US 20220062375A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2022/0062375 A1

STINCHCOMB et al. (43) Pub. Date: Mar. 3, 2022

(54) **COMPOSITIONS AND METHODS FOR DENGUE VIRUS CHIMERIC CONSTRUCTIONS IN VACCINES**

(71) Applicants: **TAKEDA VACCINES, INC.**, Cambridge, MA (US); **THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT**, Atlanta, GA (US)

(72) Inventors: **Dan T. STINCHCOMB**, Enumclaw, WA (US); **Claire KINNEY**, Fort Collins, CO (US); **Richard M. KINNEY**, Fort Collins, CO (US); **Jill A. LIVENGOOD**, Fort Collins, CO (US)

(73) Assignees: **TAKEDA VACCINES, INC.**, Cambridge, MA (US); **THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT**, Atlanta, GA (US)

(21) Appl. No.: **17/478,537**

(22) Filed: **Sep. 17, 2021**

Related U.S. Application Data

(60) Continuation of application No. 16/561,755, filed on Sep. 5, 2019, now abandoned, which is a continuation of application No. 15/492,981, filed on Apr. 20, 2017, now Pat. No. 10,449,231, which is a division of application No. 14/209,808, filed on Mar. 13, 2014, now Pat. No. 9,783,579.

(60) Provisional application No. 61/800,204, filed on Mar. 15, 2013.

Publication Classification

(51) **Int. Cl.**

A61K 38/16	(2006.01)
A61K 31/7048	(2006.01)
A61K 39/12	(2006.01)
A61K 31/713	(2006.01)
C07K 14/005	(2006.01)
C07K 14/18	(2006.01)
C07K 19/00	(2006.01)
C12N 15/09	(2006.01)
C12N 15/861	(2006.01)

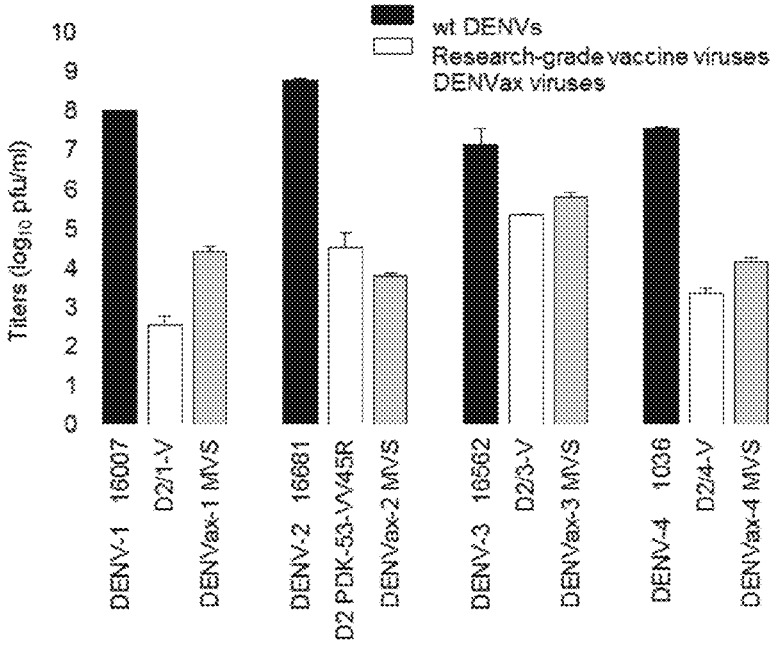
(52) **U.S. Cl.**

CPC ***A61K 38/162*** (2013.01); ***A61K 31/7048*** (2013.01); ***A61K 39/12*** (2013.01); ***A61K 31/713*** (2013.01); *A61K 2039/5254* (2013.01); ***C07K 14/1825*** (2013.01); ***C07K 19/00*** (2013.01); ***C12N 15/09*** (2013.01); ***C12N 15/8613*** (2013.01); ***C07K 14/005*** (2013.01)

(57) **ABSTRACT**

Embodiments herein report compositions, uses and manufacturing of dengue virus constructs and live attenuated dengue viruses. Some embodiments concern a composition that includes, but is not limited to, a tetravalent dengue virus composition. In certain embodiments, compositions can include constructs of one or more serotypes of dengue virus, such as dengue-1 (DEN-1) virus, dengue-2 (DEN-2) virus, dengue-3 (DEN-3) or dengue-4 (DEN-4) virus constructs. In other embodiments, constructs disclosed herein can be combined in a composition to generate a vaccine against more one or more dengue virus constructs that may or may not be subsequently passaged in mammalian cells.

Specification includes a Sequence Listing.



Genetic variations among D2/4 chimeras (compared to wt D2 16681 and D4-1036)

Genome	D2			junction prM	D4				junction
Genes	NCR	C			E				
Mutation types*	***PDK-53***	seed	Eng	MluI	seed	Marker	Eng	Eng	NgoMIV
Genome NT position	C57T	A225T	A396C	A453G	C647G/C	A1401G	C2027T	A2275	TG2380/1CC
Protein-AA position	NCR	C-silent	C-R100S	prM-silent	prM-T79R/T	E-silent	E-A364V	E-M447L	E-V482A
D2-16681	C	A	A(R)	A					TG(V)
D2-PDK-53	***T***	-	-						
D4-1036					G(T)	A	C(A)	A(M)	
Cloned D2/4-V1 (pD2/4-VP1)	***T***	-	C(S)	g	-	g	T(V)	C(L)	CC(A)
DENVax-4 (MVS)	***T***	t	C(S)	g	-	g	T(V)	C(L)	CC(A)

"-": same as wt D2 16681 or D4 1036; small nt letter: silent mutation in open reading region

*: PDK-53: D2-PDK-53 specific genotype (VS 16681), ***Italics: major attenuation PDK-53 loci***; Seed: mutations found only in specified virus seed and not in the original clone; Eng: Engineered mutations for the D2/4 clones; Mlu and NgoMV: D2/4 junction engineered RE sites;

**: C8571T (PDK-53 silent mutation) was not included in most D2/4 chimeric clones

FIG. 1

D2																		
NS1		NS2A			NS3			NS4A				NS4B	NS5					
PDK-53	seed	seed		PDK-53	***PDK-53***	seed	PDK-53	seed	PDK-53			seed	PDK53	seed				
G2579A	A3674G	A3773A/G		C4018T	A5270T	C5391T	T5547C	C6437T	G6599C			T7026C/T	C8571T**	A9750C				
NS1-G53D	NS2A-D66G	NS2A-K99K/R		NS2A-L181F	NS3-E250V	NS3-silent	NS3-silent	NS4A-A21V	NS4A-G75A			NS4B-silent	NS5-silent	NS5-silent				
G(G0 ***A(D)***	A(D) -	A(K) -		C(L) T(F)	A(E) ***T(V)***	C -	T c	C(A) -	G(G) C(A)			T -	C t	A -				
A(D)	-	-		T(F)	***T(V)***	-	c	-	C(A)			-	-	-				
A(D)	G(G)	A/G(K/R)		T(F)	***T(V)***	t	c	T(V)	C(A)			c/t	-	c				

FIG. 1
(continued)

Fig. 2

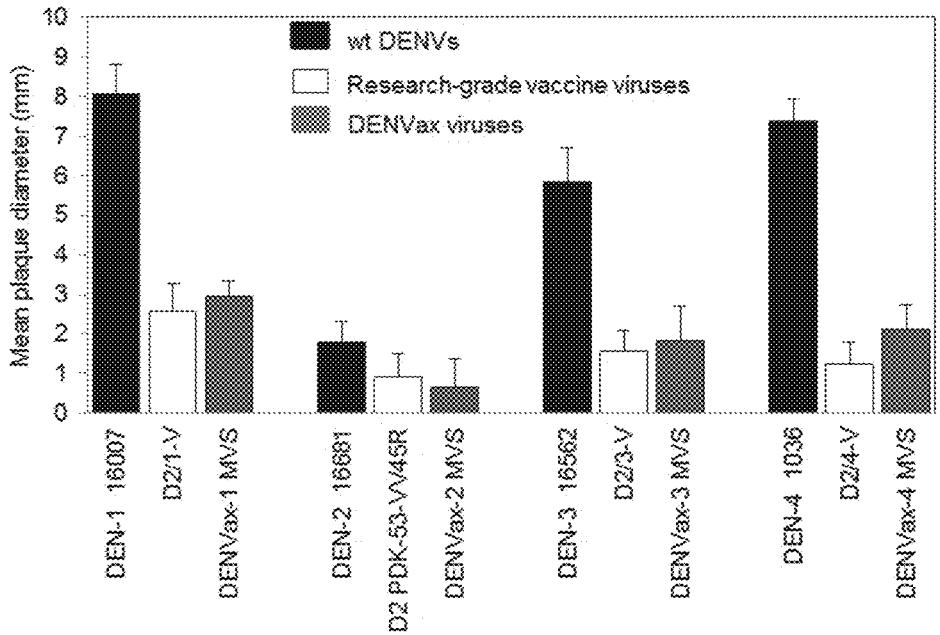

wt DENVs
Research-grade vaccine viruses
DENVax viruses
Mean plaque diameter (mm)
0
1
2
3
4
5
6
7
8
9
10
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-V/V45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 3

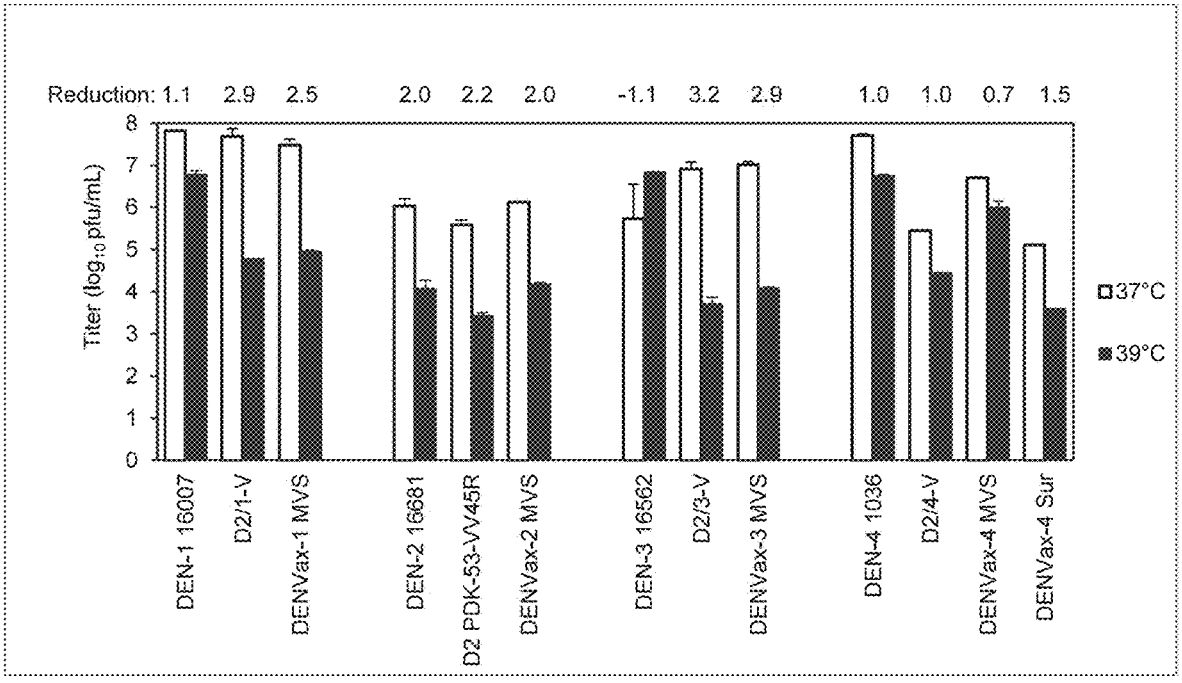

Reduction: 1.1 2.9 2.5 2.0 2.2 2.0 -1.1 3.2 2.9 1.0 1.0 0.7 1.5
Titer (log_{10}pfu/mL)
0
1
2
3
4
5
6
7
8
37°C
39°C
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 Sur

Fig. 4

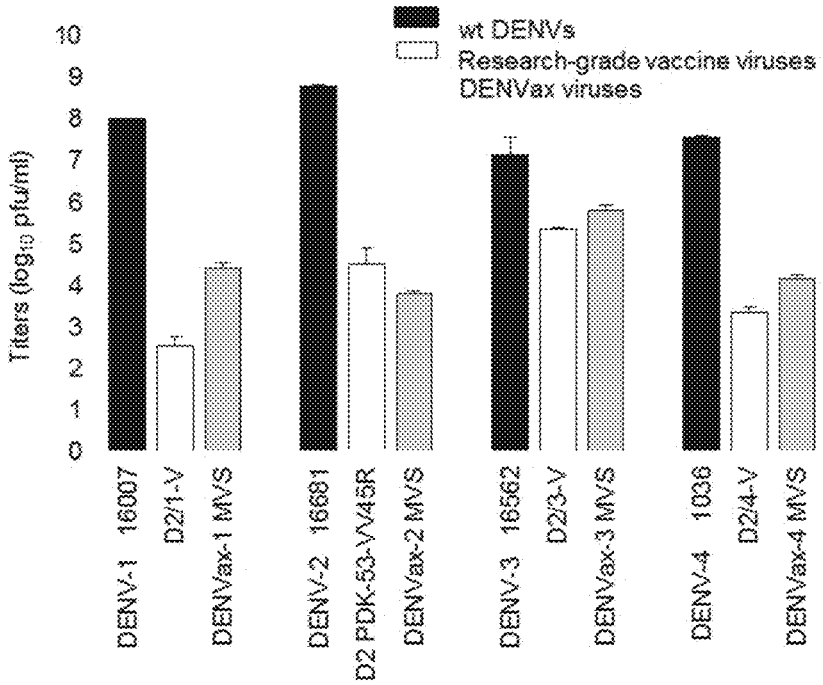

wt DENVs
Research-grade vaccine viruses
DENVax viruses
Titers (log_{10} pfu/ml)
0
1
2
3
4
5
6
7
8
9
10
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENV-4 1036
D2/4-V
DENVax-4 MVS

Fig. 5A

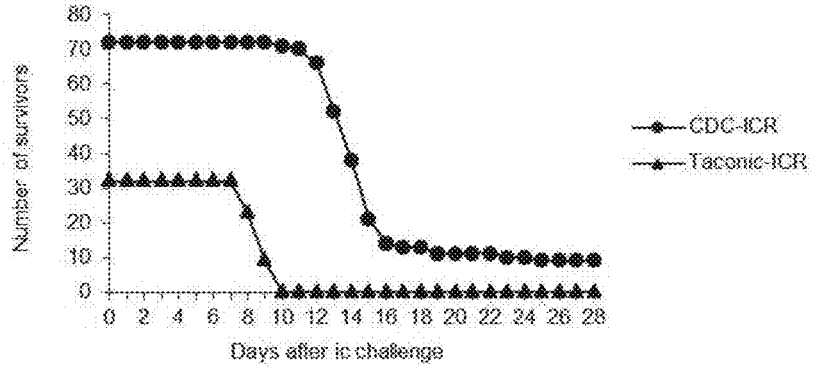


Fig. 5B

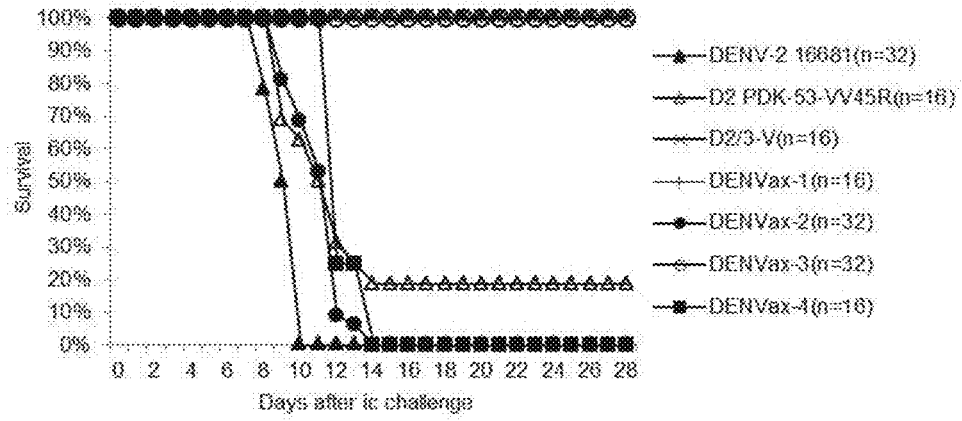


Fig. 5C

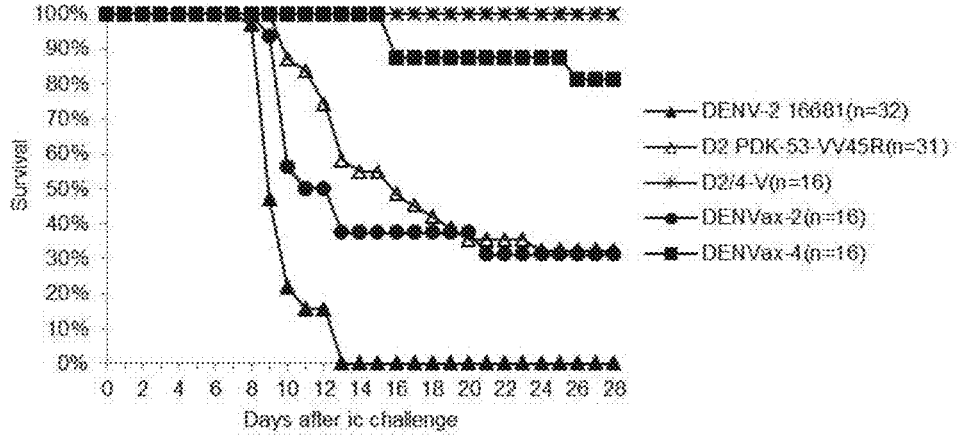


Fig. 6

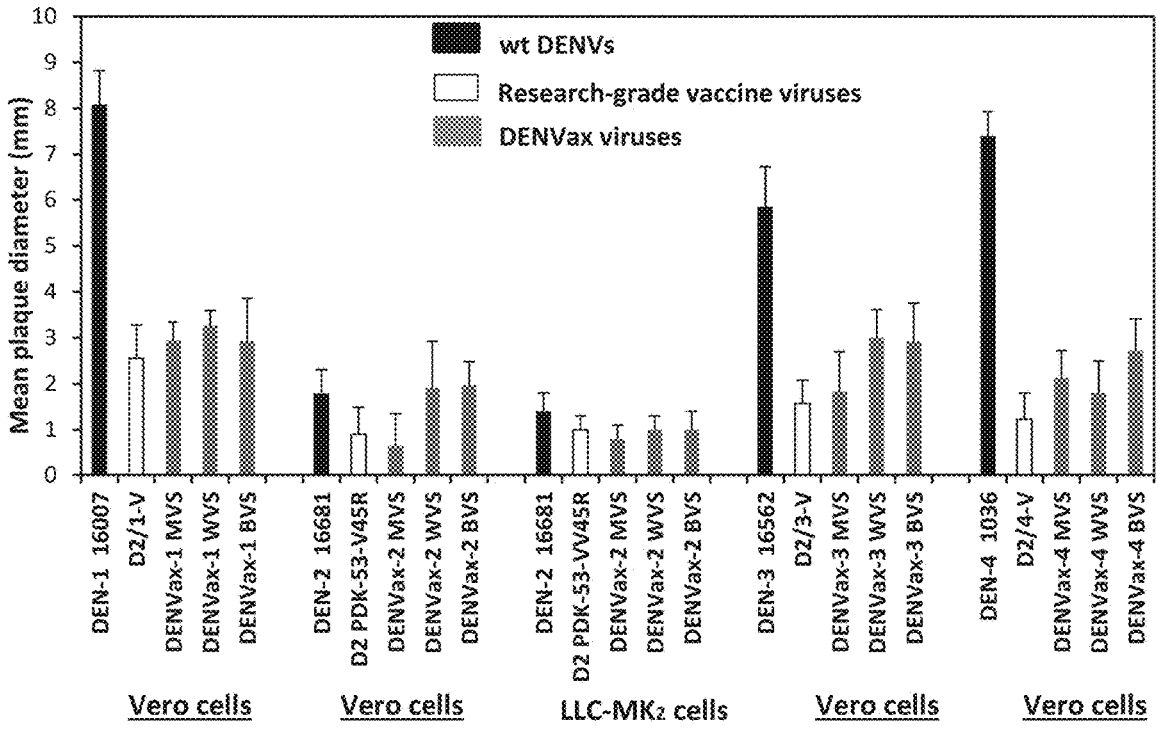

Mean plaque diameter (mm)
0
1
2
3
4
5
6
7
8
9
10
wt DENVs
Research-grade vaccine viruses
DENVax viruses
DEN-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DEN-2 16681
D2 PDK-53-V45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DEN-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS
Vero cells
Vero cells
LLC-MK2 cells
Vero cells
Vero cells

Fig. 7

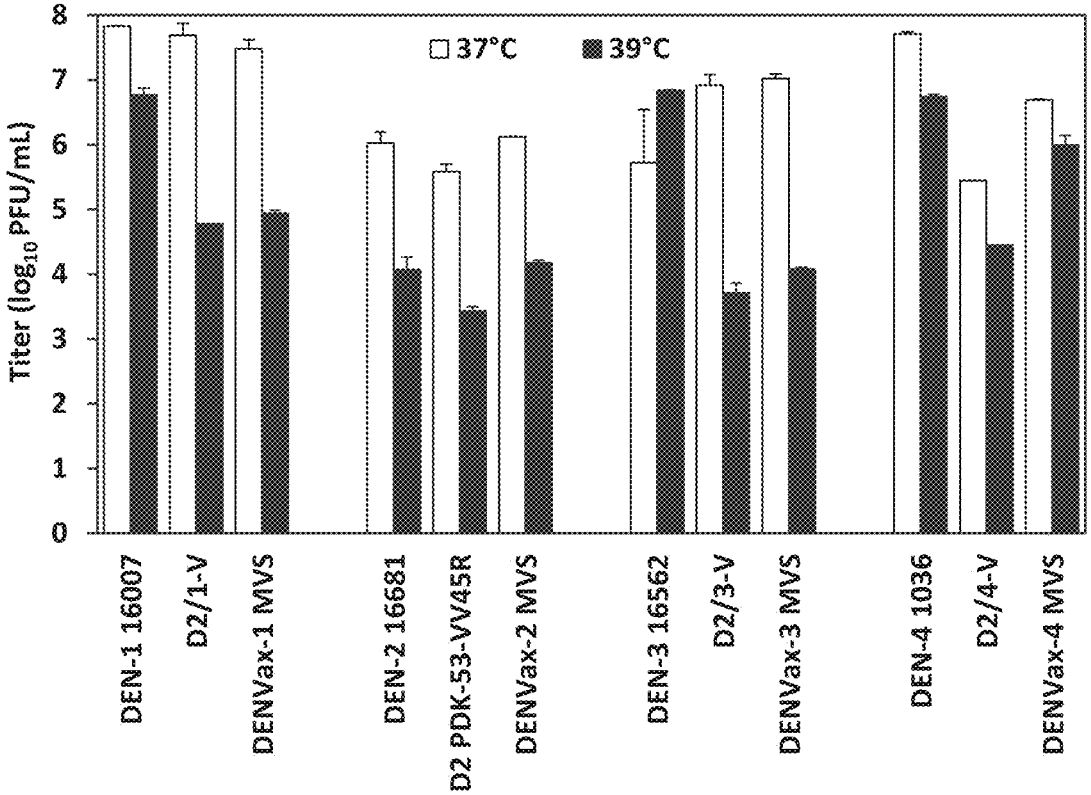
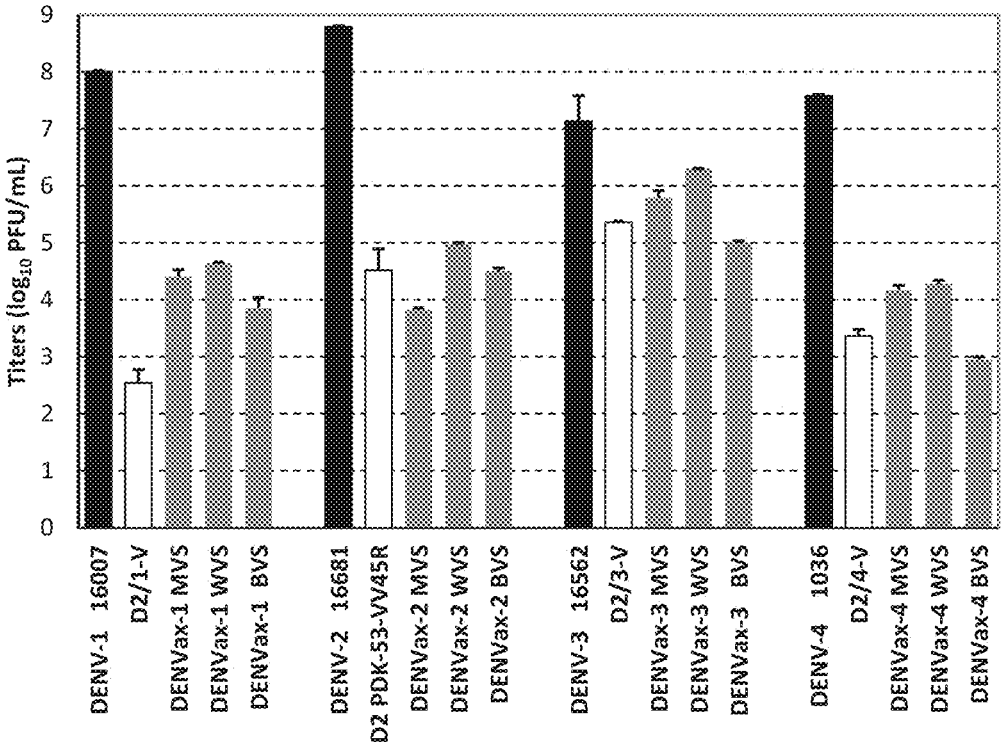

Titer (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
37°C
39°C
DEN-1 16007
D2/1-V
DENVax-1 MVS
DEN-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DEN-3 16562
D2/3-V
DENVax-3 MVS
DEN-4 1036
D2/4-V
DENVax-4 MVS

Fig. 8


Titers (log_{10} PFU/mL)
0
1
2
3
4
5
6
7
8
9
DENV-1 16007
D2/1-V
DENVax-1 MVS
DENVax-1 WVS
DENVax-1 BVS
DENV-2 16681
D2 PDK-53-VV45R
DENVax-2 MVS
DENVax-2 WVS
DENVax-2 BVS
DENV-3 16562
D2/3-V
DENVax-3 MVS
DENVax-3 WVS
DENVax-3 BVS
DENV-4 1036
D2/4-V
DENVax-4 MVS
DENVax-4 WVS
DENVax-4 BVS

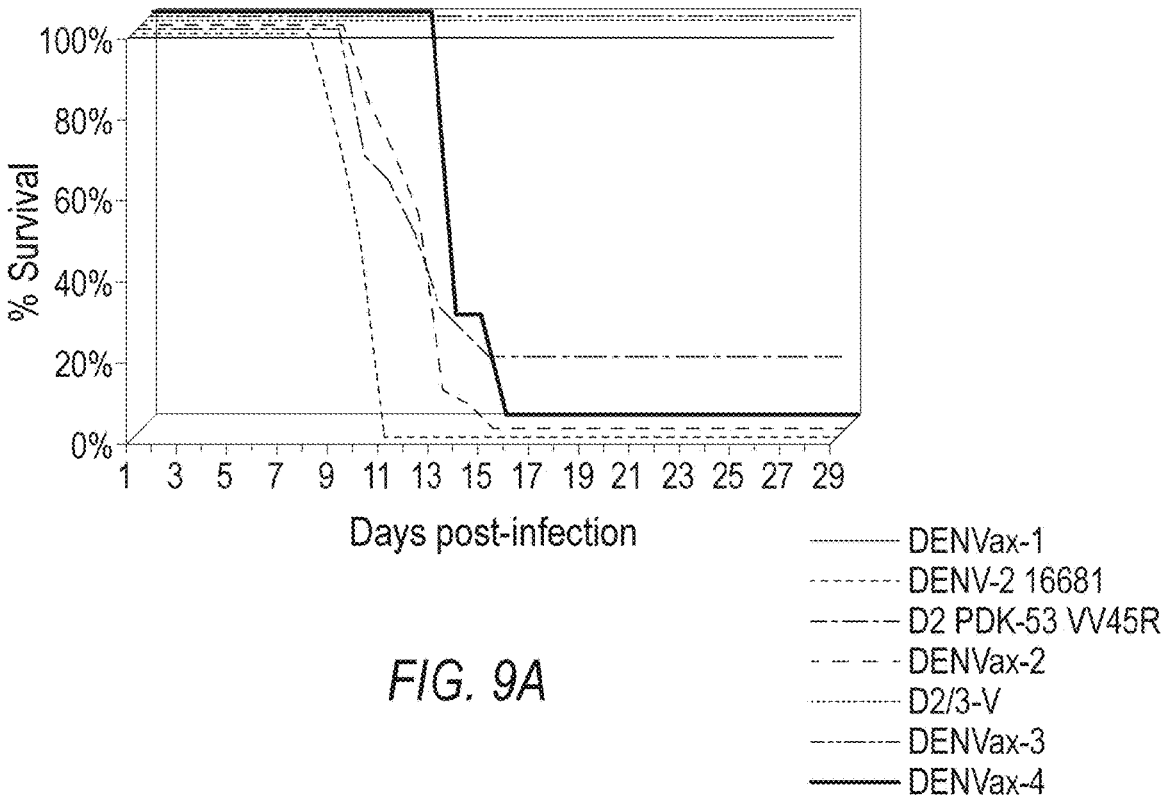

100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENVax-1
DENV-2 16681
D2 PDK-53 VV45R
DENVax-2
D2/3-V
DENVax-3
DENVax-4

FIG. 9A

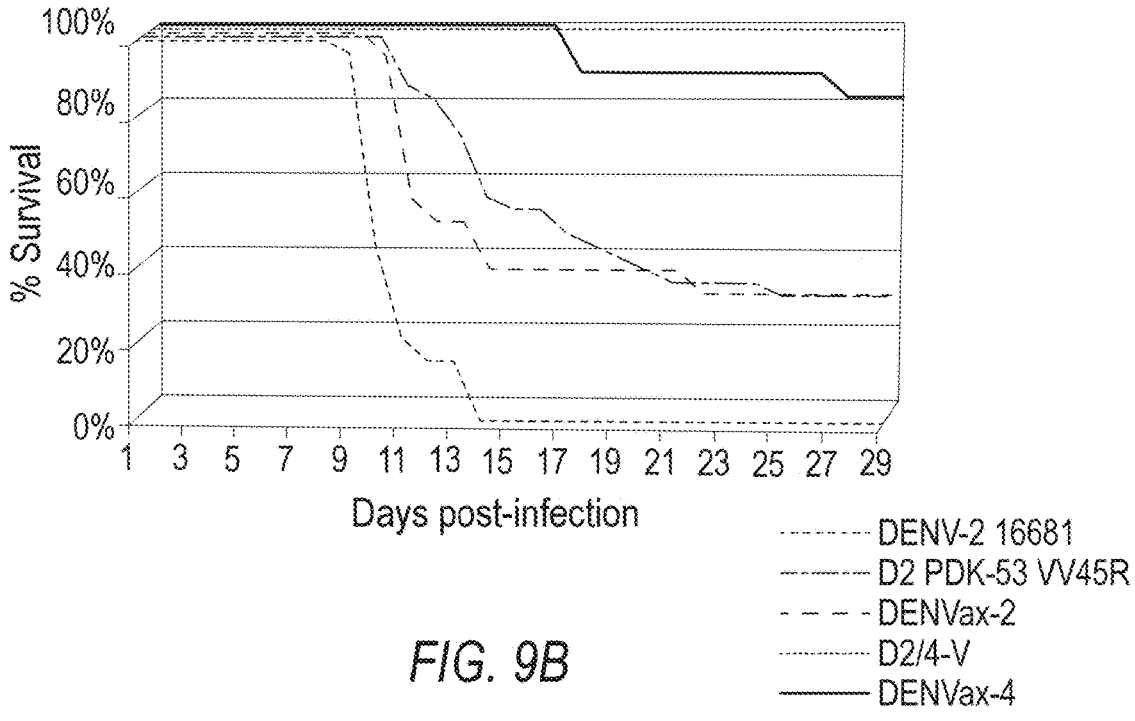

100%
80%
60%
40%
20%
0%
% Survival
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
Days post-infection
DENV-2 16681
D2 PDK-53 VV45R
DENVax-2
D2/4-V
DENVax-4

FIG. 9B

Serotype	Strain	virus origin	C57T 5′NCR	A524T prM-D29V	T900C[a] M (silent)
DENV-2	16681	isolate from human	C	A	T
	PDK-53	PDK cell pass of 16681	*T*	*T*	-
	PDK-53-V(VV45R)	Recombinant PDK-53-V	*T*	*T*	**c**
	PDK-53-E(VE48R)	Recombinant PDK-53-E	*T*	*T*	**c**

Underlined Mutations: the 3 most important attenuation loci of PDK-53

Italics font: PDK-53 specific sequence (change from 16681)

Bold font: Different nt sequence between PDK-53 and clone-derived V or E virus

[a]Engineered silent clone marker to differenciate original PDK-53 and recombinant (clone-derived) viruses

FIG. 10

C2055T E (silent)	G2579A NS1-G53D	C4018T NS2A-L181	A5270T NS3-E250V	T5547C NS3 (silent)	G6599C NS4A-G75A	C8571T NS5 (silent)
C	G	C	A	T	G	C
t	*A*	*T*	*T/A mix*	*c*	*C*	*t*
t	*A*	*T*	**T**	*c*	*C*	**C**
t	*A*	*T*	**A**	*c*	*C*	**C**

FIG. 10
(continued)

COMPOSITIONS AND METHODS FOR DENGUE VIRUS CHIMERIC CONSTRUCTIONS IN VACCINES

CROSS REFERENCE TO PRIOR APPLICATIONS

[0001] This patent application is a continuation of U.S. patent application Ser. No. 16/561,755, filed Sep. 5, 2019, which is a U.S. continuation of U.S. patent application Ser. No. 15/492,981, filed on Apr. 20, 2017, which is a U.S. divisional application that claims priority to U.S. patent application Ser. No. 14/209,808, filed Mar. 13, 2014, which claims the benefit of U.S. Provisional Patent Application No. 61/800,204, filed Mar. 15, 2013, the disclosures of which are all incorporated herein by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. R43 AI084291-01 awarded by the National Institutes of Health. The Government has certain rights in the invention.

