

CAVELIER

ABOGADOS

www.cavelier.com
cavelier@cavelier.com

EDIFICIO SISKI
CARRERA 4 No. 72 - 35
BOGOTA 8, COLOMBIA

1149
000

Teléfono: (57-1) 347 3611
Telefax: (57-1) 211 8650
(57-1) 540 0860
Fax in U.S.A.: (1-305) 381 9660

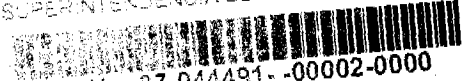
Señora Jefe de la

DIVISION DE NUEVAS CREACIONES

Superintendencia de Industria y Comer

E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No 07-044491-00002-0000

FECHA: 07-02-20 17:04:20 Dep. 2020 NUECREACIONE
TIT. 11 PATENTE-YO Eve: 378 FASENACIONALI
VAL. 114 RESPUESTAREQUE Folios: 18

Trámite : 011

Evento : 378

Actuaci

Referencia : Expediente No. 07.044.491

Asunto : Solicitud de patente para: **VECTORES**
QUIMERICOS

Solicitante : **MERIAL LIMITED**

Fecha solicitud: 4 de mayo de 2007

1. Yo, Emilio FERRERO, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al Oficio No. 8896, notificado por fijación en lista del 27 de julio de 2007.

2. Mediante el oficio No. 8896 se puso en conocimiento del solicitante el resultado del examen de forma realizado por ese Despacho, requiriéndole para completar el siguiente requisito:

2.1. Cesión: Para atender a la observación hecha por su Despacho, presento junto con este escrito el documento en que consta la cesión del derecho a la patente de los inventores Jiansheng Yao, Sheena May Loosmore y Jean-Christophe Francis Audonnet a favor de la sociedad **MERIAL LIMITED**, debidamente legalizado por Apostilla. Acompaño la traducción oficial al español de este documento.

TABLE 3. Reversibility of negative-strand synthesis

Expt	Incubation conditions	% of labeled RF RNA in negative-strand RNA ^a			
		<i>ts17</i>	<i>ts133</i>	SIN HR	24R1
1	Maintained at 30°C	1.9	1.8		
	Shifted to 40°C at 6 h p.i.	13.8	13.6		
	Shifted to 40°C at 6 h p.i., returned to 30°C at:				
	7 h p.i.	0.9	3.7		
	8 h p.i.	1.1			
2	9 h p.i.	0.9	4.3		
	Maintained at 30°C	2.0		1.4	4.8
	Shifted to 40°C at 6 h p.i.	11.2		1.8	16.7
	Shifted to 40°C at 6 h p.i., returned to 30°C at 9 h p.i.	2.4		<1.0	3.6

^a The values shown are averages of multiple hybridizations of RFs from two to five experiments.

pendent on the length of time spent at 40°C, since similar levels were reached after 1, 2, or 3 h of incubation.

To test the reversibility of negative-strand synthesis, cells were infected with each virus at a multiplicity of infection of 100 and were treated and labeled as described above except that cultures in experiment 1 were labeled from 9.5 to 10.5 h p.i. and cultures in experiment 2 were labeled from 9.5 to 11.5 h p.i. The cells were harvested by lysis at 0°C in buffer containing 1% Triton X-100 and scraped from the dish, the nuclei were removed by centrifugation at 1,000 rpm, and the supernatant was adjusted to 1% lithium dodecyl sulfate–100 µg of proteinase K per ml before extraction with phenol and chloroform and ethanol precipitation of the nucleic acids. The viral replicative forms (RFs) were purified, and the amount of labeled minus strands in the RF RNA was determined as described previously (25). Reactivation of *ts17* and *ts133* negative-strand synthesis was also temperature dependent and fully reversible in cultures treated with cycloheximide from the time of the shift to 40°C (Table 3). Reversal did not depend on the length of time spent at 40°C before shift-down. Table 3 also shows that this pattern resembled that for the nsP4 mutant 24R1.

Polyprotein processing. Although nsP1234 and nsP123 polyproteins synthesized at 40°C by *ts133* (Fig. 2) and *ts17* (data not shown; 11, 28) were not cleaved or poorly cleaved at 40°C compared with parental proteins, processing occurred when the cultures were shifted down and chased at 30°C. Because proteolytic processing of the nsPs was reversible, these mutations most likely affect the activity of nsP2 protease rather than the cleavage sites in the polyprotein. To mimic conditions used to demonstrate reactivation of negative-strand synthesis, we determined whether nonstructural polyproteins synthesized at 30°C were cleaved at 40°C to yield mature nsPs. As shown in Fig. 3A, the nsP2 defects in *ts17* and *ts133* allowed efficient processing of polyproteins at 30°C but reduced processing at 40°C. Moreover, immunoprecipitation (Fig. 3B) and quantitation of labeled *ts17* nsPs found that compared with the 30°C chase period, only 18 to 50% as much nsP34, nsP3, and nsP4 were present after the 40°C chase, while nsP2 levels were unchanged from levels present at the end of the 30°C pulse period. In summary, our analysis did not support the concept (3) that reactivation of negative-strand synthesis resulted from specific cleavage at 40°C of *ts17* polyproteins.

