGCCGCA
 2850 GGACATGCCG GTACTGACAC ACCAAATCAT GTATGTACCA CCTGGTGGTC

| | | | | | | |
|----|------|-------------|--------------|-------------|--------------|-------------|
| | 2900 | CAGTACCAA | CAGTGTGGAC | GATTACGCAT | GGCAAAC TTC | GA CTAACCCA |
| | 2950 | AGTGTCTTTT | GGACTGAGGG | CAATGCCCCA | CCGCGTATGT | CCATACCA TT |
| | 3000 | CATAAGCATA | GGGAATGCAT | ACAGCAACTT | TTATGATGGG | TCCTCGCA CT |
| | 3050 | TCTTACAATA | TGGGGTATAT | GGCTACAACA | CATTAAACAA | CATGGGGAAA |
| 5 | 3100 | TTATACGTAC | GCCATGTGAA | CAACCACACA | CCATACCAA | TGACCAGTAC |
| | 3150 | GGTTAGTGTG | TACTTTAAAC | CCAAACATGT | CAGAGCGTGG | GTGCCGAGAC |
| | 3200 | CACCACGTCT | GTGCCCTTAC | AAAATATGCAT | GGAACGTTAA | CTTTGAACCA |
| | 3250 | ACAAACGTAA | CTGATTCAAG | ATCAAGTATC | ACATATATTC | CTGAGACGGT |
| | 3300 | CAAACCAGAC | CTATCAAAAAG | CTGGAGCTTT | CGGCCACCAG | TCCGGTGTCTG |
| | 3350 | TTTATGTGGG | TAAC TACAGA | GTGGTGAATA | GGCACCTCGC | CACGCACAAC |
| 10 | 3400 | GA CTGGCAAA | ACTGTGTGTG | GGAAGACTAC | AACAGAGACC | TCCTTGTGAG |
| | 3450 | CACCACCACA | GCCCATGGGT | GTGACACCAT | AGCCAGATGC | CAGTGCACAA |
| | 3500 | CAGGCGTGTA | CTTTTGTGCC | TCAAGGAACA | AACACTACCC | AGTCACCTTT |
| | 3550 | GAGGGGCCAG | GCCTGGTGGA | AGTTCAGGAG | AGTGAGTACT | ACCCAAAAAG |
| | 3600 | ATACCAATCC | CATGTGCTTC | TAGCTGCAGG | ATTTTCTGAA | CCAGGCATT |
| | 3650 | GTGGTGGAA | TCTCAGGTGT | GAACATGGTG | TCATCGGTAT | CGTCACCATG |
| 15 | 3700 | GGTGGAGAGG | GGGTGCTTGG | GT TGGCCGAC | GTCCGAGACC | TACTGTGGTT |
| | 3750 | AGAGGATGAT | GCCATGGAAC | AGGGCGTAAG | AGACTATGTT | GAACAACTAG |
| | 3800 | GAAATGCTTT | TGGCTCAGGT | TTCACCAACC | AAATTTGTGA | ACAAGTCAAC |
| | 3850 | TCCTCTCAAAG | AGTCACTGGT | TGGACAGGAC | TCCATTCTTG | AGAATCCCT |
| | 3900 | TAAAGCCCTA | GT TAAAGATTA | TCTCAGCACT | GGTCATTGTA | GTGAGAAATC |
| | 3950 | ACGATGACCT | CATCAGAGTG | ACTGCCACTC | TAGCCCTCAT | TGGTTGCACC |
| 20 | 4000 | TCTTCTCCAT | GGCGGTGGCT | CAAACAGAAA | GTGTCACAAT | ATTATGGAA |
| | 4050 | ACCCATGGCT | GAGCGACAAA | ACAATGGCTG | GCTCAAGAAG | TTCATGAGA |
| | 4100 | TGACCAATGC | CTGCAAGGGC | ATGGAGTGGA | TAGCCATCAA | AATTCAAAAA |
| | 4150 | TTTATTGAGT | GGCTTAAAGT | CAAGATCTAC | CAGAAGTGTA | GGAAAAACAT |
| | 4200 | GAGTTCTCTA | ACAGACTATA | ACA ACTACCA | CTCTTGGAAG | AGTCAGATTG |
| | 4250 | CCACCATAGA | ACAAAGTGCA | CCATCGCAGA | GTGACCAGGA | GCAACTGTTT |
| | 4300 | TCCAATGTCC | AGTACTTCGC | CCACTATTGC | AGAAAAGTATG | CGCCACTGTA |
| 25 | 4350 | TGCAGCTGAG | GCAAAGAGAG | TGTTCTCCCT | TGAGAAGAAA | ATGAGCAATT |
| | 4400 | ACATACAGTT | CAAGTCCAAA | TGCCGTATTG | AGCCTGTATG | TTTGCTCNTA |
| | 4450 | CATGGCAGCC | CAGGGGCCGG | AAAATCCGTG | GCCACCAACC | TGATTGGCAG |
| | 4500 | ATCACTCGCT | GAAAACTCA | ACAGCTCAGT | GTACTCCCTA | CCACCAGACC |
| | 4550 | CAGATCACTT | TGATGGCTAC | AAACAGCAAG | CGGTCGTGAT | CATGGATGAT |
| 30 | 4600 | CTATGCCAAA | ATCCTGATGG | AAAAGATGTG | TCATTGTTCT | GTCAAATGGT |
| | 4650 | TTCCAGTGTG | GACTTTGTAC | CACCGATGGC | TGCGCTAGAG | GAGAAAGGCA |
| | 4700 | TTCTGTTTAC | CTCCCGTTT | GTCTGGCAT | CAACCAATGC | TGGGTCCATC |
| | 4750 | AATGCACCAA | CTGTGTCAGA | CAGCAGAGCC | CTCGCTAGGA | GATTCCACTT |
| | 4800 | TGACATGAAC | ATTGAAGTCA | TTTCCATGTA | CAGTCAAAAT | GGCAAGATCA |
| | 4850 | ACATGCCCAT | GTCAAGTTAAG | ACGTGTGATG | AAGAGTGTG | TCCAGTCAAC |
| | 4900 | TTCAAGAGGT | GCTGCCGCT | GGTGTGTGGA | AAGGCCATGC | AGTTCATTGA |
| 35 | 4950 | CAGAAGAACT | CAAGTTAGAT | ACTCGCTGGA | CATGCTAGTT | ACTGAGATGT |
| | 5000 | TTAGGGAGTA | CAACCACAGA | CACAGTGTGG | GAGCCACCCT | TGAGGCTCTG |
| | 5050 | TTCCAAGGGC | CACCAGTCTA | CAGAGAGATC | AAAATTAGTG | TCGCACCAGA |
| | 5100 | GACACCACCA | CCACCAGCTA | TTGCTGACTT | ACTGAAATCA | GTGGACAGTG |
| | 5150 | AAGCTGTGAG | AGAGTACTGC | AAAGAAAAGG | GATGGCTTGT | GCCAGAGATC |
| | 5200 | AACTCCACCC | TACAAATTGA | GAAGCATGTG | AGCCGGGCAT | TCATCTGTCT |
| 40 | 5250 | GCAAGCACTA | ACCACGTTTG | TTTCAGTTGC | TGGAATAATA | TACATTATTT |
| | 5300 | ACAAGCTATT | TGCAGGTTTC | CAAGGCGCAT | ACACAGGGAT | GCCCAACCAG |
| | 5350 | AAACCCAAGG | TGCCCAACCCT | GAGACAAGCC | AAAGTGCAAG | GCCCAGCGTT |
| | 5400 | TGAGTTTGCT | GTGGCGATGA | TGAAGAGGAA | CTCCAGTACA | GTGAAAACCG |
| | 5450 | AGTACGGTGA | GTTCAACCATG | CTTGGCATTT | ATGACAGGTG | GGCGGTGTTA |
| | 5500 | CCACGCCACG | CCAAACCTGG | CCCAACCATC | TTGATGAATG | ACCAGGAAGT |
| 45 | 5550 | CGGCGTGTG | GATGCCAAGG | AACTAGTGGA | TAAGGATGGG | ACAAACCTAG |
| | 5600 | AACTGACACT | CCTGAAGCTC | AACAGTAA TG | AGAAGTTT CAG | AGACATCAGA |
| | 5650 | GGGTTCTTAG | CCAAAGAAGA | GGTTGAGGTG | AATGAAGCTG | TCCTAGCAAT |
| | 5700 | AAACACAAGC | AAGTTCCCCA | ACATGTACAT | ACCAGTGGGC | CAGGTGACTG |
| | 5750 | ACTACGGGTT | CCTGAACCTG | GGTGGGACGC | CCACTAAGAG | AATGCTCATG |
| | 5800 | TACAACCTCC | CCACTAGAGC | AGGTCAAGTG | GGTGGTGTCC | TCATGTCCAC |
| 50 | 5850 | TGGGAAAGTC | CTGGGGATAC | ATGTTGGTGG | GAATGGTCAT | CAAGGGTTCT |
| | 5900 | CAGCAGCACT | CCTCAAGCAC | TACTTCAACG | ATGAACAAGG | TGAAATGAG |
| | 5950 | TTCAATTGAGA | GCTCAAAGGA | CGCGGGGTTT | CCTATCATCA | ACACACCCAG |
| | 6000 | CAAGACCAAA | CTGGAACCAA | GTGTCTTCCA | CCAGTGTGTTG | AAGGCAACAA |
| | 6050 | AGAACCCAGC | AGTCTTCAGA | AATGGTGATC | CACGACTCAA | AGCCAACTTT |
| | 6100 | GAGGAGGCCA | TCTTCTCCAA | ATACATTGGC | AATGTCAACA | CGCATGTGGA |
| 55 | 6150 | TAGGTACATG | TTGGAAGCTG | TGGACCATTA | TGCAGGACAA | CTGGCTACTC |
| | 6200 | TGGACATCAG | CACGGAACCA | ATGAAGCTGG | AGGATGCCGT | GTATGGTACA |
| | 6250 | GAGGGGCTGG | AAGCACTAGA | CCTAACAACC | AGTGCAGGCT | ACCCTTATGT |