FIELD

[0003] Embodiments herein report compositions, methods, uses and manufacturing procedures for dengue virus constructs and vaccine compositions thereof. Some embodiments concern a composition that includes, but is not limited to, chimeric flavivirus virus constructs that alone or in combination with other constructs can be used in a vaccine composition. In certain embodiments, compositions can include constructs of more than one serotypes of dengue virus, such as dengue-1 (DEN-1) virus, dengue-2 (DEN-2) virus, dengue-3 (DEN-3) virus and/or dengue-4 (DEN-4) virus. In other embodiments, manufacturing strategy that can improve the safety and genetic stability of recombinant live-attenuated chimeric dengue vaccine (DENVax) viruses. Certain embodiments include at least one live, attenuated dengue virus in combination with dengue virus chimeric constructs identified to be both safe and effective in vaccine compositions where the constructs have undergone additional passages in cell cultures.

BACKGROUND

[0004] Infection with dengue virus can lead to a painful fever of varying severity. To date, four serotypes of dengue virus have been identified: dengue-1 (DEN-1), dengue-2 (DEN-2), or dengue-3 (DEN-3) in combination with dengue-4 (DEN-4). Dengue fever is caused by infection of a dengue virus. Other subtypes may be discovered in the future (e.g. DEN-5). Dengue virus serotypes 1-4 can also cause dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). The most severe consequences of infection, DHF and DSS, can be life threatening. Dengue viruses cause 50-100 million cases of debilitating dengue fever, 500,000 cases of DHF/DSS, and more than 20,000 deaths each year. To date, there is no
effective vaccine to protect against dengue fever and no drug treatment for the disease. Mosquito control efforts have been ineffective in preventing dengue outbreaks in endemic areas or in preventing further geographic spread of the disease. It is estimated that 3.5 billion people are threatened by infection with dengue virus. In addition, dengue virus is a leading cause of fever in travelers to endemic areas, such as Asia. Central and South America, and the Caribbean.

[0005] All four dengue virus serotypes are endemic throughout the tropical regions of the world and constitute the most significant mosquito-borne viral threat to humans in tropical regions, worldwide. Dengue viruses are transmitted to humans primarily by *Aedes aegypti* mosquitoes. Infection with one dengue virus serotype results in life-long protection from re-infection by that serotype, but does not prevent secondary infection by one of the other three dengue virus serotypes. In fact, previous infection with one dengue virus serotype leads to an increased risk of severe disease (DHF/DSS) upon secondary infection with a different serotype. The development of an effective vaccine represents an important approach to the prevention and control of this global emerging disease. Multiple immunizations make complete vaccine coverage difficult both for public health efforts in dengue virus endemic countries as well as travelers.

SUMMARY

[0006] Embodiments herein concern compositions, methods and uses of chimeric dengue virus constructs. In some embodiments, a composition can include chimeric dengue virus constructs having an attenuated dengue virus backbone with structural genes from at least one other dengue virus serotype. Other embodiments concern at least one live, attenuated virus in combination with one or more chimeric dengue viruses. Other embodiments can include a composition of chimeric dengue viruses having a modified DEN-2 backbone (e.g. PDK-53 as a starting backbone in P1 (passage-1) and passage variability (after passage and growth in vitro on a permissive cell line) as indicated for P2, P3, . . . P8 . . . P10 etc.) and one or more structural components of DEN-1, DEN-2, DEN-3 or DEN-4. In other embodiments, an immunogenic composition is generated where when introduced to a subject, the composition produces an immune response to one or more dengue viruses in the subject. Therefore, constructs contemplated herein can be generated and passaged in vitro, and each of the passages provides an attenuated dengue virus contemplated of use in a pharmaceutically acceptable vaccine composition. In certain embodiments a live, attenuated virus can be a live, attenuated dengue-2 virus alone or in combination with one or more chimeric dengue viruses.

[0007] In certain examples, chimeric dengue virus constructs of dengue virus serotypes can include passage 7 (P7) live, attenuated viruses or chimeric viruses having nucleic acid sequences identified by SEQ ID NOS: 1, 4, 7 and 10 or polypeptide sequences indicated by SEQ ID NOS: 2, 3, 5, 6, 8, 9, 11 and 12. It is contemplated herein that any of the passages for any of the live, attenuated viruses described herein can be used in an immunogenic composition to induce immune responses to the represented dengue viruses (e.g. serotypes 1-4). In accordance with these embodiments, an immunogenic composition that includes a P-8 isolated live, attenuated virus can be administered to a subject to induce an immunogenic response against one or more dengue virus serotypes depending on the construct selected. In addition, a live, attenuated virus can be combined with one or more of these chimeric viruses. This is contemplated for each of the live, attenuated viruses isolated/produced in each subsequent cell passages (e.g. African Green Monkey Vero cell production, hereinafter: Vero cells). It is contemplated

herein that any cell line (e.g. GMP-produced cell bank. FDA or EMA-approved) capable of producing dengue viruses is of use to passage any of the viral constructs at a manufacturing scale or as appropriate contemplated herein for subsequent use in a vaccine or immunogenic composition against Dengue virus.

[0008] In other embodiments, compositions contemplated herein can be combined with other immunogenic compositions against other Flaviviruses such as West Nile virus, Japanese encephalitis or any other flavivirus chimeric construct and/or live, attenuated virus. In certain embodiments, a single composition can be used against multiple flaviviruses.

[0009] In certain embodiments, an immunogenic composition of the present invention can include chimeric dengue viruses against one or more of DEN-1, DEN-2, DEN-3 and/or DEN-4, alone or in combination with a live, attenuated dengue virus composition.

[0010] In other embodiments, a construct can include a construct having adaptive mutations in the structural or non-structural regions of the virus that increase growth or production without affecting attenuation or safety of the virus when introduced to a subject. In certain embodiments, any of the contemplated chimeric dengue virus constructs can include a live, attenuated DEN-2 virus having specific mutations used as a backbone where the live attenuated DEN-2 PDK virus further includes structural proteins of one or more of prM (premembrane) and E (envelope) structural proteins of the other dengue virus serotypes. In addition, a DEN-2 backbone can include additional mutations in order to increase production of or enhance the immune response to a predetermine composition in a subject upon administration (e.g. chimeric Dengue virus 2/1, 2/3 or 2/4).

[0011] In some embodiments, structural protein genes can include prM and E genes of DEN-1, DEN-2, DEN-3 or DEN-4 on a DEN-2 backbone having one or two mutations that are part of a live, attenuated dengue virus. For example, a dengue construct, in certain embodiments can include those constructs termed DENVax-1-A, DENVax-2-F, DENVax-3-F, and DENVax-4-F (see Example section) where the DEN-2 backbone has one or more mutations (e.g. not found in the P1 or other previous passaged virus or PDK-53) from the DEN-2 live, attenuated virus previously demonstrated to be safe and effective to induce an immune response. The DEN-2 live, attenuated virus of the instant application is an improved version of the originally used DEN-2 live, attenuated virus. A chimeric construct of the instant invention can include a modified attenuated DEN-2 PDK-53 backbone, having one or more structural proteins of the second dengue virus serotype wherein the structural proteins can include additional mutations to increase an immunogenic response to the chimeric construct. In some embodiments, certain mutations acquired by attenuated DEN-2 PDK-53 can produce a conservative amino acid change or not in a constructs different from the P1 construct which can result in desirable traits for production etc.

[0012] In other embodiments, a live, attenuated DEN-2 genome can be used to generate constructs of dengue virus serotype 1 (DEN-1) and dengue virus serotype 3 (DEN-3), dengue virus serotype 4 (DEN-4) where one or more structural protein genes of the DEN-2 viral genome can be replaced by one or more structural protein genes of DEN-1, DEN-3 or DEN-4, respectively. In some embodiments, a structural protein can be the C, prM or E protein of a second dengue virus. In certain embodiments, structural protein genes include the prM and E genes of DEN-1, DEN-3 or DEN-4. These hybrid viruses express the surface antigens of DEN-1. DEN-3 or DEN-4 while retaining the attenuation phenotypes of the parent attenuated DEN-2.

[0013] Constructs disclosed herein can include chimeric constructs of DEN-4, DEN-2, DEN-1, and DEN-3 expressing surface antigens of DEN-1, DEN-3 and DEN-4 using attenuated DEN-2 virus as a backbone.

[0014] In certain embodiments, compositions of the instant invention can include a composition that comprises a single chimeric dengue virus construct disclosed herein and a pharmaceutically acceptable carrier or excipient. Alternatively, compositions of the instant invention can include a composition that comprises two or more, or three or more chimeric dengue virus constructs disclosed herein, and a pharmaceutically acceptable carrier or excipient. In accordance with these embodiments, a one or more dengue virus chimeric constructs contemplated herein can be combined with one or more, live attenuated dengue viruses. In certain embodiments, a live, attenuated virus can be a live, attenuated DEN-2 virus wherein additional mutations in the NCR, NS1 regions or other regions increase the immune response, increase viral growth or other improvement for an improved live, attenuated dengue virus.

[0015] In certain embodiments, the attenuation loci, nucleotide 5'NCR-57-T, NS1-53-Asp. and NS3-250-Val, of the DENV-2 vaccine have been previously determined, and all of these changes are shared by the common PDK-53 virus-specific genetic background of the four DENVax viruses. The genetic sequence of the three attenuation loci as well as the previously established in vitro and in vivo attenuation phenotypes of these vaccine candidates were carefully monitored for the cGMP-manufactured DENVax seeds. This report describes strategies used to generate master virus seeds (MVS) as well as their genetic and phenotypic characterization of use in the manufacture of dengue virus vaccine compositions. These MVS can be used for manufacture of clinical materials and ultimately commercial vaccine supplies.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The following drawings form part of the present specification and are included to further demonstrate certain embodiments. Some embodiments may be better understood by reference to one or more of these drawings alone or in combination with the detailed description of specific embodiments presented.

[0017] FIG. **1** represents an exemplary chart reflecting an exemplary chimeric construct of the instant invention, DEN-2/DEN-4 compared to previously generated constructs and wild type dengue viruses.

[0018] FIG. **2** represents an exemplary histogram plot comparing various responses using a live, attenuated DEN-2 backbone (with additional mutations) and a second dengue virus serotype as structural components substituted for the dengue-2 structural components (e.g. DENVax-1 MVS). This plot illustrates plaque sizes of the DENVax MVS. Wild-type Dengue viruses and previously published research-grade vaccine candidate viruses were included for control and comparison. This plot illustrates improved production of the dengue virus constructs compared to control dengue virus chimeric constructs.

[0019] FIG. **3** represents an exemplary histogram plot that represents temperature sensitivities of DENVax MVS (Master Virus Seed). Wild type dengue viruses and previously published research-grade vaccine candidate viruses were included for comparison with the MVS grade.

[0020] FIG. **4** represents an exemplary histogram plot that represents viral growth of DENVax MVS in C6/36 cells compared to controls. Wild-type dengue viruses and research-grade vaccine candidate viruses were included for comparison with the DENVax MVS.

[0021] FIGS. **5**A-**5**C represent exemplary plots of neurovirulence in newborn mice. FIG. **5**A shows pooled results of several experiments summarizing the neurovirulence of wt DENV-2 16681 virus in CDC-ICR (n=72) and Taconic-ICR (n=32) newborn mice challenged ic with 10^4 pfu of the virus. FIG. **5**B shows neurovirulence of DENVax MVS tested in Taconic-ICR mice with a dose of 10^4 pfu. FIG. **5**C shows neurovirulence of DENVax MVS tested in Taconic-ICR mice with a dose of 10^3 pfu. The numbers of animals tested per group in one experiment (n=16) or two pooled experiments (n=31 or 32) are indicated.

[0022] FIG. **6** represents an exemplary histogram illustrating plaque size of the DENVax MVS, WVS, and BVS. Mean plaque diameters±SD (error bars) of the virus plaques in Vero or LLC-MK_2 cells under agarose overlay measured on day 9 pi. Wild type DENVs and previously published research-grade vaccine candidate viruses were included for control and comparison.

[0023] FIG. **7** represents an exemplary histogram plot illustrating growth of DENVax MSV, WVS, and BVS in C6/36 cells at two incubation temperatures to verify their retention of this in vitro attenuation marker after large scale manufacturing.

[0024] FIG. **8** represents an exemplary histogram plotting restricted growth of DENVax MVS, WVS, and BVS in C6/36 cells. Mean titers±SD (error bars) of the viruses replicated in C6/36 cells 7 days pi. The wt Dengue viruses and previously published research-grade vaccine candidate viruses were included for comparison.

[0025] FIGS. **9**A-**9**B represent exemplary graphs of data of neurovirulence of DENVax MVS in newborn ICR mice. FIG. **9**A shows IC inoculations of the virus at dose of 10^4 PFU. FIG. **9**B shows IC inoculation of the virus at dose of 10^3 PFU.

[0026] FIG. **10** represents an exemplary chart comparing new live, attenuated viruses to previously generated live, attenuated dengue viruses.

DEFINITIONS

[0027] As used herein, "a" or "an" may mean one or more than one of an item.

[0028] As used herein the specification, "subject" or "subjects" may include, but are not limited to, mammals such as humans or mammals, domesticated or wild, for example dogs, cats, other household pets (e. g. hamster, guinea pig, mouse, rat), ferrets, rabbits, pigs, horses, cattle, prairie dogs, wild rodents, or zoo animals.

[0029] As used herein, the terms "virus chimera," "chimeric virus," "flavivirus chimera" and "chimeric flavivirus" can mean a construct comprising a portion of the nucleotide sequence of a dengue-2 virus and further nucleotide sequence that is not from dengue-2 virus or is from a different flavivirus. A "dengue chimera" comprises at least two different dengue virus serotypes but not a different flavivirus. Thus, examples of other dengue viruses or flaviviruses include, but are not limited to, sequences from dengue-1 virus, dengue-3 virus, dengue-4 virus, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus, yellow fever virus and any combination thereof.

[0030] As used herein. "nucleic acid chimera" can mean a construct of the invention comprising nucleic acid comprising a portion of the nucleotide sequence of a dengue-2 virus and further nucleotide sequence that is not of the same origin as the nucleotide sequence of the dengue-2 virus. Correspondingly, any chimeric flavivirus or flavivirus chimera disclosed herein can be recognized as an example of a nucleic acid chimera.

[0031] As used herein, "a live, attenuated virus" can mean a wild-type virus, mutated or selected for traits of use in vaccine or other immunogenic compositions wherein some traits can include reduced virulence, safety, efficacy or improved growth etc.

DESCRIPTION

[0032] In the following sections, various exemplary compositions and methods are described in order to detail various embodiments. It will be obvious to one skilled in the art that practicing the various embodiments does not require the employment of all or even some of the specific details outlined herein, but rather that concentrations, times and other specific details may be modified through routine experimentation. In some cases, well-known methods or components have not been included in the description.

[0033] In accordance with embodiments of the present invention, there may be employed conventional molecular biology, protein chemistry, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Animal Cell Culture, R. I. Freshney, ed., 1986).

[0034] Embodiments herein concern compositions, methods and uses for inducing immune responses against one or more dengue virus serotypes in a subject, individually or simultaneously. In accordance with these embodiments, attenuated dengue viruses and nucleic acid chimeras are generated and used in vaccine compositions disclosed herein. Some embodiments concern modified or mutated dengue constructs or chimeras. Other embodiments concern introducing mutations to modify the amino acid sequences of structural proteins of dengue viruses wherein the mutation increase immunogenicity to the virus.

[0035] Live, attenuated dengue viruses of all four serotypes have been developed by passaging wild-type viruses in cell culture. These are some of the most promising live, attenuated vaccine candidates for immunization against flavivirus and in particular dengue virus infection and/or disease. These vaccine candidates have been designated by a combination of their dengue serotype, the cell line through which they were passaged and the number of times they were passaged. Thus, a dengue serotype 1 wild-type virus passaged in PDK cells 13 times is designated as DEN-1 PDK-13 virus. Other vaccine candidates are DEN-2 PDK-53, DEN-3 PGMK-30/FRhL-3 (e.g. thirty passages in primary green monkey kidney cells, followed by three passages in fetal rhesus lung cells and DEN-4 PDK-48). These four

candidate vaccine viruses were derived by tissue culture passage of wild-type parental DEN-1 16007, DEN-2 16681, DEN-3 16562 and DEN-4 1036 viruses, respectively.

[0036] In certain embodiments, live, attenuated dengue-2 PDK-53 vaccine virus contained a mixture of viruses, with the population containing varying nucleotide differences. After genetic characterization of the attenuating mutations, certain attenuating characteristics were outlined and engineered into a cDNA infectious clone. RNA was transcribed from this infectious clone and introduced into Vero cells as a passage 1 of the newly characterized and derived PDK-53-Vero-DEN-2-P 1 virus (see for example, Table 1). This attenuated virus was created for each DEN serotype, but for DEN-1, DEN-3 and DEN-4, the prM and E genes were engineered into 3 separate cDNA infectious clones, thus generating four separate PDK-53-Vero viruses (termed herein as: PDK-53-Vero-DEN-2-P 1, PDK-53-Vero-DEN-1-P 1, PDK-53-Vero-DEN-3-P 1, and PDK-53-Vero-DEN-4-P 1). These attenuated vaccine virus strains were passaged in Vero cells 10 times (Table 1), and each separate lineage acquired mutations upon their adaptation to grow in Vero cells (Table 3). Certain embodiments here are directed to derivation and uses for these live, attenuated dengue viruses.

[0037] Previous human clinical trials with these attenuated viruses have indicated that DEN-2 PDK-53 has the lowest infectious dose (50% minimal infectious dose of 5 plaque forming units or PFU) in humans, is strongly immunogenic, and produces no apparent safety concerns. The DEN-1 PDK-13, DEN-3 PGMK-30/FRhL-3 and DEN-4 PDK-48 vaccine virus candidates have higher 50% minimal infectious doses of 10,000, 3500, and 150 PFU, respectively, in humans. Although only one immunization with monovalent DEN-2 PDK-53 virus or DEN-4 PDK-48 virus was required to achieve 100% seroconversion in human subjects, a booster was needed to achieve the same seroconversion rate for DEN-1 PDK-13 and DEN-3 PGMK-30/FRhL-3 viruses, which have the two highest infectious doses for humans.

[0038] DEN-2 PDK-53 virus vaccine candidate, also abbreviated PDK-53, has several measurable biological markers associated with attenuation, including temperature sensitivity, small plaque size, decreased replication in mosquito C6136 cell culture, decreased replication in intact mosquitoes, loss of neurovirulence for suckling mice and decreased incidence of viremia in monkeys. Clinical trials of the candidate PDK-53 vaccine have demonstrated its safety and immunogenicity in humans. Furthermore, the PDK-53 vaccine induces dengue virus-specific T-cell memory responses in human vaccine recipients. Some embodiments herein describe an improvement on the DEN-2 PDK-53 used in chimeric constructs disclosed herein.

[0039] Immunogenic flavivirus chimeras having a dengue-2 virus backbone and at least one structural protein of another dengue virus serotype can be used for preparing the dengue virus chimeras and methods for producing the dengue virus chimeras are described. The immunogenic dengue virus chimeras are provided, alone or in combination, in a pharmaceutically acceptable carrier as immunogenic compositions to minimize, inhibit, or immunize individuals against infection by one or more serotypes, such as dengue virus serotypes DEN-1, DEN-2, DEN-3 and DEN-4, alone or in combination. When combined, the immunogenic dengue virus chimeras may be used as multivalent vaccines (e.g. bi-, tri- and tetravalent) to confer simultaneous protection against infection by more than one species or strain of flavivirus. In certain embodiments, the dengue virus chimeras are combined in an immunogenic composition useful as a bivalent, trivalent or tetravalent vaccine against the known dengue virus serotypes or confer immunity to other pathogenic flaviviruses by including nucleic acids encoding one or more proteins from a different flavivirus.

[0040] In some embodiments, avirulent, immunogenic dengue virus chimeras provided herein contain the nonstructural protein genes of the attenuated dengue-2 virus (e.g. PDK-53), or the equivalent thereof, and one or more of the structural protein genes or immunogenic portions thereof of the flavivirus against which immunogenicity is to be induced in a subject. For example, some embodiments concern a chimera having attenuated dengue-2 virus PDK-53 genome as the viral backbone, and one or more structural protein genes encoding capsid, premembrane/membrane, or envelope of the PDK-53 genome, or combinations thereof, replaced with one or more corresponding structural protein genes from DEN-1. DEN-3 or DEN-4 or other flavivirus to be protected against, such as a different flavivirus or a different dengue virus serotype. In accordance with these embodiments, a nucleic acid chimera disclosed herein can have functional properties of the attenuated dengue-2 virus and is avirulent, but expresses antigenic epitopes of the structural gene products of DEN-1. DEN-3 or DEN-4 in addition to other flaviviruses and is immunogenic (e.g. induces an immune response to the gene products in a subject). Then, these DNA constructs are used to transcribe RNA from an infectious clone, this RNA is introduced into Vero cells again producing a new progeny virus at P1. These new progeny viruses are distinguishable from PDK-53. (See e.g. P1-P10).

[0041] In another embodiment, a nucleic acid chimera can be a nucleic acid chimera having, but not limited to, a first nucleotide sequence encoding nonstructural proteins from an attenuated dengue-2 virus, and a second nucleotide sequence encoding a structural protein from dengue-4 virus alone or in combination with another flavivirus. In other embodiments, the attenuated dengue-2 virus can be vaccine strain PDK-53 having one or more mutated amino acids (see Examples). These additional mutations confer desirable traits of use as live, attenuated dengue-2 or as chimeric constructs described herein. Some embodiments include structural proteins of one or more of C, prM or E protein of a second dengue virus.

[0042] Other aspects include that chimeric viruses can include nucleotide and amino acid substitutions, deletions or insertions for example, in the control PDK-53 dengue-2 genome to reduce interference with immunogenicity responses to a targeted dengue virus serotype. These modifications can be made in structural and nonstructural proteins alone or in combination with the example modifications disclosed herein and can be generated by passaging the attenuated virus and obtaining an improved composition for inducing an immune response against one or more dengue virus serotypes.

[0043] Certain embodiments disclosed herein provide for method for making the chimeric viruses of this invention using recombinant techniques, by inserting the required substitutions into the appropriate backbone genome. Other embodiments herein concern passaging a confirmed (e.g. safe and effective) live, attenuated chimeric virus for additional improvements. In certain embodiments, a dengue-2 backbone used herein can include one or more mutations

presented in Table 3. In other embodiments, a dengue-dengue chimera of the instant application can include one or more mutations as presented in Table 3. In yet other embodiments, a dengue-dengue chimera can include all of the mutations for each chimera as represented in Table 3 for Den-2/Den-1, Den-2/Den-3 or Den-2/Den-4. Pharmaceutical compositions that include a live, attenuated virus represented by the constructs of Table 3 are contemplated. For example, mono-, di-, tri- or tetravalent compositions are contemplated of use herein using chimeras and live, attenuated dengue-2 viruses as presented in Table 3.

[0044] In certain embodiments, a live, attenuated DEN-2 variant contemplated herein can be formulated into a pharmaceutical composition wherein the pharmaceutical composition can be administered alone or in combination with dengue-dengue chimeras or dengue-flavivirus chimeras. In certain embodiments, a bi-, tri or tetravalent compositions can be administered in a single application or in multiple applications to a subject.

Flavivirus Chimeras

[0045] Dengue virus types 1-4 (DEN-1 to DEN-4) are mosquito-borne flavivirus pathogens. The flavivirus genome contains a 5'-noncoding region (5'-NC), followed by a capsid protein (C) encoding region, followed by a premembrane/membrane protein (prM) encoding region, followed by an envelope protein (E) encoding region, followed by the region encoding the nonstructural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5) and finally a 3' noncoding region (3'NC). The viral structural proteins are C, prM and E, and the nonstructural proteins are NS1-NS5. The structural and nonstructural proteins are translated as a single polyprotein and processed by cellular and viral protesases.

[0046] Flavivirus chimeras can be constructs formed by fusing non-structural protein genes from one type, or serotype, of dengue virus or virus species of the flaviviridae, with protein genes, for example, structural protein genes, from a different type, or serotype, of dengue virus or virus species of the flaviviridae. Alternatively, a flavivirus chimera of the invention is a construct formed by fusing non-structural protein genes from one type, or serotype, of dengue virus or virus species of the flaviviridae, with further nucleotide sequences that direct the synthesis of polypeptides or proteins selected from other dengue virus serotypes or other viruses of the flaviviridae.

[0047] In other embodiments, avirulent, immunogenic flavivirus chimeras provided herein contain the nonstructural protein genes of the attenuated dengue-2 virus, or the equivalent thereof, and one or more of the structural protein genes, or antigenic portions thereof, of the flavivirus against which immunogenicity is to be conferred. Suitable flaviviruses include, but are not limited to those listed in Table 1.

[0048] Other suitable dengue viruses for use in constructing the chimeras can be wild-type, virulent DEN-1 16007. DEN-2 16681. DEN-3 16562 and DEN-4 1036 and attenuated, vaccine-strain DEN-1 PDK-13, DEN-2 PDK-53, DEN-3 PMK-30/FRhL-3 and DEN-4 PDK-48. Genetic differences between the DEN-1, DEN-2, DEN-3 and DEN-4 wild type/attenuated virus pairs are contemplated along with changes in the amino acid sequences encoded by the viral genomes.

[0049] Sequence listings for DEN-2 PDK-53 correspond to the DEN-2 PDK-53-V variant, wherein genome nucleotide position 5270 is mutated from an A to a T and amino acid position 1725 of the polyprotein or amino acid position 250 of the NS3 protein contains a valine residue. The DEN-2 PDK-53 variant without this nucleotide mutation, DEN-2 PDK-53-E, differs from PDK-53-V only in this one position. DEN-2 PDK-53-E has an A at nucleotide position 5270 and a glutamate at polyprotein amino acid position 1725, NS3 protein amino acid position 250. It is understood that embodiments herein include modified PDK 53 that include one or more passages in a separate host cell (e.g. Vero cells, see Table 1) where desirable traits of use in vaccine compositions contemplated herein are generated.

[0050] In certain embodiments, designations of the chimeras can be based on the DEN-2 virus-specific infectious clone modified backbones and structural genes (prM-E or C-prM-E) insert of other dengue viruses or other flaviviruses. DEN-2 for the dengue-2 backbone, followed by the strain from which the structural genes are inserted. One DEN-2 backbone variant is reflected in the next letter after the number designation. One particular DEN-2 backbone variant from which the chimera was constructed is indicated by the following letter placed after a hyphen, parent 16681 (P). PDK-53-E (E), or PDK-53-V (V); the last letter indicates the C-prM-E structural genes from the parental (P) strain or its vaccine derivative (V) or the prM-E structural genes from the parental (P) or its vaccine derivative (V1). For example; DEN-2/1-VP denotes the chimera comprising the attenuated DEN-2 PDK-53V backbone comprising a valine at NS3-250 and the C-prM-E genes from wild-type DEN-1 16007; DEN-2/1-VV denotes the DEN-2 PDK-53V backbone with the vaccine strain of dengue-1, DEN-1 PDK-13; DEN-2/1-VP1 denotes the DEN-2 PDK-53V backbone and the prM-E genes from wild-type DEN-1 16007; DEN-2/3-VP1 denotes the DEN-2 PDK-53V backbone and the prM-E genes from wild-type DEN-3 0.16562; DEN-2/4VP1 denotes the DEN-2 PDK-53V backbone and the prM-E genes from wild-type DEN-4 1036. Other chimeras disclosed herein are indicated by the same manner.

[0051] In one embodiment, chimeras disclosed herein contain attenuated dengue-2 virus PDK-53 genome as the viral backbone, in which the structural protein genes encoding C, prM and E proteins of the PDK-53 genome, or combinations thereof, can be replaced with the corresponding structural protein genes from dengue-1, dengue-3 or dengue-4 virus and optionally, another flavivirus to be protected against, such as a different flavivirus or a different dengue virus strain.

[0052] In the nonstructural protein regions, a Gly-to-Asp (wild type-to-PDK-53) mutation was discovered at nonstructural protein NS1-53 (genome nucleotide position 2579); a Leu-to-Phe (wild type-to-PDK-53) mutation was discovered at nonstructural protein NS2A-181 (genome nucleotide position 4018); a Glu-to-Val (wild type-to-PDK-53) mutation was discovered at nonstructural protein NS3-250 (genome nucleotide position 5270); and a Gly-to-Ala mutation (wild type-to-PDK-53) was discovered at nonstructural protein NS4A-75 (genome nucleotide position 6599). The live, attenuated DEN-2 virus of the instant invention further includes mutations as presented in any chimera or live, attenuated dengue-2 virus of Table 3.

[0053] PDK-53 virus strain has a mixed genotype at genome nucleotide 5270. A significant portion (approximately 29%) of the virus population encodes the non-mutated NS3-250-Glu that is present in the wild type DEN-2 16681 virus rather than the NS3-250-Val mutation. As both

genetic variants are avirulent, this mutation may not be necessary in an avirulent chimera.

[0054] Previously, it was discovered that avirulence of the attenuated PDK-53 virus strain can be attributed to mutations in the nucleotide sequence encoding nonstructural proteins and in the 5' noncoding region. For example, a single mutation at NS1-53, a double mutation at NS1-53 and at 5'NC-57, a double mutation at NS1-53 and at NS3-250 and a triple mutation at NS1-53, at 5'NC-57 and at NS3-250, result in attenuation of the DEN-2 virus. Therefore, the genome of any dengue-2 virus containing such non-conservative amino acid substitutions or nucleotide substitutions at these loci can be used as a base sequence for deriving the modified PDK-53 viruses disclosed herein. Another mutation in the stem of the stem/loop structure in the 5 noncoding region will provide additional avirulent phenotype stability, if desired. Mutations to this region disrupt potential secondary structures important for viral replication. A single mutation in this short (only 6 nucleotide residues in length) stem structure in both DEN and Venezuelan equine encephalitis viruses disrupts the formation of the hairpin structure. Further mutations in this stem structure decrease the possibility of reversion at this locus, while maintaining virus viability.

[0055] Mutations disclosed herein can be achieved by any method known in the art including, but not limited to, naturally-occurring or selected clones having additional features once passaged in a cell line of interest (e.g. Vero cells). It is understood by those skilled in the art that the virulence screening assays, as described herein and as are well known in the art, can be used to distinguish between virulent and avirulent backbone structures.

Construction of Flavivirus Chimeras

[0056] Flavivirus chimeras described herein can be produced by splicing one or more of the structural protein genes of the flavivirus against which immunity is desired into a PDK-53 dengue virus genome backbone, or other methods known in the art, using recombinant engineering to remove the corresponding PDK-53 gene and replace it with a dengue-1, dengue-3 or dengue-4 virus gene or other gene known in the art.

