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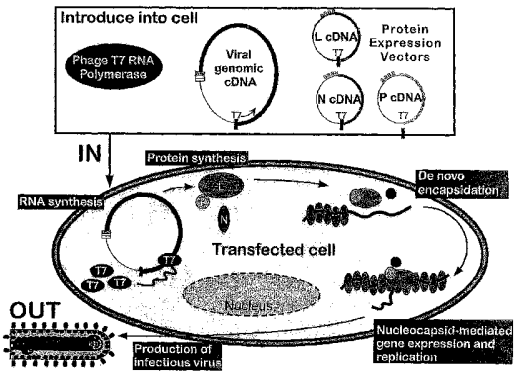
[Continued on next page]

(54) **Title:** IMPROVED METHOD FOR THE RECOVERY OF NON-SEGMENTED, NEGATIVE-STRANDED RNA VIRUSES FROM cDNA



(57) **Abstract:** Methods for producing infectious, non-segmented, negative-stranded RNA viruses of the Order *Mononegavirales* are provided that involve coexpression of a viral cDNA along with essential viral proteins, N, P, and L in a host cell transiently transfected with an expression vector encoding an RNA polymerase. In alternate methods, after the host cell is transfected with a viral cDNA expression vector and one or more vectors encoding the RNA polymerase, N protein, P protein, and L protein, the host cell is exposed to an effective heat shock under conditions sufficient to increase recovery of the recombinant virus. In other alternate embodiments, the host cells are transferred after viral rescue begins into co-culture with a plaque expansion cell, typically a monolayer of expansion cells, and the assembled infectious, non-segmented, negative-stranded RNA virus is recovered from the co-culture. Also provided within the invention are compositions for producing infectious, non-segmented, negative-stranded RNA virus of the Order *Mononegavirales,* recombinant viruses produced using the foregoing methods and compositions, and immunogenic compositions and methods employing the recombinant viruses. In additional embodiments, the methods and compositions of the invention are employed to produce growth- or replication-defective non-segmented negative-stranded RNA viruses and subviral particles.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 A61K39/12 C12N7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/00883 A (AMERICAN CYANAMID COMPANY; PARKS, CHRISTOPHER, L; SIDHU, MOHINDERJIT,) 3 January 2002 (2002-01-03) * entire document, in particular the cited passages *claims 1,17,46-48 page 18, line 22 - page 19, line 22; examples 4.1,5 page 31, lines 21-26; example 4.1 page 32, lines 5-11 page 32, lines 13-25; example 3.1.4 page 33, lines 6-16 page 33, line 28 - page 34, line 7 ----- -/--	1-111, 176-226

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
4 July 2005	14. 11. 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Brenz Verca, S

INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/63064 A (AMERICAN CYANAMID COMPANY; PARKS, CHRISTOPHER, L; SIDHU, MOHINDERJIT,) 9 December 1999 (1999-12-09) claim 44 page 15, line 5 - page 17, line 6 page 21, line 22 - page 22, line 16	1-111, 176-226
X	DAS SUBASH C ET AL: "Improved technique for transient expression and negative strand virus rescue using fowlpox T7 recombinant virus in mammalian cells" JOURNAL OF VIROLOGICAL METHODS, vol. 89, no. 1-2, September 2000 (2000-09), pages 119-127, XP002334309 ISSN: 0166-0934 the whole document	1,2, 19-22, 24,25, 58,60, 62-64, 66, 68-72, 78-81, 83,84
A	HARTY RONALD N ET AL: "Vaccinia virus-free recovery of vesicular stomatitis virus" JOURNAL OF MOLECULAR MICROBIOLOGY AND BIOTECHNOLOGY, vol. 3, no. 4, October 2001 (2001-10), pages 513-517, XP009050017 ISSN: 1464-1801 the whole document	
A	MURPHY B R ET AL: "Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics" JOURNAL OF CLINICAL INVESTIGATION, NEW YORK, NY, US, vol. 110, no. 1, July 2002 (2002-07), pages 21-27, XP002982924 ISSN: 0021-9738 the whole document	
A	COLLINS P L ET AL: "Respiratory Syncytial Virus: Reverse Genetics and Vaccine Strategies" VIROLOGY, ACADEMIC PRESS,ORLANDO, US, vol. 296, no. 2, 10 May 2002 (2002-05-10), pages 204-211, XP004467197 ISSN: 0042-6822 the whole document	

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SKIADOPOULOS M H ET AL: "GENERATION OF A PARAINFLUENZA VIRUS TYPE 1 VACCINE CANDIDATE BY REPLACING THE HN AND F GLYCOPROTEINS OF THE LIVE-ATTENUATED PIV3 CP45 VACCINE VIRUS WITH THEIR PIV1 COUNTERPARTS" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 18, no. 5/6, 14 October 1999 (1999-10-14), pages 503-510, XP001000985 ISSN: 0264-410X the whole document	
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A	ROBERTS A ET AL: "Attenuated vesicular stomatitis viruses as vaccine vectors" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 73, no. 5, May 1999 (1999-05), pages 3723-3732, XP002266675 ISSN: 0022-538X the whole document	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/018305

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-111, 176-226

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-111, 176-226

Methods of producing an infectious non-segmented negative-stranded RNA virus of the Order Mononegavirales; compositions for producing an infectious non-segmented negative- stranded RNA virus of the Order Mononegavirales; methods of producing a growth- or replication-defective non-segmented negative- stranded RNA virus of the Order Mononegavirales; composition for producing a growth- or replication-defective non-segmented negative- stranded RNA virus of the Order Mononegavirales.

Inventions 2-94: claims 112-175, 227-229

Infectious non-segmented negative-stranded RNA virus of the order Mononegavirales; immunogenic composition comprising said virus; method of stimulating the immune system of a mammalian comprising the administration of said virus, where each invention encompasses a different virus according to the following list :
Invention 2: Measles Virus in example IV and IX
Inventions 3-6: Parainfluenza Viruses (PIV) of table 5 in example V
Inventions 7-56: Respiratory Syncytial Viruses (RSV) of table 6 in example VIII
invention 57: Canine Distemper Virus (Onderstepoort strain) of table 7 in example IX
invention 58: Mumps Virus (Jeryl Lynn strain) of table 7 in example IX
invention 59: Respiratory synytial virus rA2cp deltaSH/M2-404/L248-404 of table 7 in example IX
invention 60: Bovine PIV (Shipping Fever strain) of table 7 in example IX
Inventions 61-64: chimeric bovine-human PIV of table 7 in example IX
Inventions 65-80: Vesicular Stomatitis Viruses (VSV) of table 8 in example IX
Inventions 81-84: VSV comprising gag constructs of table 9 in example IX
Inventions 85-87: h/bPIV of table 9 in example IX
Inventions 88-93: VSV expressing influenza virus glycoproteins of table 9 in example IX
Invention 94: bPIV expressing corona virus glycoprotein of table 9 in example IX

INTERNATIONAL SEARCH REPORT

ation on patent family members

International Application No
PCT/US2004/018305

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