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6300 TGCCCTGGGC ATCAAGAAGA GAGACATCCT ATCTAAGAAG ACCAGGGACC
 6350 TCACTAAGTT GAAAGAATGC ATGGACAAGT ATGGCCTAAA CCTGCCAATG
 6400 GTAACCTATG TGAAAGATGA GCTCAGATCT GCAGAGAAGG TGGCCAAAGG
 5 6450 AAAATCCAGG CTTATTGAAG CTTCAGTTT GAATGACTCA GTGGCAATGA
 6500 GACAGACATT TGGAAACCTG TACAAAACCT TCCACCTCAA CCCAGGCATT
 6550 GTGACGGGCA GTGCAGTTGG GTGTGACCCA GATCTGTTTT GGAGCAAGAT
 6600 ACCAGTCATG TTGGATGGAC ATCTCATAGC TTTTGATTAC TCAGGCTATG
 6650 ATGCTAGCCT CAGCCCAGTG TGGTTTGCAT GTCTGAAACT GCTCCTAGAG
 6700 AAGCTTGGGT ACACACACAA GGAAACAAAC TACATAGATT ACCTCTGCAA
 10 6750 CTCCCACCAC CTGTACAGAG ACAAACACTA CTTTGTGCGA GGTGGTATGC
 6800 CATCAGGGTG TTCTGGCACC AGCATCTTTA ACTCAATGAT TAACAACATC
 6850 ATAATCAGGA CACTCATGCT GAAAGTGTAC AAGGGCATTG ACTTGGACCA
 6900 ATTCAAGATT ATTGCCTATG GTGATGATGT GATTGCTTCC TACCCGTGGC
 6950 CCATTGATGC TTCCCTGCTA GCTGAAGCAG GAAAAGATTA TGGTTTGATC
 15 7000 ATGACACCAG CAGATAAAGG AGAGTGCTTC AATGAAGTCA ACTGGACGAA
 7050 TGTCACCTTC CTGAAAAGGT ACTTTAGAGC AGATGAGCAA TACCCATTCC
 7100 TGGTCCACCC TGTTATGCCC ATGAAAGACA TCCATGAATC TATTAGATGG
 7150 ACCAAAGATC CAAAGAACAC CCAAGATCAT GTGCGCTCGC TGTGCCTATT
 7200 GGCTTGGCAC AATGGGGAGC ACGAATATGA GGAGTTCATT CGCAAAATCA
 7250 GAAAGCGTGC CAGTTGGACG CTGTTTGACC CTACCTGCGT TTTCAACCCCT
 20 7300 GCGCAGGAAG TGGTTGGACT CCTTTTAAAA TAAAGCACAa TTTAGTAAAT
 7350 TTGAATTGGC TTAACCCTAC CGCACTAACC GAACTAGATA ACGGTGCGGT
 7400 AGGGGTAAAT TCTCCGCAT TCGGTGCGGTC GAGG

Appendix 3: Seq ID No 3

25 **Sequence of the modified virus after propagation for 12 months**

[0095]

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|----|------|-------------|------------|------------|-------------|-------------|
| | 0 | UUAAAACAGC | CUGUGGGUUG | UUCCCACCCA | CAGGGCCCAC | UGGGCGCUAG |
| | 50 | CACACUGGUA | UCACGGUACC | CUUGUGCGCC | UGUUUUUAUCU | UCCCCUCCCC |
| 5 | 100 | ACUGUAACUU | AGAAGAAUGA | CAUAAACGGU | CAACAGAUAG | CUCAGUACAC |
| | 150 | CAACUGAGCC | CCGACCAAGC | ACUUCUGUUA | CCCCGGACCG | AGUAACAAUA |
| | 200 | GGCUGCUCGC | GCGGCUGAAG | GUGAAAACGU | UCGUUACCCG | GCCAAUUACU |
| | 250 | UCGAGAAACC | UAGUACCACC | AUGAAGGUUG | CGCAGCGUUU | CGCUCCGCAC |
| | 300 | AACCCCAGUG | UAGAUCAGGU | CGAUGAGUCA | CCGCACUCCC | CACGGGCGAC |
| 10 | 350 | CGUGGCGGUG | GCUGCGCUGG | CGGCCUGCCU | AUGGGGCAAC | CCAUGGGACG |
| | 400 | CUUCAAUACU | GACAUGGUGC | GAAGAGUCUA | UUGAGCUAAU | UGGUAGUCCU |
| | 450 | CCGGCCCCUG | AAUGCGGCUA | AUCCUAACUG | CGGGGCAAGU | GCCCACAAAC |
| | 500 | CAGUGGGUGG | CUUGUCGUAA | CGGGUAACCC | UGCAGCGGAA | CCGACUACUU |
| 15 | 550 | UGGGUGUCCG | UGUUUCCUUU | UAUUCUUAUU | CUGGCUGCUU | AUGGUGACAA |
| | 600 | UUGAGAGAUU | GUUGCCAUAU | AGCUAUUGGA | UUGGCCAUCU | GGUGAGUAAC |
| | 650 | AGAGCAAUCA | UAUUCCUCUU | UGUUGGAUUU | AUACCACUUG | AUUCCACUAG |
| | 700 | UUACAACACU | CUGCUACAUA | UUAAUUGCUU | AAAUACAAGA | AGAUGGGAGC |
| | 750 | ACAAGUAUCG | ACACAAAAGA | CUGGUGCACA | CGAGACCGGU | UUGAGCGCUA |
| 20 | 800 | ACGGACACUC | UAUCAUUCAC | UAUACCAACA | UCAACUACUA | CAAAGAUGCA |
| | 850 | GCAUCCAACU | CAGCCAACAG | GCAGGAUUUC | ACCCAGGAUC | CAGGUAAGUU |
| | 900 | CACUGAACCG | GUCAAGGAUA | UCAUGAUCAA | AUCGAUGCCC | GCCCUAAACU |
| | 950 | CACCGUCCGC | GGAGGAGUGC | GGGUACAGCG | ACAGGGUGAG | AUCCCUAACG |
| 25 | 1000 | YUCGGCAACU | CAACCAUAC | CACUCAAGAA | AGUGCAAACG | UAGUUGUUGG |
| | 1050 | CUAUGGCAGG | UGGCCAGAGU | ACUUGAAAGA | UGAAGAAGCU | ACUGCGGAAG |
| | 1100 | AUCAGCCAAC | ACAACCCGAU | GUAGCCACAU | GCAGGUUCUA | CACGUUGGAA |
| | 1150 | UCCGUCCAGU | GGGAGAAAAA | UAGCGCUGGA | UGGUGGUGGA | AGUUCCCCGA |
| 30 | 1200 | AGCACUUAAG | GACAUGGGCC | UCUUUGGUCA | GAACAUGCUU | UACCACUAUC |
| | 1250 | UCGGUAGAGC | AGGCUACACU | AUACAUGUGC | AGUGCAAACG | AUCCAAAUUU |
| | 1300 | CAUCAGGGCU | GUCUACUUGU | UGUCUGUGUA | CCUGAAGCUG | AGAUGGGGUG |
| | 1350 | UUCCAGACG | GACAAAGAGG | UUGCUGCGAU | GAACCUCACG | AAGGGUGAAA |
| | 1400 | CGGCGCACAA | GUUUGAACCA | ACCAAACCA | CAGGCGGCCA | CACAGUGCAA |
| 35 | 1450 | UCCAUAUGUGU | GCAACGCGGG | UAUGGGCAUC | GGCGUGGGGA | ACCUCACCAU |
| | 1500 | CUACCCUCAC | CAGUGGAUCA | ACUUGCGCAC | UAAUAACUGC | GCUACAAUUG |
| | 1550 | UGAUGCCGUA | UAUAAAUUA | GUACCCAUGG | AUAACAUGUU | UAGGCACUAC |
| | 1600 | AAUUUCACGC | UAAUGGUGAU | CCCAUUUGCA | CCCCUGGAUU | ACAAUGCCCA |
| 40 | 1650 | AGCAUCUGAG | UACGUACCUG | UAACUGUCAC | AAUAGCCCCA | AUGUGUGCAG |
| | 1700 | AAUACAAUGG | UUURAGGCUG | GCUUACCAGC | AAGGGCUGCC | AGUGCUAAAU |
| | 1750 | ACACCGGGAA | GCAAUCAGUU | UAUGACAUCG | GAUGAUUUUC | AAUCCCCUUC |
| | 1800 | GGCUAUGCCA | CAAUUUGAUG | UGACUCCGCA | CAUGGACAUC | CCAGGUGAAG |
| 45 | 1850 | UGCACAACCU | CAUGGAGAUU | GCAGAAGUUG | AUUCGGUGGU | ACCUGUUAAC |
| | 1900 | AACACUGCGG | CCAAUCUGCA | AAGCAUGGAC | GCAUAUCACA | UAGAGGUGAA |
| | 1950 | CGCAGGAAAU | CACCAAGGUG | AAAAGAUAAU | CGCUUCCAG | AUACAACCCG |
| | 2000 | GGCUGGAUUC | AGUGUUUAAG | AGAACACUGC | UAGGUGAAGU | GCUCAAUUUAU |
| 50 | 2050 | UACGCGCACU | GGUCAGGGAG | CAUUAAGCUA | ACAUUCACAU | UUUGUGGUUC |
| | 2100 | AGCAAUGGCC | ACGGGCAAGC | UACUCUUAGC | AUACUCCCCA | CCUGGCGCCG |
| | 2150 | AUGUACCGGC | UAGCAGAAAG | CAGGCAAUGM | UGGGAACCCA | UAUCAUCUGG |
| | 2200 | GACUUAGGGC | UGCAAUCCAG | UUGCGUUCUA | UGUAUUCCA | GGAUCAGUCA |
| 55 | 2250 | GACACAUUAU | CGCCUAGUGC | AACAGGAUGA | GUACACCAGC | GCCGGCAAUG |