[0057] Alternatively, using the sequences provided in the sequence listing, the nucleic acid molecules encoding the flavivirus proteins may be synthesized using known nucleic acid synthesis techniques and inserted into an appropriate vector. Avirulent, immunogenic virus is therefore produced using recombinant engineering techniques known to those skilled in the art.

[0058] A target gene can be inserted into the backbone that encodes a flavivirus structural protein of interest for DEN-1, DEN-3, DEN-4 or other flavivirus. A flavivirus gene to be inserted can be a gene encoding a C protein, a PrM protein and/or an E protein. The sequence inserted into the dengue-2 backbone can encode both PrM and E structural proteins. The sequence inserted into the dengue-2 backbone can encode all or one of C, prM and E structural proteins.

[0059] Suitable chimeric viruses or nucleic acid chimeras containing nucleotide sequences encoding structural proteins of other flaviviruses or dengue virus serotypes can be evaluated for usefulness as vaccines by screening them for the foregoing phenotypic markers of attenuation that indicate avirulence and by screening them for immunogenicity. Antigenicity and immunogenicity can be evaluated using in vitro or in vivo reactivity with flavivirus antibodies or immunoreactive serum using routine screening procedures known to those skilled in the art.

Dengue Virus Vaccines

[0060] In certain embodiments, chimeric viruses and nucleic acid chimeras can provide live, attenuated viruses useful as immunogens or vaccines. Some embodiments include chimeras that exhibit high immunogenicity to dengue-4 virus while producing no dangerous pathogenic or lethal effects.

[0061] To reduce occurrence of DHF/DSS in subjects, a tetravalent vaccine is needed to provide simultaneous immunity for all four serotypes of the virus. A tetravalent vaccine is produced by combining a live, attenuated dengue-2 virus of the instant application with dengue-2/1, dengue-2/3, and dengue-2/4 chimeras described above in a suitable pharmaceutical carrier for administration as a multivalent vaccine.

[0062] The chimeric viruses or nucleic acid chimeras of this invention can include structural genes of either wild-type or live, attenuated virus in a virulent or an attenuated DEN-2 virus backbone. For example, the chimera may express the structural protein genes of wild-type DEN-4 1036 virus, its candidate vaccine derivative in either DEN-2 backgrounds.

[0063] Viruses used in the chimeras described herein can be grown using techniques known in the art. Virus plaque titrations are then performed and plaques counted in order to assess the viability and phenotypic characteristics of the growing cultures. Wild type viruses can be passaged through cultured cell lines to derive attenuated candidate starting materials.

[0064] Chimeric infectious clones can be constructed from the various dengue serotype clones available. The cloning of virus-specific cDNA fragments can also be accomplished, if desired. The cDNA fragments containing the structural protein or nonstructural protein genes are amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) from dengue virus RNA with various primers. Amplified fragments are cloned into the cleavage sites of other intermediate clones. Intermediate, chimeric dengue virus clones are then sequenced to verify the accuracy of the inserted dengue virus-specific cDNA.

[0065] Full genome-length chimeric plasmids constructed by inserting the structural protein and/or nonstructural protein gene region of dengue serotype viruses into vectors are obtainable using recombinant techniques well known to those skilled in the art.

Nucleotide and Amino Acid Analysis

[0066] The NS1-53 mutation in the DEN-2 PDK-53 vaccine virus is significant for the attenuated phenotype of this virus, because the NS1-53-Gly of the DEN-2 16681 virus is conserved in nearly all flaviviruses, including the tick-borne viruses, sequenced to date. DEN-4 vaccine virus can also contain an amino acid mutation in the NS1 protein at position 253. This locus, which is a Gln-to-His mutation in DEN-4 PDK-48 vaccine virus, is Gin in all four wild serotypes of dengue virus. This Gin residue is unique to the dengue viruses within the flavivirus genus. The NS1 protein is a glycoprotein that is secreted from flavivirus-infected cells. It is present on the surface of the infected cell and NS1-specific antibodies are present in the serum of virus-infected individuals. Protection of animals immunized with

NS1 protein or passively with NS1-specific antibody has been reported. The NS1 protein appears to participate in early viral RNA replication.

[0067] The mutations that occurred in the NS2A, NS2B, NS4A, and NS4B proteins of the DEN-1, -2, -3 and -4 attenuated strains are conservative in nature. The NS4A-75 and NS4A-95 mutations of DEN-2 and DEN-4 vaccine viruses, respectively, occurred at sites of amino acid conservation among dengue viruses, but not among flaviviruses in general.

[0068] The flaviviral NS3 protein possesses at least two recognized functions: the viral proteinase and RNA helicase/NTPase. The 698-aa long (DEN-2 virus) NS3 protein contains an amino-terminal serine protease domain (NS3-51-His, -75-Asp, -135-Ser catalytic triad) that is followed by sequence motifs for RNA helicase/NTPase functions (NS3-196-GAGKT (SEQ ID NO:147), -284-DEAH, -459-GRIGR). None of the mutations in the NS3 proteins of DEN-1, DEN-2, or DEN-3 virus occurred within a recognized motif. The NS3-510 Tyr-to-Phe mutation in DEN-1 PDK-13 virus was conservative. Since the wild-type DEN-2, -3 and -4 viruses contain Phe at this position, it is unlikely that the Tyr-to-Phe mutation plays a role in the attenuation of DEN-1 virus. The NS3-182 Glu-to-Lys mutation in DEN-1 PDK-13 virus occurred at a position that is conserved as Asp or Glu in most mosquito-borne flaviviruses and it may play some role in attenuation. This mutation was located 15 amino acid residues upstream of the GAGKT helicase motif. As noted in previous reports, the NS3-250-Glu in DEN-2 16681 virus is conserved in all mosquito-borne flaviviruses except for yellow fever virus.

[0069] Nucleic acid probes selectively hybridize with nucleic acid molecules encoding the DEN-1, DEN-3 and DEN-4 viruses or complementary sequences thereof. By "selective" or "selectively" is meant a sequence which does not hybridize with other nucleic acids to prevent adequate detection of the dengue virus. Therefore, in the design of hybridizing nucleic acids, selectivity will depend upon the other components present in a sample. The hybridizing nucleic acid should have at least 70% complementarity with the segment of the nucleic acid to which it hybridizes. As used herein to describe nucleic acids, the term "selectively hybridizes" excludes the occasional randomly hybridizing nucleic acids, and thus, has the same meaning as "specifically hybridizing." The selectively hybridizing nucleic acid of this invention can have at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, and 99% complementarity with the segment of the sequence to which it hybridizes, preferably 85% or more.

[0070] Sequences, probes and primers which selectively hybridize to the encoding nucleic acid or the complementary, or opposite, strand of the nucleic acid are contemplated. Specific hybridization with nucleic acid can occur with minor modifications or substitutions in the nucleic acid, so long as functional species-specific hybridization capability is maintained. By "probe" is meant nucleic acid sequences that can be used as probes or primers for selective hybridization with complementary nucleic acid sequences for their detection or amplification, which probes can vary in length from about 5 to 100 nucleotides, or preferably from about 10 to 50 nucleotides, or most preferably about 18-24 nucleotides.

[0071] if used as primers, the composition preferably includes at least two nucleic acid molecules which hybridize to different regions of the target molecule so as to amplify a desired region. Depending on the length of the probe or primer, the target region can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of detecting the presence of the dengue virus, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes is at least enough to distinguish hybridization with a nucleic acid from other organisms.

[0072] Nucleic acid sequences encoding the DEN-4, DEN-3 or DEN-1 virus (e.g. structural elements) can be inserted into a vector, such as a plasmid, and recombinantly expressed in a living organism (e.g. into a dengue-2 backbone) to produce recombinant dengue virus peptides and/or polypeptides and/or viruses.

Nucleic Acid Detection Methods

[0073] A rapid genetic test that is diagnostic for each of the vaccine viruses described herein is provided by the current invention. This embodiment of the invention enhances analyses of viruses isolated from the serum of vaccinated humans who developed a viremia, as well as enhancing characterization of viremia in nonhuman primates immunized with the candidate vaccine viruses.

[0074] These sequences include a diagnostic TaqMan probe that serves to report the detection of the cDNA amplicon amplified from the viral genomic RNA template by using a reverse-transciptase/polymerase chain reaction (RT/PCR), as well as the forward and reverse amplimers that are designed to amplify the cDNA amplicon, as described below. In certain instances, one of the amplimers has been designed to contain a vaccine virus-specific mutation at the 3'-terminal end of the amplimer, which effectively makes the test even more specific for the vaccine strain because extension of the primer at the target site, and consequently amplification, will occur only if the viral RNA template contains that specific mutation.

[0075] Automated PCR-based nucleic acid sequence detection system can be used, or other known technology for nucleic acid detection. The TaqMan assay is a highly specific and sensitive assay that permits automated, real time visualization and quantitation of PCR-generated amplicons from a sample nucleic acid template. TaqMan can determine the presence or absence of a specific sequence. In this assay, a forward and a reverse primer are designed to anneal upstream and downstream of the target mutation site, respectively. A specific detector probe, which is designed to have a melting temperature of about 10.degree. C. higher than either of the amplimers and containing the vaccine virus-specific nucleotide mutation or its complement (depending on the strand of RT/PCR amplicon that is being detected), constitutes the third primer component of this assay.

[0076] A probe designed to specifically detect a mutated locus in one of the vaccine viral genomes will contain the vaccine-specific nucleotide in the middle of the probe. This probe will result in detectable fluorescence in the TaqMan assay if the viral RNA template is vaccine virus-specific. However, genomic RNA templates from wild-type DEN viruses will have decreased efficiency of probe hybridization because of the single nucleotide mismatch (in the case of the parental viruses DEN viruses) or possibly more than one mismatch (as may occur in other wild-type DEN viruses) and will not result in significant fluorescence. The DNA

polymerase is more likely to displace a mismatched probe from the RT/PCR amplicon template than to cleave the mismatched probe to release the reporter dye (TaqMan Allelic Discrimination assay, Applied Biosystems).

[0077] One strategy for diagnostic genetic testing makes use of molecular beacons. The molecular beacon strategy also utilizes primers for RT/PCR amplification of amplicons, and detection of a specific sequence within the amplicon by a probe containing reporter and quencher dyes at the probe termini. In this assay, the probe forms a stem-loop structure. The molecular beacons assay employs quencher and reporter dyes that differ from those used in the TaqMan assay.

Pharmaceutical Compositions

[0078] Embodiments herein provide for administration of compositions to subjects in a biologically compatible form suitable for pharmaceutical administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the active agent (e.g. pharmaceutical chemical, protein, gene, of the embodiments) to be administered in which any toxic effects are outweighed by the therapeutic effects of the active agent. Administration of a therapeutically active amount of the therapeutic compositions is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regima may be adjusted to provide the optimum therapeutic response.

[0079] In one embodiment, the compound (e.g. pharmaceutical chemical, protein, peptide etc. of the embodiments) may be administered in a convenient manner, for example, subcutaneous, intravenous, by oral administration, inhalation, intradermal, transdermal application, intravaginal application, topical application, intranasal or rectal administration. Depending on the route of administration, the active compound may be contained in a protective buffer (e.g. FTA, F127/trehalose/albumin). In one embodiment, a composition may be orally administered. In another embodiment, the composition may be administered intravenously. In one embodiment, the composition may be administered intranasally, such as inhalation. In yet another embodiment, the composition may be administered intradermally using a needle-free system (e.g. Pharmajet®) or other intradermal administration system.

[0080] A composition may be administered to a subject in an appropriate carrier or diluent, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. The term "pharmaceutically acceptable carrier" as used herein is intended to include diluents such as saline and aqueous buffer solutions. It may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. The active agent may also be administered parenterally, or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms or other stabilizing formulation (e.g. FTA).

[0081] Pharmaceutical compositions suitable for injectable use may be administered by means known in the art. For example, sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion may be used. In all cases, the composition can be sterile and can be fluid to the extent that easy syringability exists. It might be stable under the conditions of manufacture and storage and may be preserved against the contaminating action of microorganisms such as bacteria and fungi. The pharmaceutically acceptable carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of microorganisms can be achieved by heating, exposing the agent to detergent, irradiation or adding various antibacterial or antifungal agents.

[0082] Sterile injectable solutions can be prepared by incorporating active compound (e.g. a compound that induces an immune response to one or more dengue virus serotypes) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[0083] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above. It is contemplated that compositions are especially suitable for intramuscular, subcutaneous, intradermal, intranasal and intraperitoneal administration. A particular ratio may be sought such as a 1:1, 1:2 or other ratio (e.g. PFUs of a given dengue virus serotype)

[0084] The active therapeutic agents may be formulated within a mixture predetermined ratios. Single dose or multiple doses can also be administered on an appropriate schedule for a given situation (e.g. prior to travel, outbreak of dengue fever).

[0085] In another embodiment, nasal solutions or sprays, aerosols or inhalants may be used to deliver the compound of interest. Additional formulations that are suitable for other modes of administration include suppositories and pessaries.

[0086] Certain formulations can include excipients, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like.

[0087] A pharmaceutical composition may be prepared with carriers that protect active ingredients against rapid elimination from the body, such as time-release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others are known.

[0088] Pharmaceutical compositions are administered in an amount, and with a frequency, that is effective to inhibit or alleviate side effects of a transplant and/or to reduce or prevent rejection. The precise dosage and duration of treatment may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Dosages may also vary with the severity of the condition. A pharmaceutical composition is generally formulated and administered

to exert a therapeutically useful effect while minimizing undesirable side effects. In general, dose ranges from about 10^2 to 10^6 PFU can be administered initially and optionally, followed by a second administration within 30 days or up to 180 days later, as needed. In certain embodiments, a subject can receive dual administration of a mono, bi-, tri or tetravalent composition disclosed herein wherein the composition is a single composition mixture or has predetermined compositions of different dengue virus serotypes. In some embodiments, a DEN2/4 chimera can be present in higher concentrations than other dengue virus serotypes such as a live, attenuated dengue-1.

[0089] It will be apparent that, for any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

[0090] In one embodiment, a composition disclosed herein can be administered to a subject subcutaneously or intradermally.

[0091] The pharmaceutical compositions containing live, attenuated dengue viruses may be administered to individuals, particularly humans, for example by subcutaneously, intramuscularly, intranasally, orally, topically, transdermally, parenterally, gastrointestinally, transbronchially and transalveolarly. Topical administration is accomplished via a topically applied cream, gel, rinse, etc. containing therapeutically effective amounts of inhibitors of serine proteases. Transdermal administration is accomplished by application of a cream, rinse, gel, etc. capable of allowing the inhibitors of serine proteases to penetrate the skin and enter the blood stream. In addition, osmotic pumps may be used for administration. The necessary dosage will vary with the particular condition being treated, method of administration and rate of clearance of the molecule from the body.

[0092] In certain embodiments of the methods of the present invention, the subject may be a mammal such as a human or a veterinary and/or a domesticated animal or livestock or wild animal.

Therapeutic Methods

[0093] In one embodiment of the present invention, methods provide for inducing an immune response to dengue virus serotype(s) using a mono, bi-, tri or tetravalent formulation of live, attenuated and/or chimeric viral constructs contemplated herein.

[0094] Embodiments of the present invention is further illustrated by the following non-limiting examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention or the scope of the appended claims.

EXAMPLES

[0095] The following examples are included to demonstrate certain embodiments presented herein. It should be appreciated by those of skill in the art that the techniques disclosed in the Examples which follow represent techniques discovered to function well in the practices disclosed herein, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in particular embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope herein.

Example 1

[0096] In some exemplary methods, compositions used to generate as referred to herein as "master virus seeds (MVS)" are disclosed. These compositions may be derived from one or more live, attenuated dengue viruses, such as DEN-1, DEN-2, DEN-3, and DEN-4. In certain methods, compositions may be derived from one or more live attenuated Dengue viruses that include but are not limited to, specific constructs disclosed herein referred to as DENVax-1, DENVax-2, DENVax-3, and DENVax-4. In other exemplary methods, strategies used to generate and characterize these compositions are provided. In yet other embodiments, tetravalent dengue virus formulations and genetic and phenotypic characterization of these formulations are provided.

Production and Analysis of Pre-Raster DENVax Viruses

[0097] Certain procedures were performed to generate pre-master dengue virus seeds, such as serial amplification and purification of dengue viruses (e.g. DENVax). First, DENVax viruses were re-derived by transfection of viral RNA transcribed from the full-length recombinant DENVax cDNA into production-certified cells (e.g. Vero cells), resulting in P1 (passage 1) virus seed. The four P1 viruses from each of dengue-1 to dengue-4 were then amplified and plaque purified to obtain the candidate pre-master vaccine P7 seeds (see Table 1). Certain tests were performed to analyze passages of dengue viruses. For example, full-length genome sequencing demonstrated that all four of the P2 (passage 2) seed viruses were genetically identical to their homologous progenitor, research-derived, research-grade candidate vaccine virus. The original plaque phenotypes were also retained in the P2 viruses. Six plaque purified viruses (P3 A-F) were isolated for each serotype of dengue virus (e.g. DENVax1-4) from the P2 seeds, and each isolated plaque was directly plaque purified two more times. The third plaque purification (P5) of each virus was amplified twice (P6 A-F and P7 A-F) in Vero cells to produce the potential pre-master P7 DENVax seeds (Table 1).

TABLE 1

Example of a cGMP Rederivation of DENVax Viruses in WCB-Vero Cells

Passage	Seed Production/Purification	Characterizations
P1	Transfect WCB-Vero with transcribed viral RNAs	Plaque titrate
P2	Amplify P1 virus	Full genome sequence
P3	Pick 6 plaques (A-F)/serotype from P2 plaque assay	Plaque purification
P4	Pick plaques A-F from P3 plaque assay	Plaque purification
P5	Pick plaques A-F from P4 plaque assay	Plaque purification
P6	Amplify P5 A-F plaques	Plaque titrate
P7	Pre-master seeds: Amplify P6 A-F	Full genome sequence, TaqMAMA, Plaque phenotypes
P8*	MVS: Amplify selected P7 virus seed	Full genetic and phenotypic characterization

TABLE 1-continued

Example of a cGMP Rederivation of DENVax Viruses in WCB-Vero Cells

Passage	Seed Production/Purification	Characterizations
P9	WVS: Amplify P8 Master Seed viruses	Full genome sequence, TaqMAMA
P10	BVS: Amplify P9 Working Seed viruses	Full genome sequence, TaqMAMA

*One optimal P7 seed (A, B, C, D, E, or F) was selected based on the genetic and plaque analysis to make P8 MVS

[0098] Some tests were further performed to characterize P7 DENVax seeds, such as analysis of genome sequences and plaque phenotypes of the P7 seeds, and comparison with P2 seeds (Table 2). Plaque phenotypes of the P7 viruses were generally similar to those of the P2 seeds. In some exemplary experiments, virus titers were monitored. Virus titers reached over 6.0 log pfu/ml for most of the P7 seeds, except for 5 viruses. Genome sequencing of more than 60 candidate vaccine virus seeds after 10 or more serial passages in Vero cells identified no reversion event at NS1-53 and NS3-250 of the three major attenuation determinants of the DENV-2 PDK-53 genetic vector, suggesting that these 2 loci are quite stable in candidate vaccine virus seeds. All sequence chromatograms of the 24 candidate strains generated from both forward and reverse sequencing for these two sites were homogenous without any minor nucleotide populations evident at the NS1-53 and NS3-250 genetic loci. In contrast to the NS1 and NS3 sites, different levels of reversions at the 5'NCR-57 attenuation locus were identified from multiple serially passaged research grade vaccine viruses, suggesting this locus might not be as stable as NS1 and NS3 after multiple passages in cell culture. Therefore, a sensitive mismatch amplification assay (TaqMAMA) was developed to accurately measure the reversion rate at the 5'NCR-57 locus by real-time RT-PCR. In some studies, the 5'NCR-57 reversion rates of all 24 of the P7 seeds were measured by the TaqMAMA. Depending on the concentration of the input viral RNA for each virus in the assay, the sensitivity limit of the TaqMAMA ranged between 0.01% and 0.07% reversion, which is much more sensitive than the 10-30% reversion sensitivity limit detectable by consensus genome sequence analysis. The resulting data illustrates that 15 of the 24 P7 viruses had minimal or undetectable reversion (<0.07%), one virus (DENVax-3-D) had almost 100% reversion, and 8 viruses (1 DENVax-1, 1 DENVax-2, 2 DENVax-3, and 4 DENVax-4) had partial reversion ranging from 0.08% to 12.85% (Table 2). Full-length genome sequencing was conducted for 16 of the 24 P7 viruses with low levels of 5'NCR57 reversion as measured by TaqMAMA. All the sequenced viruses maintained the other two DENVax attenuation determinants (NS1-53. NS3-250), and all had acquired additional mutations that were not present in the original, engineered recombinant cDNA clones (Table 2). In one exemplary target vaccine composition, DENVax-1-A, DENVax-2-F, DENVax-3-F, and DENVax-4-F were selected as target pre-master seed for each serotype because their genotypes and plaque phenotypes most closely resembled those of the originally designed vaccine recombinants. The DENVax-1-A, DENVax-2-F, and DENVax-4-F had two non-synonymous mutations, and the DENVax-3-F had one. The evidence suggests these additional mutations observed in these 4 pre-master seeds do not cause safety concerns or immunogenicity alterations for the viruses. These pre-master seeds were further amplified to generate the MVS (master seed, designated as P7, Table 1).

[0099] Exemplary methods provided herein used purified in-vitro transcribed viral RNA from cloned cDNA plasmid as the pure source to transfect vaccine-certified Vero cells to generate vaccine virus. Serial plaque purifications and full-genome sequence analyses were incorporated into the manufacturing procedures to ensure manufactured vaccine seeds with optimal purity and genetic stability. Six cloned viruses were prepared as potential pre-master seeds for each serotype of DENVax. Through genomic analysis, including TaqMAMA and complete genomic sequencing, as well as characterization of viral plaque phenotypes, pre-master seeds were chosen to advance to master virus seeds production for each serotype (serotypes 1-4). The selected pre-master seeds had undetectable reversions (<0.01% or <0.07%) at the 5'NCR-57 locus, with 1 or 2 amino acid substitutions in their genomes, and retained the small plaque phenotypes previously observed.

TABLE 2

Characterizations of pre-master (P7) seeds

Virus	Clone[a]	TaqMAMA[b]	Log_{10} pfu/ml	Plaque[c]	Mutations identified in genome[d]
DENVax-1	**A**	**	6.85	P2	NS2A-116 I-L, NS2B-92 E-D, one silent
	B	*	6.93	P2	nd[e]
	C	*	6.93	D	nd
	D	**	7.02	D	C-67 K-A; one silent
	E	0.57%	7.28	P2	nd
	F	**	7.18	P2	E473 T-M; one silent
DENVax-2	A	0.03%	6.33	P2	NS1-341 K-N
	B	*	6.33	P2	E-305 K-T, two silent
	C	*	5.84	L	NS4A-18 T-A, four silent
	D	0.08%	6.20	P2	NS2B-99 I-L, one 3'NCR
	E	0.03%	6.31	P2	prM-52 K-E, NS5-412 I-V, two silent
	F	**	6.15	P2	prM-52 K-E, NS5-412 I-V
DENVax-3	A	*	6.00	P2	NS5-200 K-N, one silent, one 3'NCR
	B	0.05%	6.27	P2	NS2A-33 I-T NS2A-59 MT
	C	0.30%	6.25	P2	nd
	D	100.00%	6.27	P2	nd
	E	0.31%	6.00	P2	nd
	F	**	6.30	P2	E-223 TS, one silent

TABLE 2-continued

Characterizations of pre-master (P7) seeds

Virus	Clone[a]	TaqMAMA[b]	Log_{10} pfu/ml	Plaque[c]	Mutations identified in genome[d]
DENVax-4	A	0.47%	5.60	P2	E323 K-R/K, NS2B-21 L-F/L, NS2B-39 T-S, one silent
	B	*	5.65	D	NS2A-126 A-V; NS4A-5 N-D; NS5-383 K-R, one silent
	C	4.50%	5.90	P2	nd
	D	12.85%	5.97	D	nd
	E	0.52%	6.85	S	prM-85 E-D, NS2B-45 T-A, NS5-320 M-T, NS5-551 E-G, two silent
	F	0.02%	6.93	S	NS2A-66 D-G, NS4A-21 A-V, four silent

[a]Cloned viruses (by serial plaque purifications) selected for further development of MVS are designated bold.

[b]*: Reversion rate < 0.07% (detection limit). **: Reversion rate < 0.01% (detection limit).

[c]Plaque phenotypes: P2: similar P2 virus; L = larger than P2 virus, D = similar size, but appear somewhat different in clearness of the plaques; S = smaller than P2.

[d]Substitutions differing from the engineered DENVax cDNA clones. Amino acid mutations are listed with residue position of the virus protein and the changes (wt-mutation). Total number of silent mutations in structural and non-structural genes of each seed is listed. Mutations at non-coding region (NCR) are also noted.

[e]nd = Not done. These clones had higher 5'NCR-57 reversion rates (by TaqMAMA) than other clones, so were excluded from further sequence analysis.

Example 2

[0100] In some exemplary methods, compositions of master virus seeds, working virus seeds and bulk virus seeds as well as their genetic and phenotypic characterization are described. These compositions are provided for manufacture of clinical materials and ultimately commercial vaccine supplies. Serial plaque purifications and full-genome sequence analyses were incorporated into the manufacturing process to ensure compositions of vaccine seeds with optimal safety and genetic stability for manufacture of clinical trial materials.

Production and Manufacturing Quality Controls for MVS, WVS, and BVS

[0101] In some studies, MVS of the 4 DENVax were produced by amplifying the pre-master P7 seed in certified Vero cells. In other studies. MVS were used to make large amount of WVS in cell factories. Further, the BVS stocks of DENVax were amplified from the WVS and were formulated into tetravalent drug product mixtures to be used for human clinic trials. Quality controls for product release were performed in some exemplary methods, including, but not limited to, testing all of the MVS, WVS, and BVS for identity, infectious titer, sterility, mycoplasma, and in vitro and in vivo adventitious agents. All seeds passed the virus identity test using serotype-specific RT-PCR assays, which showed positive amplification corresponding to its serotype and negative for heterologous serotypes (data not shown). No detectable mycoplasma or adventitious agents were detected in the MVS, WVS, or BVS stocks.

Genetic Analysis of the MVS, WVS, and BVS

[0102] In certain exemplary methods, after generation of MVS from the selected pre-MVS (P7) strains selected above were produced and the respective viral RNA was sequenced again. Full-length genome sequencing revealed that the MVS for DENVax-1 was identical to its pre-master seed, while the WVS and subsequent BVS acquired 2 additional substitutions at E-483 and NS4B-108 (see Tables 2 and 3). The Ala substitution at E-483 represented part of the genotype in the MVS, but became the dominant genotype in BVS. DENVax-2 and DENVax-3 were identical to their respective pre-master seeds (Table 2 and 3). The DENVax-2 MVS was identical to its pre-master seed, and the WVS and BVS had 2 additional mutations at NS4A-36 and NS4B-111. Both mutations were partial in WVS and were the major genotype in the BVS. The MVS of DENVax-3 was again identical to the pre-master seed, but the WVS and BVS contained an additional aa substitution at NS4A-23. The DENVax-4 MVS acquired an additional amino acid mutation, at locus NS2A-99 (from Lys to Lys/Arg mixed genotype) during production of the MVS (Table 3). Its WVS and BVS retained the NS2A-99 Lys/Arg mixed genotype, and the BVS had an extra NS4B-2384 Scr/Phe mixed genotype. Consensus sequence results also confirmed that MVS, WVS as well as BV retained the three genetic determinants of attenuation at the 5'NCR-57, NS1-53, and NS3-250 loci. Analysis of the least stable attenuating locus by TaqMAMA demonstrated that the 5'NCR-57 reversion rate between <0.7% to and 0.13% among MVS, 50.07% among WVS, and between <0.07 and 0.21% among BVS. A 3% reversion at the 5'NCR-57 locus was considered the maximum permissible rate for acceptance of a vaccine lot (Table 3).

TABLE 3

Nucleotide and amino acid substitutions in DENVax seeds

DENVax	Nucleotides	Amino Acids	Pre-master	MVS[a]	WVS[a]	BVS[a]
DENVax-1	2384 G-C	E-483 Gly-Ala	-	-	**Gly/Ala**	**Ala**
	3823 A-C	NS2A-116 Ile-Leu	Leu	Leu	Leu	Leu
	4407 A-T	NS2B-92 Glu-Asp	Asp	Asp	Asp	Asp
	7148 C-T	NS4B-108 Thr-Ile	-	-	**Ile**	**Ile**
	7311 A-G	silent	G	G	G	G
	TaqMAMA 5'NCR-57 reversion %[b]		--	-	-	-

TABLE 3-continued

Nucleotide and amino acid substitutions in DENVax seeds

DENVax	Nucleotides	Amino Acids	Pre-master	MVS[a]	WVS[a]	BVS[a]
DENVax-2	592 A-G	prM-52 Lys-Glu	Glu	Glu	Glu	Glu
	6481 G-C	NS4A-36 Ala-Pro	-	-	**Ala/Pro**	**Pro**
	7156 C-T	NS4B-111 Leu-Phe	-	-	**Leu/Phe**	**Phe**
	8803 A-G	NS5-412 Ile-Val	Val	Val	Val	Val
	TaqMAMA 5'NCR-57 reversion %[b]		--	-	0.07%	0.21%
DENVax-3	1603 A-T	E-223 Thr-Ser	Ser	Ser	Ser	Ser
	6436 G-A	NS4A-23 Asp-Asn	-	-	**Asn**	**Asn**
	7620 A-G	silent	G	G	G	G
	TaqMAMA 5'NCR-57 reversion %[b]		--	-	-	-
DENVax-4	225 A-T	silent	T	T	T	T
	3674 A-G	NS2A-66 Asp-Gly	Gly	Gly	Gly	Gly
	3773 A-A/G	NS2A-99 Lys-Lys/Arg	-	**Lys/Arg**	**Lys/Arg**	**Lys/Arg**
	5391 C-T	silent	T	T	T	T
	6437 C-T	NS4A-21 Ala-Val	Val	Val	Val	Val
	7026 T-C	silent	T/C	T/C	T/C	T/C
	7538 C-C/T	NS4B-238 Ser-Ser/Phe	-	-	**Ser/Phe**	**Ser/Phe**
	9750 A-C	silent	C	C	C	C
	TaqMAMA 5'NCR-57 reversion %[b]		-	0.13%	-	-

[a]Bold: Changes started at MVS stocks.

[b]"--" indicates reversion rate <0.01% (detection limit), "-" indicates reversion rate <0.07% (detection limit)

[0103] Full-genome sequence analysis revealed that an additional amino acid mutation developed in the DENVax-4 MVS, while the other three DENVax MVS lots retained the consensus genome sequence of their pre-master seeds. Overall, from deriving of the P1 seeds to the pre-master (P7) weeds, only 1 or 2 non-synonymous mutations occurred in a given seed. From P1 to MVS (P8) seeds, 2 to 7 nucleotide substitutions were identified in any given DENVax seed and only 2 to 3 of these substitutions resulted in amino acid changes. Thus, minor changes occurred. RNA viruses are error-prone in their genome replication, so genetic substitutions in flavivirus genome during cell passages are not unexpected. None of the silent mutations in the MVS were within the 5' or 3'NCR that may affect virus replication. Only the change in prM-52 Lys-Glu of the DENVax-2, and the substitution in NS2A-66 Asp-Gly of DENVax-4 are not conservative changes. The NS2A-66 mutation of the DENVax-4 is in the nonstructural backbone part of the DENV-2 PDK-53. Although NS2A-66 locus is usually Asp among various strains of DENV-2, it is usually Gly for DENV-4. It is possible that the Asp to Gly change in the DENVax-4 is relevant for fitness of the DENVax-4 in Vero cells. The DENVax-2 prM-52 mutation resides in the C-terminal portion of the prM that is cleaved out from the mature virus particles. In some exemplary methods, phenotypic characterization was performed to confirm that none of the mutations in the MVS seeds significantly altered the attenuation phenotypes of the vaccine.

[0104] The DENVax viruses demonstrated high genetic stability during the manufacturing process. The three defined DENV-2 PDK-53 attenuation loci located in 5'NCR, NS1-53, and NS3-250 remained stable in the consensus genome sequence upon serial passage of the DENVax from pre-Master strains to bulk vaccine preparations. The highly sensitive TaqMAMA of the 5'NCR-57 locus demonstrated minimal or undetectable reversion in the MVS, WVS (P9/Working), and BVS (Bulk Virus Seed for vaccines) of dengue virus serotypes. The 5'NCR-57 reversion rates of the DENVax BVS preparations (P10-equivalent) were significantly lower than the 5'NCR-57 reversion rates that evolved in research-grade vaccine candidates after 10-serial passages in Vero cells (4-74% reversion). The strategy for large-scale manufacturing of the DENVax seeds provided herein resulted in a genetically stable vaccine seed which retained the attenuation markers in the candidate vaccine viruses.