Recombinants possessing both mutant nsP2 and nsP4. Our data suggested that nsP2 and nsP4 interact functionally,

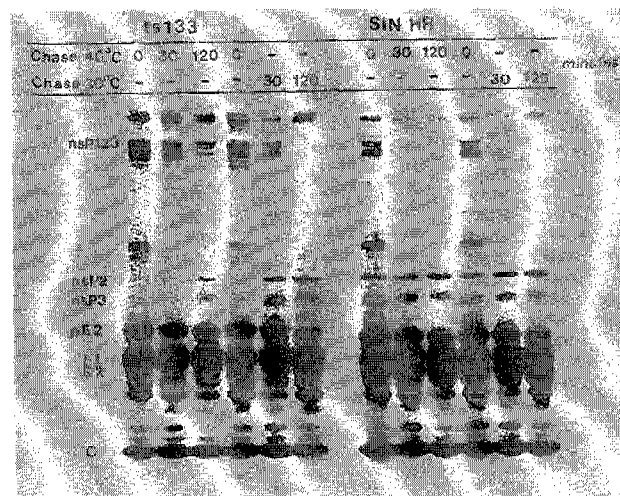


FIG. 2. Assay determining whether polyproteins produced at 40°C by *ts133*-infected cells were cleaved at 30°C but not at 40°C. Chicken embryo fibroblast cells infected with *ts133* or SIN HR were maintained at 30°C until 6 h p.i., when they were shifted to 40°C, treated with hypertonic salt medium (0.335 M NaCl in DMEM lacking methionine and containing 5% dialyzed fetal bovine serum and 22 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.4]) for 40 min, and given a 30-min pulse of [³⁵S]methionine (50 µCi/ml). Duplicate cultures were chased at either 40 or 30°C for the times indicated. Samples were electrophoresed on 5 to 10% polyacrylamide gels in Laemmli buffer (20) and fluorographed. An autoradiograph of the gel is shown.

since mutations in the two proteins show similar phenotypes. Furthermore, the conditional lethality of *ts118* requires mutations in both nsP2 and nsP4 (9). To probe such interactions, we created a recombinant genome that contained both the *ts17* nsP2 and 24R1 nsP4 lesions and tested them as follows. Infected cultures were shifted to 40°C at 6 h p.i. in the presence of cycloheximide (100 µg/ml) and labeled with [³H]uridine (200 µCi/ml in the presence of 20 µg of actinomycin D per ml) from 6.5 to 8.5 h p.i. Purified RF RNA was denatured and hybridized to an excess of purified SIN virion plus strands as described previously (25). Viruses possessing both mutant nsP2 and nsP4 genes (Toto:*ts17*:24R1) were viable and expressed all of the *ts17* phenotypes (Table 4). The double mutants also resumed negative-strand synthesis when shifted to 40°C but at higher levels typical of 24R1 viruses which possessed only the mutation in nsP4 (31). In cells infected with *ts17*, 24R1, and Toto:*ts17*:24R1, negative strands synthesized at 40°C were stable and accumulated as double-stranded RNA at a rate that was linear but higher for Toto:*ts17*:24R1 and 24R1 than for *ts17* (Fig. 4A). For all three viruses, significant fractions of the replicative intermediate and RF populations reactivated negative-strand transcription (Fig. 4B). Consistently, we observed in multiple experiments the accumulation of negative strands by the double mutant Toto:*ts17*:24R1 that was the sum of the amounts accumulated by 24R1 and *ts17*, and we concluded there was an additive effect when replication complexes contained both mutant nsP2 and nsP4 and that the nsP2 defect did not interfere with or alter significantly the expression of the nsP4 defect or vice versa. However, replication complexes possessing mutant nsP4 were more efficient at reactivating negative-strand synthesis than were those containing only mutant nsP2. These results suggest that the nsPs