| | | | | | | | |
|----|------|------------|-------------|-------------|-------------|-------------|------------|
| | 2300 | UCACCU | GUGCUG | GUAUCAGACA | GGUAUAGUGG | UUCCACCCGG | CACACCCAAC |
| | 2350 | AAGUGUGUCG | UCCUGUGCUU | UGUGUCAGCG | UGUAAUGACU | UCUCCGUGCG | |
| | 2400 | CAUGCUGCGU | GACACACCAU | UCAUCGGCCA | AACAACACUG | CUACAAGGUG | |
| 5 | 2450 | AUACGGACGU | GGCCGUCAAC | AAUGCAGUAG | CCAGGGUAGC | UGAUACAAUU | |
| | 2500 | GCCAGUGGGC | CCAGCAACUC | CACUAGCAUU | CCUGCACUAA | CCGCAGUUGA | |
| | 2550 | GACUGGGCAC | ACAUCACAGG | UAGAGCCUAG | UGAUACAAUG | CAAACACGGC | |
| | 2600 | AUGUAAAGAA | CUACCAUUCG | CGAUCUGAAU | CAACAAUAGA | GAACUUC'CUU | |
| | 2650 | AGCCGGUCGG | CCUGUGUAUA | UWUUGAAGAS | UACUUUACCA | AAGAUCAAGA | |
| 10 | 2700 | CAGCGCCAAU | AGGUACAUGU | CAUGGACUAA | AAAUGCUAGA | AGGAUGGUGC | |
| | 2750 | AAUUGAGGCG | AAAGUUUGAA | CUGUUCACAU | ACAUGCGGUU | UGAUUUGGAG | |
| | 2800 | AUCACAUUUG | UUAUCACUAG | UAGACAACUG | CCUGGGACUA | GCAUCGCGCA | |
| | 2850 | AGACAUGCCG | CCACUGACAC | ACCAAUUCAU | GUAUUAUACCC | CCUGGUGGUC | |
| | 2900 | CARUACCAAA | CAGUGUGACC | GAUUUUGCAU | GGCAAACUUC | GACUAAUCCA | |
| 15 | 2950 | AGUAUCUUUU | GGACUGAGGG | CAAUGCCCCC | CCGCGUAUGU | CCAUACCAUU | |
| | 3000 | UAUAAGCAUA | GGGAAUGCAU | ACAGCAACUU | UUAUGACGGA | UGGUCGCACU | |
| | 3050 | UCUCACAAAA | UGGGGUUAUAC | GGCUACAAUG | CAUUAACAA | CAUGGGCAAA | |
| | 3100 | UUAUACGCAC | GCCAUGUGAA | CAAAGACACA | CCGUACCAGA | UGUCCAGUAC | |
| 20 | 3150 | GAUUCGUGUG | UACUUUAAAC | CCAAACAUAU | CAGAGUGUGG | GUGCCAAGAC | |
| | 3200 | CACCACGUUU | GUGCCCCUAU | AUUAUUAUCUA | GUAACGUUAA | CUUUGACCCA | |
| | 3250 | ACCAACCUAA | CUGAUUCAAG | AUCAAGUAUA | ACAUUUGUGC | CAGACACUAU | |
| | 3300 | CCGUCCGGAA | GUCCGUACAG | CUGGAAAAUU | CGGCCACCAG | UCCGGUGCUG | |
| | 3350 | UUUACGUGGG | UAAUUACAGA | AUAGUGAACA | GGCACCUCGC | CACGCACAAC | |
| 25 | 3400 | GACUGGCAAA | ACUGUGUGUG | GGAAGACUAC | AACAGAGACC | UCCUUGUGAG | |
| | 3450 | CACCACUACA | GCCCAUGGGU | GUGACACUAA | AGCCAGAUGU | CAGUGCACAG | |
| | 3500 | CAGGCGUAUA | UUUUUGUGCC | UCAAGGAACA | AACAUUACCC | AGUCACCUUC | |
| | 3550 | GAGGGGCCAG | GCUUGGUGGA | AGUUCAGGAG | AGCGAGUACU | ACCCAAAAAG | |
| | 3600 | AUAUCAGUCC | CACGUGCUUC | UAGCUGCAGG | AUUUUCUGAA | CCGGGCGAUU | |
| 30 | 3650 | GUGGCGGAAU | CCUCAGAUGU | CAACACGGCG | UGAUCGGUAA | CGUCACCAUG | |
| | 3700 | GGUGGAGAGG | GGGUCGUUGG | GUUUGCCGAC | GUCAGAGACC | UACUGUGGUU | |
| | 3750 | AGAGGAUGAU | GCCAUGGAAC | AGGGCGUAAG | AGACUAUGUU | GAACAACUAG | |
| | 3800 | GAAAUUCUUU | CGGCUCAGGU | UUCACCAAUC | AAAUUUGUGA | ACAGGUCAAC | |
| | 3850 | CUCCUCAAA | AGUCAUUGGU | UGGACAGGAU | UCUAUUCUGG | AAAAAUCCCU | |
| 35 | 3900 | UAAGGCUCUA | GUUAAGAUUA | UCUCAGCACU | GGUCGUUGUA | GUGAGAAAUC | |
| | 3950 | ACGAUGAUCU | CAUAACGGUU | ACCGCCACUC | UAGCUUUAAU | UGGUUGCACC | |
| | 4000 | UCUUCUCCGU | GGCGGUGGCU | CAAGCAGAAG | GUGUCACAAU | AUUAUGGAAU | |
| | 4050 | ACCCAGGGCC | GAGCGACAAA | ACAAUAGCUG | GCUCAAGAAG | UUUACUGAGA | |
| 40 | 4100 | UGACCAACGC | CUGCAAGGGC | AUGGAGUGGA | UAGCCAUAAA | AAUUCAAAAG | |
| | 4150 | UUUAUUGAGU | GGCUUAAAGU | CAAGAUUCUG | CCGGAAGUGA | AGGAAAAACA | |
| | 4200 | CGAGUUCUC | AACAGGCUAA | AGCAAUACC | ACUCCUAGAG | AGCCAGAUUG | |
| | 4250 | CAACCAUAGA | GCAGAGUGCA | CCAUCGCAGA | GUGAUCAAGA | GCAACUCUUC | |
| | 4300 | UCCAACGUCC | AGUACUUCGC | CCAUUAUUGC | AGAAAGUAUG | CGCCAUUGUA | |
| 45 | 4350 | CGCUGCCGAG | GCGAAGAGAG | UGUUCUCACU | UGAGAAGAAA | AUGAGCAACU | |
| | 4400 | ACAUACAGUU | CAAGUCCAAA | UGCCGUUAUG | AGCCUGUAUG | CUUACUCCUA | |
| | 4450 | CAUGGCAGCC | CAGGGGCCGG | AAAGUCCGUG | GCCACCAACU | UGAUUGGCAG | |
| | 4500 | AUCCCUUCGA | GAAAAACUCA | ACAGCUCUGU | AUACUCCCUA | CCACCAGACC | |
| | 4550 | CCGACCACUU | UGACGGCUAC | AAGCAGCAAG | CGGUCGUGAU | CAUGGAUGAC | |
| 50 | 4600 | UUAUGCCAAA | AUCCUGAUGG | AAAAGAUGUC | UCACUAUUUU | GUCAGAUGGU | |
| | 4650 | UUCUAGCGUG | GACUUUGUAC | CACCGAUGGC | UGCGCUAGAG | GAAAAAGGAA | |
| | 4700 | UCCUAUUUAC | CUCCCCGUUC | GUGUUGGCAU | CAACCAACGC | UGGGUCCAUC | |
| | 4750 | AAUGCACCCA | CUGUGUCUGA | CAGCAGAGCG | CUCGCUAGGA | GAUUCCACUU | |
| | 4800 | UGACAUGAAC | AUUGAAGUCA | UUUCUAUGUA | CAGUCAAAC | GGCAAGAUCA | |
| 55 | 4850 | ACAUGCCCAU | GUCAGUUAUA | ACAUGUGAUG | AAGAGUGUUG | UCCAGUUAAC | |
| | 4900 | UUCAAAAGGU | GCUGCCCGUU | GGUGUGUGGG | AAGGCYAUGC | AAUUCAUUGA | |