Plaque Phenotype of DENVax MVS

[0105] In one exemplary method, plaque phenotypes of the DENVax MVS were compared with wild type Dengue viruses and their homologous research-grade chimeric viruses in Vero cells (FIG. **2**). All of the MVS of DENVax-1, -2, and -3 produced plaques that were significantly smaller than their wild type homologs and very similar (within 0.4-mm differences) to their homologous research-grade viruses in Vero cells. DENVax-4 MVS was also significantly smaller than the wild type DENV-4, but was slightly larger (0.9 mm difference) than the original lab derived D2/4-V chimera.

[0106] FIG. **2** represents an exemplary histogram illustrating plaque sizes of the DENVax MVS in contrast with control wild type viruses and research-grade vaccine candidate viruses. Mean plaque diameters (mm)±SD (error bars) of the virus plaques in Vero cells under agarose overlay measured on day 9 pi. The wild type DEN viruses, represented by black bars, and previously published research-grade vaccine candidate viruses, represented by white bars, were included for control and comparison to the DENVax master vaccine seeds represented by grey bars.

Temperature Sensitivity of DENVax MVS

[0107] In another exemplary method, temperature sensitivity was tested in Vero cells for the DENVax MVS and compared with their homologous wild type and the original research-grade chimeric vaccine virus. The wild type (wt) DENV-3 16562 was not temperature sensitive. The wt dengue virus serotype 1 and dengue virus serotype-4 were moderately temperature sensitive at 39° C. (titers were approximately 1.0 $\log_{10}$ pfu/ml lower at 39° C. than at 37° C., FIG. **3**). Wt Dengue virus serotype-2 16681 was the most temperature sensitive of the wt Dengue viruses tested, and resulted in a 100-fold titer drop at 39° C. DENVax-1, -2, and

-3 were as temperature sensitive as their original homologous research-grade chimeric vaccine viruses (FIG. **2**). Titers at 39° C. dropped between 2.0 and 3.0 $\log_{10}$ pfu/ml for these DENVax strains. DENVax-4 also was temperature sensitive, demonstrating a 5-fold reduction in titer. However, the original research-grade D2/4-V demonstrated about a 10-fold reduction in titer. The final stabilized DENVax-4 MVS contained F127 (and other agents known to stabilize these formulations (FTA)), which was shown to enhance thermal stability of the Dengue viruses. The presence of the F127 in DENVax-4 MVS likely contributed to the less pronounced temperature sensitivity of the virus in the Vero culture assay. In a separate experiment, temperature sensitivity of an MSV-derived DENVax-4 strain in the absence of F127 was further evaluated. To remove the F127 from the strain, viral RNA was isolated from a DENVax-4 bulk virus preparation and was transfected into Vero cells. This DENVax-4 virus appeared to be as temperature sensitive as the D2/4 V research strain (titer reduced 1.5 $\log_{10}$ pfu/ml) on day 3 pi in the absence of F127 (FIG. **3**).

[0108] FIG. **3** illustrates an exemplary histogram illustrating temperature sensitivities of DENVax MVS. The wild type Dengue viruses and previously published research-grade vaccine candidate viruses were included for comparison. The DENVax-4 MVS contains additional F-127 that can mask the temperature sensitivity results of the virus in this assay. A separate experiment analyzing a surrogate DENVax-4 in the absence of F127 was also included. Mean titers±SD (error bars) of the viruses replicated in Vero cells at 37° C. or 39° C.

DENVax MVS Replication in Mosquito C636 Cells

[0109] In some exemplary methods, the DENVax MVS were grown in C6/36 cells to verify their retention of the in vitro attenuation phenotype, with the knowledge that the research-grade chimeric vaccine viruses retained the attenuation phenotype of the backbone DENV-2 PDK53 virus in these mosquito cells. Compared to the wt Dengue viruses, DENVax-1. DENVax-2 and DENVax-4 MVS showed significant growth reduction (at least 3 $\log_{10}$ pfu/ml reduction) in C6/36 cells on day 6 pi (FIG. **4**). The DENVax-3 MSV also exhibited reduced growth compared to the wt DENV-3 16562, but the reduction was not as marked (1-2 $\log_{10}$ pfu/mL reduction). However, the C6/36 titers of the DENVax-3 seed lots were similar (within 1 $\log_{10}$ pfu/ml difference) to the C6/36 titer of the original research-grade chimeric D2/3-V vaccine virus.

[0110] FIG. **4** illustrates an exemplary histogram plotting restricted growth of DENVax MVS (grey bars) in C6/36 cells in comparison with wt Dengue viruses (black bars) and research-grade vaccine viruses (white bars). Mean titers±SD (error bars) of the viruses replicated in C6/36 cells 6 days pi.

Virus Infection, Dissemination, and Transmission Rates in Whole Mosquitoes

[0111] In some exemplary methods, the infection and dissemination rates of the DENVax were compared with their parental wt Dengue viruses. In certain exemplary experiments, oral infection experiments were conducted in *Ae. aegypti* mosquitoes. Infectious blood meals were back-titrated to measure the virus titers and only the experiments with similar virus titers in the blood meal (less than 1 $\log_{10}$ pfu/mi differences) between parental Dengue viruses and DENVax for each serotype were included for comparisons in Table 4. DENVax-1, DENVax-2, and research-grade D2 PDK-53-VV45R did not infect mosquitoes through oral feeding, which is significantly different ($p<0.0001$) from their parental viruses, DENV-1 16007 (44% infection) and DENV-2 16681 (43.3% infection). Because no mosquito was infected by DENVax-1 and -2, there was little to no dissemination concern for these two vaccine viruses. While DENVax-4 did infect some mosquitoes through oral feeding (2 out of 55), the infection rate was significantly lower ($p<0.05$) than its parental wt virus, DENV-4 1036 (8 out of 50). DENVax-3 did not infect any mosquitoes in two experiments with blood meal viral titers of 5.2±0.02 $\log_{10}$ pfu/mil (Table 4), and in a separate experiment with blood meal viral titer of 6.0 $\log_{10}$ pfu/ml, only 1 out of 30 mosquitoes became infected (data not shown). However, wt Dengue virus-3 16562 also had a very low infection rate (8%) at 5.2 logo pfu/ml, and the rate did not increase in a separate experiment with a higher blood meal viral titer at 6.2 $\log_{10}$ pfu/ml (3%, 1 positive out of 30 mosquitoes, data not shown). Although the wild type (wt) Dengue virus-3 and Dengue virus-4 had significantly lower infection rates than the wt Dengue virus-1 and Dengue virus-2, the mean virus titers in the infected mosquitoes were similar (3.1 to 3.9 $\log_{10}$ pfu/mosquito). In contrast, the DENVax-4 titers from the two infected mosquitoes were both minimal (0.7 $\log_{10}$ pfu/mosquito), which was 1,000-fold lower than the titer from the mosquitoes infected by wt Dengue virus serotype-4 1036 (3.9 f 1.5 pfu/mosquito).

[0112] For those mosquitoes that were infected, dissemination out of the midgut could be assessed by determining whether virus was present in the legs. The four parental DENVs resulted in dissemination rates ranging between 36.3% and 62.5%, and their mean virus titers (in $\log_{10}$ pfu) from the legs were between 0.9±0.3 and 2.2±0.7 (excluding negative samples). Neither of the two DENVax-4 infected mosquitoes resulted in virus dissemination to the legs (Table 4). While disseminated virus was detectable in the legs, none of the four wt Dengue viruses was detectable in saliva of orally infected mosquitoes, suggesting that oral feeding conditions may not be sufficiently sensitive to measure the transmission rate of these DENVs. Therefore, in other exemplary methods, highly stringent artificial mosquito infections by direct IT inoculation were subsequently performed (Table 4). Except for DENVax-4, all viruses (wt and DENVax) achieved 100% infection of the IT inoculated *Ae. aegypti*. The DENVax-4 inoculum had a slightly lower viral titer than the other three viral inocula, but it still successfully infected 70% of the inoculated mosquitoes. Despite the high body infection rates achieved by IT inoculation, all four DENVax viruses exhibited significantly lower ($p<0.005$) or non-detectable transmission rates (0-10%) compared to the wt Dengue viruses (43-87%, Table 4). The DENVax viruses demonstrated little to no infection and dissemination after oral feeding, and the highly stringent IT results affirmed the minimal transmission capacity of these DENVax viruses in *Ae. aegypti*.

TABLE 4

Virus infection, dissemination, and transmission rates in whole mosquitoes

	Oral Feed					IT inoculation				
Virus	Blood Meal[a] Mean ± SD	Infection[b] % (P/N)	Body Titer[c] Mean ± SD	p[d]	Dissemination[e] % (P/N)[f]	Inoculum pfu/dose	Infection[b] % (P/N)	Body Titer[c] Mean ± SD	Saliva[f] % (P/N)	p[d]
DENV-1 16007	6.6	44.0% (11/25)	3.6 ± 1.5		36.3% (4/11)	53.9	100% (30/30)	4.7 ± 0.48	43% (13/30)	
DENVax-1	6.9	0% (0/30)	NA	<0.0010	NA	67.8	100% (30/30)	3.4 ± 0.39	10% (3/30)	<0.005
DENV-2 16681	6.6	43.3% (13/30)	3.1 ± 1.5		38.5% (5/13)	67.8	100% (30/30)	5.2 ± 0.34	87% (26/30)	
D2 PDK53-VV45R	6.4	0% (0/30)	NA	<0.0001	NA	56.4	100% (30/30)	4.0 ± 0.20	0% (0/30)	<0.0001
DENVax-2	6.4	0% (0/30)	NA	<0.0001	NA	52.7	100% (30/30)	3.5 ± 0.27	7% (2/30)	<0.0001
DENV-3 16562	5.2	8% (2/25)	3.8 ± 0.2		50% (1/23)	34.0	100% (30/30)	4.2 ± 0.50	67% (20/30)	
DENVax-3	5.2 ± 0.02	0% (0/50)	NA	0.108	NA	37.3	100% (30/30)	3.3 ± 0.36	3% (1/30)	<0.0001
DENV-4 1036	5.8 ± 0.5	16% (8/50)	3.9 ± 1.5		62.5% (5/8)	69.4	100% (30/30)	5.2 ± 0.45	70% (21/30)	
DENVax-4	5.4 ± 0.4	3.6% (2/55)	0.7 ± 0.0	0.033	0% (0/2)	11.8	70% (21/30)	1.1 ± 0.46	0% (0/21)	<0.0001

[a]Virus titers or Mean ± standard deviation if from more than 1 experiment in blood meal (log_{10} pfu/ml) by back titration

[b]Rate of virus detected in mosquito bodies. P/N = positive/total mosquitoes

[c]Mean virus titers ± standard deviation (log_{10} pfu/mosquito) in mosquito body, only positive sample are included for calculation

[d]Statistic analysis of the differences between wt DENV and DENVax by Fisher Exact probability

[e]Rate of virus detected in legs of the positively infected mosquitoes

[f]Rate of virus detected in saliva of the positively infected mosquitoes. Used to measure transmission efficiency

[0113] Vector competence is an important safety component for live-attenuated flavivirus vaccine viruses. Previously, the research-grade DENV-2 PDK-53-VV45R virus and wt derivatives were tested in *Ae. aegypti*, and found that the NS1-53-Asp attenuating mutation was the dominant determinant for impaired mosquito replication. The other two major attenuation loci of the DENV-2 PDK-53 vaccine, nucleotide 5'NCR-57-T and NS3-250-Vail, also exhibited some inhibiting effect on replication in mosquitoes-, thus providing additional, redundant restrictions for mosquito vector competence. Some exemplary methods described herein were used to test the mosquito oral and IT infection and replication for all four DENVax strains. DENVax-1, -2, and -3 did not infect any *Ae. aegypti* mosquitoes through oral infection (Table 4). The DENVax-4 infected only 3.6% of orally exposed mosquitoes, a level significantly lower than that of the wt DENV-4 with a replicative mean titer in the mosquito bodies lower than that of wt DENV-4 infected mosquitoes. Surprisingly, DENVax-4 was detected in the legs of the infected mosquitoes, suggesting that DENVax-4 was not able to disseminate from the mosquito midgut following oral infection. The infection rates for the DENVax-1, -2, and -4 were all significantly less than their wt counterparts, but the difference was not significant between DENVax-3 and wt DENV-3 16562 due to the very low infection rates for both viruses. Compared to other wt strains of DENV assessed in *Ae. aegypti* collected from the same Mae Sot Province, Thailand, the parental wt Dengue virus strains used for engineering DENVax appeared to have lower infectious and dissemination rates by oral infection. The wt DENV-1 PUO359, DENV-2 PUO218, DENV-3 PaH881/88, and DENV-4 1288 used for engineering the Yellow Fever (YF) 17D vaccine-based ChimeriVax-DEN vaccines had infection rates ranging 47-77%. In contrast, the YF 17D vaccine cannot infect *Ae. aegypti*. Although the ChimeriVax strains contained the prM-E from these highly infectious wt DENV, the ChimeriVax retain the mosquito attenuation phenotype of their YF 17D replicative backbone. Results provided herein also indicated that the mosquito attenuation of DENV-2 PDK-53 backbone was maintained in the DENVax strains. In addition, using the wt Dengue virus strains with lower mosquito-infectivity in constructs included in compositions described herein provides an additional safety feature.

[0114] The oral infection results illustrate that the DENVax had minimum mosquito infectivity and dissemination capacity. In addition, the more sensitive and stringent IT infection experiments were performed to further analyze the potential of DENVax to be transmitted by *Ae. aegypti*. The IT results demonstrated that all four DENVax viruses had non-detectable or minimal mosquito transmission potential compared to their wt counterparts. DENVax transmission could only theoretically occur if (1) vector feeds on a vaccine with a sufficient viremia titer to infect mosquito midgut, (2) the virus is capable of replicating in the midgut epithelium and able to subsequently disseminate out of the midgut, and (3) the disseminated virus can replicate in salivary gland and expectorate sufficient virus in saliva for transmission. The threshold of human viremia required to infect mosquitoes has not been established adequately, but human viremia can be 10^6-10 mosquito infectious $dose_{50}$ (MID_{50})/ml after natural wt DENV infection. This MID_{50} was based on direct IT inoculation of mosquitoes with diluted human plasma. Analysis of DENVax in nonhuman primates indicated that viremia titers following DENVax immunization were very low (less than 2.4 log_{10} pfu/ml) and lasted for 2-7 days. Given the low viremia levels and the low mosquito infection, dissemination, and transmission capac-

ity of DENVax, it is unlikely that these vaccine viruses could be transmitted by mosquitoes in nature or cause viremia.

[0115] Therefore, it is proposed that any of the passages of any of the serotypes (P1-P10) could be used in a composition to generate a safe and effective vaccine against one, two, three or all four dengue virus serotypes.

Neurovirulence in Suckling Mice

[0116] The original research-grade vaccine viruses were highly attenuated for neurovirulence in newborn ICR mice maintained in-house at DVBD/CDC. All of these mice survived ic (intracerebral) challenge with 10^4 pfu of each vaccine virus. The wt Dengue virus serotype-2 16681 virus, on the other hand, resulted in 62.5%-100% mortality in these CDC-LCR mice in various experiments. In some experiments, commercial ICR mice obtained from Taconic Labs (Taconic-ICR) were used to study neurovirulence in newborn mice. It was observed that newborn Taconic-ICR mice were significantly more susceptible to Dengue virus serotype-2 infection than the previous CDC-LCR mice. FIG. **5**A summarizes the neurovirulence of wt Dengue virus serotype-2 16681 in CDC-ICR colony and Taconic-ICR, newborn mice challenged ic with 10^4 pfu of the virus. The Taconic-ICR mice (100% mortality in 32 mice, average survival time of 8.3±0.5 days) were more susceptible to ic Dengue virus serotype-2 16681 challenge than the previous CDC-ICR mice (91% fatalities in 72 mice, average survival time of 14.6±2.3 days).

[0117] In other exemplary methods, in order to evaluate neurovirulence of the DENVax MVS, the Taconic-ICR mice initially were challenged ie (intracerebrally) with a dose of approximately 10^4 pfu of wt Dengue virus serotype-2 16681, D2 PDK-53 VV45R, D2/3-V, or DENVax 1-4 virus in one (n=16) or two (n=31-32) experiments (FIG. **5**B). At this dose, D2/3-V research grade virus, as well as DENVax-1, and DENVax-3 MVS exhibited fully attenuated neurovirulence phenotypes (no illness or mortality). As expected, wt Dengue virus serotype-2 was found to be "fatal", with average mouse survival time (AST) of 8.3±0.8 days. In these Dengue virus serotype-2-sensitive Taconic-ICR mice, the D2 PDK-53-VV45R research grade virus resulted in 81.3% mortality. The DENVax-2 MVS and DENVax-4 MVS were uniformly fatal in the Taconic-ICR, showing AST values of 9.8±1.7, 10.2±1.4, and 11.3±0.4 days, respectively.

[0118] In some exemplary methods, the neurovirulence of wt Dengue virus serotype-2 16681 virus was compared with that of D2 PDK-53 VV45R, DENVax-2 MVS and DENVax-4 MVS, as well as D2/4-V research grade virus, at a 10-fold lower dose (10-pfu, FIG. **5**C). The wt Dengue virus serotype-2 retained a uniformly fatal neurovirulent phenotype, with AST of 9.0±1.4 days, at this lower challenge dose. The other 4 viruses exhibited intermediate neurovirulence phenotypes, and the degree of neurovirulence was serotype-specific. The D2 PDK-53-VV45R virus and its DENVax-2 MVS cognate showed significant attenuation (32.3% survival with AST of 13.1±3.8 days and 31.2% survival with AST of 10.5±3.4 days, respectively). Both the DENVax-4 MVS and the research grade D2/4-V virus were highly attenuated for neurovirulence (81.3% survival with AST of 18.8±5.8 days and 100% survival, respectively). The results suggested that MVS of DENVax-1 and -3 exhibited complete attenuation of neurovirulence, while DENVax-2 and -4 MVS lots retained attenuation phenotypes that closely resembled their homologous research-grade virus vaccine candidates.

[0119] FIGS. **5**A-**5**C represent exemplary graphs illustrating neurovirulence in newborn mice tested with various compositions including wt Dengue virus serotype-2 and different attenuated Dengue viruses. Pooled results of numerous experiments summarizing the neurovirulence of wt Dengue virus serotype-2 16681 virus in CDC-ICR (n=72) and Taconic-ICR (n=32) newborn mice challenged ic with 10^4 pfu of the virus (A). Neurovirulence of DENVax MVS tested in Taconic-ICR mice with a dose of 10^4 pfu (B) or 10^3 pfu (C). The numbers of animals tested per group in one experiment (n=16) or two pooled experiments (n=31 or 32) are indicated.

Plaque Phenotype of WVS, and BVS

[0120] Certain studies were performed to compare plaque phenotypes of WVS and BVS with MVS, wt Dengue viruses and their homologous lab derived, research-grade chimeras in Vero cells (FIG. **6**). Mean plaque sizes were calculated from 10 plaques for each vaccine virus, but from reduced numbers of wt DENV-1, -3, and -4. All of the MVS viruses of DENVax-1, -2, and -3 produced plaques that were significantly smaller than their wt homologs and very similar (within 0.4-mm differences) to their homologous research-grade viruses in Vero cells. DENVax-4 MVS was also significantly smaller than the wt DENV-4, but was slightly (0.9 mm) larger than the original lab derived D2/4-V chimera. With the exception of the DENVax-2, all of the WVS and BVS of the DENVax-1, -3, -4 retained significantly smaller plaque sizes than those produced from their wt homologs. The DENVax-2 WVS and BVS produced plaques that were similar to the plaques of wt DENV-2 virus in Vero cells, but when tested in LLC-MK_2 cells all of the DENVax-2 manufactured seeds produced plaques that were somewhat smaller than those of the wt DENV-2 (1.4 t 0.4) and similar to the lab derived D2 PDK-53-VV45R (1.0±0.3) (FIG. **6**).

[0121] Evaluation of the phenotypic markers of viral attenuation, including small plaque phenotype, temperature sensitivity, reduced replication in mosquito cells, reduced infection/dissemination/transmission by mosquitoes, and reduced neurovirulence in newborn ICR mice, were assessed for the compositions of MVS stocks. Results indicated that all of the DENVax retained the expected attenuation phenotypes similar to the original research-grade vaccine viruses. Given the mutations responsible for attenuation are conserved in all MVS, WVS and BV, it can be expected the attenuated phenotypes to be retained in the material manufactured for human clinical testing.

[0122] FIG. **6** represents an exemplary histogram illustrating plaque size of the DENVax MVS, WVS, and BVS. Mean plaque diameters±SD (error bars) of the virus plaques in Vero or LLC-MK_2 cells under agarose overlay measured on day 9 pi. The wt DENVs and previously published research-grade vaccine candidate viruses were included for control and comparison.

Virus Replication in Mosquito C6/36 Cells

[0123] Previous studies demonstrated that the research-grade PDK-53-based chimeric vaccine viruses retained the attenuation phenotype of the backbone DENV-2 PDK53

virus in C6/36 cells. In some exemplary methods, the DENVax MSV, WVS, and BVS were grown in C6/36 cells to verify their retention of this in vitro attenuation marker after large scale manufacturing. Compared to the wt Dengue viruses, except for DENVax-3, the manufactured seeds showed marked growth reduction (at least 3 $\log_{10}$ PFU/ml reduction) in C6/36 cells on day 6 pi (FIG. **7**). The DENVax-3 seeds also exhibited reduced growth compared to the wt DENV-3 16562, but the reduction was not as marked (1-2 $\log_{10}$ PFU/ml reduction). However, the titers of the DENVax-3 seed lots were similar (within 1 $\log_{10}$ PFU/ml difference) to the original research-grade chimeric D2/3-V vaccine virus.

[0124] FIG. **8** represents an exemplary histogram plotting restricted growth of DENVax MVS, WVS, and BVS in C6/36 cells. Mean titers±SD (error bars) of the viruses replicated in C6/36 cells 7 days pi. The wt Dengue viruses and previously published research-grade vaccine candidate viruses were included for comparison.

Neurovirulence in Suckling Mice

[0125] Additional experiments were performed to analyze neurovirulence in newborn ICR mice. At an intracranial dose of 10^4 PFU, the survival rates for wt DENV-2 16681 and the D2 PDK-53-VV45R were 0% and 18.8%, respectively (FIG. **9**A) in the ICR mice, but were about 20% for wt DENV-2 16681 and 100% for the D2 PDK-53-VV45R in the CDC ICR mice. In this study, DENVax-1 and DENVax-3 MVS were attenuated (100% survival) for the mice at a dose of 10^4 PFU, but the MVS of DENVax-2 and DENVax-4 caused 100% mortality at the dose of over 10^4 PFU (FIG. **5**A). However, when tested at a dose of 10, PFU of virus, the DENVax-2 (31.3% survival) and DENVax-4 (81.3% survival) showed reduced neurovirulence relative to wt Dengue virus serotype-2 16681 (0% survival), and their survival rates were similar to those of the research-grade vaccine candidates D2 PKD-53-VV45R (32.3%) and D2/4-V (100%), respectively (FIG. **9**B). Although, wt DENV-1, -3, or -4 were not included for comparison in this study, previous work demonstrated that wt DENV-1 16007 was attenuated in the CDC-ICR mice by the ic route, while both wt DENV-3 16562 and DENV-4 1036 were highly virulent (0% survival) for the CDC-ICR mice. It is likely that these 3 wt DENV would exhibit similar or greater virulence in the more susceptible Taconic ICR mice. Therefore, inclusion of these wt Dengue viruses for comparison with their homologous DENVax MVSs was considered to be uninformative. This study indicated that all 4 DENVax MVSs and original laboratory derived candidate vaccine viruses exhibit comparable mouse attenuation phenotypes relative to the wt DENV-2 16681.

[0126] FIGS. **9**A-**9**B represent exemplary graphs of data of neurovirulence of DENVax MVS in newborn ICR mice. (A) IC inoculations of the virus at dose of 10^4 PFU. (B) IC inoculation of the virus at dose of 10^3 PFU

[0127] All seed lots of the DENVax were tested for the identity, sterility, and freedom from undesirable agents. Full-genome sequence analysis revealed that one extra amino acid mutation evolved in the DENVax-4 MVS, while the other 3 DENVax MVSs retained the consensus genome sequence of their pre-master seeds. In WVS lots, the DENVax-3 acquired an extra amino acid mutation and the other 3 serotypes accumulated 2 extra amino acid substitutions, relative to their pre-master seeds. Genome sequences of all the 4 BVS lots were identical to their WVS lots. Overall from the P2 seeds to the pre-master (P7) seeds, only 1 or 2 non-silent mutations occurred in a given seed. Between pre-master and BCS (P10) seeds, only 1 to 2 nucleotide substitutions were observed, all of which occurred in NS2A, 4A, or 4B, with the exception of single nucleotide change resulting in a conserved glycine and alanine at residue E-483. From P2 to BVS (P10) seeds, total 3 to 8 nucleotide substitutions were identified in any given DENVax seed, and only 2 to 4 of these substitutions resulted in amino acid changes. None of the silent mutations in the BVS were within the 5'- or 3'-NCR region which may affects virus replication. These results suggest that the DENVax viruses were genetically highly stable during manufacture. The three defined DENV-2 PDK-53 attenuation loci located in 5'NCR, NS1-53, and NS3-250 remained unchanged in the consensus genome sequence upon serial passage of the DENVax to generate BVS stocks. The highly sensitive TaqMAMA of the 5'-NCR-57 locus showed minimal or undetectable reversion in the MVS, WVS, and BVS of DENVax. The highest reversion rate of 0.21% was identified in the DENVax-2 BVS. The reversion rates of the P10-equivalent BVS (<0.07% to 0.21%) were significantly lower than the reversion rates that evolved in other vaccine candidates after serial passages in Vero cells (4-74% reversion by P10). This suggests that this strategy for large scale manufacturing of the DENVax seeds is successful, regarding maintaining genetic stability and retention of attenuation markers in the candidate vaccine viruses.

[0128] Since MVS stocks disclosed herein will be used for future manufacturing of WVS and BVS lots, full panels of virus attenuation phenotype evaluations, including small plaque phenotype, temperature sensitivity, reduced replication in mosquito cells, reduced infection/dissemination/transmission in whole mosquitoes, and reduced neurovirulence in newborn ICR mice, were conducted for all MVS or their equivalent surrogate stocks. For the WVS and BVS stocks, plaque size, infectivity in mosquito cells, were also performed to confirm their attenuations. Results indicated that all the MVS stocks of the 4 serotypes of DENVax retained the expected attenuation phenotypes, such as small plaques, reduced replication in C6/36 cells, and reduced mouse neurovirulence, similar to the original lab-derived vaccine viruses (FIGS. **6**, **8**, and **9**). Except for the DENVax-4, all other 3 MVS stocks of DENVax were TS at 39° C. as shown in FIGS. **3** and **7**.

[0129] For the WVS and BVS stocks, two attenuation phenotypes, small plaques and restricted replication in C6/36 cells, were analyzed and confirmed. Since there are very little genetic changes between the MVS and BVS, it was expected that they would retain the attenuation phenotypes as MVS. In addition to the experiments described in this report, safety and immunogenicity of the manufactured DENVax in Ag129 mice and nonhuman primate have been tested.

[0130] Exemplary methods are provided herein to demonstrate manufacture of DENVax MVS, WVS, and BVS stocks under cGMP. The BVS stocks were used to formulate the tetravalent DENVax currently in human clinical trial evaluations. A unique manufacture strategy to optimize the genetic stability and safety of the manufactured MVS was provided in some exemplary methods. Since the main attenuation loci of the DENVax have been well characterized previously and a highly sensitive and quantifiable SNP

assay, TaqMAMA was developed to integrate genome sequence and the TaqMAMA to identify optimal pre-master seeds for making the MVS. The genetic and phenotypic characterizations of the MVS were fully analyzed to confirm that these viruses retained desirable attenuations for safety of the vaccine. This may be the only live, attenuated viral vaccine that can be efficiently analyzed for all the major attenuation genetic loci during manufacturing from pre-master all the way to BVS stocks. Results provided herein exemplified the advantage of strategically designed live-attenuated vaccines in vaccine safety.

[0131] FIG. **10** represents an exemplary table comparing new live, attenuated viruses to previously generated live, attenuated dengue viruses. Mutations are indicated where different from a control virus (e.g. 16681), or other live, attenuated dengue-2 viruses.

Materials and Methods

Viruses and Cells

[0132] DENV-1 16007, DENV-2 16681, DENV-3 16562, and DENV-4 1034 served as wild-type (wt) DENV controls, and they were the parental genotype viruses for the four recombinant DENVax vaccine candidates. DENVax progenitor research-grade viruses, designated as D2/1-V, D2 PDK-53-VV45R, D2/3-V, and D2/4-V, were prepared and characterized previously. Vero (African green monkey kidney) cells used for making the master and working cell banks for vaccine production were originated from the American Type Culture Collection (ATCC) CCL81 cell line that has been characterized by the World Health Organization (WHO) for vaccine manufacture (WCB-Vero cells).

Derivation of Live Recombinant DENVax Viruses from cDNA Clones

[0133] To re-derive the candidate vaccine viruses under cGMP manufacturing conditions, the previously engineered DENV infectious cDNA clones, pD2-PDK-53-VV45R, pD2/1-V, pD2/4-V, and in vitro-ligated pD2/3-V containing the fill genome-length viral cDNAs were used to make fresh viral RNA transcripts by in vitro transcription as described previously. Briefly, XbaI-linearized DENV genomic cDNAs were treated with proteinase K, extracted with phenol/chloroform and precipitated in ethanol to remove any residual proteins, and then suspended in RNase-free Tris-EDTA buffer prior to transcription. The in vitro transcription was conducted using the AmpliScribe T7 High Yield Transcription kit (Epicentre Technologies) following the manufacturer's recommended protocol. The RNA A-cap analog, m7G(5')ppp(5')A (New England BioLabs), was incorporated during the 2-hr transcription reaction to add the 5'-terminal A-cap to the RNA transcript. The samples were then treated with DNase I to digest the template cDNA, followed by low pH phenol/chloroform extraction and ethanol precipitation to remove residual DNA and proteins. The purified RNA transcripts, suspended in RNase-free water, were distributed in 20-μl aliquots and stored at −80° C. until ready for transfection of cells. The integrity and concentration of the RNA transcripts were analyzed by agarose gel electrophoresis. Each 20-μl aliquot was estimated to contain sufficient genome-length viral RNA to permit transfection of 0.4-1×10^7 production-certified Vero cells by electroporation.

[0134] Transfection of each RNA transcript into WCB-Vero cells was performed in the cGMP facility at Shantha Biotechnics. DENVax RNA transcripts were thawed, mixed with 400 μl of the Vero cell suspension (1×10^7 cells/ml), and transferred to a pre-chilled sterile electroporation cuvette (4-mm gap) for electroporation by a Gene Pulser Xcell total system (BioRad Laboratories). Each sample was pulsed once at 250V/∞ Ohms/500 μF, incubated for 10-15 min at room temperature, transferred to a 75-cm^2 flask containing 30 ml of cell growth medium (MEM with 10% FBS), and incubated at 36° C.±PC, 5% CO_2 for 6 to 11 days. The culture medium was harvested, clarified by centrifugation, stabilized, and stored in small aliquots below −60° C. The viral titers of candidate vaccine stocks (termed P1 for passage level 1) resulting from transfection were determined by plaque titration assay in Vero cells and used for further propagation of the DENVax seeds.

Manufacture of DENVax Virus Seeds

[0135] P1 virus seeds were used to propagate DENVax pre-master, master, working, and bulk virus seed lots through a strategy designed to ensure the optimal genetic stability and safety of the manufactured lots. This strategy included three serial plaque purifications, as well as genetic analyses of viruses at various passage levels to select the optimal clonal virus population for continued seed production (Table 1). Briefly, the P1 seeds harvested from transfected cells were amplified once by infection of Vero cells at a MOI of 0.001 to generate the P2 seeds. Aliquots of the P2 seed stocks were evaluated by plaque morphology and complete viral genomic sequencing. The genetically confirmed P2 stocks were plated on Vero cell monolayers with overlay medium as described in the plaque titration section below to generate well-isolated plaques. After visualization with neutral red, six individual plaques from each of the 4 serotypes of vaccine viruses were isolated (plaque clones A to F) and mixed into 0.5 ml of culture medium (passage P3). Each of the six plaque suspensions was subjected to two additional rounds of plaque purification, resulting in twice- and thrice-plaque purified virus seeds at passages P4 and P5, respectively. The P5 viruses were amplified through two sequential Vero passages to produce P7 seed stocks.