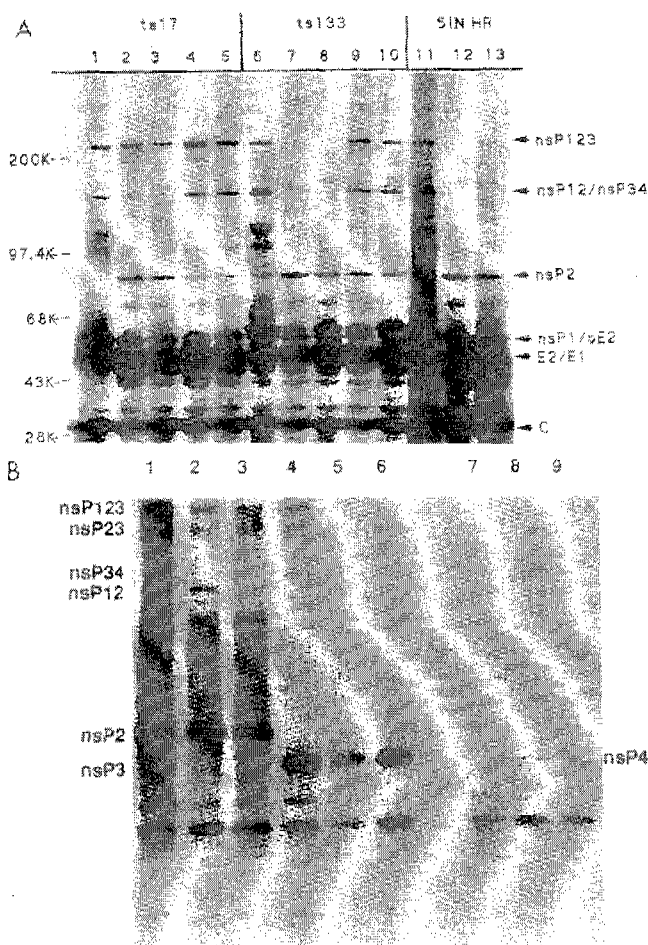


FIG. 3. Assays determining whether polyproteins produced at 30°C by *ts17*- and *ts133*-infected cells were cleaved in the presence or absence of cycloheximide at 30°C but not at 40°C. (A) Chicken embryo fibroblast cells in 35-mm-diameter petri dishes were infected with *ts17*, *ts133*, or SIN HR at a multiplicity of infection of 100 and maintained at 30°C. At 4 h p.i., the cultures were incubated in medium lacking methionine and containing 2 µg of actinomycin D per ml, 5% dialyzed fetal bovine serum, and 22 mM HEPES (pH 7.4) for 2 h and then treated for 90 min with the same medium containing 0.335 M NaCl. Cultures were pulsed with [³⁵S]methionine (50 µCi/ml) for 30 min at 30°C, after which they were either harvested immediately (lanes 1, 6, and 11) or chased for 60 min at 30°C in the absence (lanes 2, 7, and 12) or presence (lanes 3, 8, and 13) of 100 µg of cycloheximide per ml. Duplicate cultures were shifted to 40°C and chased at 40°C for a further 60 min in the absence (lanes 4 and 9) or presence (lanes 5 and 10) of cycloheximide. Samples were electrophoresed together with molecular weight markers on 5 to 10% polyacrylamide gels in Laemmli buffer (20). (B) Immunoprecipitation of *ts17* nsPs. Cultures of *ts17*-infected cells were treated with hypertonic medium from 5 to 6 h p.i. and then subjected to a 1-h pulse of [³⁵S]methionine (200 µCi/ml) at 30°C. Duplicate cultures were harvested immediately after the pulse (lanes 1, 4, and 7) or were chased in the presence of cycloheximide from 7 to 8 h p.i. at 40°C (lanes 2, 5, and 8) or at 30°C (lanes 3, 6, and 9). Samples were denatured and reacted with nsP2 antibodies (lanes 1 to 3), nsP3 antibodies (lanes 4 to 6), or nsP4 antibodies (lanes 7 to 9) as described previously (2). An autoradiograph of the resulting gel is shown.

retain and express individual functions when assembled together in transcriptionally active complexes (2).

To conclude, we report that defective nsP2 proteins in preformed replication complexes were responsible for loss

TABLE 4. Evidence that recombinant viruses with nsP2 and nsP4 mutations expressed conditionally lethal nsP2 phenotypes but levels of negative-strand synthesis typical of mutant nsP4

Virus ^a	Growth	RNA negative	26 mRNA synthesis	% labeled RF RNA in negative strands (range) ^b
Totol101	wt ^c	No	wt	4.1 (2-6)
24R1	wt	No	wt	27.6 (19-34)
<i>ts17</i>	<i>ts</i>	Yes	<i>ts</i>	15.9 (12-20)
<i>ts17</i> :24R1.1	<i>ts</i>	Yes	<i>ts</i>	29.2 (17-39)
<i>ts17</i> :24R1.2	<i>ts</i>	Yes	<i>ts</i>	34.1 (32-35)

^a The *ts17*:24R1.1 and *ts17*:24R1.2 viruses were derived from two independently isolated hybrid clones.

^b The range of values is from four individual determinations, whose average values are listed.

^c wt, wild type.

of subgenomic synthesis and reactivation of negative-strand synthesis by *ts17* and *ts133*. The *ts17* and *ts133* mutations did not affect the putative nsP2 helicase activity because transcription was not inhibited after a shift to 40°C (19). Second, reactivation of negative-strand synthesis, once ceased in infected cells, was induced by alterations in either of two different and stable proteins, nsP2 or nsP4 (25), and not by cleavage of a nsP34 precursor as proposed by deGroot et al. (3). Reactivation of negative-strand synthesis caused neither an increase in the rate of positive-strand synthesis nor formation of additional replication complexes (this study; 25, 31). Therefore, we argue that reactivation must be due to the replacement of the preferred template of the replication complex, the negative strand, with a positive-strand template that in turn is copied into a negative strand. Thus, reactivation results from template switching by previously formed replication complexes. In addition, our results suggest that nsP2 and nsP4 function differently in template recognition and initiation of negative-strand synthesis, since the amount of negative-strand synthesis was greater when replication complexes contained both mutant nsP2 and nsP4. Not all nsP2 mutations conferring *ts* 26S mRNA synthesis reactivated negative-strand synthesis (10, 17, 28). Reversibility of reactivation suggests that these particular nsP2 and nsP4 defects induce changes in protein interactions, possibly with limiting host factors (15, 21, 25, 26), or changes in folding or conformation of the nsPs that modify the structure or affinity of the positive-strand polymerase to resemble the negative-strand polymerase and thus allow recognition of the 3' promoter on a positive strand. We interpret such findings as supporting our model (25, 29, 31): a replication complex is formed by the association of newly synthesized nsPs with a positive-strand template and synthesizes a negative strand. The newly created negative strand becomes the preferred template of the replication complex. Both nsP2 and nsP4 would function normally to fix the negative strand as the stable template of alphavirus replication complexes. Because both nsPs are essential for negative-strand synthesis, subsequent modifications that restrict their activity to positive-strand synthesis would shorten the functional half-life of the negative-strand polymerase. This, in turn, would convert the replication complex from negative-strand to positive-strand synthesis and thereby lead to overproduction of positive strands relative to negative strands in infected cells and to cessation of negative-strand synthesis if no new replication complexes form. Given their resemblance to SIN, the mechanism for cessation of negative-strand synthesis may be common to other superfamily members. The