| | | | | | | |
|----|------|------------|------------|------------|------------|-------------|
| | 4950 | UAGGAGAACU | CAAGUUAGAU | AUUCGCUGGA | CAUGCUAGUU | ACUGAAAUGU |
| | 5000 | UUAGGGAGUA | UAACCAUAGA | CACAGUGUGG | GAGCCACUCU | UGAAGCUCUG |
| | 5050 | UUCCAAGGGC | CACCAGUCUA | CAGAGAGAUC | AAAAUCAGCG | UCGCCCCAGA |
| 5 | 5100 | GACACCCCCA | CCACCAGCUA | UUGCUGAUUU | ACUGAAAUCA | GUGGACAGUG |
| | 5150 | AAGCUGUGAG | GGAAUACUGC | AAGGAGAGAG | GGUGGCUUGU | GCCAGAGAUC |
| | 5200 | AAUUCUACCC | UACAAAUAGA | GAAGCAUGUG | AGUAGAGCAU | UCAUAUGUUU |
| | 5250 | ACAAGCCCUA | ACCACGUUUG | UUUCAGUUGC | UGGUAAUAUA | UACAUUAUUU |
| 10 | 5300 | ACAAAUUAUU | UGCAGGUUUC | CAAGGCGCCU | ACACAGGGAU | GCCCAACCAG |
| | 5350 | AAACCUAAGG | UGCCCACCCU | GAGACAGGCC | AAAGUACAGG | GCCCAGCGUU |
| | 5400 | UGAGUUCGCU | GUGGCGAUGA | UGAAAAGGAA | CGCCAGUACA | GUAAAAACCG |
| | 5450 | AGUACGGUGA | AUUCACCAUG | CUUGGCAUUU | ACGACAAGUG | GGCGGUGUUA |
| | 5500 | CCGCGCCACG | CCAAGCCUGG | CCCCACCAUC | UUGAUGAAUG | AUCAGGAAGU |
| 15 | 5550 | CGGCGUGUUG | GAUGCCAAGG | AACUAGUUGA | UAAAGAUGGG | ACAAAUCUAG |
| | 5600 | AAUUGACUCU | CCUGAAGCUC | AACCGUAACG | AAAAGUUCAG | AGAUUUUAGG |
| | 5650 | GGGUUUCUAG | CAAGAGAAGA | GGUUGAAGUG | AAUGAAGCUG | UCCUAGCAAU |
| | 5700 | AAAUACAAGC | AAAUUCCCUA | ACAUGUACAU | ACCAGUGGGC | CAGGUGACUG |
| 20 | 5750 | ACUACGGGUU | UCUGAACCUG | GGAGGGACUC | CCACGAAGAG | AAUGCUC AUG |
| | 5800 | UAUAACUUEC | CAACUAGAGC | AGGUCAGUGU | GGAGGUGUCC | UCAUGUCAAC |
| | 5850 | AGGGAAAGUC | CUGGGAUAC | AUGUAGGAGG | GAAUGGACAU | CAAGGGUUCU |
| | 5900 | CAGCGGCACU | CCUCAGGCAC | UACUUAACG | AGGAGCAGGG | UGAAAUAGAA |
| | 5950 | UUCAUUGAGA | GCUCAAAGGA | CGCGGGAUUC | CCUGUGAUCA | ACACUCCAG |
| 25 | 6000 | UAAGACAAAA | UUGGAACCAA | GUGUGUUUCA | CCAGGUGUUC | GAGGGCAACA |
| | 6050 | AGGAACCAGC | GGUCCUUAGA | AAUGGGGACC | CACGACUCAA | AGCCAACUUC |
| | 6100 | GAGGAAGCAA | UCUUCUCCAA | GUACAUUGGC | AAUGUCAACA | CGCAUGUAGA |
| | 6150 | UGAGUACAUG | UUGGAGGCUG | UGGACCAUUA | UGCAGGACAA | CUAGCUACUC |
| 30 | 6200 | UGGACAUCAG | UACGGAGCCC | AUGAAGCUAG | AGGACGCCGU | GUAUGGUACA |
| | 6250 | GAGGGGCUGG | AAGCACUAGA | CCUAACCACC | AGUGCAGGCU | ACCCUUACGU |
| | 6300 | GGCCCUGGGC | AUCAAGAAAA | GAGAUUUUCU | AUCUAAGAAG | ACUAAAGACC |
| | 6350 | UCACUAAGUU | GAAGGAAUGC | AUGGACAAAU | AUGGCCUAAA | UUUGCCAAUG |
| | 6400 | GUAACCUACG | UCAAGAUGA | GUUGAGAUCU | GCUGAGAAGG | UGGCCAAGGG |
| 35 | 6450 | AAAAUCCAGG | CUUAUUGAGG | CUUCUAGUCU | CAAUGACUCA | GUAGCAAUGA |
| | 6500 | GGCAAACAUU | UGGAAAUUUA | UAUAAGACCU | UUCACCUCAA | CCCGGGCAUC |
| | 6550 | GUUACGGGCA | GUGCUGUUGG | GUGUGAUCCA | GAUGUGUUUU | GGAGCAAGAU |
| | 6600 | CCCUGUUAUG | CUUGAUGGAC | AUCUCAUAGC | UUUUGACUUA | UCAGGCUAUG |
| 40 | 6650 | ACGCUAGCCU | CAGCCCAGUG | UGGUUUGCAU | GUUUGAAACU | UCUCCUAGAG |
| | 6700 | AAACUAGGGU | AUACAAACAA | GGAAACAAAC | UACAUAGAUU | ACCUCUGUAA |
| | 6750 | UUCCCAUCAC | CUGUAUAGAG | ACAAGCACUA | CUUUGUAAGA | GGCGGUAUGC |
| | 6800 | CAUCAGGGUG | UUCAGGCACC | AGCAUAUUUA | AUUCCAUGAU | UAACAACAUC |
| 45 | 6850 | AUAAUCAGGA | CUCUCAUGCU | GAAGGUUUUA | AAAGGCAUUG | AUUUGGACCA |
| | 6900 | AUUCAGAAUG | AUUGCCUAUG | GGGAUGAUGU | GAUUGCUUCC | UAUCCGUGGC |
| | 6950 | CUAUCGAUGC | UUCGUGUUA | GCUGAAGCAG | GAAAAGAUUA | UGGUUUAAUC |
| | 7000 | AUGACCCCAG | CAGACAAAGG | CGAGUGCUUC | AACGAGGUAA | CCUGGACGAA |
| | 7050 | UGUGACCUUU | CUGAAAAGGU | ACUUUAGGGC | AGAUGAGCAA | UACCCAUUUC |
| 50 | 7100 | UGGUCCAUC | UGUUAUGCCA | AUGAAGGACA | UACAUGAGUC | CAUUAGGUGG |
| | 7150 | ACCAAAGAUC | CCAAGAACAC | ACAGGAUCAU | GUGCGCUCGC | UGUGCCUAUU |
| | 7200 | GGCUUGGCAC | AACGGGGAGC | AAGAAUAUGA | GGAGUUUAUU | CGCAAGAUCA |
| | 7250 | GAAGCGUGCC | CGUUGGGCGC | UGCUUGACCC | UACCCGCUUU | UUCAACACUG |
| | 7300 | CGCAGGAAGU | GGCUGGACUC | CUUUUAAAAU | UAGAGCAUAA | UUAGUAAAUC |
| 55 | 7350 | AUAAUUGGCU | UAACCCUACC | GCAUGAACCG | AACUUGAUAA | AAGUGCGGUA |
| | 7400 | GGGGUAAAUU | CUCCGCAUUC | GGUGCGG | | |

Appendix 4: **Seq ID No 4**

Comparison of genomes of the unmodified (native) virus and modified virus

5

[0096]

N: Unmodified (native) virus

M: Modified virus

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EP 2 826 856 B9

1 50
N TTTAAACAGC CTGTGGGTTG TTCCCACCCA CAGGGCCCAC TGGGCGCTAG
M
5
51 100
N CACACTGGTA TCACGGTACC TTTGTGCGCC TGTTTTATAT CCCCCTCCCC
M C.....C. T.....
10
101 150
N ACTGTAACTT AGAGAAATCA CATAAACGAT CAATAGAAGG CGCAGCACAC
MAG...G.G. ...C...TA. .T...T....
15
151 200
N CAGCTGAGTC TTGACCAAGC ACTTCTGTTT CCCC GGACTG AGTATCAATA
M ..A.....C. CC.....AC.A.....
20
201 250
N GACTGCTCAC GCGGTTGAAG GAGAAAACGT TCGTTACCCG GCCAACTACT
M .G.....G.C......T.....T....
25
251 300
N TCGAGAAACC TAGTACCACC ATGAAAGTTG CGCAGTGTTT CGCTCAGCAC
MG....C....C....
30
301 350
N AACCCAGTG TAGATCAGGT CGATGAGTCA CCGCATTCCC CACGGGCGAC
MC.....
35
351 400
N CGTGGCGGTG GCTGCGTTGG CGGCCTGCCT ATGGGGCAAC CCATGGGACG
MC.....
40
401 450
N CTTCAATACT GACATGGTGC GAAGAGTCTA TTGAGCTAGT TGGTAGTCCT
MA.....
45
451 500
N CCGGCCCCCTG AATGCGGCTA ATCCTAACTG CGGAGCAAGT GCCCACAAC
MG.....
50
501 550
N CAGTGGGTAG CTTGTCGTAA CGGGCAACTC TGCAGCGGAA CCGACTACTT
MG.T...C.
55
551 600
N TGGGTGTCCG TGTTTCCTTT TATTCTTATT CTGGCTGCTT ATGGTGACAA
M
601 650
N TTGAGAGATT GTTACCATAT AGCTATTGGA TTGGCCATCC GGTGACTAAC
MR.....R..TG....

EP 2 826 856 B9

| | | | | |
|----|---|-------------|------------|------------------------------|
| | | 651 | | 700 |
| | N | AGAGCAATTA | TATACCTCTT | TGTTGGATTT |
| | M |C. | ...T..... |G.....G |
| 5 | | 701 | | 750 |
| | N | TTACAACACT | CTGCTACACA | TTATTTACTT |
| | M | |T. |G... ..TA..... |
| 10 | | 751 | | 800 |
| | N | ACAAGTATCA | ACACAAAAAA | CTGGTGCACA |
| | M |G |G. |C..... |
| 15 | | 801 | | 850 |
| | N | ACGGAAGCTC | CATCATTAC | TACACCAACA |
| | M |CA... | T..... | ..T..... ..C..... |
| 20 | | 851 | | 900 |
| | N | GCATCCAAC | TCAAGGATA | TCATGATCAA |
| | M | | |A..G..... |
| 25 | | 901 | | 950 |
| | N | CACCGAACCG | GGAGGAGTGT | GGGTACAGTG |
| | M |T...C |C |C..... |
| 30 | | 951 | | 1000 |
| | N | CACCGAACCG | GGAGGAGTGT | GGGTACAGTG |
| | M |T...C |C |C..... |
| 35 | | 1001 | | 1050 |
| | N | CTCGGCAACT | CAACCATTAC | CACTCAGGAG |
| | M | | |A..A..... |
| 40 | | 1051 | | 1100 |
| | N | CTATGGCGGG | TGGCCAGAGT | ACTTGAAAGA |
| | M |A.. | | |
| 45 | | 1101 | | 1150 |
| | N | ATCAACCAAC | ACAACCCGAT | GTAGCCACAT |
| | M |G..... | |R.....C.. ..T..... |
| 50 | | 1151 | | 1200 |
| | N | TCCGTCCAGT | GGGAGAAAAA | TTCCGCTGGA |
| | M | | | ..AG..... |
| 55 | | 1201 | | 1250 |
| | N | AGCACTTAAG | GACATGGGCC | TCTTTGGTCA |
| | M | | |G.....T.T. |
| | | 1251 | | 1300 |
| | N | TCGGTAGAGC | AGGCTACACT | ATACACGTGC |
| | M | | |T.... ..C.. ..T |
| | | 1301 | | 1350 |
| | N | CACCAAGGCT | GTCTACTTGT | TGTCTGTGTA |
| | M | ..T..G.... | |A.... |
| | | 1351 | | 1400 |
| | N | TTCCAAAGTG | GACGGTACTG | TAAATGAGCA |
| | M |C.GAC. | ...AAAGAG. | .TGC..C.AT .A.CC.C... A..... |
| | | 1401 | | 1450 |