[0136] Genetic analysis of the three major DENVax attenuation loci using spot sequencing and/or Taqman-based mismatched amplification mutation assay (TaqMAMA) as previously disclosed, and plaque phenotype analysis were conducted to screen all 24 P7 seeds. Seeds possessing appropriate initial characteristics were then further characterized by full genomic sequencing. As a result of these analyses, one of the 6 (clone A-F) P7 seeds of each DENVax serotype was selected to be the pre-master seed, based on the presence of the DENV-2 PDK-53 attenuating mutations, minimal genomic sequence alterations, and expected plaque phenotype. Each selected pre-master seed was expanded to master virus seed (MVS or P8) by a one-time passage of the virus at MOI of 0.001 in multiple 175 cm^2 flasks of Vero cells. Except for the DENVax-4 MVS, the master virus seeds were harvested at 8-10 days post infection (pi). The MVS stocks were harvested at 6-10 days post infection (pi), clarified by centrifugation, stabilized by the addition of sucrose/phosphate/glutamate solution (final concentration 7.5% sucrose, 3.4 mM potassium dihydrogen phosphate, 7.2 mM dipotassium hydrogen phosphate, 5.4 mM monosodium glutamate, respectively) and 0.95 to 1.90% FBS (final concentration). DENVax-4 MVS was prepared differently to optimize its yield. Briefly, multiple flasks of cells were infected with DENVax-4 pre-master seed at a MOI of 0.001

in the presence of 0.1% F-127T™, poloxamer 407, (other EO-PO block copolymers have been assessed and may substitute here, see issued patent) that have been demonstrated to enhance DENV virus thermal stability. Infectious media was harvested days 6-10 pi, and stabilized with 17% FBS (final concentration), pooled, and frozen. All four DENVax MVS stocks were stored as 1-mi aliquots below −60° C.

[0137] The DENVax working virus seeds (WVS) were prepared by one-time passage in Vero cell culture of the MVS at a MOI of 0.001. The procedures were similar to the production of MVS, except they were cultured in multiple-layer cell factories (6360 cm^2). The WVS stocks were filtered through 10 μM and 0.45 μM filters, stabilized with the same stabilizers used for the MVS, aliquoted into 30 ml PETG bottles or 2.0 ml cryovials, and stored below-60° C.

[0138] In certain methods, bulk virus seeds (BVS) were produced by infecting multiple cell factories (6360 cm^2 each) of confluent Vero cells with 90 mL of diluted WVS to attain a MOI of 0.001. A media used for dilution of the WVS inocula contained 0.1% F-127™ without scrum. After 1.5 hr adsorption, cells were washed 3 times with PBS, and 800 ml of serum-free DMEM medium was added to each cell factory, and the factories were incubated at 36(±1°) C in 5(±0.5)% CO_2. After incubation for four days, small aliquots of medium were collected for sterility testing. Viruses were harvested between day 5 and day 10 pi, and immediately clarified by filtration through a 0.45 um pore size filter, and 1 L of each clarified virus pool was stabilized by addition of 500 ml of 3×FTA buffer (final concentrations of 15% trehalose, 1.0% Pluronic® F-127™ poloxamer 407, 0.1% human albumin USP in PBS, pi 7.4). The stabilized virus was distributed into I-L PETG bottles and stored frozen below −60° C. for subsequent pooling and quality control testing. All stabilized virus harvests with a virus titer above 10^5 PFU/ml and an acceptable level of residual DNA were rapidly thawed in a water bath at 32° C., then aseptically pooled and mixed. Each pooled monovalent BVS was distributed into labeled PETG containers and stored at below −60° C. until further use.

Manufacture Product Quality Controls

[0139] The MVS, WVS, and BVS seeds were tested for identity, sterility, and detectable adventitious agents. The identity of each vaccine stock was confirmed by RT-PCR with DENVax serotype-specific primers. The amplified cDNA fragments contained the E/NS1 chimeric junction site to permit identification of each of the four DENVax serotypes. Each seed was tested in all 4 serotype-specific RT-PCR reactions to confirm viral identity and freedom from cross contamination with heterologous DENVax serotypes. Sterility testing was performed in accordance with USP 71 (United States Pharmacopeia, section 71). Mycoplasma testing was performed.

[0140] The following in vitro and in vivo tests for viral contamination were all performed using unclarified, unstabilized DENVax harvests collected during manufacture of the seeds. Harvested infectious media were first neutralized with DENV rabbit polyclonal antiserum (Inviragen) at 36±1° C. for 1 hr to inactivate the DENV. For in vitro test, the neutralized seeds were inoculated into three indicator cells lines, MRC5. VERO and MA 104, in 25 cm^2 flasks. Echo virus (CPE control) or mumps virus (hemadsorption control) were used as positive CPE or hemadsorption control, respectively. All cells were monitored daily for CPE for a total of 14 days. At the end of 14 days, the culture supernatant was removed and replaced with 10 mL of a guinea pig red blood cell (RBC) solution (3 mL of 0.5% guinea pig RBC in phosphate buffered saline, made up to 10 mL with cell growth medium). The flasks were then incubated at 5±3° C. for 30 minutes followed by incubation at room temperature for 30 minutes. The monolayers were washed with PBS and observed under 10× magnification for the presence of any star-shaped clumps of RBCs for hemadsorption.

[0141] In vivo tests for adventitious agents were performed in suckling mice, post-weaning mice and guinea pigs. Suckling mice were inoculated with 0.1 ml or 0.01 ml (10 mice in each dose group) of the DENV-antiserum neutralized seed sample through intraperitoneal (ip) injection. Similarly, 10 post-weaning mice were each inoculated ip with 0.5 ml or 0.03 ml of the sample. Guinea pigs (5/group) were each inoculated ip with 5.0 mL. Suckling mice were observed daily for morbidity and mortality for a total of 14 days following inoculation. Post-weaning mice were observed for a total of 28 days, and guinea pigs were observed for a total of 42 days following inoculation. The test articles met the acceptance criterion if ≥80% of the inoculated animals remained healthy throughout the observation period.

[0142] The in vivo testing for contaminants was also performed in embryonated chicken eggs and was conducted. For every sample, 10 embryonated hen eggs (9 days old) were each inoculated with 0.5 mL of the DENV antiserum-neutralized sample into the allantoic fluid and incubated at 35° C. for 3 days. The allantoic fluids from these 10 eggs were harvested, pooled and passaged into the allantoic fluid of 10 fresh embryonated eggs (10-11 days old; 0.5 mL/egg) and incubated at 35° C. for a further 3 days. Similarly, for each sample, 10 embryonated eggs (6-7 days old) were each inoculated with 0.5 mL per egg (DENVax-2 monovalent BVS) or 0.25 mL per egg (DENVax-1, DENVax-3 and DENVax-4 BVS) by injection into the yolk sac and incubated at 35° C. for 9 days. The yolk sacs from these 10 eggs were harvested and pooled, and a 10% suspension was passaged into the yolk sacs of 10 fresh embryonated eggs (6-7 days old; 0.5 mL/egg) and incubated at 35° C. for a further 9 days. Eggs inoculated into the allantoic fluid (both initial and passage inoculations) were observed for viability after 3 days incubation. Both pools of allantoic fluid were tested for hemagglutination activity using chicken, guinea pig and human type O erythrocytes at 4° C. and 25° C. Eggs inoculated into the yolk sack (both initial and passage inoculations) were observed for viability after 9 days of incubation.

Virus Plaque Assay and Immunofocus Assay

[0143] Virus titers were measured by plaque assay or immunofocus assay using Vero cells. Plaque assays were performed in double agarose overlays in six-well plates of confluent Vero cells as previously described, and they were also used to evaluate the plaque phenotypes of the DENVax seeds. For accurate comparison, plaque sizes of all viruses were measured and compared in the same experiment. After visualization with neutral red on day 9 pi, up to 10 well isolated plaques for each virus were measured for mean plaque size calculation. Fewer plaques were measured for wt

DENV-1, -3, and -4, whose larger plaque sizes often did not permit measurement of 10 well-separated plaques.

[0144] Because tetravalent DENVax contains all four DENV serotypes, a DENV serotype-specific immunofocus assay was developed to quantitate each DENVax component in the tetravalent formulations. Immunofocus assays of each individual DENVax MVS were compared with the plaque assays to ensure virus titration results were comparable between the two assays. The immunofocus assay was conducted in 6-well plates of confluent Vero cells infected with serially diluted viruses. Cells were overlayed with a balanced salt medium (BSS/YE-LAH medium) containing 0.7% high viscosity carboxymethyl cellulose (Sigma) and incubated for 7 days at 37° C. with 5% CO_2. After removal of overlays, cell sheets were washed 3 times with PBS, fixed with cold 80% acetone for 30 min at −20'C, washed once with PBS, and blocked with a blocking buffer containing 2.5% (w/v) nonfat dry milk, 0.5% Triton X-100, 0.05% Tween-20 in PBS at 37° C. for 30 min. Blocked cells were incubated with diluted DENV serotype-specific MAbs, IF1 (DENV-1), 3H5 (DENV-2), 8A-1 (DENV-3), or 1H10 (DENV-4) in blocking buffer at 37° C. for 1 hour or 4° C. overnight, washed 3 times with washing buffer (0.05% Tween-20 in PBS), and incubated with alkaline phosphatase- or horse radish peroxidase (HRP)-conjugated affinity-pure goat anti-mouse IgG (Jackson Immuno Research Laboratories) at 37° C. for 45-60 min. Plates were washed 3 times before the appropriate substrate, 1-Step NBT/BCIP plus suppressor (Pierce) for alkaline phosphatase or Vector-VIP kit (Vector Labs) for HRP, was added for color development. Color development was stopped by rinsing with water when the foci were fully developed. Stained immunofoci were directly visualized and counted on a light box.

Genetic Sequence

[0145] Full length genomes of the MVS and WVS were sequenced (see below). Briefly, viral RNA was extracted from DENVax seeds by using the QIAamp viral RNA kit (Qiagen), and overlapping cDNA fragments covering the entire genome were amplified using the Titan One Tube RT-PCR kit (Roche Applied Science, Inc.). The amplified cDNA fragments were gel purified before sequencing with both forward and reverse primers using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Sequence reactions were cleaned using the BigDye XTerminator Purification kit (Applied Biosystems), and run on the 3130xl Genetic analyzer (Applied Biosystems) at DVBD/CDC. The Lasergene SeqMan software (DNAStar, Inc) was used for genome analysis and comparison.

Taqman-Based Mismatch Amplification Nutation Assay (TaqMAMA)

[0146] TaqMAMA is a sensitive, quantitative single nucleotide polymorphism assay developed to permit finer assessment of the level of reversion at the 5'NC-57 locus of attenuation, and was further optimized for this study. Extracted viral RNA from MVS and WVS were analyzed by the TaqMAMA with both sets of primers/Tagman probe that are specific to wt or the vaccine 5'NC-57 region. The forward primers used to detect DENV-2 wt and vaccine sequences were D2-41-GC and D2-40-TT, respectively. The 3'-terminal nucleotide of each forward primer matched the specific 5'NCR-57 nucleotide for each virus, while the nucleotide adjacent to the 3'-terminal nucleotide in each primer differed from the DENV-2 viral genomic sequence to enhance the mismatch effect. The reverse primer, CD-207, and the Taqman probe, CD-169F, for both wt and vaccine sets were identical. Sequences of the primers and probe as well as cycling conditions were described previously. The real time RT-PCR was performed with the iQ5 or CFX-95 system (BioRad), using a BioRad iScript RT-PCR (for probes) kit, in a 25-μl reaction containing 5 μl of viral RNA template, 0.4 uM of each primer, and 0.2 uM of the probe. Triplicate reactions for each wt- and vaccine-specific assay were conducted for each sample. Genome copy numbers were determined relative to a standard curve prepared for each viral genotype, where the RNA standards were transcripts derived from plasmids containing nt 1-2670 of each genotype-specific cDNA. In addition, the specificity of the assay was confirmed by testing each RNA standard with the heterologous genotype primer/probe sets to ensure minimum cross-reactivity in every experiment. The results were reported as the percentage of viral genomes showing reversion. Previously, due to higher cross-reactive backgrounds that limited the input RNA levels for this assay, the original detection sensitivity was about 0.1% reversion (discrimination power). Since then, the assay has been further optimized using improved real-time PCR equipment and reaction kits, and the cross-reactive background was decreased considerably at much high levels (7-8 $\log_{10}$ copies) of RNA template input. This optimization resulted in significant improvement of the detection sensitivity, down to 0.01-0.07% reversion.

Virus Replication in Mosquito C6/36 Cells and Temperature Sensitivity in Mammalian Vero Cells

[0147] The replication phenotypes of the four DENVax MVS stocks and wt DENV-1, -2, -3, and -4 viruses were evaluated in C6/36 mosquito cells (*Aedes albopictus*). C6/36 cells grown in 6-well plates were infected in duplicate with each virus at a MOI of 0.001 and incubated with 4 ml/well of DMEM medium containing 2% FBS in a 5% CO_2 incubator at 28° C. Small aliquots of the culture supernatant were collected for each virus on day 6 pi, mixed with an equal volume of medium containing 40% FBS, and stored at −80° C. until ready for virus plaque titration.

[0148] Temperature sensitivity was conducted by comparing viral growth at 39° C. versus growth at 37° C. at five days pi of Vero cells in 6-well plates. Cells were infected in quadruplicate with each virus at a MOI of 0.001 at 37° C. Following adsorption of virus, the infected cultures were incubated with 4 mL/well of DMEM medium containing 2% FBS in 2 separate 5% CO_2 incubators, one set (duplicate plates) at 37° C. and the other at 39° C. Aliquots (50-μl) of the culture supernatant were collected on day 5 pi, mixed with an equal volume of DMEM containing 40% of FBS, and stored at −80° C. until ready for virus plaque titration. Incubator temperatures were calibrated with NIST-traceable factory-calibrated thermometers (−1 to 51° C.; ERTCO).

Mosquito Infection, Dissemination, and Transmission

[0149] *Aedes aegypti* mosquitoes used for the study were from a colony established in 2002 from a village near Mac Sot (16' N, 33' E), Thailand. After emerging from larvae, adult mosquitoes were maintained at 28° C. at a 16:8 (light:dark) photoperiod with 10% sucrose solution provided ad libitum. Five-to-seven day old female mosquitoes were

used for infectious blood meal feeding or intrathoracic (IT) inoculations. Aliquots of freshly cultured DENVax and wt DENV were used immediately upon harvest (without any freeze-thaw cycle) to make virus blood meals as indicated below for oral infection. Remaining virus supernatants were supplemented with FBS to a final concentration of 20%, and aliquots were stored at −80° C. for future virus plaque titration and IT inoculation experiments. The freshly prepared DENVax seeds for these experiments were amplified from the pre-master seeds in Vero cells, and were considered DENVax MVS equivalents.

[0150] Infectious blood meals were prepared by mixing fresh virus at a ratio of 1:1 with defribrinated chicken blood (Colorado Serum Company) on the day of oral infection. Mosquitoes were sugar-starved overnight and then offered the virus:blood mixture for 1 hour using a Hemotek membrane feeding system (Discovery Workshops). A 50-μl aliquot of the blood meal was retained at −80° C. for back-titration of virus doses. Fully-engorged females were sorted under cold anesthesia and placed into cartons with 10% sucrose solution provided ad libitum. Cartons were placed at 28° C. with a photoperiod of 16:8 h (light:dark). After 14 days, 25-30 mosquitoes from each virus group were anesthetized via exposure to triethylamine (Flynap®, Carolina Biological Supply Company) and one hind leg was removed and placed in 0.5 ml of DMEM with 10% FBS and 5% penicillin/streptomycin (100 U/ml and 100 μg/ml respectively). Saliva was collected by inserting the proboscis of the anesthetized mosquito into a capillary tube containing 2.5% FBS and 25% sucrose solution. Mosquitoes were allowed to salivate for at least 15 minutes and then capillary tubes and bodies were placed into separate tubes containing DMEM. Mosquito bodies, legs and saliva were stored at −80° C. until they were triturated and assayed for infectious virus. For IT inoculation, mosquitoes were cold-anesthetized and inoculated with approximately 50 pfu of virus in 0.34 μl inoculum. Inoculated mosquitoes were kept for 7 days in the same conditions as described above. Mosquitoes were then anesthetized, and their saliva and bodies were collected as described above. Samples were stored at −80° C. until further processing.

[0151] To process the samples for virus titration, body and leg samples were homogenized with copper coated BBs (Crossman Corporation, N.Y.) at 24 cycles/second for 4 min using a mixer mill, and then clarified by centrifuging at 3,000×g for 3 min. Saliva samples were centrifuged at 3.000×g for 3 minute, to expel fluid from capillary tubes. Ten-fold dilutions of the body and leg homogenates and saliva samples were tested for presence of infectious virus by plaque assay. Results from bodies, legs, and saliva were used for determining the infection, dissemination, and transmission rates, respectively.

Mouse Neurovirulence

[0152] Timed pregnant female ICR mice were obtained from Taconic Labs, and monitored several times each day to determine approximate birth times of pup litters. In a given experiment, approximately 12-24 hours after birth, two litters of eight pups per virus (n=16), was challenged with 10^3 to 10^4 pfu of virus in 20 μl of diluent by intracranial (ic) inoculation using a 30-gauge needle. Animals were monitored at least 3 times daily for at least 32 days following challenge. At the first sign of illness (rough fur, hunched back, weight loss, abnormal movement, paralysis, or lethargy) animals were euthanized by lethal anesthetization with isoflurane gas, followed by cervical dislocation. The post-infection day of euthanasia represented the "time to illness/morbidity" or "survival time" for the animal. The animal experiments were conducted following a DVBD/CDC IACUC-approved animal protocol.

Derivation of Master Seed Viruses

DENvax-1 Master Virus Seed (MVS)

[0153] Nucleotide sequence of the chimeric viral genome and deduced amino acid sequence of the translated protein are provided herein. Most of the prM-E gene (nt 457 to -2379, underlined) is wild-type (wt) DEN-1 16007 virus specific; the remaining genome is DEN-2 PDK-53 virus specific. All engineered substitutions differ from wt virus (D1 16007 or D2 16681), as well as extra mutations (changes from engineered cDNA clone) detected in the MVS are marked.

[0154] Substitutions Included in the Genome and Protein:

[0155] Junction sites between D1 (prM-E) and D2 backbone:

- **[0156]** a. MluI (nt 451-456): engineered silent mutation, nt-453 A-to-G
- **[0157]** b. NgoMIV (nt 2380-2385): engineered mutations, nt-2381/2382 TG-to-CC (resulted in E-482 Val-to-Ala change)

[0158] D2 PDK-53 virus backbone (change from wt D2 16681): all in bold

- **[0159]** a. 5'-noncoding region(NCR)-57 (nt-57 C-to-T): major attenuation locus (in red)
- **[0160]** b. NS1-53 Gly-to-Asp (nt-2579 G-to-A): major attenuation locus (in red)
- **[0161]** c. NS2A-181 Leu-to-Phe (nt-4018 C-to-T)
- **[0162]** d. NS3-250 Glu-to-Val (nt-5270 A-to-T): major attenuation locus (in red)
- **[0163]** e. nt-5547 (NS3 gene) T-to-C silent mutation
- **[0164]** f. NS4A-75 Gly-to-Ala (nt-6599 G-to-C)

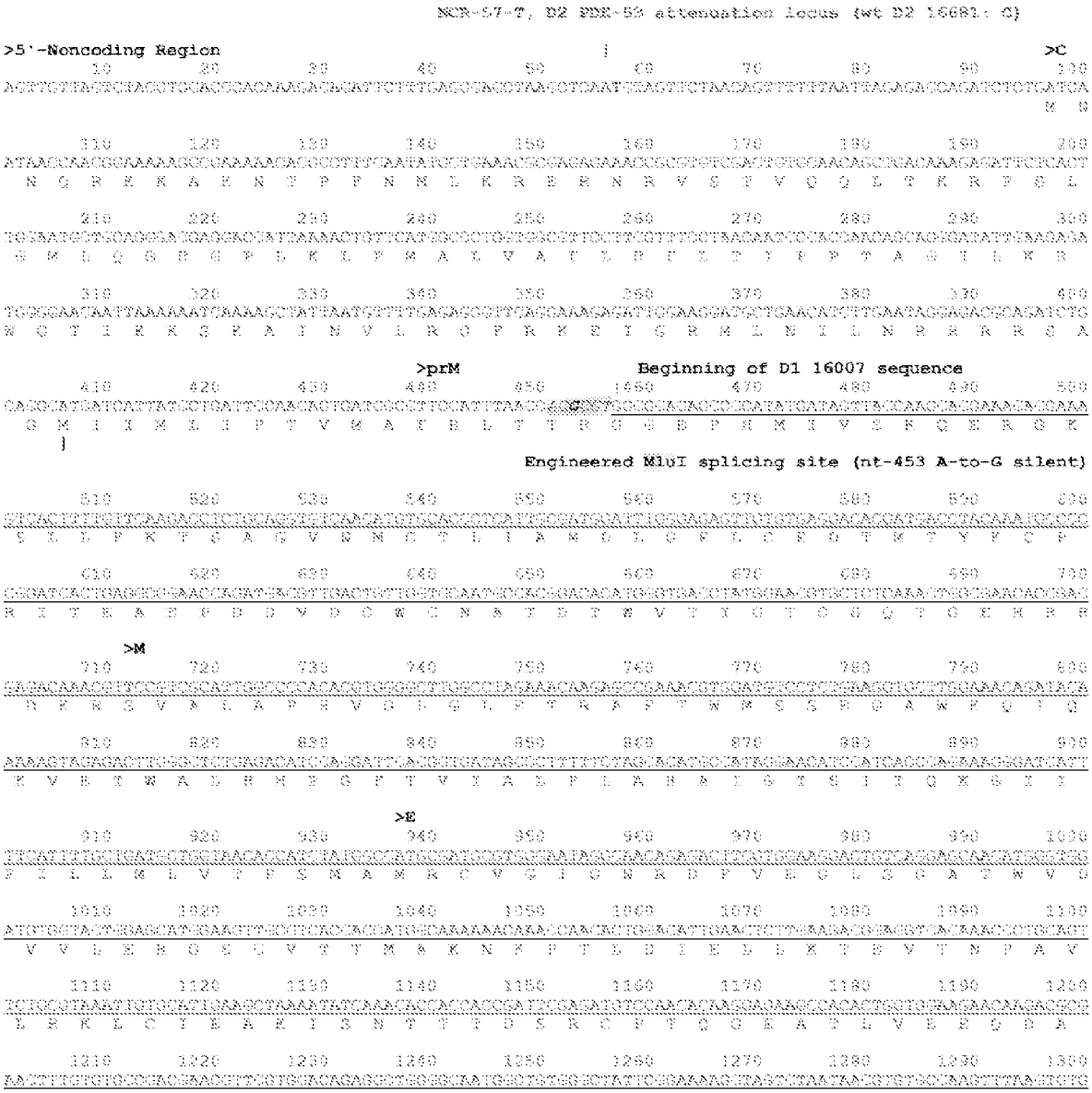
[0165] nt-8571 C-to-T silent mutation of PDK-53 is not engineered in the vaccine virus

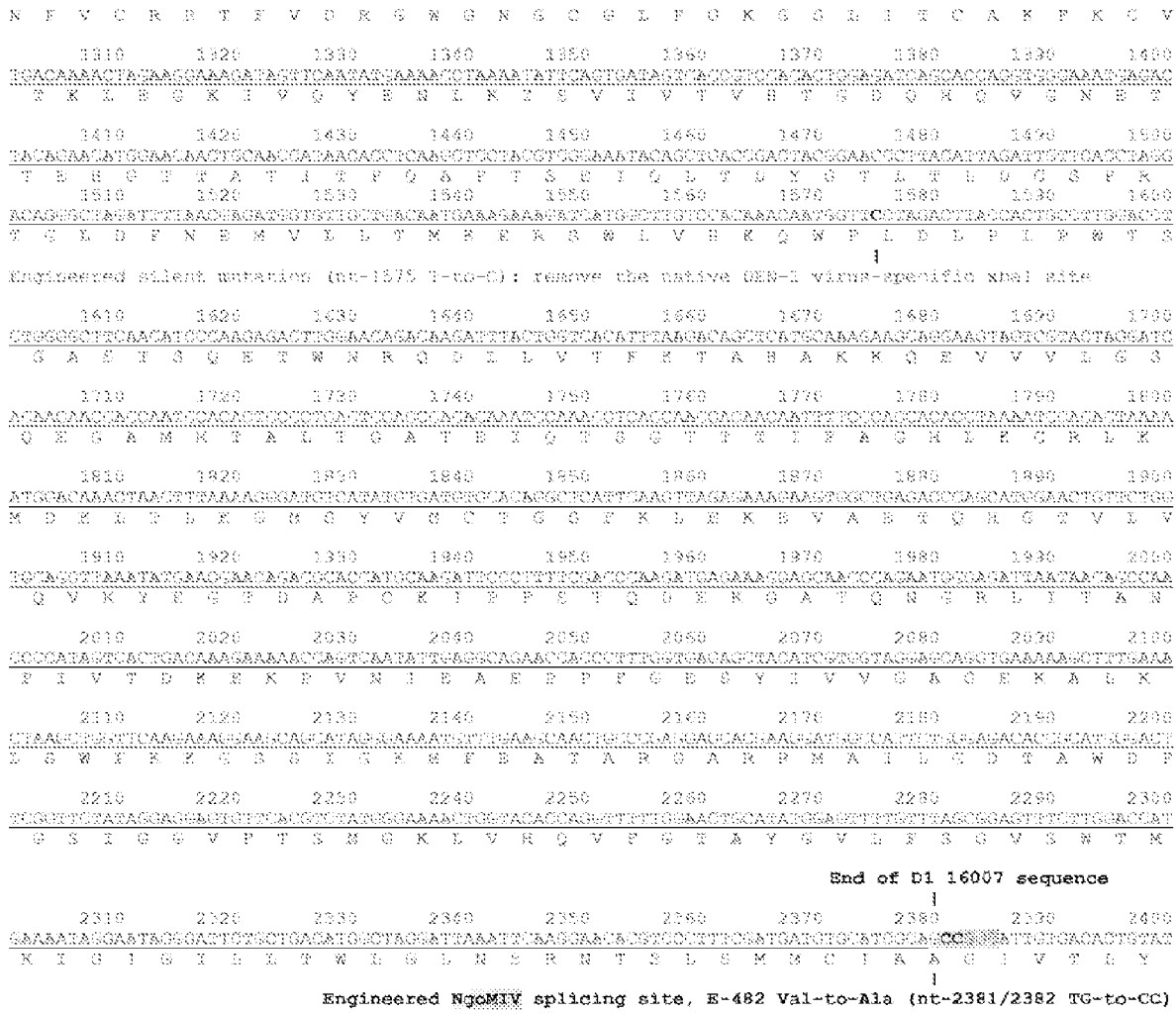
[0166] DEN-1 prM-E (change from wt D1 16007)

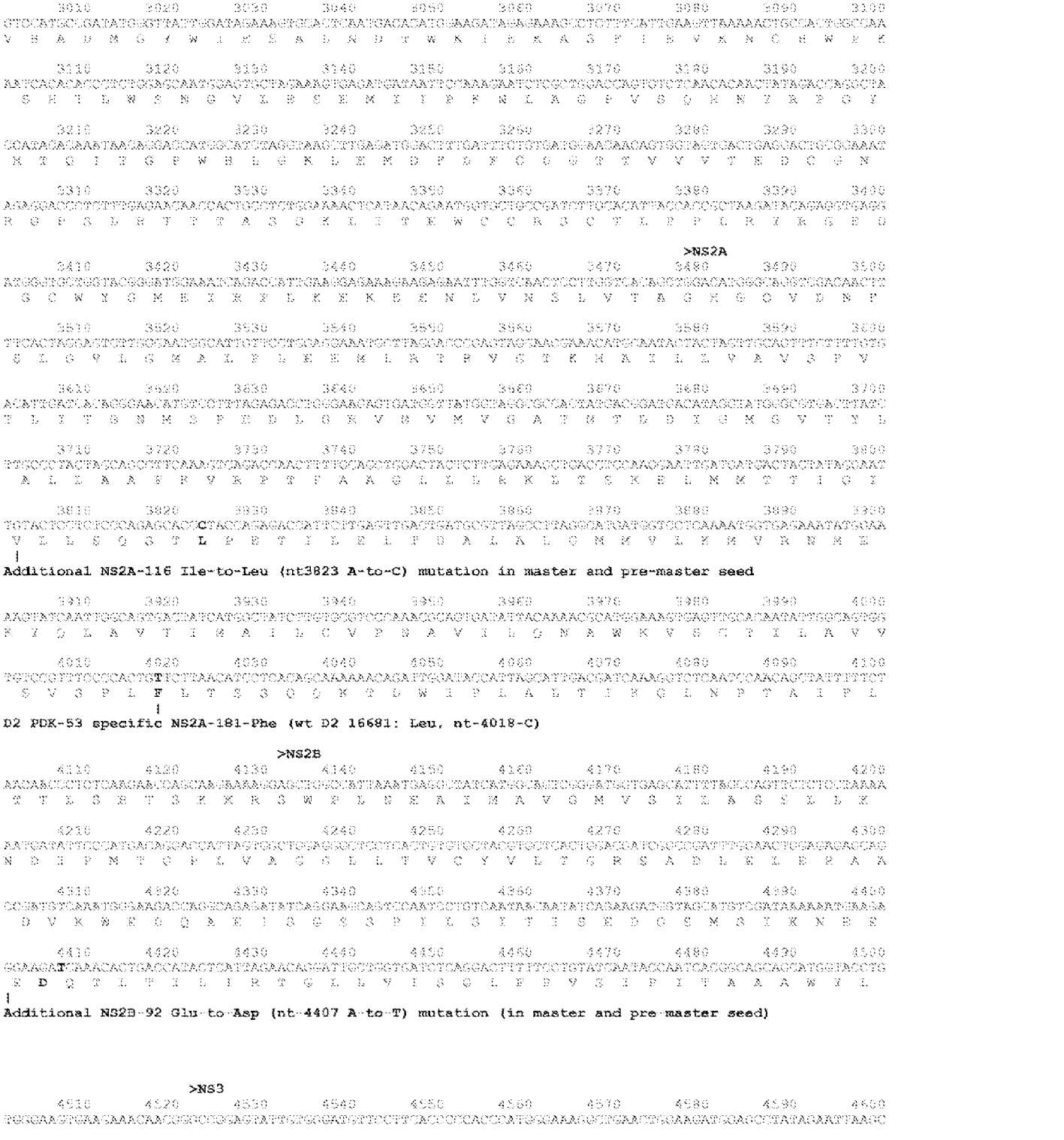
- **[0167]** a. Engineered nt-1575 T-to-C silent mutation to remove native XbaI site