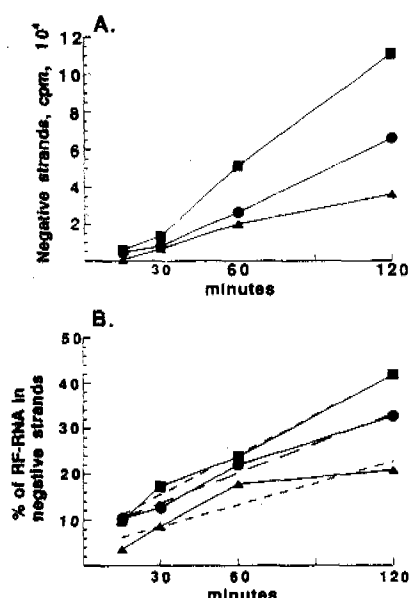


FIG. 4. Accumulation of negative strand RNA at 40°C by *ts17*, *ts24R1*, and the double mutant *Toto:ts17:R1*. Chicken embryo fibroblast cultures in 60-mm-diameter petri dishes were infected at a multiplicity of infection of 100 with *ts17* (▲), *ts24R1* (●), or *Toto:ts17:R1* (■) at 30°C and maintained at 30°C until 6 h p.i., when they were shifted to 40°C and incubated continuously in the presence of 100 µg of cycloheximide per ml. At 6.5 h p.i., the cultures were labeled at 40°C with [³H]uridine (500 µCi/ml, 2 ml per petri dish; ICN, Irvine, Calif.) and were harvested 15, 30, 60, or 120 min later. To measure negative-strand RNA synthesized at 40°C in the presence of cycloheximide, the double-stranded cores (RF RNA) of the viral replicative intermediates were purified by chromatography on CF-11, denatured, and hybridized in the presence of an excess of unlabeled genomic positive strands (25). Typically, 6×10^4 to 8×10^4 cpm in labeled RF RNA per 10⁶ infected cells is obtained after a 60-min pulse with 200 µCi of [³H]uridine per ml. The results shown represent the average values from duplicate experiments. (A) Accumulation of incorporation of [³H]uridine in the negative-strand component of RF RNAs; (B) percentage of the total labeled RF RNA that was in labeled negative-strand RNA. Because the replicative intermediates are active also in positive-strand synthesis, a value of 50% of the labeled RF RNA in negative-strand RNA is taken to mean that 100% of RFs contain newly synthesized negative strands.

intriguing aspect of such a mechanism is its stability under normal conditions but its *ts* reversibility in certain SIN nsP2 and nsP4 mutants.

We thank J. H. Strauss for providing *ts17* recombinant cDNAs and monospecific nsP antibodies, J. Lemm and C. M. Rice for providing the deletion plasmids for subcloning, and I. Dé for performing the immunoprecipitations shown in Fig. 3B.

We acknowledge support from the Public Health Service through NIAID award AI-15123.

REFERENCES

- Ahlquist, P., E. Strauss, C. Rice, J. Strauss, J. Haseloff, and D. Zimmern. 1985. Sindbis virus proteins nsP1 and nsP2 contain homology to nonstructural proteins from several RNA plant viruses. *J. Virol.* 53:536-542.
- Barton, D. J., S. G. Sawicki, and D. L. Sawicki. 1991. Solubilization and immunoprecipitation of alphavirus replication complexes. *J. Virol.* 65:1496-1506.
- deGroot, R. J., W. R. Hardy, Y. Shirako, and J. H. Strauss. 1990. Cleavage-site preferences of Sindbis virus polyproteins containing the non-structural proteinase. Evidence for temporal regulation of polyprotein processing in vivo. *EMBO J.* 9:2631-2638.
- Ding, M., and M. J. Schlesinger. 1989. Evidence that Sindbis virus nsP2 is an autoprotease which processes the virus non-structural polyprotein. *Virology* 171:280-284.
- Dominguez, G., C.-Y. Wang, and T. K. Frey. 1990. Sequence of the genome RNA of rubella virus: evidence for genetic rearrangement during togavirus evolution. *Virology* 177:225-238.
- Faragher, S. G., A. D. J. Meek, C. M. Rice, and L. Dalgarno. 1988. Genome sequences of a mouse-avirulent and a mouse-virulent strain of Ross river virus. *Virology* 163:509-526.
- Goldbach, R., O. Le Gall, and J. Wellink. 1991. Alpha-like viruses in plants. *Semin. Virol.* 2:19-25.
- Gorbalenya, A. E., E. V. Koonin, and M. M. C. Lai. 1991. Putative papain-related thiol proteases of positive-strand RNA viruses. *FEBS Lett.* 288:201-205.
- Hahn, Y. S., A. Grakoui, C. M. Rice, E. G. Strauss, and J. H. Strauss. 1989. Mapping of RNA⁻ temperature-sensitive mutants of Sindbis virus: complementation group F mutants have lesions in nsP4. *J. Virol.* 63:1194-1202.
- Hahn, Y. S., E. G. Strauss, and J. H. Strauss. 1989. Mapping of RNA⁻ temperature-sensitive mutants of Sindbis virus: assignment of complementation groups A, B, and G to nonstructural proteins. *J. Virol.* 63:3142-3150.
- Hardy, W. R., Y. S. Hahn, R. J. deGroot, E. G. Strauss, and J. H. Strauss. 1990. Synthesis and processing of the nonstructural polyproteins of several temperature-sensitive mutants of Sindbis virus. *Virology* 177:199-208.
- Hardy, W. R., and J. H. Strauss. 1988. Processing the nonstructural polyproteins of Sindbis virus: study of the kinetics in vivo by using monospecific antibodies. *J. Virol.* 62:998-1007.
- Hardy, W. R., and J. H. Strauss. 1989. Processing the nonstructural polyprotein of Sindbis virus: the nonstructural proteinase is in the C-terminal half of nsP2 and functions both in *cis* and in *trans*. *J. Virol.* 63:4653-4664.
- Hodgman, T. C. 1988. A new superfamily of replicative proteins. *Nature (London)* 332:22-23. (Erratum, 333:578.)
- Ishikawa, M., T. Meshi, T. Ohno, and Y. Okada. 1991. Specific cessation of minus-strand RNA accumulation at an early stage of tobacco mosaic virus infection. *J. Virol.* 65:861-868.
- Kamer, G., and P. Argos. 1984. Primary structural comparison of RNA-dependent polymerases from plant, animal and bacterial viruses. *Nucleic Acids Res.* 12:7269-7282.
- Keränen, S., and L. Kääriäinen. 1979. Functional defects of RNA-negative temperature-sensitive mutants of Sindbis and Semliki Forest virus. *J. Virol.* 32:19-29.
- Kinney, R., B. J. B. Johnson, J. B. Welch, K. R. Tschia, and D. W. Trent. 1989. The full length nucleotide sequences of the virulent Trinidad donkey strain of Venezuelan equine encephalitis virus and its attenuated vaccine derivative, strain TC-83. *Virology* 170:19-30.
- Kroner, P. A., B. M. Young, and P. Ahlquist. 1990. Analysis of the role of brome mosaic virus 1a protein domains in RNA replication, using linker insertion mutagenesis. *J. Virol.* 64:6110-6120.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- Lemm, J. A., R. K. Durbin, V. Stollar, and C. M. Rice. 1990. Mutations which alter the level or structure of nsP4 can affect the efficiency of Sindbis virus replication in a host-dependent manner. *J. Virol.* 64:3001-3011.
- Mi, S., and V. Stollar. 1991. Expression of Sindbis virus nsP1 and methyltransferase activity in *Escherichia coli*. *Virology* 184:423-427.
- Rice, C. M., R. Levis, J. H. Strauss, and H. V. Huang. 1987. Production of infectious RNA transcripts from Sindbis virus cDNA clones: mapping of lethal mutations, rescue of a temperature-sensitive marker, and in vitro mutagenesis to generate defined mutants. *J. Virol.* 61:3809-3819.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.