EP 2 826 856 B9

| | | | | | | |
|----|---|-------------|------------|-------------|------------|------------|
| | N | CGGATATGAA | GCTTGAACCC | ACCAGAACCA | CAGGCGTACG | CCGAGTGCAA |
| | M | ...CGCAC... | .T.....A |A..... |GC.A | .AC..... |
| | | 1451 | | | | 1500 |
| 5 | N | TCCGCAGTGT | ACAACGCGGG | TATGGGCGTC | GGCGTGGGGA | ACCTCACCAT |
| | M | ...AT..... | G..... |A.. | | |
| | | 1501 | | | | 1550 |
| 10 | N | CTTCCCTCAC | CAGTGGATCA | ACCTGCGCAC | TAACAACTGT | GCTACAATTG |
| | M | ..A..... | | ..T..... | ...T.....C | |
| | | 1551 | | | | 1600 |
| | N | TGATGCCATA | CATAAATAGT | GTACCCATGG | ATAACATGTT | TAGGCACTAC |
| | M |G.. | T.....TCA | | | |
| 15 | | 1601 | | | | 1650 |
| | N | AACTTCACGC | TAATGATGAT | CCCATTGCA | CCCCTGGATT | ACACCAACCA |
| | M | ..T..... |G.... | | | ...ATGC... |
| | | 1651 | | | | 1700 |
| 20 | N | AGCATCTACG | TACGTACCTA | TAACTGTCAC | AATAGCACCA | ATGTGTGCTG |
| | M |GA. |G | |C... |A. |
| | | 1701 | | | | 1750 |
| 25 | N | AATACAATGG | TTTGAGGCTC | GTTACCTCGC | AAGGGTTGCC | AGTGATGAAC |
| | M | | ...A.....G | .C.TA.CA.. |C.... |C.A..T |
| | | 1751 | | | | 1800 |
| | N | ACACCGGGAA | GCAATCAGTT | CCTGACATCG | GATGACTTTC | AATCACCTTC |
| | M | | | TA..... |T.... |C..... |
| 30 | | 1801 | | | | 1850 |
| | N | GGCTATGCCA | CAATTTGATG | TGACTCCAGA | CATGGACATC | CCAGGTGAAG |
| | M | | |GC. | | |
| | | 1851 | | | | 1900 |
| 35 | N | TGAACAACCT | CATGGAGATT | GCAGAGGTTG | ACTCGGTGGT | ACCTGTTAAC |
| | M | ..C..... | |A..... | .T..... | |
| | | 1901 | | | | 1950 |
| 40 | N | AACAATGAGG | CCAATCTGAA | AAGCATGGAC | GCATACCGCA | TACCGGTGAA |
| | M |C..C.. |C. | |T.A.. | ..GA..... |
| | | 1951 | | | | 2000 |
| | N | CRCAGGAAAT | CAACAAGGTG | AAAAGATATT | TGGTTTCCAA | ATACAACCCG |
| | M | | ..C..... | | C.C.....G | |
| 45 | | 2001 | | | | 2050 |
| | N | GGCTTGATTC | AGTGTTTAAG | AGAACACTGC | TAGGTGAGAT | GCTCAATTAT |
| | M |G..... | | |AG. | |
| | | 2051 | | | | 2100 |
| 50 | N | TACACGCACT | GGTCAGGGAG | CATTAAGCTA | ACATTTATGT | TTTGTGGTTC |
| | M | ...G..... | | |C.CA. | |
| | | 2101 | | | | 2150 |
| 55 | N | AGCAATGGCC | ACGGGCAAAT | TACTCTTAGC | ATACTCACCA | CCTGGCGCCG |
| | M | |GC | |C... | |
| | | 2151 | | | | 2200 |

EP 2 826 856 B9

| | | | | | | |
|----|---|------------|-------------|-------------|------------|------------|
| | N | ATGTACCGAC | TAGCAGAAAG | GAGGCAATGC | TGGGAACCCA | TGTCATCTGG |
| | M |G. | |C....A | | .A..... |
| | | 2201 | | | | 2250 |
| 5 | N | GACTTTGGGC | TGCAATCCAG | TTGTGTTCTG | TGTGTTCCAT | GGATCAGCCA |
| | M |A.... | | ...C....A | ...A..... |T.. |
| | | 2251 | | | | 2300 |
| 10 | N | GACACACTAC | AGGTTGGTGC | AGCAGGATGA | GTACACCGGC | GCCGGCTATA |
| | M |T..T | C.CC.A.... | .A..... |A.. |A..G |
| | | 2301 | | | | 2350 |
| | N | TCACCTGCTG | GTACCAAACA | AGTATAGTGG | TTCCACCCGG | CACACCCAAA |
| | M | | ...T..G... | G..... | |C |
| 15 | | 2351 | | | | 2400 |
| | N | AAGTGTGTCA | TCCTGTGCTT | TGTGTCAGCG | TGTAATGATT | TCTCCGTGAG |
| | M |G | | |C. |C. |
| | | 2401 | | | | 2450 |
| 20 | N | CATGCTGAGT | GACACACCAT | TCATCGGCCA | AACAGCACTG | CTGCAGAGCC |
| | M |C.. | | |A..... | ..A..AG.TG |
| | | 2451 | | | | 2500 |
| | N | CTGTGGAAGA | AGCTGAAGAG | AACGCAGTTG | CACGTGTGGC | TGACACAATT |
| 25 | M | A.AC...C.T | G..C.TCA.C | ..T....A. | .CA.G..A.. | ...T..... |
| | | 2501 | | | | 2550 |
| | N | GCCAGTGGGC | CCAGCAACTC | CGAGAGCGTT | CCTGCACTAA | CAGCAGTTGA |
| | M | | | .ACT...A.. | | .C..... |
| 30 | | 2551 | | | | 2600 |
| | N | GACTGGGCAC | ACATCACAGG | TAGTGCCTAG | TGACACAATG | CAAACAAGGC |
| | M | | | ...A..... | ...T..... |C... |
| | | 2601 | | | | 2650 |
| 35 | N | ATGTGAAGAA | CTACCATTCTG | AGATCTGAGT | CAACAATAGA | GAACTTCCTT |
| | M |A..... | | C.....A. | | |
| | | 2651 | | | | 2700 |
| | N | AGCAGGTCCG | CCTGTGTGTA | TATTGAAGAG | TACTATACCA | ACACTGAAAC |
| 40 | M | ...C....G. |A.. | |T..... | .AGA.C..G. |
| | | 2701 | | | | 2750 |
| | N | CAGACAAAAT | TTATACATGT | TGCCCCACTAT | AAATACTAGA | TGGATGGTGC |
| | M | ...CGCC... | AGG..... | CATGG..... |G..... | A..... |
| 45 | | 2751 | | | | 2800 |
| | N | AATTGAGGAG | AAAGTTTGAG | ATGTTACACAT | ACATGAGGTT | TGACATGGAA |
| | M |C. |A | C..... |C.... | ...T.....G |
| | | 2801 | | | | 2850 |
| 50 | N | ATCACATTTG | TTATCACTAG | TAGACAACTG | CATCGAACTA | GCATGCCGCA |
| | M | | | | .C.G.G.... |CG.... |
| | | 2851 | | | | 2900 |
| | N | GGACATGCCG | GTACTGACAC | ACCAAATCAT | GTATGTACCA | CCTGGTGGTC |
| 55 | M | A..... | CC..... | |A....C | |
| | | 2901 | | | | 2950 |
| | N | CAGTACCAAA | CAGTGTGGAC | GATTACGCAT | GGCAAACCTC | GACTAACCCA |

EP 2 826 856 B9

MAC.TT....T...

2951 3000

5 N AGTGTCTTTT GGA CTGAGGG CAATGCCCCA CCGCGTATGT CCATACCATT

M ...A.....C

3001 3050

10 N CATAAGCATA GGAATGCAT ACAGCAACTT TTATGATGGG TCCTCGCACT

M T.....C..R .GG.....

3051 3100

N TCTTACAATA TGGGGTATAT GGCTACAACA CATTAAACAA CATGGGGAAA

M ...C....A.CTGC....

15 3101 3150

N TTATACGTAC GCCATGTGAA CAACCACACA CCATACCAAA TGACCAGTAC

MC..AG..... ..G.....G. ..T.....

20 3151 3200

N GGTTAGTGTG TACTTTAAAC CCAAACATGT CAGAGCGTGG GTGCCGAGAC

M .A..C.....A.T....A....

25 3201 3250

N CACCACGTCT GTGCCCCTAC AAAAATGCAT GGAACGTAA CTTTGAACCA

MT.T .TT..AT.TA .T.....C...

30 3251 3300

N ACAAACGTAA CTGATTCAAG ATCAAGTATC ACATATATTC CTGAGACGGT

M ..C...C...AG.G. .A..C..TA.

3301 3350

N CAAACCAGAC CTATCAAAAG CTGGAGCTTT CGGCCACCAG TCCGGTGCTG

M .CGT..G..A G.CCGT.C...AAA..

35 3351 3400

N TTTATGTGGG TAACTACAGA GTGGTGAATA GGCACCTCGC CACGCACAAC

MC..... ...T..... A.A.....C.

3401 3450

N GACTGGCAAA ACTGTGTGTG GGAAGACTAC AACAGAGACC TCCTTGTGAG

M

40 3451 3500

N CACCACCACA GCCCATGGGT GTGACACCAT AGCCAGATGC CAGTGCACAA

MT...T..TG

45 3501 3550

N CAGGCGTGTA CTTTGTGCC TCAAGGAACA AACACTACCC AGTCACCTTT

MA.. T.....T.....C

3551 3600

N GAGGGGCCAG GCCTGGTGA AGTTCAGGAG AGTGAGTACT ACCCAAAAAG

MT.....C.....

50 3601 3650

N ATACCAATCC CATGTGCTTC TAGCTGCAGG ATTTTCTGAA CCAGGCGATT

M .Y.T..G... ..C.....G.....

55 3651 3700

N GTGGTGAAT CCTCAGGTGT GAACATGGTG TCATCGGTAT CGTCACCATG

MC.....A... C....C..C. .G.....