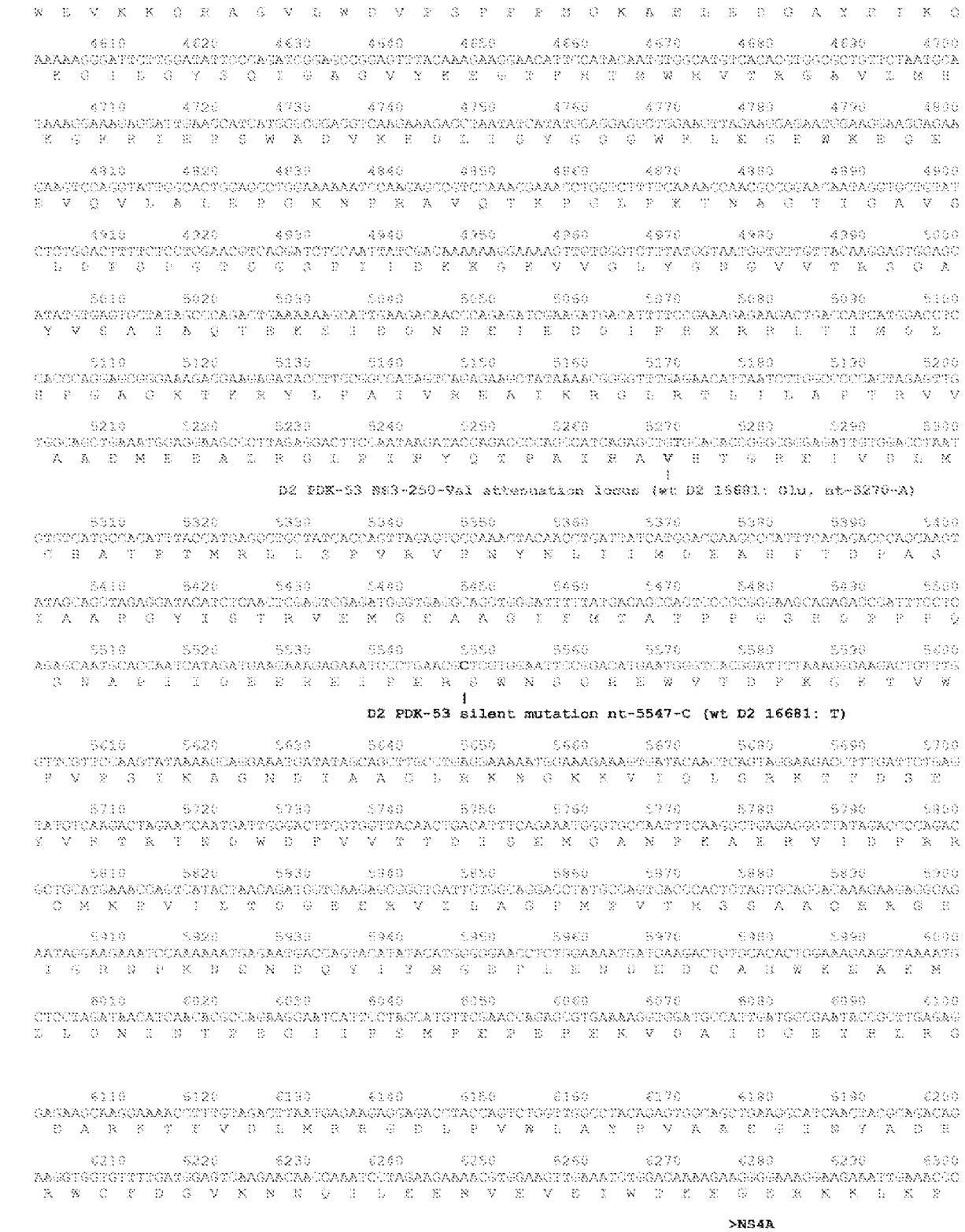
[0168] Additional substitutions found in vaccine seed (0.03% nt different from original clone)

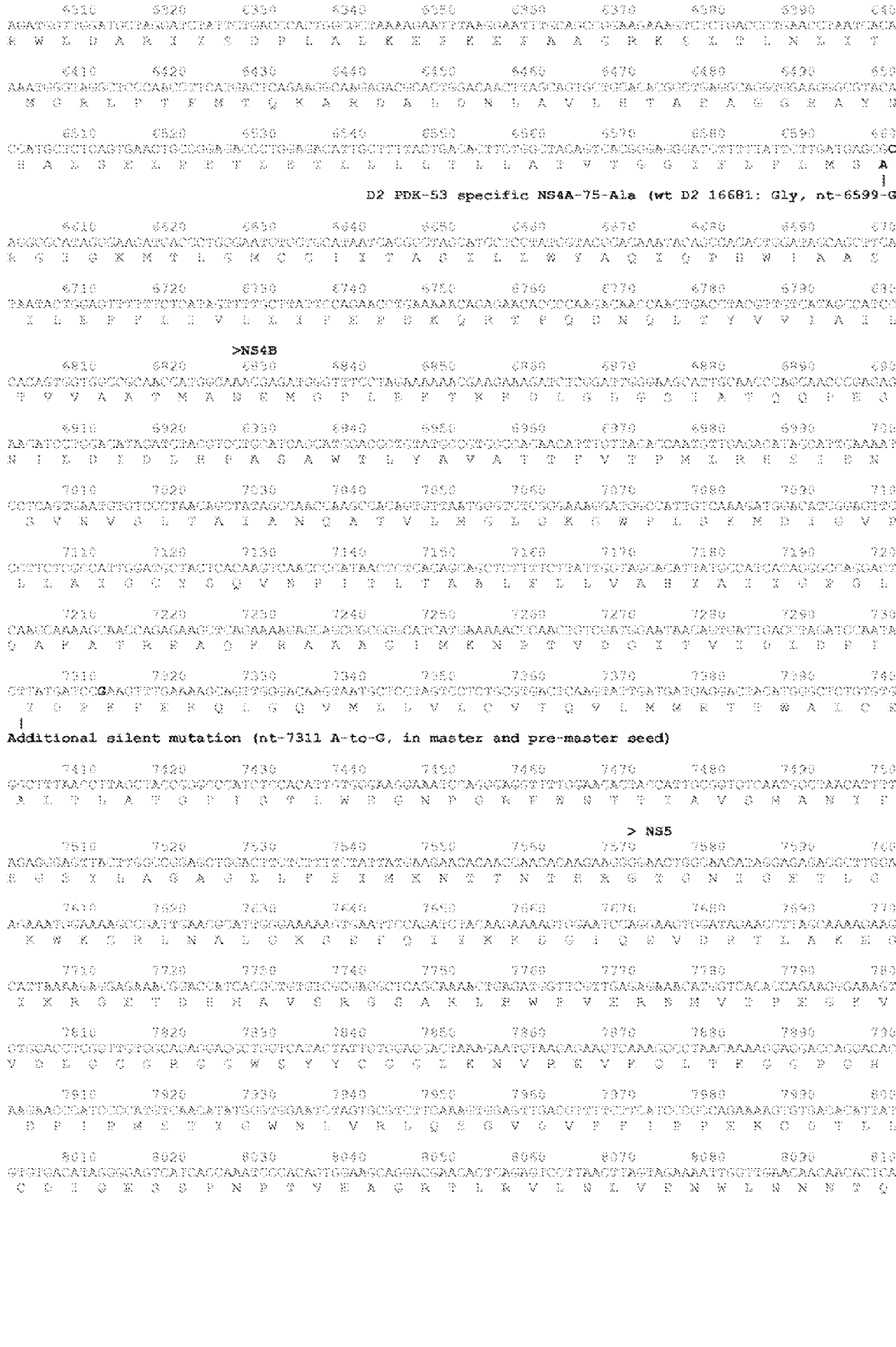
- **[0169]** a. NS2A-116 Ile-to-Leu (nt-3823 A-to-C, in bold)
- **[0170]** b. NS2B-92 Glu-to-Asp (nt-4407 A-to-T, in bold)
- **[0171]** c. nt-7311 A-to-G silent mutation (in bold)

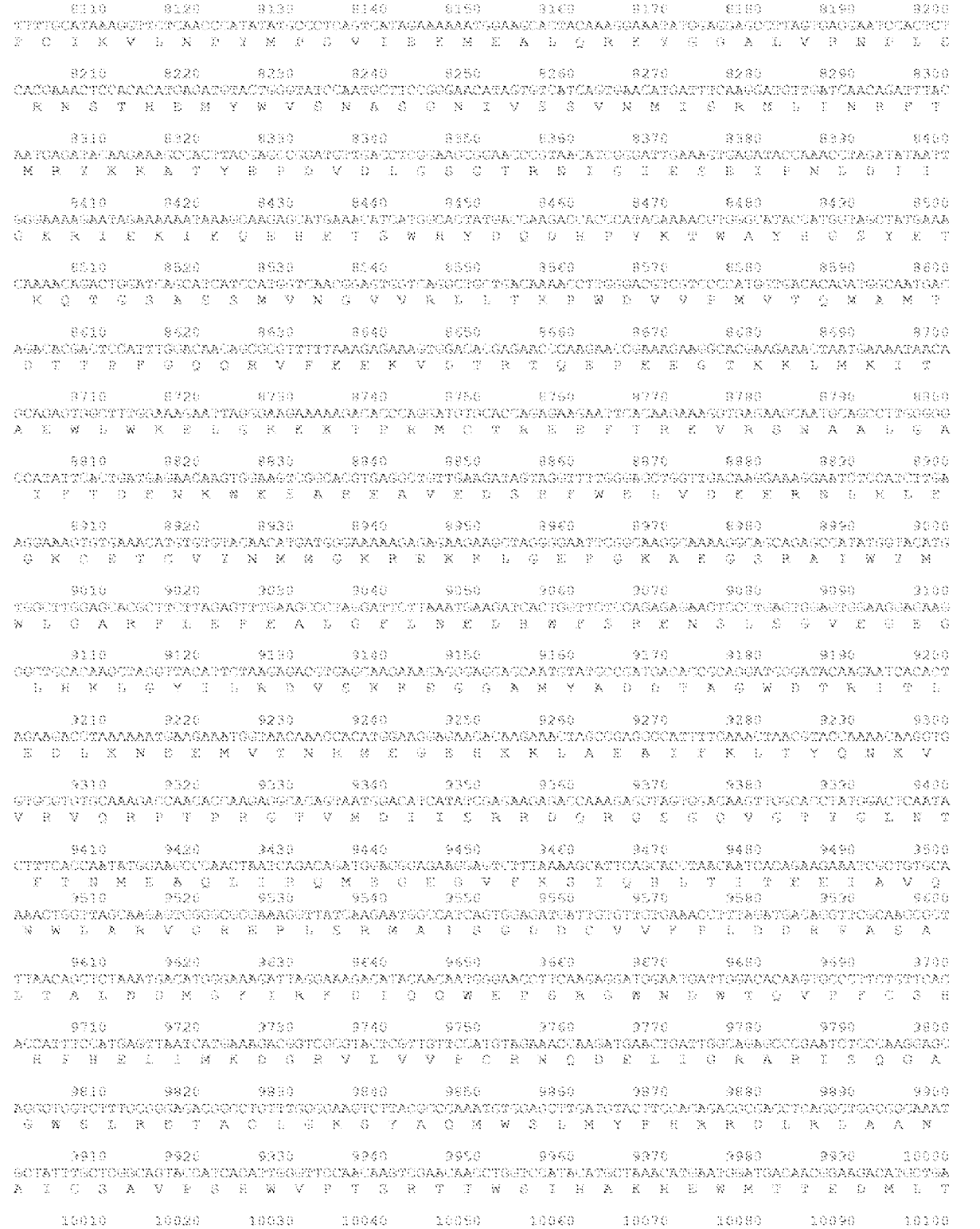

NCR-5'-7, D2 PDK-53 attenuation locus (wt D2 16681: C)
>5'-Noncoding Region
>C
>prM
Beginning of D1 16007 sequence
Engineered MluI splicing site (nt-453 A-to-G silent)
>M
>E


End of D1 16007 sequence
Engineered NgoMIV splicing site, E-482 Val-to-Ala (nt-2381/2382 TG-to-CC)
>NS1
D2 EDE-53 NS1-53-Asp attenuation locus (wt D2 16681: Gly, nt-2579-G)


>NS2A
Additional NS2A-116 Ile-to-Leu (nt3823 A-to-C) mutation in master and pre-master seed
D2 PDK-53 specific NS2A-181-Phe (wt D2 16681: Leu, nt-4018-C)
>NS2B
Additional NS2B-92 Glu-to-Asp (nt-4407 A-to-T) mutation (in master and pre-master seed)
>NS3


D2 PDK-53 N83-250-Val attenuation locus (wt D2 16681: Glu, nt-5270-A)
D2 PDK-53 silent mutation nt-5547-C (wt D2 16681: T)
>NS4A


D2 PDK-53 specific NS4A-75-Ala (wt D2 16681: Gly, nt-6599-G)
>NS4B
Additional silent mutation (nt-7311 A-to-G, in master and pre-master seed)
> NS5




>3'-Noncoding Region

[0172] DENvax-2 Master Virus Seed (MVS)

[0173] Nucleotide sequence of the recombinant viral genome and deduced amino acid sequence of the translated protein are provided herein. The engineered virus is based on D2 PDK-53 virus. All engineered substitutions that are different from wild-type DEN-2 16681 virus (also the parental virus for PDK-53), as well as extra mutations (changes from engineered cDNA clone) detected in the MVS are marked.

[0174] Substitutions Included in the Genome and Protein:

[0175] D2 PDK-53 virus backbone (change from wt D2 16681): all in bold

[0176] a. 5-noncoding region (NCR)-57 (nt-57 C-to-T): major attenuation locus (in red)

[0177] b. prM-29 Asp-to-Val (nt-524 A-to-T)

[0178] c. nt-2055 C-to-T (E gene) silent mutation

[0179] d. NS1-53 Gly-to-Asp (nt-2579 G-to-A): major attenuation locus (in red)

[0180] e. NS2A-181 Leu-to-Phe (nt-4018 C-to-T)

[0181] f. NS3-250 Glu-to-Val (nt-5270 A-to-T): major attenuation locus (in red)

[0182] g. nt-5547 (NS3 gene) T-to-C silent mutation

[0183] h. NS4A-75 Gly-to-Ala (nt-6599 G-to-C)

[0184] * nt-8571 C-to-T silent mutation of PDK-53 is not engineered in the vaccine virus

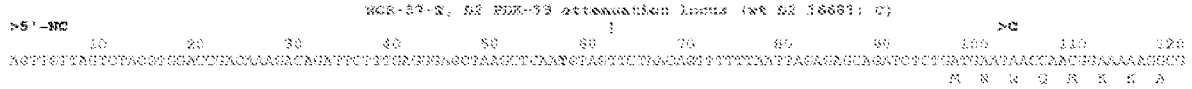
[0185] Engineered clone marker (silent mutation):

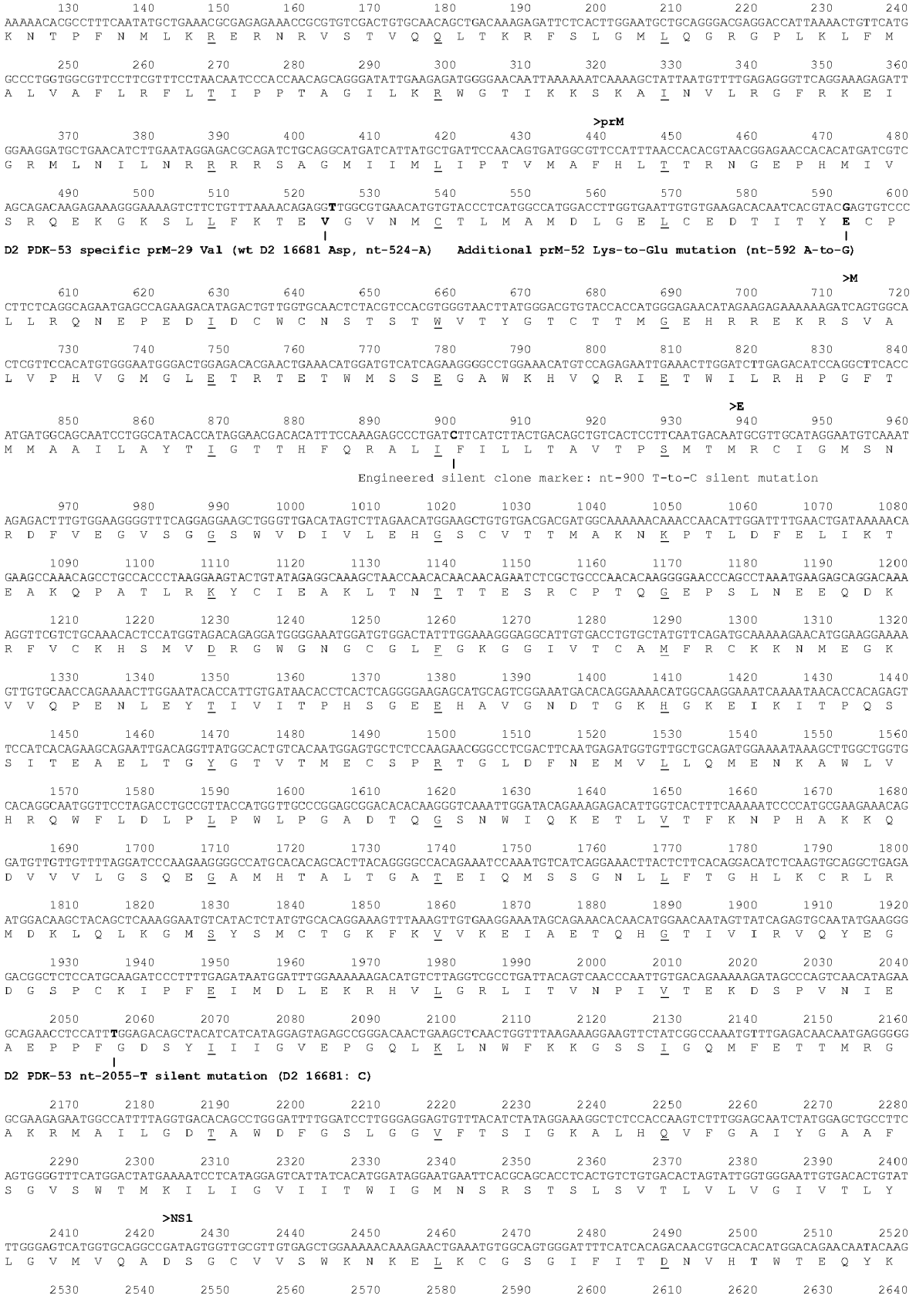
[0186] a. nt-900 T-to-C silent mutation: infectious clone marker

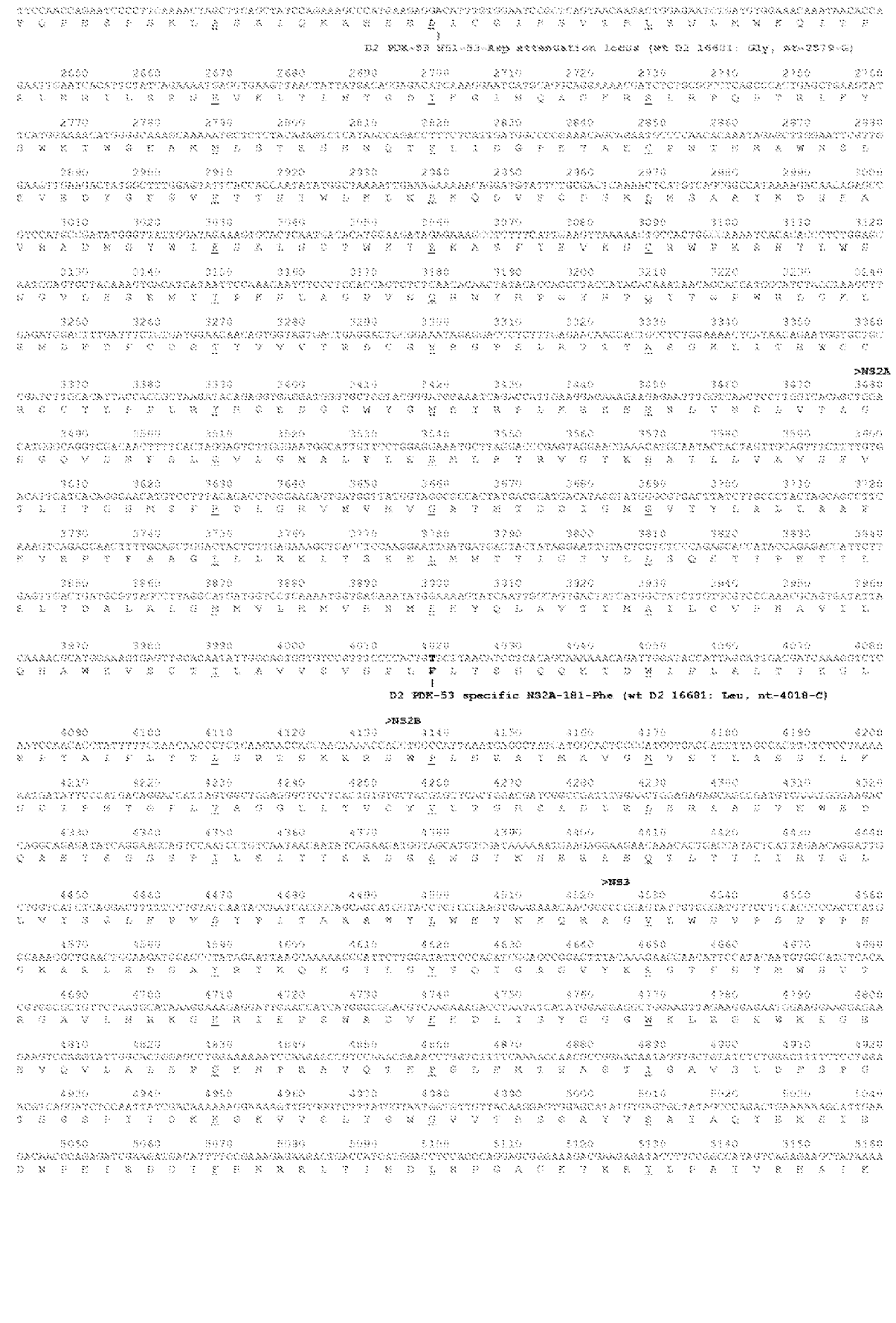
[0187] Additional substitutions found in vaccine seed (0.02% nt different from original clone)

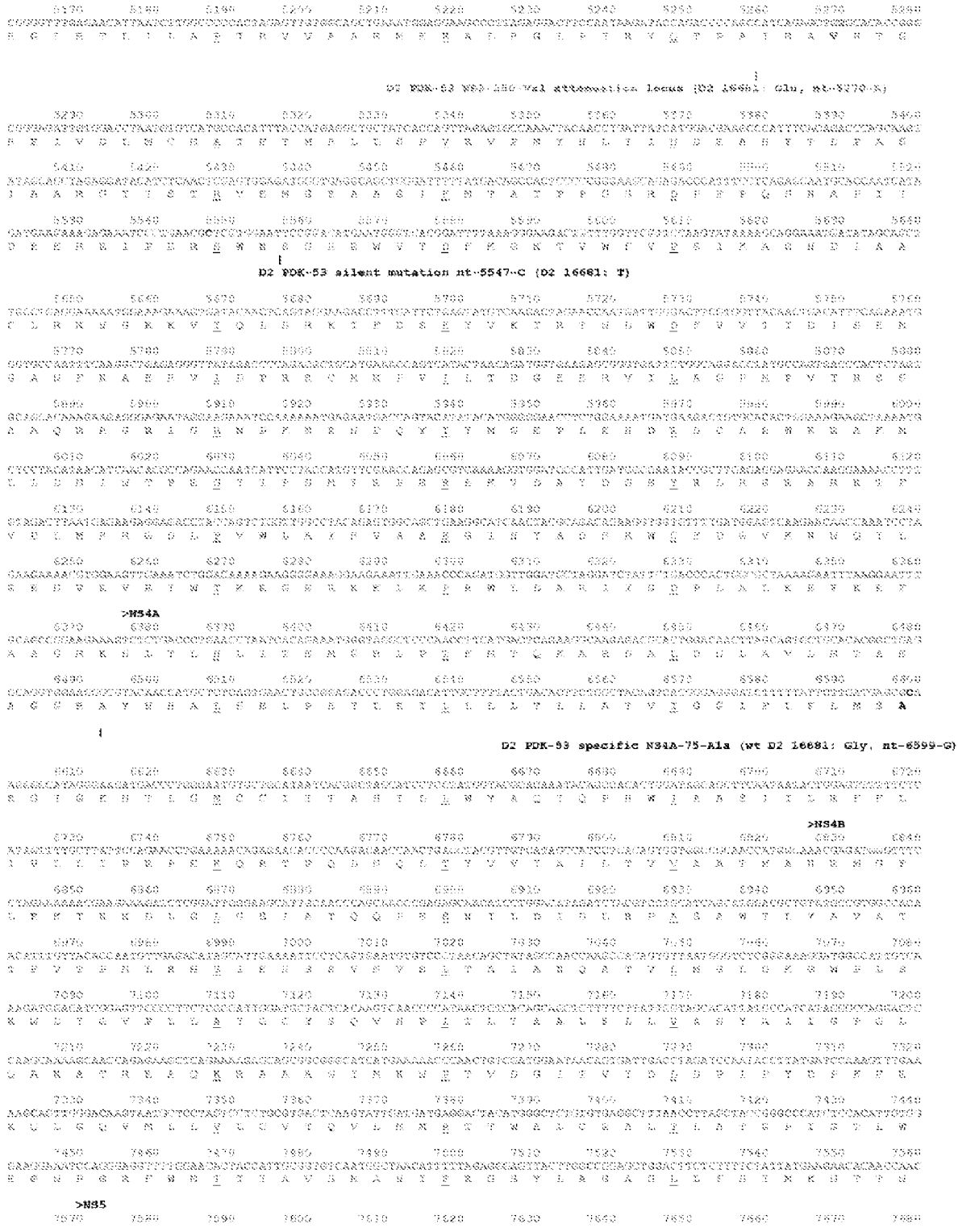
[0188] a. prM-52 Lys-to-Glu (nt-592 A-to-G), in bold

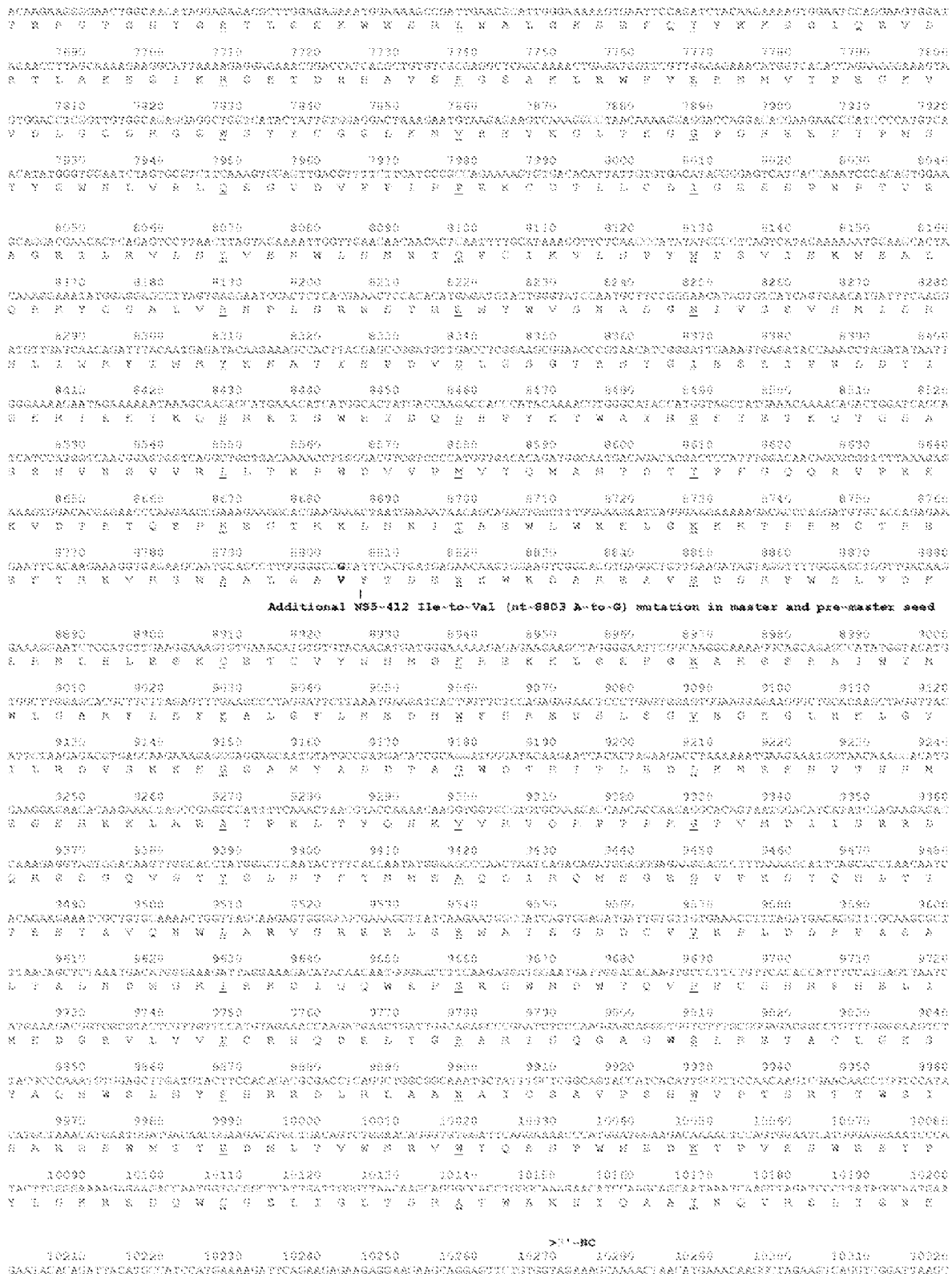
[0189] b. NS5-412 Ile-to-Val (nt-8803 A-to-G), in bold

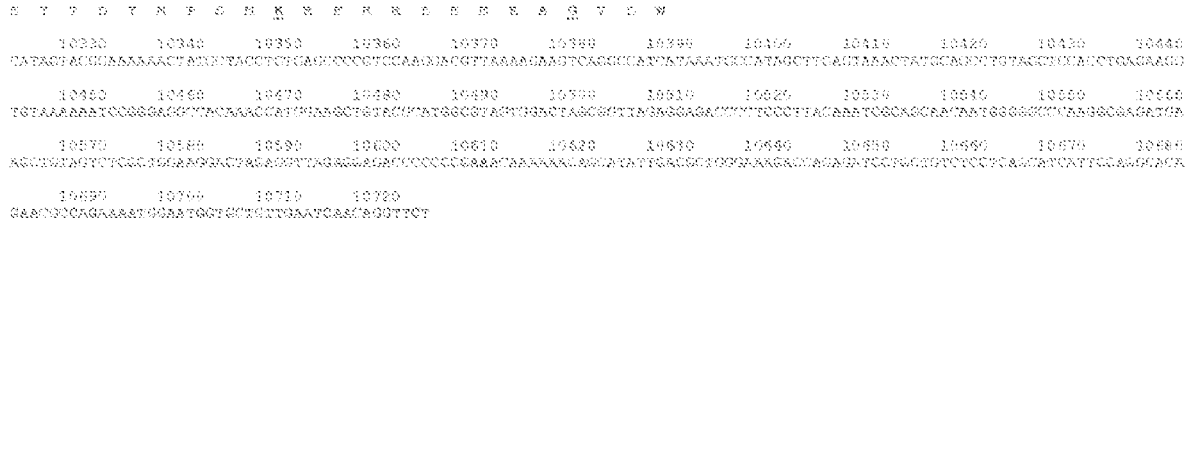

HCR-27-2, 62 PDX-93 attenuation locus (nt 62 16681: C)
>5'-NC
>C
10
20
30
40
50
60
70
80
90
100
110
120
M N K Q R K K A


>prM
D2 PDK-53 specific prM-29 Val (wt D2 16681 Asp, nt-524-A)
Additional prM-52 Lys-to-Glu mutation (nt-592 A-to-G)
>M
>E
Engineered silent clone marker: nt-900 T-to-C silent mutation
D2 PDK-53 nt-2055-T silent mutation (D2 16681: C)
>NS1


D2 PDK-53 NS1-53-Asp attenuation locus (wt D2 16681: Gly, nt-2579-G)
>NS2A
D2 PDE-53 specific NS2A-181-Phe (wt D2 16681: Leu, nt-4018-C)
>NS2B
>NS3


D2 PDK-53 silent mutation nt-5547-C (D2 16681: T)
>NS4A
D2 PDK-53 specific NS4A-75-Ala (wt D2 16681: Gly, nt-6599-G)
>NS4B
>NS5


Additional NS5-412 Ile-to-Val (nt-8803 A-to-G) mutation in master and pre-master seed



DENvax-3 Master Virus Seed (MVS)

[0190] Nucleotide sequence of the chimeric viral genome and deduced amino acid sequence of the translated protein are provided herein. Most of the prM-E gene (nt-457 to -2373, underlined) is wild-type (wt) DEN-3 16562 virus-specific; the remaining nucleotide sequence is DEN-2 PDK-53 virus-specific. The E protein of DEN-3 virus has two fewer amino acids than the E protein of DEN-2. Therefore, nt position starting from NgoMIV is 6 nt less than the original DEN-2 PDK-53 nt position. All engineered substitutions differ from wt virus (DEN-3 16562 or DEN-2 16681), as well as extra mutations (changes from engineered cDNA clone) are marked.