25. Sawicki, D. L., D. B. Barkhimer, S. G. Sawicki, C. M. Rice, and S. Schlesinger. 1990. Temperature sensitive shutoff of alphavirus minus strand RNA synthesis maps to a nonstructural protein, nsP4. *Virology* 174:43-52.
26. Sawicki, D. L., L. Kääriäinen, C. Lambek, and P. J. Gomas. 1978. Mechanism for control of synthesis of Semliki Forest virus 26S and 42S RNA. *J. Virol.* 25:19-27.
27. Sawicki, D. L., and S. G. Sawicki. 1980. Short-lived minus-strand polymerase for Semliki Forest virus. *J. Virol.* 34:108-118.
28. Sawicki, D. L., and S. G. Sawicki. 1985. Functional analysis of the A complementation group mutants of Sindbis HR virus. *Virology* 144:20-34.
29. Sawicki, D. L., and S. G. Sawicki. 1987. Alphavirus plus and minus strand RNA synthesis, p. 251-259. *In* M. Brinton and R. Ruckert (ed.), *Positive-strand RNA viruses*. Alan R. Liss, Inc., New York.
30. Sawicki, D. L., S. G. Sawicki, S. Keränen, and L. Kääriäinen. 1981. A Sindbis virus coded function for minus-strand synthesis. *J. Virol.* 39:348-358.
31. Sawicki, S. G., and D. L. Sawicki. 1986. The effect of loss of regulation of minus strand RNA synthesis on Sindbis virus replication. *Virology* 151:339-349.
32. Sawicki, S. G., D. L. Sawicki, L. Kääriäinen, and S. Keränen. 1981. A Sindbis virus mutant temperature-sensitive in regulation of minus strand synthesis. *Virology* 115:507-512.
33. Shirako, Y., B. Niklasson, J. M. Dalrymple, E. G. Strauss, and J. H. Strauss. 1991. Structure of the Ockelbo virus genome and its relationship to other Sindbis viruses. *Virology* 182:753-764.
34. Shirako, Y., and J. H. Strauss. 1990. Cleavage between nsP1 and nsP2 initiates the processing pathway of Sindbis virus nonstructural polyprotein P123. *Virology* 177:54-64.
35. Strauss, E. G., R. deGroot, R. Levinson, and J. H. Strauss. 1992. Identification of the active site residues in the nsP2 proteinase of Sindbis virus. *Virology* 191:932-940.
36. Strauss, E. G., R. Levinson, C. M. Rice, J. Dalrymple, and J. H. Strauss. 1988. Nonstructural proteins nsP3 and nsP4 of Ross River and O'Nyong-nyong viruses: sequence and comparison with those of other alphaviruses. *Virology* 164:265-274.
37. Strauss, E. G., C. M. Rice, and J. H. Strauss. 1984. Complete nucleotide sequence of the genomic RNA of Sindbis virus. *Virology* 133:92-110.
38. Strauss, J. H., and E. G. Strauss. 1990. Alphavirus proteinases. *Semin. Virol.* 1:347-356.
39. Takkinen, K. 1986. Complete nucleotide sequence of the non-structural protein genes of Semliki Forest virus. *Nucleic Acids Res.* 14:5667-5682.