EP 2 826 856 B9

| | | | | |
|----|---|-------------|-------------|----------------|
| | | 3701 | | 3750 |
| | N | GGTGGAGAGG | GGGTCGTTGG | GTTTGCCGAC |
| | M | | | ...A..... |
| 5 | | 3751 | | 3800 |
| | N | AGAGGATGAT | GCCATGGAAC | AGGGCGTAAG |
| | M | | | |
| 10 | | 3801 | | 3850 |
| | N | GAAATGCTTT | TGGCTCAGGT | TTCACCAACC |
| | M | | C..... |T..... |
| 15 | | 3851 | | 3900 |
| | N | CTCCTCAAAG | AGTCACTGGT | TGGACAGGAC |
| | M | |T.... |T..T..... |
| 20 | | 3901 | | 3950 |
| | N | TAAAGCCCTA | GTAAAGATTA | TCTCAGCACT |
| | M | ...G..T... | |R..... |
| 25 | | 3951 | | 4000 |
| | N | ACGATGACCT | CATCACAGTG | ACTGCCACTC |
| | M |T.. | ...A..G..T | ..C..... |
| 30 | | 4001 | | 4050 |
| | N | TCTTCTCCAT | GGCGGTGGCT | CAAACAGAAA |
| | M |G. | | ...G.....G |
| 35 | | 4051 | | 4100 |
| | N | ACCCATGGCT | GAGCGACAAA | ACAATGGCTG |
| | M |G...C | |A..... |
| 40 | | 4101 | | 4150 |
| | N | TGACCAATGC | CTGCAAGGGC | ATGGAGTGGA |
| | M |C.. | |A.. |
| 45 | | 4151 | | 4200 |
| | N | TTTATTGAGT | GGCTTAAAGT | CAAGAT-CTA |
| | M | | |T..G |
| 50 | | 4201 | | 4250 |
| | N | TGAGTTCCCTC | AACAGACTAT | AACAACCTACC |
| | M | C..... |G...A | ..G...T.... |
| 55 | | 4251 | | 4300 |
| | N | GCCACCATAG | AACAAAGTGC | ACCATCGCAG |
| | M | ..A..... | ..G..G..... |T..A. |
| 60 | | 4301 | | 4350 |
| | N | TTCCAATGTC | CAGTACTTCG | CCCACTATTG |
| | M | C.....C... | |T..... |
| 65 | | 4351 | | 4400 |
| | N | ATGCAGCTGA | GGCAAAGAGA | GTGTTCTCCC |
| | M | .C..T..C.. | A..G..... |A. |
| 70 | | 4401 | | 4450 |
| | N | TACATACAGT | TCAAGTCCAA | ATGCCGTATT |
| | M | | | |

EP 2 826 856 B9

| | | | | |
|----|---|------------|-------------|--------------------|
| | | 4451 | | 4500 |
| | N | ACATGGCAGC | CCAGGGGCCG | GAAAATCCGT |
| | M | |G..... | T..... |
| 5 | | 4501 | | 4550 |
| | N | GATCACTCGC | TGAAAACTC | AACAGCTCAG |
| | M |C..... | A..... |T. .R..... |
| 10 | | 4551 | | 4600 |
| | N | CCAGATCACT | TTGATGGCTA | CAAACAGCAA |
| | M | ..C..C.... |C..... | ...G..... |
| 15 | | 4601 | | 4650 |
| | N | TCTATGCCAA | AATCCTGATG | GAAAAGATGT |
| | M | CT..... | | C...C.A..T |
| 20 | | 4651 | | 4700 |
| | N | TTTCCAGTGT | GGACTTTGTA | CCACCGATGG |
| | M |T..C.. | | |
| 25 | | 4701 | | 4750 |
| | N | ATTCTGTTCA | CCTCCCCGTT | TGTCCTGGCA |
| | M | ..C..A..T. | | C..GT..... |
| 30 | | 4751 | | 4800 |
| | N | CAATGCACCA | ACTGTGTCAG | ACAGCAGAGC |
| | M |C |T. |G..... |
| 35 | | 4801 | | 4850 |
| | N | TTGACATGAA | CATTGAAGTC | ATTTCCATGT |
| | M | | |T.... |
| 40 | | 4851 | | 4900 |
| | N | AACATGCCCA | TGTCAGTTAA | GACGTGTGAT |
| | M | | | A..A..... |
| 45 | | 4901 | | 4950 |
| | N | CTTCAAGAGG | TGCTGCCCGC | TGGTGTGTGG |
| | M |A... |T |Y... ..A..... |
| 50 | | 4951 | | 5000 |
| | N | ACAGAAGAAC | TCAAGTTAGA | TACTCGCTGG |
| | M | .T..G..... | | ..T..... |
| 55 | | 5001 | | 5050 |
| | N | TTTAGGGAGT | ACAACCACAG | ACACAGTGTG |
| | M | | .T.....T.. |T. |
| | | 5051 | | 5100 |
| | N | GTTCCAAGGG | CCACCAGTCT | ACAGAGAGAT |
| | M | | |C..C |
| | | 5101 | | 5150 |
| | N | AGACACCACC | ACCACCAGCT | ATTGCTGACT |
| | M |C.. | |T. |
| | | 5151 | | 5200 |
| | N | GAAGCTGTGA | GAGAGTACTG | CAAAGAAAAG |
| | M | | .G..A..... | ...G..G.GA |
| | | 5201 | | 5250 |

EP 2 826 856 B9

| | | | | | | |
|----|---|------------|------------|-------------|------------|-------------|
| | N | CAACTCCACC | CTACAAATTG | AGAAGCATGT | GAGCCGGGCA | TTCATCTGTC |
| | M | ...T..T... |A. | | ...TA.A... |A...T |
| | | 5251 | | | | 5300 |
| 5 | N | TGCAAGCACT | AACCACGTTT | GTTTCAGTTG | CTGGAATAAT | ATACATTATT |
| | M | .A.....C.. | | |T..... | |
| | | 5301 | | | | 5350 |
| 10 | N | TACAAGCTAT | TTGCAGGTTT | CCAAGGCGCA | TACACAGGGA | TGCCCCAACCA |
| | M |AT... | |C | | |
| | | 5351 | | | | 5400 |
| | N | GAAACCCAAG | GTGCCCACCC | TGAGACAAGC | CAAAGTGCAA | GGCCCCAGCGT |
| | M |T... | |G.. |A..G | |
| 15 | | 5401 | | | | 5450 |
| | N | TTGAGTTTGC | TGTGGCGATG | ATGAAGAGGA | ACTCCAGTAC | AGTGAAAACC |
| | M |C.. | |A..... | ..G..... | ...A..... |
| | | 5451 | | | | 5500 |
| 20 | N | GAGTACGGTG | AGTTCACCAT | GCTTGGCATT | TATGACAGGT | GGGCGGTGTT |
| | M | | .A..... | | ..C....A.. | |
| | | 5501 | | | | 5550 |
| | N | ACCACGCCAC | GCCAAACCTG | GCCCAACCAT | CTTGATGAAT | GACCAGGAAG |
| 25 | M | ...G..... |G.... |C..... | | ..T..... |
| | | 5551 | | | | 5600 |
| | N | TCGGCGTGTT | GGATGCCAAG | GAAGTAGTGG | ATAAGGATGG | GACAAACCTA |
| | M | | |T. |A..... |T... |
| 30 | | 5601 | | | | 5650 |
| | N | GAAGTGACAC | TCCTGAAGCT | CAACAGTAAT | GAGAAGTTCA | GAGACATCAG |
| | M | ...T....T. | |C....C | ..A..... |T..T.. |
| | | 5651 | | | | 5700 |
| 35 | N | AGGGTTCCTA | GCCAAAGAAG | AGGTTGAGGT | GAATGAAGCT | GTCTTAGCAA |
| | M | G.....T... | ..A.G..... |A.. | | |
| | | 5701 | | | | 5750 |
| | N | TAAACACAAG | CAAGTTCCCC | AACATGTACA | TACCAGTGGG | CCAGGTGACT |
| 40 | M |T..... | ...A.....T | | | |
| | | 5751 | | | | 5800 |
| | N | GACTACGGGT | TCCTGAACCT | GGGTGGGACG | CCCACTAAGA | GAATGCTCAT |
| | M | | .T..... | ...A.....T |G.... | |
| | | 5801 | | | | 5850 |
| 45 | N | GTACAACTTC | CCCACTAGAG | CAGGTCAGTG | TGGTGGTGTC | CTCATGTCCA |
| | M | ...T..... | ..A..... | | ...A..... |A. |
| | | 5851 | | | | 5900 |
| | N | CTGGGAAAGT | CCTGGGGATA | CATGTTGGTG | GGAATGGTCA | TCAAGGGTTC |
| 50 | M | .A..... |A... |A..A. |A.. | |
| | | 5901 | | | | 5950 |
| | N | TCAGCAGCAC | TCCTCAAGCA | CTACTTCAAC | GATGAACAAG | GTGAAATAGA |
| | M |G.... |G... | | ..G..G..G. | |
| 55 | | 5951 | | | | 6000 |
| | N | GTTTATTGAG | AGCTCAAAGG | ACGCGGGGTT | CCCTATCATC | AACACACCCA |

EP 2 826 856 B9

M A.....A...G.G...T....

6001 6050

5 N GCAAGACCAA ACTGGAACCA AGTGTCTTCC ACCAG-TGTT TGAAGGCAAC

M .T.....A..T.....G..T.G....C..G.....

6051 6100

10 N AAAGAACCCA GCAGTCCTCA GAAATGGTGA TCCACGACTC AAAGCCAACT

M ..G.....-..G.....T.G..C.....

6101 6150

N TTGAGGAGGC CATCTTCTCC AAATACATTG GCAATGTCAA CACGCATGTG

M .C.....A..A.....G.....A

6151 6200

15 N GATGAGTACA TGTGGAAGC TGTGGACCAT TATGCAGGAC AACTGGCTAC

MG.....A.....

6201 6250

20 N TCTGGACATC AGCACGGAAC CAATGAAGCT GGAGGATGCC GTGTATGGTA

MT.....G..C.....A....C....

6251 6300

N CAGAGGGGCT GGAAGCACTA GACCTAACAA CCAGTGCAGG CTACCCTTAT

MC.....C

6301 6350

25 N GTTGCCCTGG GCATCAAGAA GAGAGACATC CTATCTAAGA AGACCAGGGA

M ..G.....A....T..T.....T.AA..

6351 6400

30 N CCTCACTAAG TTGAAAGAAT GCATGGACAA GTATGGCCTA AACCTGCCAA

MG....A.....TT.....

6401 6450

35 N TGGTAACCTA TGTGAAAGAT GAGCTCAGAT CTGCAGAGAA GGTGGCCAAA

MC..C.....T.G....T.....G

6451 6500

N GGAAATCCA GGCTTATTGA AGCTTCCAGT TTGAATGACT CAGTGGCAAT

MG.....T...C.C.....A.....

6501 6550

40 N GAGACAGACA TTTGGAAACC TGTACAAAAC CTTCCACCTC AATCCAGGCA

M ...G..A...TT.A..T..G..T.....G....

6551 6600

45 N TTGTGACGGG CAGTGCAGTT GGGTGTGACC CAGATCTGTT TTGGAGCAAG

M .C..T.....T...T.....G....

6601 6650

N ATACCAGTCA TGTGATGG ACATCTCATA GCTTTTGATT ACTCAGGCTA

M ..C..T..T..C.T.....C..T.....

6651 6700

50 N TGATGCTAGC CTCAGCCCAG TGTGGTTTGC ATGTCTGAAA CTGCTCCTAG

M ...C.....T.....T.....