Substitutions Included in the Genome and Protein

[0191] Junction sites:

[0192] a. MluI (nt 451-456): engineered silent mutation, nt-453 A-to-G

[0193] b. NgoMIV (nt 2374-2379): engineered mutations, nt-2375/2376 TG-to-CC (resulted in E-480 Val-to-Ala change)

[0194] D2 PDK-53 virus backbone (change from wt D2 16681): in bold

[0195] a. 5'-noncoding region(NCR)-57 (nt-57 C-to-T): major attenuation locus (in red)

[0196] b. NS1-53 Gly-to-Asp (nt-2573 G-to-A): major attenuation locus (in red)

[0197] c. NS2A-181 Leu-to-Phe (nt-4012 C-to-T)

[0198] d. NS3-250 Glu-to-Val (nt-5264 A-to-T): major attenuation locus (in red)

[0199] e. nt-5541 (NS3 gene) T-to-C silent mutation

[0200] f. NS4A-75 Gly-to-Ala (nt-6593 G-to-C)

[0201] * nt-8565 C-to-T silent mutation of PDK-53 is not engineered in the vaccine virus

[0202] Engineered mutation in DEN-3 prM-E (change from wt D3 16562)

[0203] a. Engineered nt-552 C-to-T silent mutation: clone marker

[0204] b. Engineered E-345 His-to-Leu (nt-1970 A-to-T) for efficient replication in cultures

[0205] Additional substitutions found in vaccine seed (0.02% nt different from original clone)

[0206] a. E-223 Thr-to-Ser mutation (nt-1603 A-to-T, in bold)

[0207] b. nt-7620 A-to-G silent mutation (in bold)

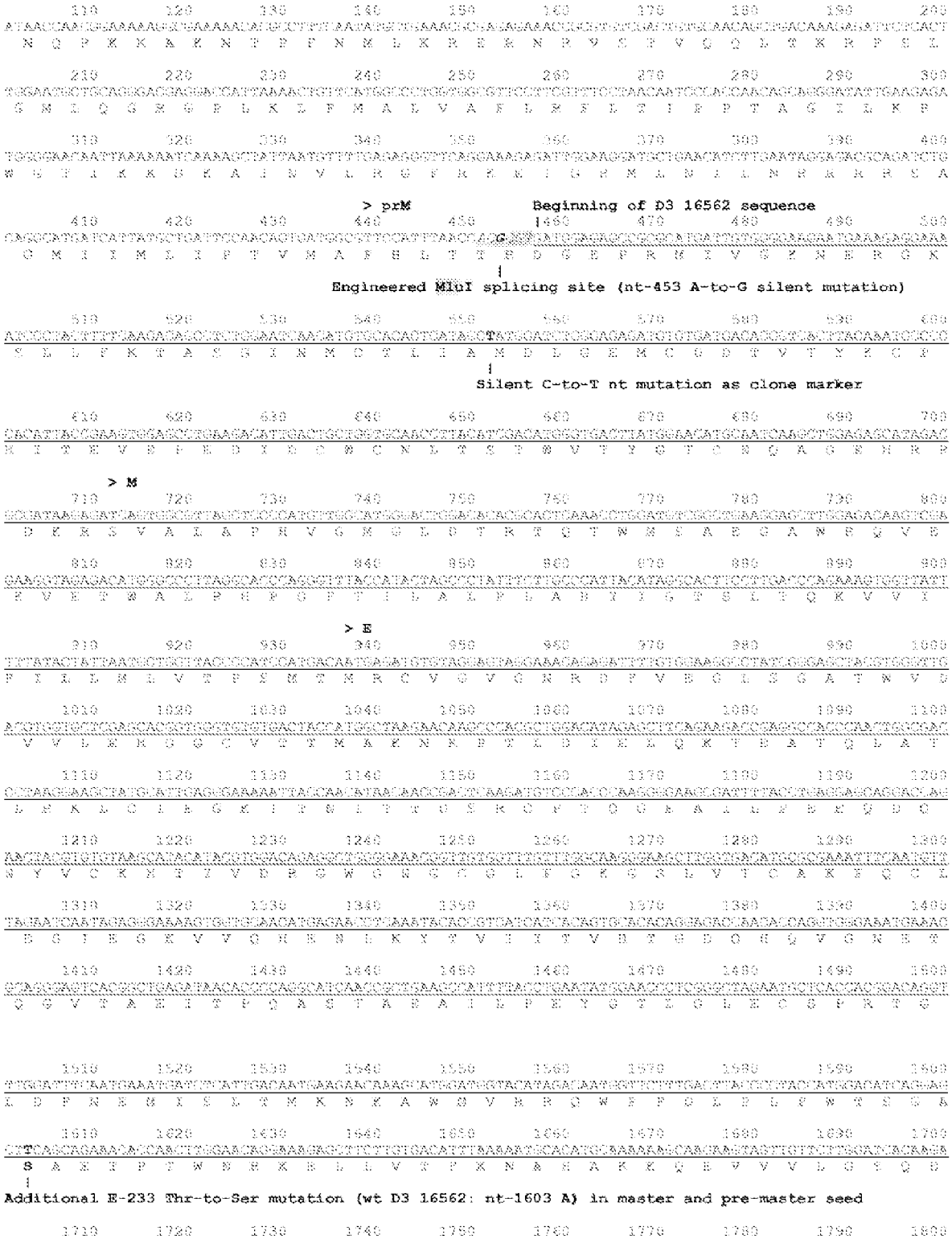
NCR-57-8, D2 PDR-53 attenuation locus (wt D2 16681:C)

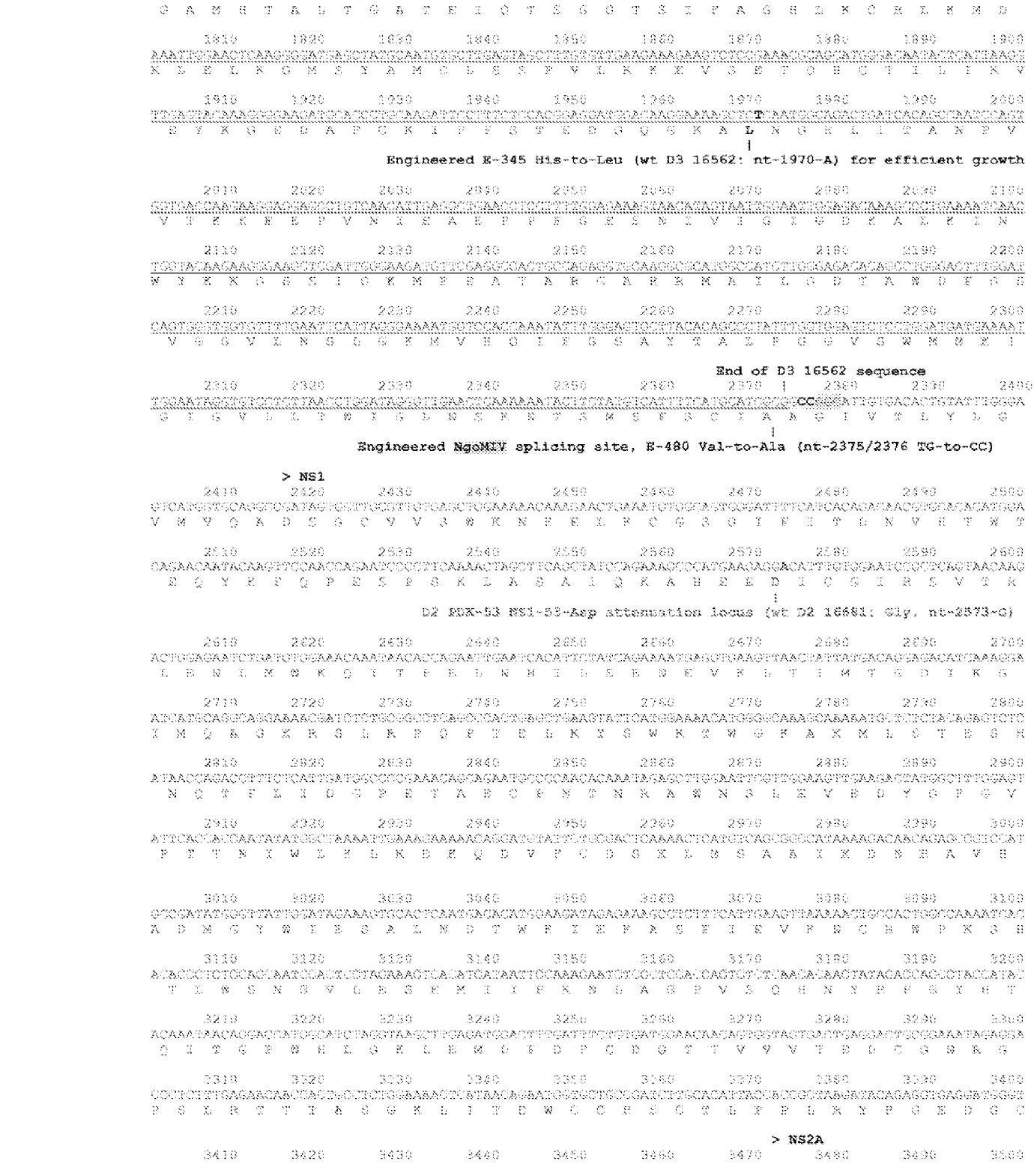
> 5'-Noncoding Region > C

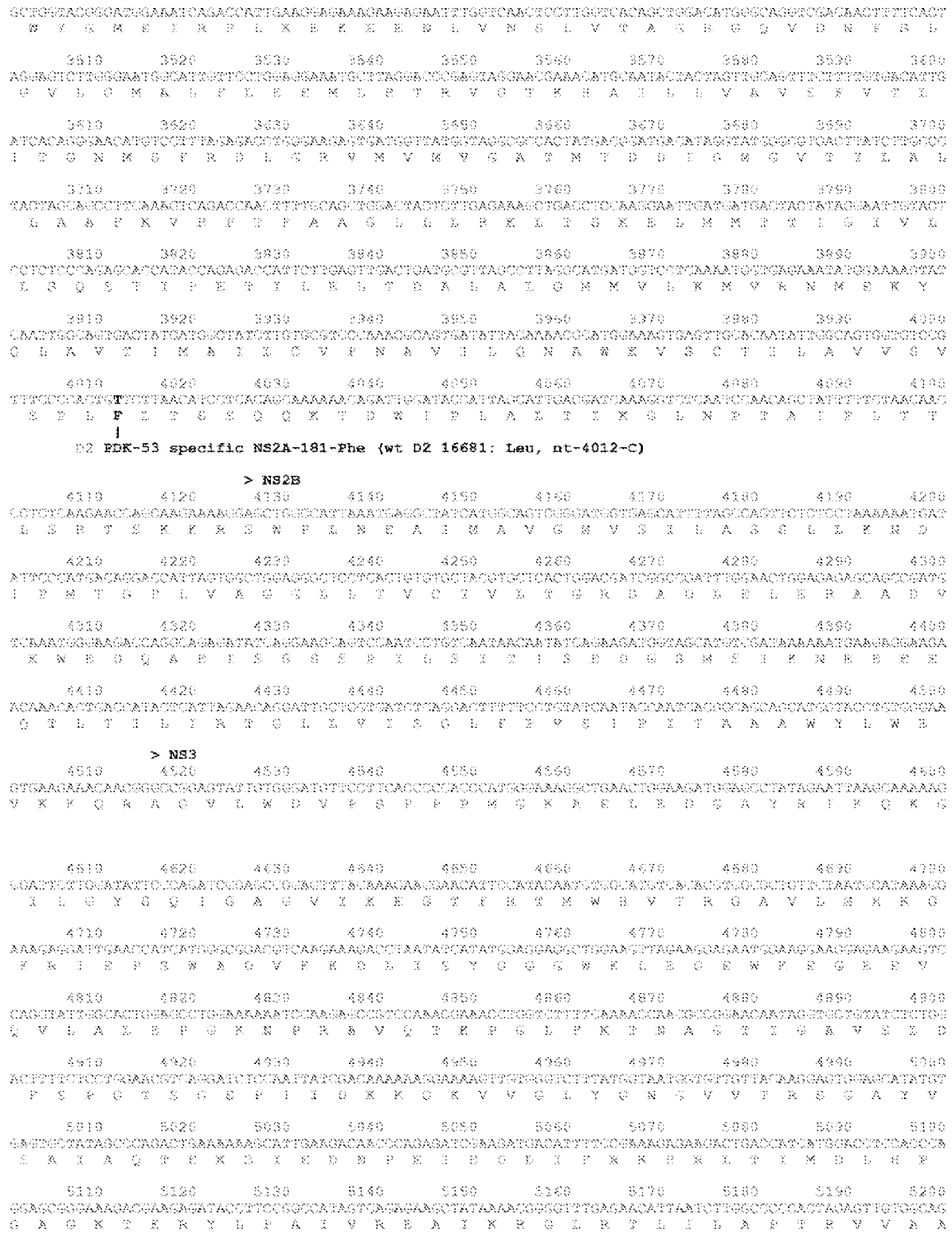
10 20 30 40 50 60 70 80 90 100

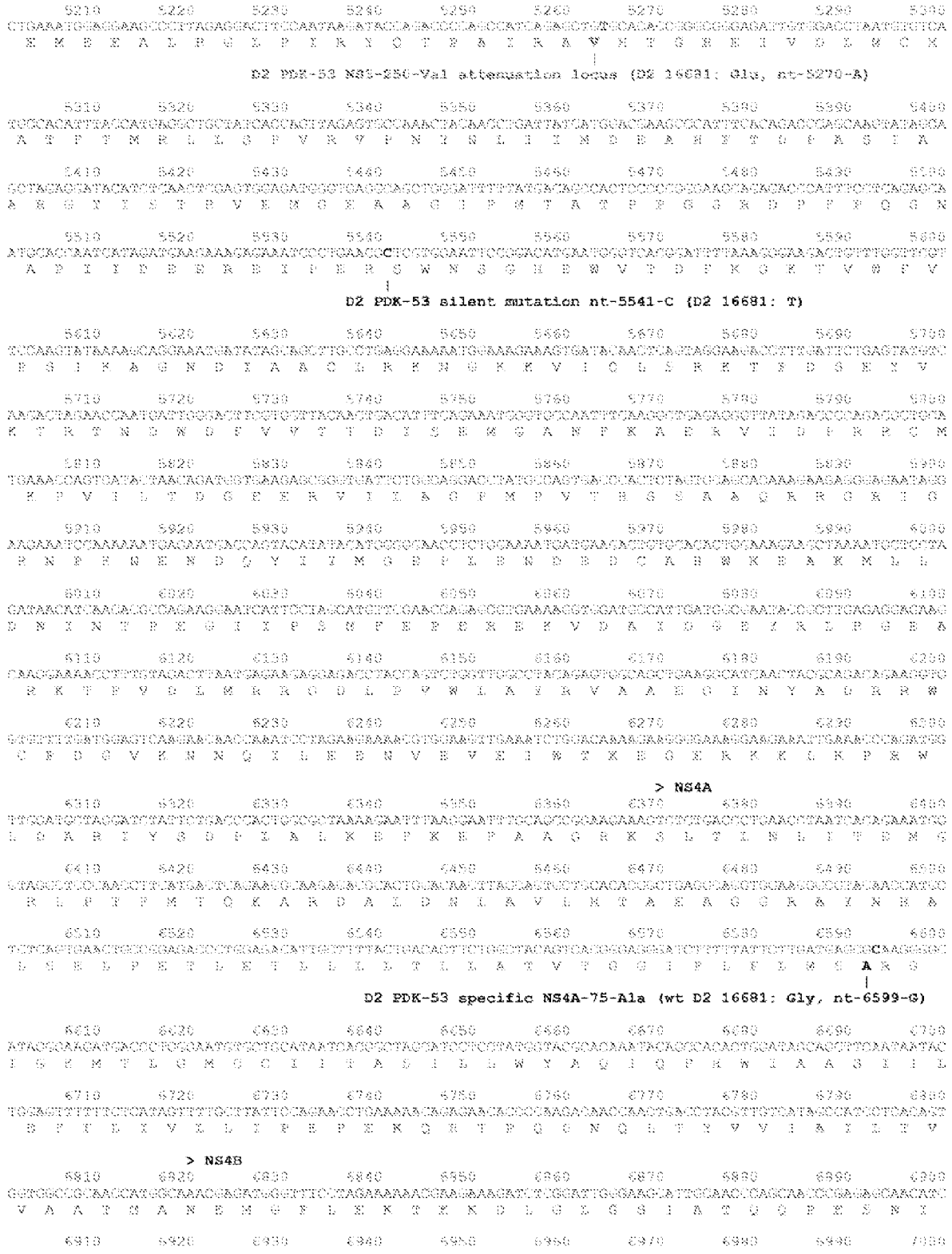
ACTTGTTAGTCTACGTGGACCGACAAAGACAGATTCTTTGAGGCAGTAAGTTCAATCTAGTTCTAACAGTTTTTTAATTAGACAGCAGATCTCTGATGA

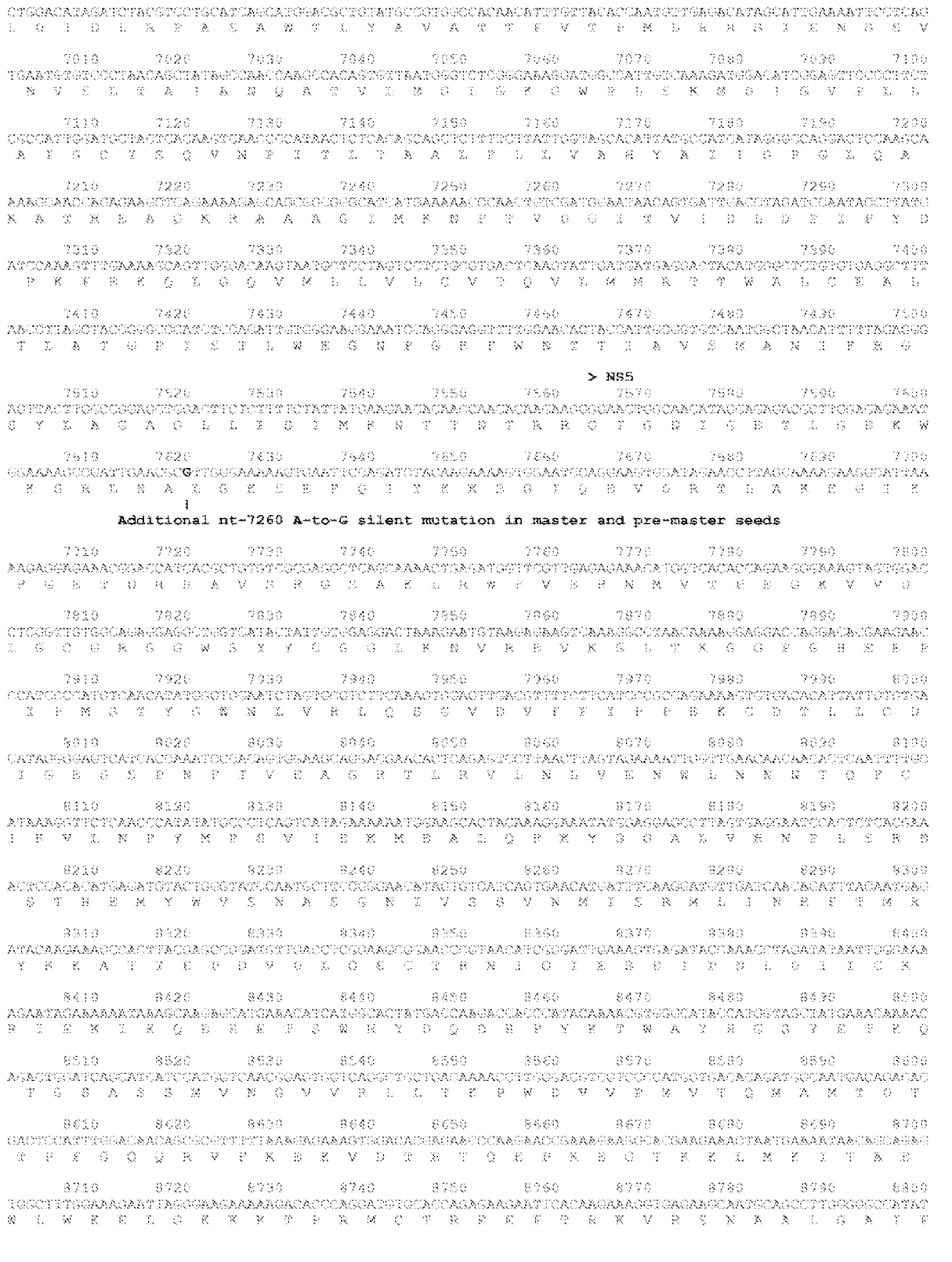
N N

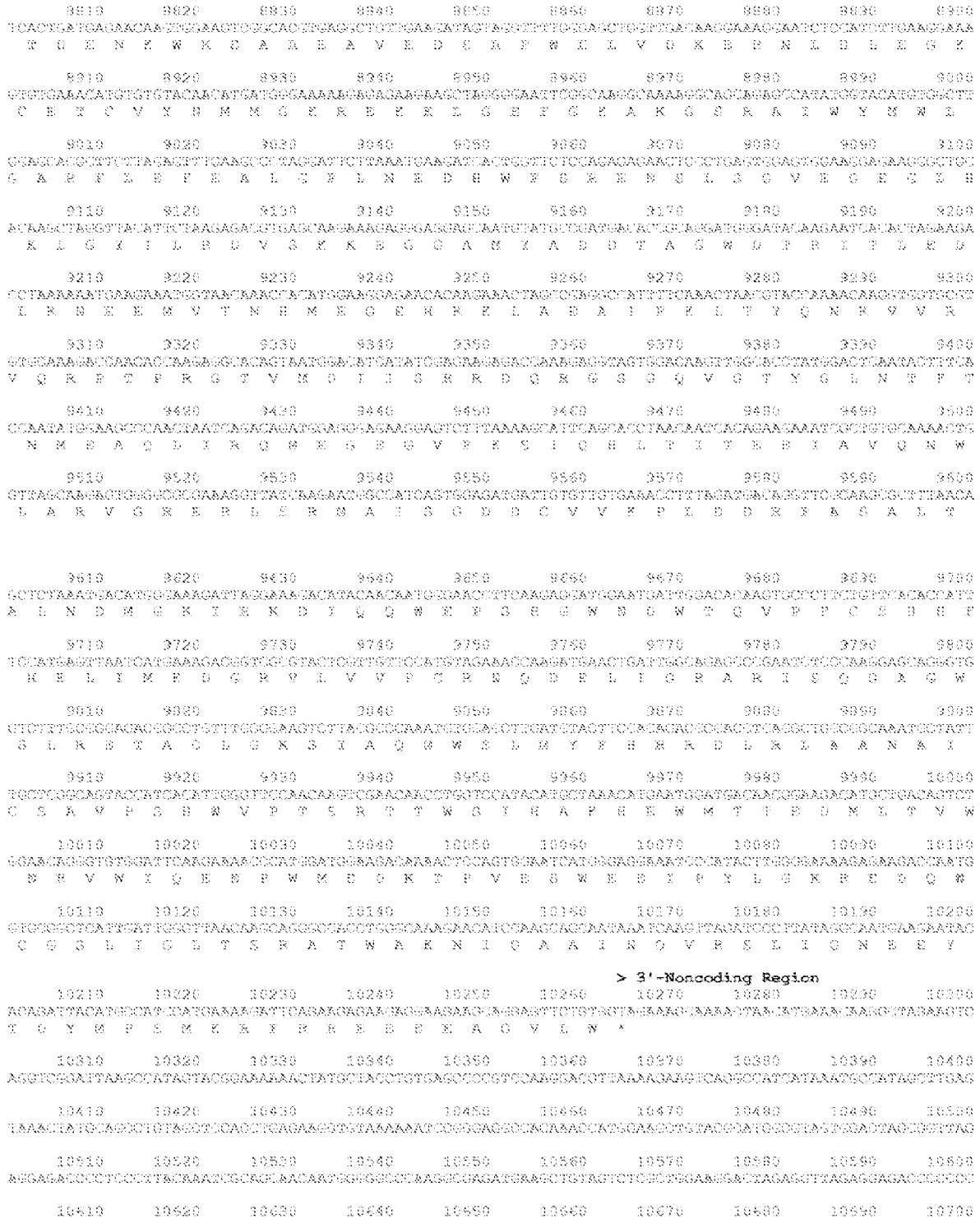

> prM
Beginning of D3 16562 sequence
Engineered MluI splicing site (nt-453 A-to-G silent mutation)
Silent C-to-T nt mutation as clone marker
> M
> E
Additional E-233 Thr-to-Ser mutation (wt D3 16562: nt-1603 A) in master and pre-master seed


Engineered E-345 His-to-Leu (wt D3 16562: nt-1970-A) for efficient growth
End of D3 16562 sequence
Engineered NgoMIV splicing site, E-480 Val-to-Ala (nt-2375/2376 TG-to-CC)
> NS1
D2 NGK-53 NS1-53-Asp attenuation locus (wt D2 16681: Gly, nt-2373-G)
> NS2A


52 PDK-53 specific NS2A-181-Phe (wt DZ 16681: Leu, nt-4012-C)
> NS2B
> NS3


D2 PDK-53 NS1-250-Val attenuation locus (D2 16681: Glu, nt-5270-A)
D2 PDK-53 silent mutation nt-5541-C (D2 16681: T)
> NS4A
D2 PDK-53 specific NS4A-75-Ala (wt D2 16681: Gly, nt-6599-G)
> NS4B


> NS5
Additional nt-7260 A-to-G silent mutation in master and pre-master seeds



> 3'-Noncoding Region

DENvax-4 Master Virus Seed (MVS)

[0208] Nucleotide sequence of the chimeric viral genome and deduced amino acid sequence of the translated protein. Most of the prM-E gene (nt-457 to -2379, underlined) is wild-type (wt) DEN-4 1036 virus-specific; the remaining nucleotide sequence is DEN-2 PDK-53 virus-specific. All engineered substitutions differ from wt virus (DEN-3 16562 or DEN-2 16681), as well as extra mutations (changes from engineered cDNA clone) are marked.

Substitutions Included in the Genome and Protein:

[0209] Junction sites:

[0210] a. MluI (nt 451-456): engineered silent mutation, nt-453 A-to-G

[0211] b. NgoMIV (nt 2380-2385): engineered mutations, nt-2381/2382 TG-to-CC (resulted in E-482 Val-to-Ala change)

[0212] D2 PDK-53 virus backbone (change from wt D2 16681)

[0213] a. 5'-noncoding region(NCR)-57 (nt-57 C-to-T): major attenuation locus (in red)

[0214] b. NS1-53 Gly-to-Asp (nt-2579 G-to-A): major attenuation locus (in red)

[0215] c. NS2A-181 Leu-to-Phe (nt-4018 C-to-T, in bold)

[0216] d. NS3-250 Glu-to-Val (nt-5270 A-to-T): major attenuation locus (in red)

[0217] e. nt-5547 (NS3 gene) T-to-C silent mutation (in bold)

[0218] f. NS4A-75 Gly-to-Ala (nt-6599 G-to-C, in bold)

[0219] * nt-8571 C-to-T silent mutation of PDK-53 is not engineered in the vaccine virus

[0220] Engineered substitutions in cDNA clone

[0221] a. Engineered C-100 Arg-to-Ser (nt-396 A-to-C): may improve viral replication in culture

[0222] b. Engineered nt-1401 A-to-G silent mutation

[0223] c. Engineered E-364 Ala-to-Val (nt-2027 C-to-T): may improve viral replication in culture

[0224] d. Engineered E-447 Met-to-Leu (nt-2275 A-to-C): may improve viral replication in culture

[0225] Additional substitutions found in vaccine seed (0.06% nt different from original clone)

[0226] a. nt-225 (C gene) A-to-T silent mutation (in bold)

[0227] b. NS2A-66 Asp-to-Gly (nt-3674 A-to-G) mutation (in bold)

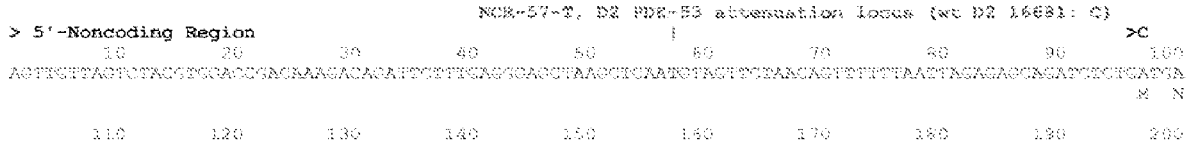
[0228] c. NS2A-99 Lys-to-Lys/Arg mix (nt-3773 A-to-A/G mix, in bold)

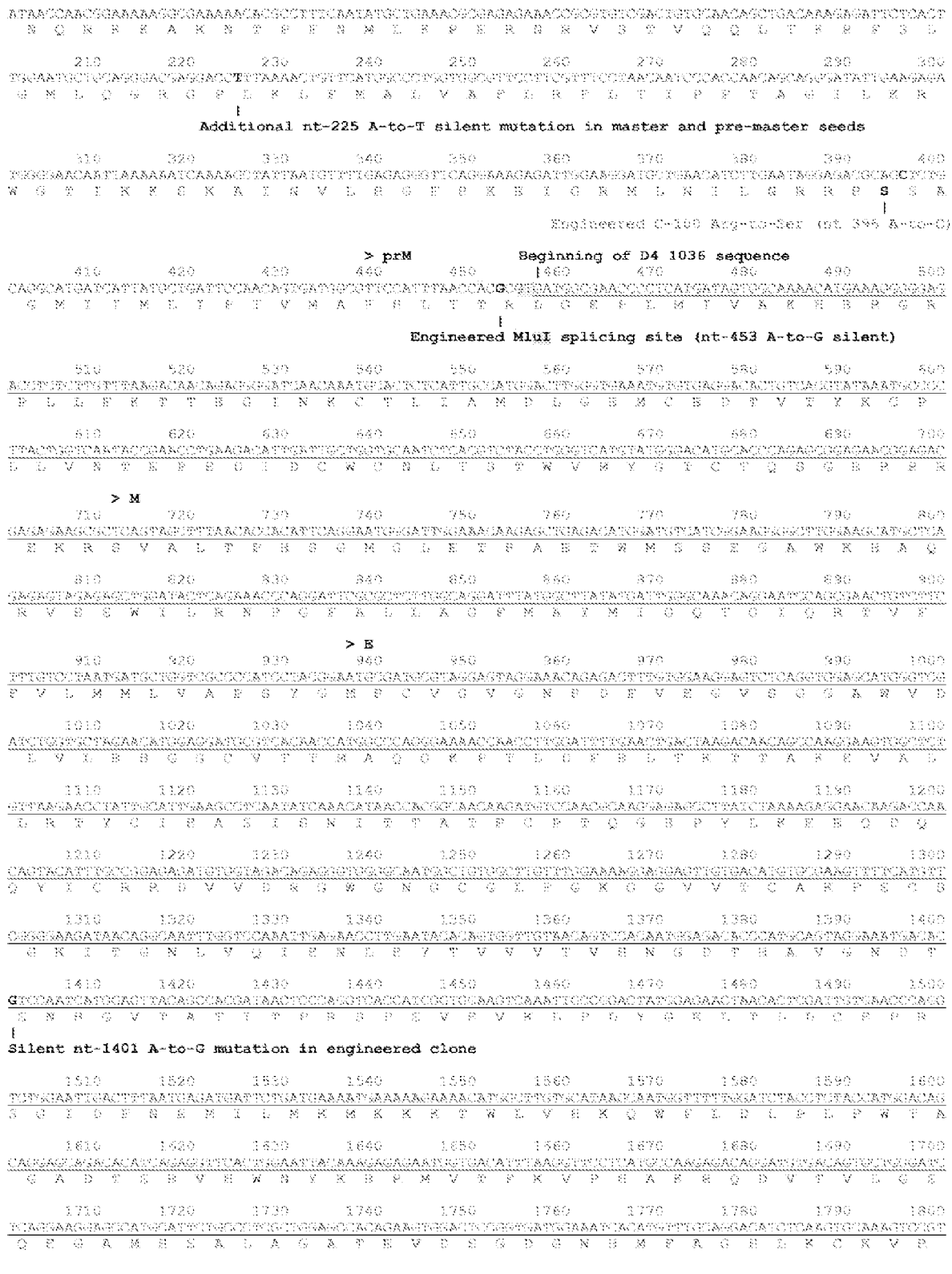
[0229] d. nt-5391 C-to-T (NS3 gene) silent mutation (in bold)