A 7066500

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 07, 2007

**THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE
RECORDS OF THIS OFFICE OF A DOCUMENT RECORDED ON
JUNE 15, 2005.**

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office



T. Lawrence

T. LAWRENCE
Certifying Officer

4/15/05

35
010

06-23-2005

Form PTO-1595 (Rev. 10/02) OMB No. 0651-0027 (exp. 6/30/2005) Tab settings ⇌ ⇌ ⇌ ▼ ▼		REC 103026388	U.S. DEPARTMENT OF COMMERCE U.S. Patent and Trademark Office
To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.			
1. Name of conveying party(ies): YAO, Jiansheng LOOSMORE, Sheena May AUDONNET, Jean-Christophe Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No		2. Name and address of receiving party(ies) Name: Merial Limited Internal Address: _____ Street Address: 3239 Satellite Blvd. City: Duluth State: GA Zip: 30096 Additional name(s) & address(es) attached? ☐ Yes ☒ No	
3. Nature of conveyance: ☒ Assignment ☐ Merger ☐ Security Agreement ☐ Change of Name ☐ Other _____ Execution Date: 02/18/2005			
4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application is: 10/05/2004 A. Patent Application No.(s) 10/958,267 B. Patent No.(s) _____ Additional numbers attached? ☐ Yes ☒ No			
5. Name and address of party to whom correspondence concerning document should be mailed: Name: Dr. Judy Jarecki-Black Internal Address: Merial Limited Street Address: 3239 Satellite Blvd. City: Duluth State: GA Zip: 30096		6. Total number of applications and patents involved: ☐ 7. Total fee (37 CFR 3.41).....\$ _____ ☐ Enclosed ☒ Authorized to be charged to deposit account 8. Deposit account number: 502354	
DO NOT USE THIS SPACE			
9. Signature. Dr. Judy Jarecki-Black Name of Person Signing		Signature 15 June 2005 Date	
Total number of pages including cover sheet, attachments, and documents: 5			

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents & Trademarks, Box Assignments
Washington, D.C. 20231

06/22/2005 ECDUPER 00000102 502354 10958267

01 FC:8021

40.00 DA

PATENT
REEL: 016702 FRAME: 0812

011
~~54~~

ASSIGNMENT

● We, Jiansheng YAO, 74 Loganberry Crescent, North York, Ontario, M2H 3H1, Canada, Sheena May LOOSMORE, 70 Crawford Rose Drive, Aurora, Ontario L4G 4R4, Canada and Jean-Christophe Francis AUDONNET, 19, rue de Créqui Lyon, France 69006 have made certain inventions or discoveries (or both) set forth in an application for Letters Patent of the United States of America entitled: **CHIMERIC VECTORS** and **MERIAL LIMITED**, a company limited by shares Registered in England And Wales No. 3332751, with registered office at P.O. BOX 327, Sandringham House, Sandringham Avenue, Harlow business Park, Harlow Essex CM19 5TG, England, and domesticated in Delaware, USA as **MERIAL LLC**, with a place of business at 3239 Satellite Blvd., Duluth, GA 30096-4640, USA, and which, together with its successors and assigns is hereinafter called "Assignee," is desirous of acquiring the title, rights, benefits and privileges hereinafter recited, and of confirming the same or any part thereof heretofore acquired by Assignee.

Now, therefore, for valuable consideration furnished by Assignee to us, receipt and sufficiency of which we hereby acknowledge, we hereby, without reservation:

1. Assign and convey to and confirm in Assignee the entire right, title and interest in and to said inventions and discoveries, said application for Letters Patent of the United States of America, any and all other applications for Letters Patent on said inventions and discoveries in whatsoever countries, including all divisional, renewal, substitute, continuation and Convention applications based in whole or in part upon said inventions or discoveries or upon said applications, and any and all Letters Patent and reissues and extensions of Letters Patent granted for said inventions and discoveries or upon said applications, and every priority right that is or may be predicated upon or arise from said inventions, said discoveries, said applications and said Letters Patent;

2. Authorize Assignee to file patent applications in any or all countries on any or all of said inventions and discoveries in our names or in the name of Assignee or otherwise as Assignee may deem advisable, under the International Convention or otherwise;

3. Authorize and request the Commissioner of Patents of the United States of America and the empowered officials of all other governments to issue or transfer all said Letters Patent to Assignee, as assignee of the entire right, title and interest therein or otherwise as Assignee may direct;

4. Warrant that we have not knowingly conveyed to others any right in said inventions, discoveries, applications or patents or any license to use the same or to make, use or sell anything embodying or utilizing any of said

~~505~~

- inventions or discoveries; and that we have good right to assign the same to
- Assignee without encumbrance;

5. Bind our heirs and legal representatives, as well as ourselves to do, upon Assignee's request and at its expense, but without additional consideration to us or them, all acts reasonably serving to assure that the said inventions and discoveries, the said patent applications and the said Letters Patent shall be held and enjoyed by Assignee as fully and entirely as the same could have been held and enjoyed by us or our heirs or representatives if this assignment had not been made; and particularly to execute and deliver to Assignee all lawful application documents including petitions, specifications, and oaths, and all assignments, disclaimers, and lawful affidavits in form and substance as may be requested by Assignee; to communicate to Assignee all facts known to us relating to said inventions and discoveries or the history thereof; and to furnish Assignee with any and all documents, photographs, models, samples and other physical exhibits in our control or in the control of our heirs or legal representatives and which may be useful for establishing the facts of our conceptions, disclosures, and reduction to practice of said inventions and discoveries.