6701 6750

55 N AGAAGCTTGG GTACACACAC AAGGAAACAA ACTACATAGA TTACCTCTGC

MA..A..T..A.....T

EP 2 826 856 B9

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45
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55

```

        6751                                     6800
N  AACTCCCACC ACCTGTACAG AGACAAACAC TACTTTGTGC GAGGTGGTAT
M  ..T.....T. ....T... ..G... ..AA ....C.....

        6801                                     6850
N  GCCATCAGGG TGTCTGGCA CCAGCATCTT TAACTCAATG ATTAACAACA
M  ..... ..A.... ..A.. ...T..C... .....

        6851                                     6900
N  TCATAATCAG GACACTCATG CTGAAAGTGT ACAAGGGCAT TGAATTGGAC
M  ..... ..T..... ..G..T. .T..A..... ..T.....

        6901                                     6950
N  CAATTCAGGA TTATTGCCTA TGGTGATGAT GTGATTGCTT CCTACCCGTG
M  .....A. .G..... ..G..... .....T.....

        6951                                     7000
N  GCCATTGAT  GCTTCCCTGC TAGCTGAAGC AGGAAAAGAT TATGGTTTGA
M  ...T..C... ..G...T ..... ..A.

        7001                                     7050
N  TCATGACACC AGCAGATAAA GGAGAGTGCT TCAATGAAGT CAACTGGACG
M  .....C.. .....C... ..C..... ....C..G.. A.C.....

        7051                                     7100
N  AATGTCACCT TCCTGAAAAG GTACTTTAGA GCAGATGAGC AATACCCATT
M  .....G.... .T..... ..G .....

        7101                                     7150
N  CCTGGTCCAC CCTGTTATGC CCATGAAAGA CATCCATGAA TCTATTAGAT
M  T.....T ..... .A.....G.. .....G .....G.

        7151                                     7200
N  GGACCAAAGA TCCAAAGAAC ACCCAAGATC ATGTGCGCTC GCTGTGCCCTA
M  ..... ..C..... ..A..G.... .....

        7201                                     7250
N  TTGGCTTGGC ACAATGGGGA GCACGAATAT GAGGAGTTCA TTCGCAAAAT
M  ..... ..C..... ..A..... ..T. ....G..

        7251                                     7300
N  CAGAAAGCGT GCCAGTTGGA CGCTGTTTGA CCCTACCTGC GTTTTCAACC
M  .....-.... ...C.....G .....C.... .....C.. T.....A

        7301                                     7350
N  CTGCGCAGGA AGTGGTTGGA CTCCTTTTAA AATAA-AGCA CAATTTAGTA
M  ..... ..C..... ..... ..T.G.... T....-....

        7351                                     7400
N  AATTTGAATT GGCTTAACCC TACCGCACTA ACCGAACTAG ATAACGGTGC
M  ...CAT.... .....TG. ....T. ....AA....

        7401                                     7437
N  GGTAGGGGTA AATTCTCCGC ATTCGGTGCG GTCGAGG
M  ..... ..-

```

Sequence identity: 90,3%

Appendix 5: Seq ID No 5

Comparison of amino acid sequences of the unmodified (native) virus and modified virus

5 [0097]

N: Unmodified (native) virus

M: Modified virus

```

10      1                                     50
      N MGAQVSTQKT GAHETXLSAN GSSIIHYTNI NYYKDAASNS ANRQDFTQDP
      M ..... .H.....

15      51                                     100
      N GKFTEPVKDI MIKSMPALNS PTVEECGYSD RVRISITLGNS TITTQESANV
      M ..... .SA..... .L.....

20      101                                    150
      N VVGYGWPEY LKDEEATAED QPTQPDVATC RFYTLESVQW EKNSAGWWWK
      M .....R.....

25      151                                    200
      N FPEALKDMGL FGQNMHYHYL GRAGYTIHVQ CNASKFHQGC LLVVCVPEAE
      M .....L.....

30      201                                    250
      N MGCSKVDGTV NEQELTEGET DMKLEPTRTT GVRRVQSAVY NAGMGVGVGN
      M ....QT.KE. AAMN..K... AH.F...K.. .GHT...I.C .....I....

35      251                                    300
      N LTIFPHQWIN LRTNNCATIV MPYINSVPMD NMFRHYNFTL MMIPFAPLDY
      M ...Y..... .V.....

40      301                                    350
      N TNQASTYVPI TVTIAPMCAE YNGLRLVTSQ GLPVMNTPGS NQFLTSDDFQ
      M NA...E...V .....AYQ. ....L..... .M.....

45      351                                    400
      N SPSAMPQFDV TPDMDIPGEV NNLMEIAEVD SVVPVNNNEA NLKSMDAYRI
      M ..... .H..... H.....TA. ..Q.....H.

50      401                                    450
      N PVNXGNQQGE KIFGFQIQPG LDSVFKRTL GEMLNYYTHW SGSIKLTFFM
      M E.....H... ..A..... ..V....A.. .....T.

55      451                                    500
      N CGSAMATGKL LLAYSPPGAD VPTSREAML GTHVIWDFGL QSSCVLCVPW
      M ..... ..A...Q..M ...I...L.. .....I..

      501                                    550
      N ISQTHYRLVQ QDEYTGAGYI TCWYQTSIVV PPGTPKKCVI LCFVSACNDF
      M .....S..NV .....G... ..N...V .....

      551                                    600
      N SVSMLSDTPF IGQTALLQSP VEEAEENAVA RVADTIASGP SNSESVPALT
      M ..R..R.... ....T...GD TDV.VN.... ....T.I....

      601                                    650

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EP 2 826 856 B9

N AVETGHTSQV VPSDTMQTRH VKNYHSRSES TIENFLSRSA CVYIEEYYTN
M E..... .FTK

651 700

5 N TETRONLYML PTINTRWMVQ LRRKFEMFTY MRFDMEITFV ITSROLHRTS
M DQDSA.R..S W...ARR... ..L... ..PG..

701 750

10 N MPQDMPVLTH QIMYVPPGGP VPNSVDDYAW QTSTNPSVFW TEGNAPPRMS
M IA....P... ..I..... ..T.F.. ..I.. ..

751 800

15 N IPFISIGNAY SNFYDGSSH F LQYGVYGYNT LNNMGKLYVR HVNNHTPYQM
M W... S.N.....AA. ...KD.....

801 850

N TSTVSVYFKP KHVRAWVPRP PRLCPYKNAW NVNFEPTNVT DSRSSITYIP
M S..IR..... ..I.V..... ..IKSSD...L.V.

851 900

20 N ETVKPDLSKA GAFGHQSGAV YVGNIRVVNR HLATHNDWQN CVWEDYNRDL
M D.IR.EVRT. .K..... ..I... ..

901 950

25 N LVSTTTAHGC DTIARCQCTT GYFPCASRNK HYPVTFEGPG LVEVQSEYY
M A

951 1000

N PKRYQSHVLL AAGFSEPGDC GGILRCEHGV IGIVTMGGEG VVGFDVDRDL
M ...X..... ..Q... ..

1001 1050

30 N LWLEDDAMEQ GVRDYVEQLG NAFGSGFTNQ ICEQVNLKE SLVGQDSILE
M

1051 1100

35 N KSLKALVKII SALVIVVRNH DDLITVTATL ALIGCTSSPW RWLKQKVSQY
M X..... .

1101 1150

40 N YGIPMAERQN NGWLKKFTEM TNACKGMEWI AIKIQKFIEW LKVKIYQKCR
MR..... .S..... ..LPEVK

1151 1200

N KNMSSSTDYN NYHSWKSQIA TIEQSAPSQS DQEQLFSNVQ YFAHYCRKYA
M EKHEFLNRLK QLPLE.... .

1201 1250

45 N PLYAAEAKRV FSLEKKMSNY IQFKSKCRIE PVCLLXHGSP GAGKSVATNL
M L.....

1251 1300

50 N IGRSLAEKLN SSVYSLPPDP DHFDGYKQQA VVIMDDLQON PDGKDVSLFC
M

1301 1350

N QMVSSVDFVP PMAALEEKGI LFTSPFVLAS TNAGSINAPT VSDSRALARR
M

1351 1400

55 N FHFDMNIEVI SMYSQNGKIN MPMSVKTCD E ECCPVNFKRC CPLVCGKAMQ

EP 2 826 856 B9

M
1401 1450
5 N FIDRRRTQVRY SLDMLVTEMF REYNHRHSVG ATLEALFQGP PVYREIKISV
M
1451 1500
N APETPPPPAI ADLLKSVDSE AVREYCKEKG WLVPEINSTL QIEKHVSRAF
MR.....
10 1501 1550
N ICLQALTTFFV SVAGIIYIIY KLFAGFQGAY TGMPNQKPKV PTLRQAKVQG
M
15 1551 1600
N PAFEFAMM KRNSSTVKTE YGEFTMLGIY DRWAVLPRHA KPGPTILMND
MA.....K.....
20 1601 1650
N QEVGVLDLDAKE LVDKDGNTLE LTLKLNSNE KFRDIRGFLA KEEVEVNEAV
MR.. R.....
25 1651 1700
N LAINTSKFPN MYIPVGQVTD YGFLNLGGTP TKRMLMYNFP TRAGQCGGVL
M
30 1701 1750
N MSTGKVLGIH VGGNGHQGFS AALLKHYFND EQGEIEFIES SKDAGFPIIN
MR....EV..
1751 1800
N TPSKTKLEPS VFHQCLKATK NPAVLRNGDP RLKANFEEAI FSKYIGNVNT
MVFEGN. E.....
35 1801 1850
N HVDEYMLEAV DHYAGQLATL DISTEPMKLE DAVYGTEGLE ALDLTTSAGY
M
1851 1900
N PYVALGIKKR DILSKKTRDL TKLKECMDKY GLNLPMVTYV KDELRSAEKV
MK.....
40 1901 1950
N AKGKSRLIEA SSLNDSVAMR QTFGNLYKTF HLNPGIVTGS AVGCDDPLFW
MV..
45 1951 2000
N SKIPVMLDGH LIAFDYSGYD ASLSPVWFAC LKLLLEKLG Y THKETNYIDY
MN.....
2001 2050
N LCNSHHLYRD KHYFVRGGMP SGCSGTSIFN SMINNIIIRT LMLKVYKGID
M
50 2051 2100
N LDQFRIIAYG DDVIASYPWP IDASLLAEAG KDYGLIMTPA DKGEFCNEVN
MM.....T
55 2101 2150
N WTNVTFLKRY FRADEQYPFL VHPVMPMKDI HESIRWTKDP KNTQDHRVSL
M

2151 2196
 N CLLAWHNGEH EYEEFIRKIR KRASWTLFDP TCVFNPAQEV VGLLLK
 MQ SVPVGRCLTL PAFSTLRRKW LDSF--

Sequence identity: 91%

Claims

1. Modified enterovirus of ECHO 7 type **characterized by** a genome sequence that has at least 85%, preferably at least 95%, still more preferably at least 99% of sequence identical to Seq ID No 1 ; and wherein changes in the sequence of the genome of the modified enterovirus are not larger than 0.7% after continuous propagation of the modified enterovirus in cell cultures for 12 months.
2. Modified enterovirus of ECHO 7 type, **characterized by** the genome sequence of Seq ID No 1.
3. Method for manufacturing a modified enterovirus of ECHO 7 type by modification of native ECHO 7 virus, identified by genome sequence Seq ID No 2, wherein the modification comprises conducting the virus adaptation in cancer cells, attenuated by an anti-cancer agent, subsequently passaging the modified virus in human embryonal fibroblast culture, propagating the modified virus in human melanoma cells, and subsequently passaging the modified virus in human embryonal fibroblast culture, optionally treated by ribavirin, and isolation and purification of the virus.
4. Method of claim 3, wherein the procedure of propagating the virus in cancer cells and subsequently passaging the modified virus in human embryonal fibroblast culture is repeated several times.
5. Method of claim 3 or 4, wherein the procedure of propagating the modified virus in human melanoma cells, and subsequently passaging the modified virus in human embryonal fibroblast culture is repeated several times.
6. Method of claim 3, 4 or 5, wherein the virus adaptation is conducted in cancer cells of at least two different cancers, such as human breast adenocarcinoma cells and gastric adenocarcinoma cells.
7. Method of any of claims 3-6, wherein the modification gives a modified enterovirus of ECHO 7 type, which is **characterized by** a genome sequence that has at least 85%, preferably at least 95%, still more preferably at least 99% of sequence identical to Seq ID No 1; and wherein in the modified enterovirus changes in the sequence of the genome are not larger than 0.7% after continuous propagation of the modified enterovirus in cell cultures for 12 months
8. Method of claim 7, wherein the modification gives a modified enterovirus of ECHO 7 type, which is **characterized by** a genome sequence of Seq ID No 1.
9. Method of claim 7 or 8, wherein the changes are one nucleotide replacements, which consist partly of mute mutations without change of corresponding amino acid.
10. The virus of claim 1 or 2 for use in treating oncological diseases.
11. The virus for use of claim 10, wherein the oncological disease is selected from the group consisting of: melanoma, gastric cancer, intestinal cancer, human breast cancer, prostate cancer, pancreatic cancer, lung cancer, kidney cancer, bladder cancer, lymphosarcoma, uterine cancer, angiosarcoma, rhabdomyosarcoma.
12. The virus for use of claim 10 wherein the oncological disease is melanoma.

Patentansprüche

1. Modifiziertes Enterovirus vom Typ ECHO 7, **gekennzeichnet durch** eine Genomsequenz, die mindestens 85%, vorzugsweise mindestens 95%, noch mehr bevorzugt mindestens 99% sequenzidentisch mit Seq ID Nr. 1 ist; und worin Änderungen in der Genomsequenz des modifizierten Enterovirus nach kontinuierlicher Vermehrung des modifizierten Enterovirus in Zellkulturen über 12 Monate nicht größer als 0,7% sind.

2. Modifiziertes Enterovirus vom Typ ECHO 7, **gekennzeichnet durch** die Genomsequenz Seq ID Nr. 1.
3. Verfahren zur Herstellung eines modifizierten Enterovirus vom Typ ECHO 7 durch Modifikation eines nativen ECHO-7-Virus, identifiziert durch Genomsequenz Seq ID Nr. 2, worin die Modifikation die Durchführung der Virusadaption in durch ein Anti-Krebsmittel abgeschwächten Krebszellen umfasst, anschließendes Passagieren des modifizierten Virus in humaner embryonaler Fibroblastenkultur, Vermehren des modifizierten Virus in humanen Melanomzellen und anschließendes Passagieren des modifizierten Virus in humaner embryonaler Fibroblastenkultur, gegebenenfalls behandelt mit Ribavirin und Isolierung und Reinigung des Virus.
4. Verfahren nach Anspruch 3, worin die Maßnahme zur Vermehrung des Virus in Krebszellen und anschließendes Passagieren des modifizierten Virus in humaner embryonaler Fibroblastenkultur mehrfach wiederholt werden.
5. Verfahren nach Anspruch 3 oder 4, worin die Maßnahme zur Vermehrung des modifizierten Virus in humanen Melanomzellen und anschließendes Passagieren des modifizierten Virus in humaner embryonaler Fibroblastenkultur mehrfach wiederholt werden.
6. Verfahren nach Anspruch 3,4 oder 5, worin die Adaption des Virus in den Krebszellen von mindestens zwei verschiedenen Krebsarten durchgeführt wird, wie z.B. humanen Mamma-Adenokarzinom- und gastrischen Adenokarzinomzellen.
7. Verfahren nach einem der Ansprüche 3-6, worin die Modifikation ein modifiziertes Enterovirus vom Typ ECHO 7 ergibt, welches durch eine Genomsequenz gekennzeichnet ist, die mindestens 85%, vorzugsweise mindestens 95%, noch mehr bevorzugt mindestens 99% sequenzidentisch mit Seq ID Nr. 1 ist; und worin Änderungen in der Genomsequenz des modifizierten Enterovirus nach kontinuierlicher Vermehrung des modifizierten Enterovirus in Zellkulturen über 12 Monate nicht größer als 0,7% sind.
8. Verfahren nach Anspruch 7, worin die Modifikation ein modifiziertes Enterovirus vom Typ ECHO 7 ergibt, welches durch die Genomsequenz Seq ID Nr. 1 gekennzeichnet ist.
9. Verfahren nach Anspruch 7 oder 8, worin die Änderungen Ein-Nukleotid-Austausche sind, die teilweise aus stummen Mutationen ohne Änderung der entsprechenden Aminosäure bestehen.
10. Das Virus nach Anspruch 1 oder 2 zur Verwendung in der Behandlung von onkologischen Erkrankungen.
11. Das Virus zur Verwendung nach Anspruch 10, worin die onkologische Erkrankung aus der Gruppe ausgewählt ist, bestehend aus: Melanom, Magenkrebs, Darmkrebs, humanem Brustkrebs, Prostatakrebs, Bauchspeicheldrüsenkrebs, Lungenkrebs, Nierenkrebs, Blasenkrebs, Lymphosarkom, Gebärmutterkrebs, Angiosarkom, Rhabdomyosarkom.
12. Das Virus zur Verwendung nach Anspruch 10, worin die onkologische Erkrankung ein Melanom ist.

Revendications

1. Entérovirus modifié de type ECHO 7 **caractérisé par** une séquence du génome qui présente au moins 85 %, préférentiellement au moins 95 %, plus préférentiellement encore au moins 99 % de séquence identique à SEQ ID NO : 1 ; et les changements dans la séquence du génome de l'entérovirus modifié n'étant pas de plus de 0,7 % après la propagation continue de l'entérovirus modifié dans des cultures cellulaires pendant 12 mois.
2. Entérovirus modifié de type ECHO 7, **caractérisé par** la séquence du génome de SEQ ID NO : 1.
3. Procédé de fabrication d'un entérovirus modifié de type ECHO 7 par modification du virus ECHO 7 natif, identifié par la séquence du génome SEQ ID NO : 2, la modification comprenant la conduction d'une adaptation du virus dans des cellules cancéreuses, atténuées par un agent anticancéreux, repiquage subséquent du virus modifié dans une culture de fibroblastes embryonnaires humains, propagation du virus modifié dans des cellules de mélanome humain, et repiquage subséquent du virus modifié dans une culture de fibroblastes embryonnaires humains, optionnellement traitée par de la ribavirine, et isolement et purification du virus.

4. Procédé selon la revendication 3, dans lequel la procédure de propagation du virus dans des cellules cancéreuses et de repiquage subséquent du virus modifié dans une culture de fibroblastes embryonnaires humains est répétée plusieurs fois.
- 5 5. Procédé selon la revendication 3 ou 4, dans lequel la procédure de propagation du virus modifié dans des cellules de mélanome humain, et de repiquage subséquent du virus modifié dans une culture de fibroblastes embryonnaires humains est répétée plusieurs fois.
- 10 6. Procédé selon la revendication 3, 4 ou 5, dans lequel l'adaptation du virus est conduite dans des cellules cancéreuses d'au moins deux cancers différents, telles que des cellules d'adénocarcinome du sein humain et des cellules d'adénocarcinome gastrique.
- 15 7. Procédé selon l'une quelconque des revendications 3 à 6, dans lequel la modification donne un entérovirus modifié de type ECHO 7, qui est **caractérisé par** une séquence du génome qui présente au moins 85 %, de préférence au moins 95 %, de façon encore davantage préférée au moins 99 % d'identité avec SEQ ID NO : 1; et dans lequel dans l'entérovirus modifié les changements dans la séquence du génome ne sont pas de plus de 0,7 % après la propagation en continu de l'entérovirus modifié dans des cultures cellulaires pendant 12 mois.
- 20 8. Procédé selon la revendication 7, dans lequel la modification donne un entérovirus modifié de type ECHO 7, qui est **caractérisé par** une séquence du génome de SEQ ID NO : 1.
9. Procédé selon la revendication 7 ou 8, dans lequel les changements sont des remplacements d'un nucléotide, qui consistent partiellement en des mutations muettes sans changement de l'acide aminé correspondant.
- 25 10. Virus selon la revendication 1 ou 2 pour une utilisation dans le traitement de maladies oncologiques.
- 30 11. Virus pour une utilisation selon la revendication 10, la maladie oncologique étant choisie dans le groupe constitué de : mélanome, cancer gastrique, cancer intestinal, cancer du sein humain, cancer de la prostate, cancer pancréatique, cancer du poumon, cancer du rein, cancer de la vessie, lymphosarcome, cancer utérin, angiosarcome, rhabdomyosarcome.
- 35 12. Virus pour une utilisation selon la revendication 10, la maladie oncologique étant un mélanome.

REFERENCES CITED IN THE DESCRIPTION

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