In testimony of which we have affixed our signatures.

Jiansheng Yao

Date of Signature

Signature:

Jiansheng YAO

74 Loganberry Crescent, North York,
Ontario, M2H 3H1, Canada

Date of Signature

Signature:

Sheena May LOOSMORE

70 Crawford Rose Drive
Aurora, Ontario L4G 4R4

Date of Signature

Signature:

Jean-Christophe Francis AUDONNET

19, rue de Créqui
Lyon, France 69006

- inventions or discoveries; and that we have good right to assign the same to Assignee without encumbrance;

5. Bind our heirs and legal representatives, as well as ourselves to do, upon Assignee's request and at its expense, but without additional consideration to us or them, all acts reasonably serving to assure that the said inventions and discoveries, the said patent applications and the said Letters Patent shall be held and enjoyed by Assignee as fully and entirely as the same could have been held and enjoyed by us or our heirs or representatives if this assignment had not been made; and particularly to execute and deliver to Assignee all lawful application documents including petitions, specifications, and oaths, and all assignments, disclaimers, and lawful affidavits in form and substance as may be requested by Assignee; to communicate to Assignee all facts known to us relating to said inventions and discoveries or the history thereof; and to furnish Assignee with any and all documents, photographs, models, samples and other physical exhibits in our control or in the control of our heirs or legal representatives and which may be useful for establishing the facts of our conceptions, disclosures, and reduction to practice of said inventions and discoveries.

In testimony of which we have affixed our signatures.

Date of Signature Signature: **Jiansheng YAO**
74 Loganberry Crescent, North York,
Ontario, M2H 3H1, Canada

Oct 12, 2004 *Sheena May Loosmore*
Date of Signature Signature: **Sheena May LOOSMORE**
70 Crawford Rose Drive
Aurora, Ontario L4G 4R4

Date of Signature Signature: **Jean-Christophe Francis AUDONNET**
19, rue de Créqui
Lyon, France 69006

inventions or discoveries; and that we have good right to assign the same to Assignee without encumbrance;

5. Bind our heirs and legal representatives, as well as ourselves to do, upon Assignee's request and at its expense, but without additional consideration to us or them, all acts reasonably serving to assure that the said inventions and discoveries, the said patent applications and the said Letters Patent shall be held and enjoyed by Assignee as fully and entirely as the same could have been held and enjoyed by us or our heirs or representatives if this assignment had not been made; and particularly to execute and deliver to Assignee all lawful application documents including petitions, specifications, and oaths, and all assignments, disclaimers, and lawful affidavits in form and substance as may be requested by Assignee; to communicate to Assignee all facts known to us relating to said inventions and discoveries or the history thereof; and to furnish Assignee with any and all documents, photographs, models, samples and other physical exhibits in our control or in the control of our heirs or legal representatives and which may be useful for establishing the facts of our conceptions, disclosures, and reduction to practice of said inventions and discoveries.

In testimony of which we have affixed our signatures.

Date of Signature

Signature:

Jiansheng YAO

74 Loganberry Crescent, North York,
Ontario, M2H 3H1, Canada

Date of Signature

Signature:

Sheena May LOOSMORE

70 Crawford Rose Drive
Aurora, Ontario L4G 4R4

OCTOBER 13, 2004

Date of Signature

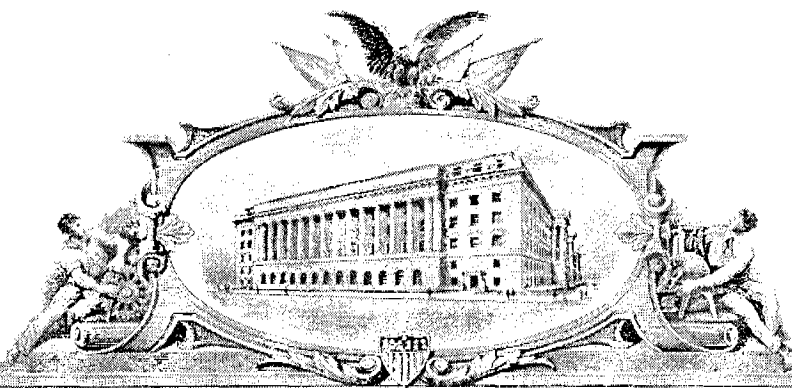
Signature:

Jean-Christophe Francis AUDONNET

119, rue de Créqui
Lyon, France 69006

516

TRADUCCIÓN OFICIAL No. 05.25/07a realizada por Diego Vargas, Traductor Oficial, Resolución No. 0001 de 1999 del Ministerio de Justicia y registrado ante el Ministerio de Relaciones Exteriores de Colombia.



A 431748

LOS ESTADOS UNIDOS DE AMÉRICA

A TODOS A QUIENES LOS PRESENTES LLEGAREN:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS

07 de Mayo de 2007

LA PRESENTE ES PARA CERTIFICAR QUE LA ADJUNTA ES UNA COPIA FIEL DE LOS ARCHIVOS DE ESTA OFICINA DE UN DOCUMENTO INSCRITO EL 15 de JUNIO de 2005.



Por autorización del
Subsecretario de Comercio para la Propiedad
Intelectual y Director de la Oficina de
Marcas y Patentes de los Estados Unidos

Firma Ilegible

T. LAWRENCE
Funcionario de Autenticaciones

Sello: * Oficina de Marcas y Patentes * Estados Unidos de América *

06-23-2005

Formulario PTO-1595 (Rev 6-93)		CÓDIGO DE BARRAS 103026388							
Al Honorable Comisionado de Marcas y Patentes: Por favor registre los documentos adjuntos originales o copias de los mismos:									
1. Nombre de la(s) parte(s) transferentes: YAO, Jiansheng LOOSMORE, Sheena May AUDONNET, Jean-Christophe Francis Se adjunta(n) nombre(s) adicionales de las partes transferentes? ☐ Sí ☒ No		2. Nombre y dirección de la(s) parte(s) receptora: Nombre: <u>Merial Limited</u> Dirección Interna: _____ Dirección: <u>3239 Satellite Blvd.</u> Ciudad: <u>Duluth</u> Estado: <u>GA</u> Zona Postal: <u>30096</u> Nombre(s) y dirección(es) adicionales adjuntos? ☐ Sí ☒ No							
3. Naturaleza de Transferencia: ☒ Cesión ☐ Fusión ☐ Acuerdo de Garantía ☐ Cambio de Nombre ☐ Otro _____ Fecha de Celebración: <u>02/18/2005</u>									
4. Número(s) de Solicitud o Patente(s): Si este documento se presenta conjuntamente con una nueva solicitud, la fecha de celebración de la solicitud es: <u>10/05/2004</u> A. Solicitud Patente No(s). <u>10/958,267</u> B. Patente No.(s) _____ Números adicionales anexos? ☐ Sí ☒ No									
5. Nombre y dirección de la parte a quien se debe enviar la documentación concerniente: Nombre: <u>Dr. Judy Jarecki-Black</u> Dirección Interna: _____ Dirección: <u>3239 Satellite Road</u> Ciudad: <u>Duluth</u> Estado: <u>GA</u> Zona Postal: <u>30096</u>		6. Número total de solicitudes y patentes involucradas: 7. Tasa total (37CFR3.41): <u>\$40.00 (Dólares)</u> ☐ Adjunta ☒ Se autoriza a ser cargada a la cuenta de depósito 8. Cuenta de Depósito número: <u>502354</u>							
NO UTILICE ESTE ESPACIO									
9. Firma. <table border="0" style="width: 100%;"> <tr> <td style="width: 33%; text-align: center;"><u>Judy Jarecki-Black</u></td> <td style="width: 33%; text-align: center;"><u>Firma Ilegible</u></td> <td style="width: 33%; text-align: center;"><u>15 de Junio de 2005</u></td> </tr> <tr> <td style="text-align: center;">Nombre del signatario</td> <td style="text-align: center;">Firma</td> <td style="text-align: center;">Fecha</td> </tr> </table> Número total de páginas incluyendo cubierta, adjuntos y documento: 5				<u>Judy Jarecki-Black</u>	<u>Firma Ilegible</u>	<u>15 de Junio de 2005</u>	Nombre del signatario	Firma	Fecha
<u>Judy Jarecki-Black</u>	<u>Firma Ilegible</u>	<u>15 de Junio de 2005</u>							
Nombre del signatario	Firma	Fecha							

Envíe por correo los documentos a registrar con la información de cubierta requerida a:
 Commissioner of Patents & Trademarks, Box Assignments
 Washington, D.C. 20231

06/22/2005 ECOOPER 00000102 502354 10958267
 01 FC:8021 40.00 DA

PATENTE
 ROLLO: 016702 CUADRO: 0812

CESIÓN

Nosotros, **Jiansheng YAO**, 74 Loganberry Crescent, North York, Ontario, M2H 3H1, Canadá, **Sheena May LOOSMORE**, 70 Crawford Rose Drive, Aurora, Ontario L4G 4R4, Canadá y **Jean-Christophe Francis AUDONNET**, 19, rue de Créqui Lyon, Francia 69006 hemos realizado

ciertas invenciones o descubrimientos (o ambos) indicados en una solicitud de patente de los Estados Unidos titulada: **VECTORES QUIMÉRICOS** y **Merial LIMITED**, una compañía limitada por acciones Registrada en Inglaterra y Gales con matrícula No. 3332751, domiciliada en P.O. Box 327, Sandringham House, Sandringham Avenue, Harlow Business Park, Harlow Essex CM19 5TG, Inglaterra, y nacionalizada en **Delaware, Estados Unidos de América como Merial LLC** y domiciliada en **3239 Satellite Boulevard, Duluth, Georgia 30096, Estados Unidos de América**, y la cual junto con sus sucesores y cesionarios se denominan en el presente "Cesionario" desea adquirir el título, derechos, beneficios y privilegios mencionados a continuación y confirmar la misma o cualquier parte de la misma hasta el momento adquirida por el Cesionario.

Por consiguiente, por contraprestación válida provista por el Cesionario a nosotros, el recibo y suficiencia de la cual por el presente reconocemos, nosotros por el presente, sin limitación:

1. Cedemos y transferimos y confirmamos en el Cesionario todo el derecho, título e interés en y a dichas invenciones y descubrimientos, dicha solicitud de patente de los Estados Unidos, así como todas las demás solicitudes de patente para dichas invenciones y descubrimientos en todos los países, incluyendo todas las solicitudes de división, renovación, sustitutivas, de continuación y de Convención que estén basadas total o parcialmente en dichas invenciones o descubrimientos o en dichas solicitudes, así como cualquier y todas las patentes y nuevas publicaciones y extensiones de patente otorgadas a dichas invenciones y descubrimientos o a dichas solicitudes, así como todos los derechos de prioridad que se puedan reivindicar o surjan de dichas invenciones, dichos descubrimientos, dichas solicitudes y dichas patentes y dichas patentes de invención;