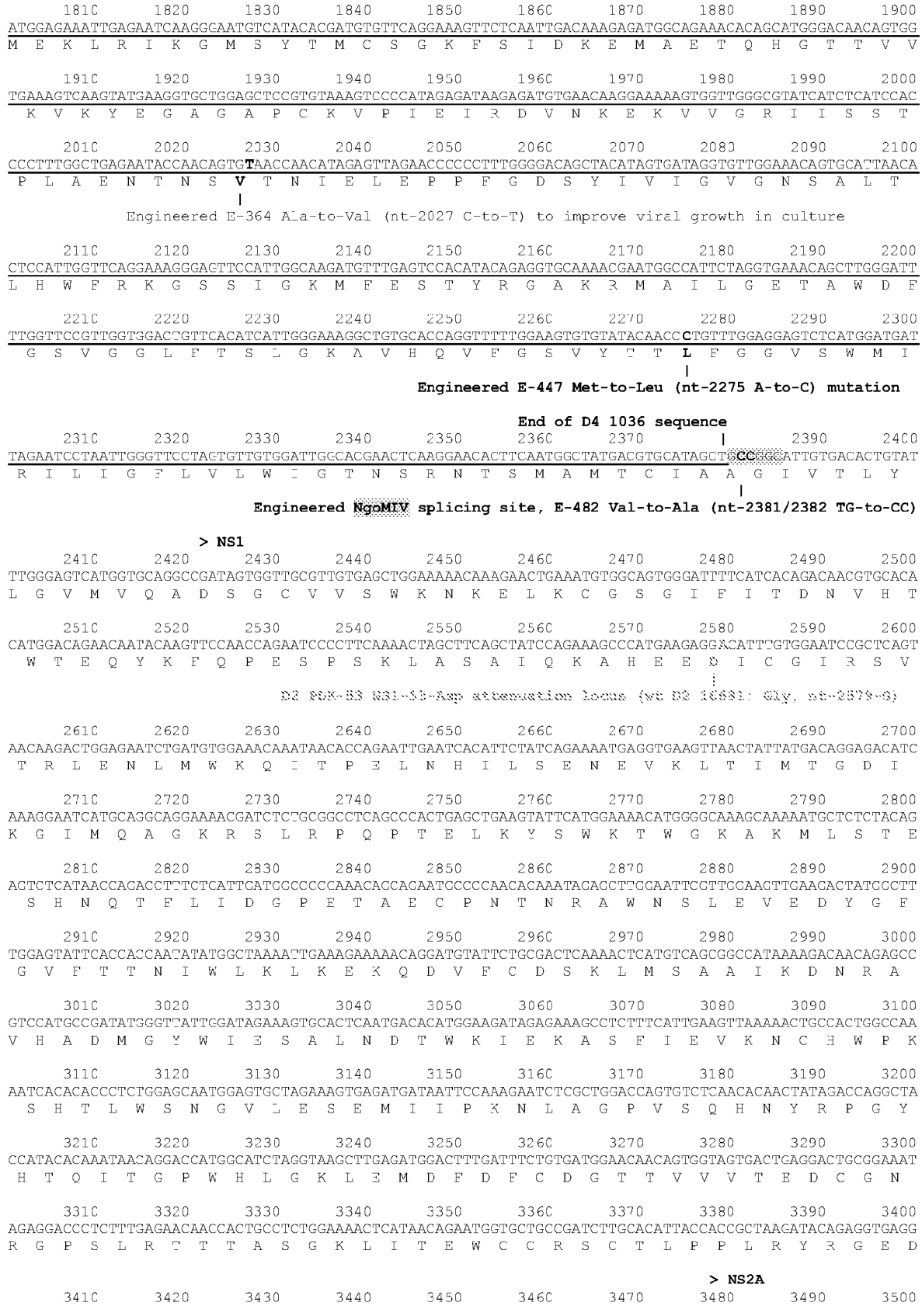
[0230] e. NS4A-21 Ala-to-Val (nt-6437 C-to-T, in bold)

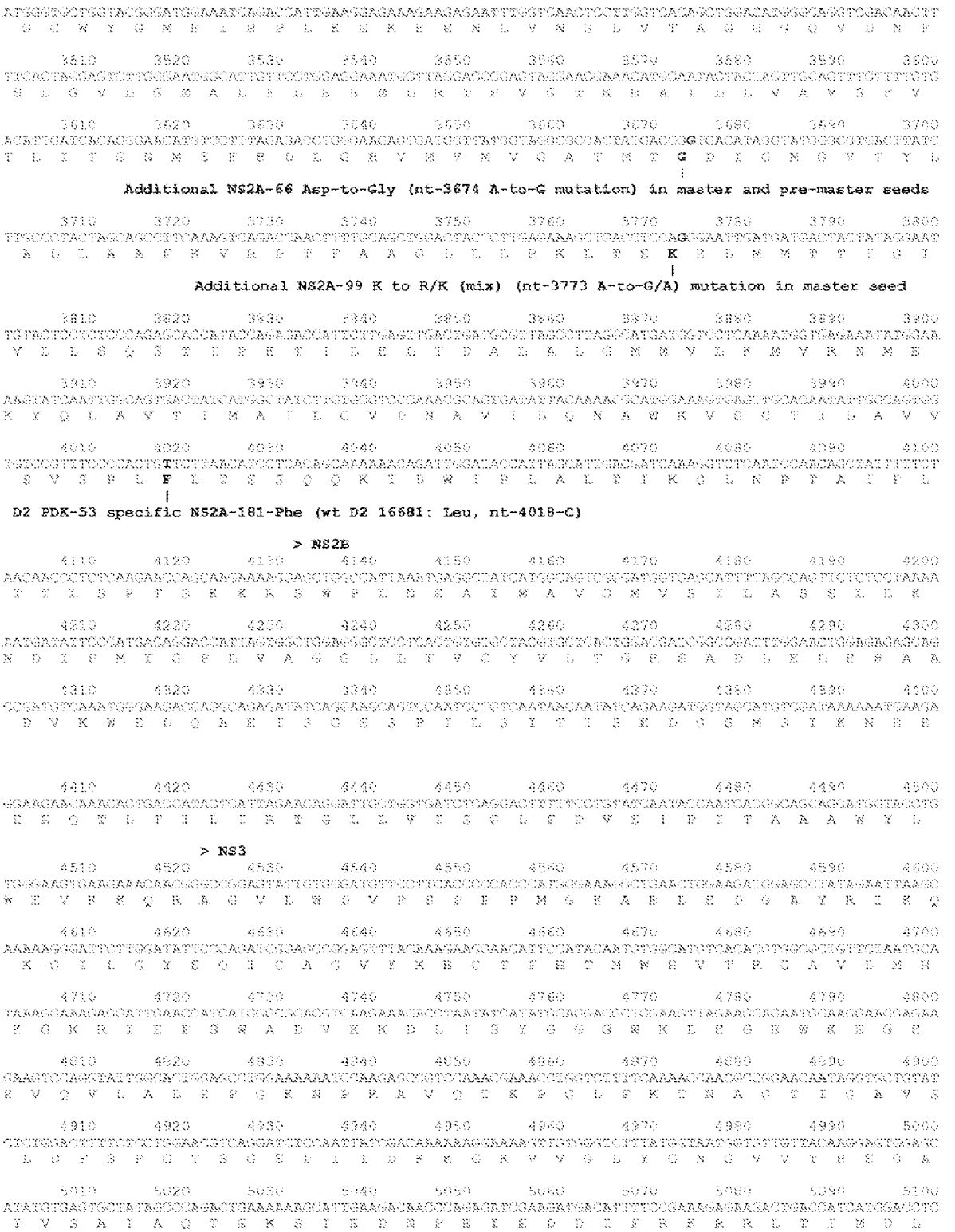
[0231] f. nt-7026 T-to-C/T mix silent mutation (in bold)

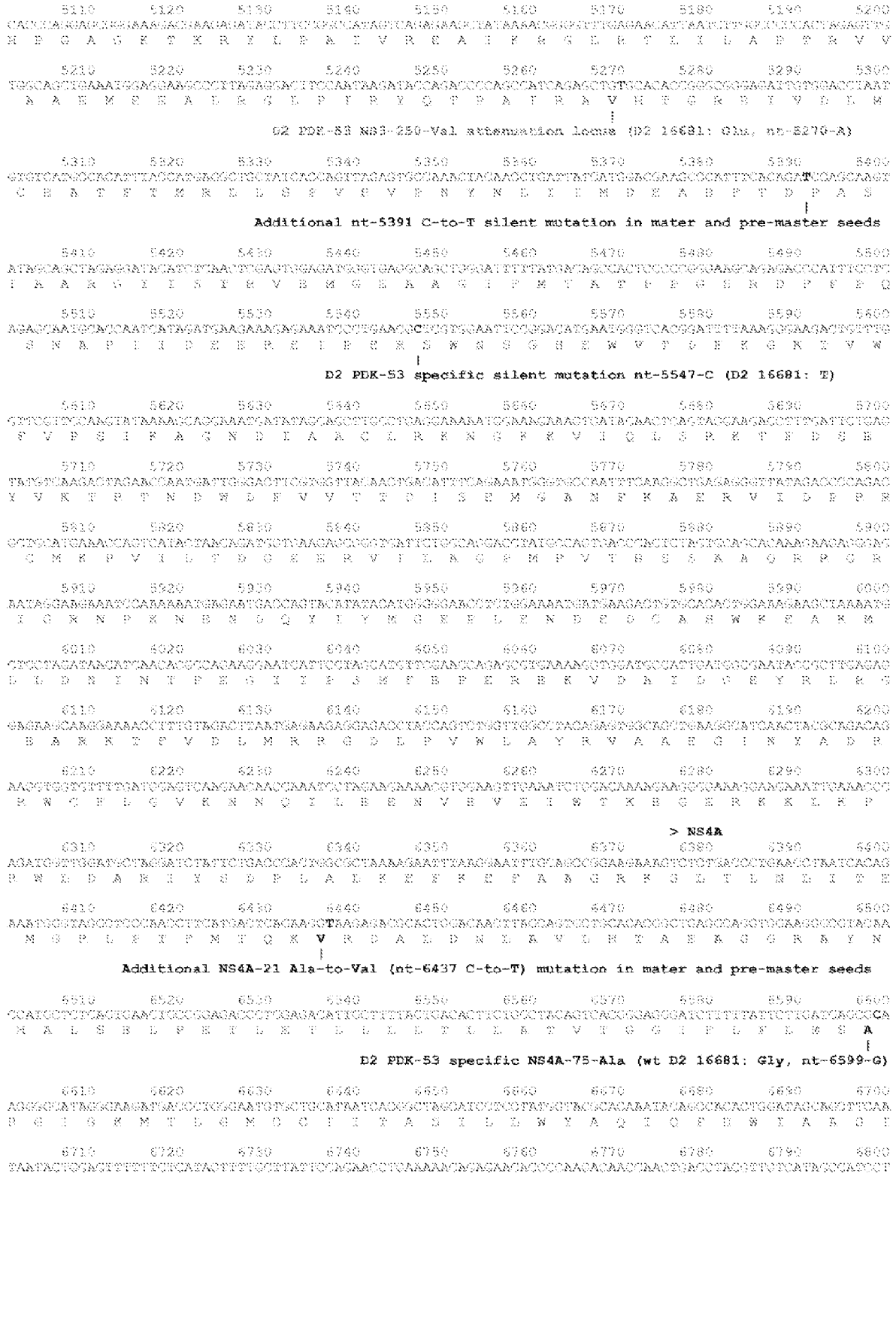
[0232] g. nt-9750 A-to-C silent mutation (in bold)

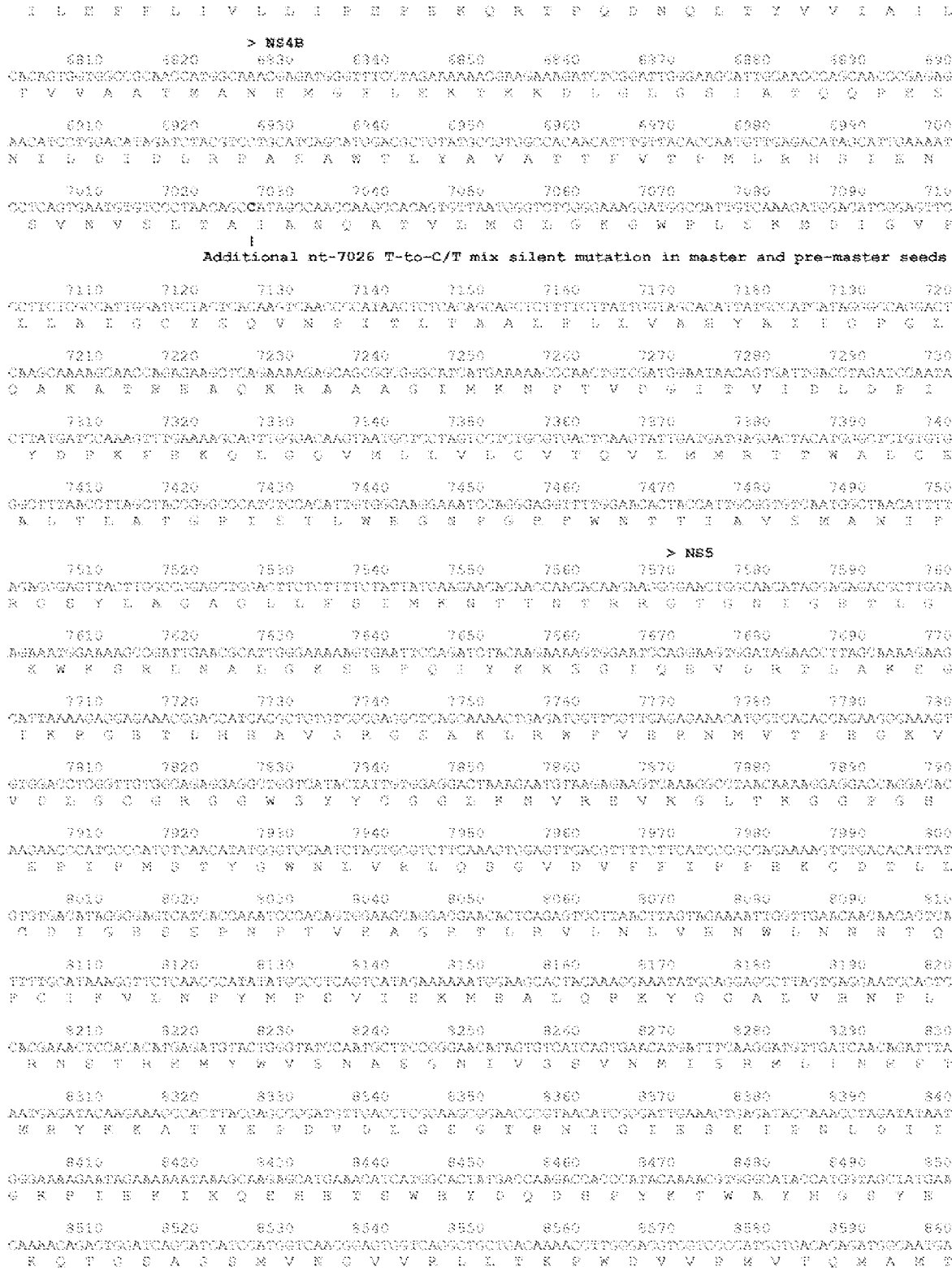

NCR-57-T, D2 PDE-E3 attenuation locus (wt D2 16681: C)
> 5'-Noncoding Region
>C
10
20
30
40
50
60
70
80
90
100
M N
110
120
130
140
150
160
170
180
190
200

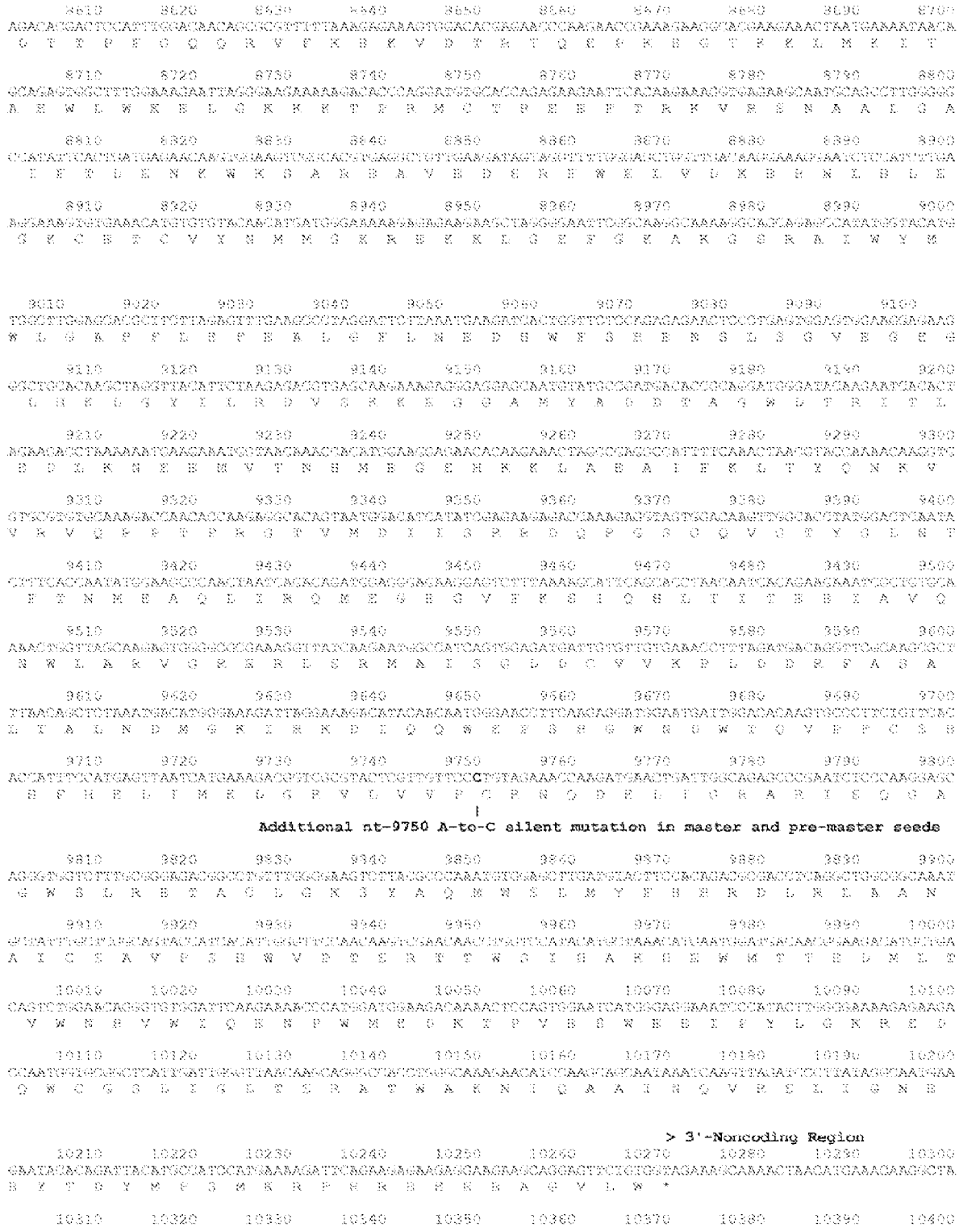

Additional nt-225 A-to-T silent mutation in master and pre-master seeds
Engineered C-180 Arg-to-Ser (nt 396 A-to-C)
> prM
Beginning of D4 1036 sequence
Engineered MluI splicing site (nt-453 A-to-G silent)
> M
> E
Silent nt-1401 A-to-G mutation in engineered clone


Engineered E-364 Ala-to-Val (nt-2027 C-to-T) to improve viral growth in culture
Engineered E-447 Met-to-Leu (nt-2275 A-to-C) mutation
End of D4 1036 sequence
Engineered NgoMIV splicing site, E-482 Val-to-Ala (nt-2381/2382 TG-to-CC)
> NS1
> NS2A


Additional NS2A-66 Asp-to-Gly (nt-3674 A-to-G mutation) in master and pre-master seeds
Additional NS2A-99 K to R/K (mix) (nt-3773 A-to-G/A) mutation in master seed
D2 PDK-53 specific NS2A-181-Phe (wt D2 16681: Leu, nt-4018-C)
> NS2B
> NS3


D2 PDK-53 NS3-250-Val attenuation locus (D2 16681: Glu, nt-5270-A)
Additional nt-5391 C-to-T silent mutation in mater and pre-master seeds
D2 PDK-53 specific silent mutation nt-5547-C (D2 16681: T)
> NS4A
Additional NS4A-21 Ala-to-Val (nt-6437 C-to-T) mutation in mater and pre-master seeds
D2 PDK-53 specific NS4A-75-Ala (wt D2 16681: Gly, nt-6599-G)


> NS4B
Additional nt-7026 T-to-C/T mix silent mutation in master and pre-master seeds
> NS5


Additional nt-9750 A-to-C silent mutation in master and pre-master seeds
> 3'-Noncoding Region

```
     10410      10420      10430      10440      10450      10460      10470      10480      10490      10500
CTTGAGTAAACTATGCAGCCTGTAGCTCCACCTGAGAAGGTGTAAAAAATCCGGCAGGCCACAAACCATGGAAGCTGTACGCATGGCGTAGTGGACTAGC

     10510      10520      10530      10540      10550      10560      10570      10580      10590      10600
GGTTAGAGGAGACCCCTCCCTTACAAATCGCAGCAACAATGGGGGCCCAAGGCGAGATGAAGCTGTAGTCTCGCTGGAAGGACTAGAGGTTAGAGGAGAC

     10610      10620      10630      10640      10650      10660      10670      10680      10690      10700
CCCCCCGAAACAAAAAACAGCATATTGACGCTGGGAAAGACCAGAGATCCTGCTGTCTCCTCAGCATCATTCCAGGCACAGAACGCCAGAAAATGGAATG

     10710      10720
GTGCTGTTGAATCAACAGGTTCT
```

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 16

<210> SEQ ID NO 1
<211> LENGTH: 10723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 1, BVS

<400> SEQUENCE: 1

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgggg gagagccgca tatgatagtt 480

agcaagcagg aaagaggaaa gtcacttttg ttcaagacct ctgcaggtgt caacatgtgc 540

accctcattg cgatggattt gggagagttg tgtgaggaca cgatgaccta caaatgcccc 600

cggatcactg aggcggaacc agatgacgtt gactgttggt gcaatgccac ggacacatgg 660

gtgacctatg gaacgtgctc tcaaactggc gaacaccgac gagacaaacg ttccgtcgca 720

ttggccccac acgtggggct tggcctagaa acaagagccg aaacgtggat gtcctctgaa 780

ggtgcttgga aacagataca aaaagtagag acttgggctc tgagacatcc aggattcacg 840

gtgatagccc ttttttctagc acatgccata ggaacatcca tcacccagaa agggatcatt 900

ttcattttgc tgatgctggt aacaccatct atggccatgc gatgcgtggg aataggcaac 960

agagacttcg tggaaggact gtcaggagca acatgggtgg atgtggtact ggagcatgga 1020

agttgcgtca ccaccatggc aaaaaacaaa ccaacactgg acattgaact cttgaagacg 1080

gaggtcacaa accctgcagt tctgcgtaaa ttgtgcattg aagctaaaat atcaaacacc 1140

accaccgatt cgagatgtcc aacacaagga gaagccacac tggtggaaga acaagacgcg 1200

aactttgtgt gccgacgaac gttcgtggac agaggctggg gcaatggctg tgggctattc 1260

ggaaaaggta gtctaataac gtgtgccaag tttaagtgtg tgacaaaact agaaggaaag 1320

atagttcaat atgaaaacct aaaatattca gtgatagtca ccgtccacac tggagatcag 1380

caccaggtgg gaaatgagac tacagaacat ggaacaactg caaccataac acctcaagct 1440

cctacgtcgg aaatacagct gaccgactac ggaaccctta cattagattg ttcacctagg 1500

acagggctag attttaacga gatggtgttg ctgacaatga aagaaagatc atggcttgtc 1560

cacaaacaat ggttcctaga cttaccactg ccttggacct ctggggcttc aacatcccaa 1620

gagacttgga acagacaaga tttactggtc acatttaaga cagctcatgc aaagaagcag 1680

gaagtagtcg tactaggatc acaagaagga gcaatgcaca ctgcgctgac tggagcgaca 1740

gaaatccaaa cgtcaggaac gacaacaatt ttcgcaggac acctaaaatg cagactaaaa 1800

atggacaaac taactttaaa agggatgtca tatgtgatgt gcacaggctc attcaagtta 1860

gagaaagaag tggctgagac ccagcatgga actgttctgg tgcaggttaa atatgaagga 1920

-continued

acagacgcac catgcaagat tccctttttcg acccaagatg agaaaggagc aacccagaat 1980

gggagattaa taacagccaa ccccatagtc actgacaaag aaaaaccagt caatattgag 2040

gcagaaccac cctttggtga gagctacatc gtggtaggag caggtgaaaa agctttgaaa 2100

ctaagctggt tcaagaaagg aagcagcata gggaaaatgt ttgaagcaac tgcccgagga 2160

gcacgaagga tggccattct gggagacacc gcatgggact tcggttctat aggaggagtg 2220

ttcacgtcta tgggaaaact ggtacaccag gtttttggaa ctgcatatgg agttttgttt 2280

agcggagttt cttggaccat gaaaatagga atagggattc tgctgacatg gctaggatta 2340

aattcaagga acacgtccct ttcgatgatg tgcatcgcag ccgccattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccctaccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

-continued

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagatcaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

-continued

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcatag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc gaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

-continued

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> SEQ ID NO 2
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 1, BVS

<400> SEQUENCE: 2

-continued

```
Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1               5                   10                  15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
            20                  25                  30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
        35                  40                  45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65                  70                  75                  80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
                85                  90                  95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
            100                 105                 110

Met Ala Phe His Leu Thr Thr Arg Gly Gly Glu Pro His Met Ile Val
        115                 120                 125

Ser Lys Gln Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ser Ala Gly
    130                 135                 140

Val Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Leu Cys Glu
145                 150                 155                 160

Asp Thr Met Thr Tyr Lys Cys Pro Arg Ile Thr Glu Ala Glu Pro Asp
                165                 170                 175

Asp Val Asp Cys Trp Cys Asn Ala Thr Asp Thr Trp Val Thr Tyr Gly
            180                 185                 190

Thr Cys Ser Gln Thr Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
        195                 200                 205

Leu Ala Pro His Val Gly Leu Gly Leu Glu Thr Arg Ala Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys Gln Ile Gln Lys Val Glu Thr Trp
225                 230                 235                 240

Ala Leu Arg His Pro Gly Phe Thr Val Ile Ala Leu Phe Leu Ala His
                245                 250                 255

Ala Ile Gly Thr Ser Ile Thr Gln Lys Gly Ile Ile Phe Ile Leu Leu
            260                 265                 270

Met Leu Val Thr Pro Ser Met Ala Met Arg Cys Val Gly Ile Gly Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
    290                 295                 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Ile Glu Leu Leu Lys Thr Glu Val Thr Asn Pro Ala Val Leu
                325                 330                 335

Arg Lys Leu Cys Ile Glu Ala Lys Ile Ser Asn Thr Thr Thr Asp Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Thr Leu Val Glu Glu Gln Asp Ala
        355                 360                 365

Asn Phe Val Cys Arg Arg Thr Phe Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Ile Thr Cys Ala Lys Phe Lys
385                 390                 395                 400

Cys Val Thr Lys Leu Glu Gly Lys Ile Val Gln Tyr Glu Asn Leu Lys
```

-continued

```
               405                 410                 415

Tyr Ser Val Ile Val Thr Val His Thr Gly Asp Gln His Gln Val Gly
           420                 425                 430

Asn Glu Thr Thr Glu His Gly Thr Thr Ala Thr Ile Thr Pro Gln Ala
       435                 440                 445

Pro Thr Ser Glu Ile Gln Leu Thr Asp Tyr Gly Thr Leu Thr Leu Asp
   450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Thr
465                 470                 475                 480

Met Lys Glu Arg Ser Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
               485                 490                 495

Pro Leu Pro Trp Thr Ser Gly Ala Ser Thr Ser Gln Glu Thr Trp Asn
           500                 505                 510

Arg Gln Asp Leu Leu Val Thr Phe Lys Thr Ala His Ala Lys Lys Gln
       515                 520                 525

Glu Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
   530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Thr Ser Gly Thr Thr Thr Ile Phe Ala
545                 550                 555                 560

Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Thr Leu Lys Gly
               565                 570                 575

Met Ser Tyr Val Met Cys Thr Gly Ser Phe Lys Leu Glu Lys Glu Val
           580                 585                 590

Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val Lys Tyr Glu Gly
       595                 600                 605

Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly
   610                 615                 620

Ala Thr Gln Asn Gly Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp
625                 630                 635                 640

Lys Glu Lys Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser
               645                 650                 655

Tyr Ile Val Val Gly Ala Gly Glu Lys Ala Leu Lys Leu Ser Trp Phe
           660                 665                 670

Lys Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly
       675                 680                 685

Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
   690                 695                 700

Ile Gly Gly Val Phe Thr Ser Met Gly Lys Leu Val His Gln Val Phe
705                 710                 715                 720

Gly Thr Ala Tyr Gly Val Leu Phe Ser Gly Val Ser Trp Thr Met Lys
               725                 730                 735

Ile Gly Ile Gly Ile Leu Leu Thr Trp Leu Gly Leu Asn Ser Arg Asn
           740                 745                 750

Thr Ser Leu Ser Met Met Cys Ile Ala Ala Ala Ile Val Thr Leu Tyr
       755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
   770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
               805                 810                 815
```

-continued

```
Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Asp Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200
```

-continued

```
Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                 1210                 1215

Leu Leu  Arg Lys Leu Thr Ser  Lys Glu Leu Met Met  Thr Thr Ile
    1220                 1225                 1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Leu Pro Glu  Thr Ile Leu
    1235                 1240                 1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                 1255                 1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                 1270                 1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                 1285                 1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                 1300                 1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                 1315                 1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                 1330                 1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                 1345                 1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                 1360                 1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                 1375                 1380

Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                 1390                 1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                 1405                 1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                 1420                 1425

Met Ser  Ile Lys Asn Glu Glu  Glu Asp Gln Thr Leu  Thr Ile Leu
    1430                 1435                 1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                 1450                 1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                 1465                 1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                 1480                 1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                 1495                 1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                 1510                 1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                 1525                 1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                 1540                 1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                 1555                 1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                 1570                 1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
```

-continued

```
    1580                1585                1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                1615                1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                1690                1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                1720                1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965
```

-continued

```
Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                 1975                 1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                 1990                 1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                 2005                 2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                 2020                 2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                 2035                 2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                 2050                 2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                 2065                 2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                 2080                 2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                 2095                 2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                 2110                 2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                 2125                 2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                 2140                 2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                 2155                 2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                 2170                 2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                 2185                 2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                 2200                 2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                 2215                 2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                 2230                 2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                 2245                 2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                 2260                 2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                 2275                 2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                 2290                 2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                 2305                 2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                 2320                 2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                 2335                 2340
```

-continued

```
Gln Val  Asn Pro Ile Thr Leu  Ile Ala Ala Leu Phe  Leu Leu Val
    2345                2350                2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                2365                2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                2380                2385

Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                2395                2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                2410                2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                2425                2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                2440                2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                2455                2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                2470                2475

Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                2485                2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                2500                2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                2515                2520

Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                2530                2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                2545                2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                2560                2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                2575                2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                2590                2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                2605                2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                2620                2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                2635                2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                2650                2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                2665                2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                2680                2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                2695                2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                2710                2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
```

-continued

```
    2720                2725                2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                2740                2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                2755                2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                2770                2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                2785                2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                2800                2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                2815                2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                2830                2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                2845                2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                2860                2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                2875                2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                2890                2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                2905                2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                2920                2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                2935                2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                2950                2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                2965                2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                2980                2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                2995                3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                3010                3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                3025                3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                3040                3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                3055                3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                3070                3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                3085                3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                3100                3105
```

-continued

```
Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                 3145                 3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                 3160                 3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                 3175                 3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                 3190                 3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                 3205                 3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                 3220                 3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                 3235                 3240

Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                 3250                 3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                 3265                 3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                 3280                 3285

His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                 3295                 3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                 3310                 3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                 3325                 3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                 3340                 3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                 3355                 3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                 3370                 3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                 3385                 3390


<210> SEQ ID NO 3
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 1, MVS

<400> SEQUENCE: 3

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1               5                  10                  15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
            20                  25                  30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
        35                  40                  45
```

-continued

```
Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65                  70                  75                  80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
                85                  90                  95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
            100                 105                 110

Met Ala Phe His Leu Thr Thr Arg Gly Gly Glu Pro His Met Ile Val
        115                 120                 125

Ser Lys Gln Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ser Ala Gly
    130                 135                 140

Val Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Leu Cys Glu
145                 150                 155                 160

Asp Thr Met Thr Tyr Lys Cys Pro Arg Ile Thr Glu Ala Glu Pro Asp
                165                 170                 175

Asp Val Asp Cys Trp Cys Asn Ala Thr Asp Thr Trp Val Thr Tyr Gly
            180                 185                 190

Thr Cys Ser Gln Thr Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
        195                 200                 205

Leu Ala Pro His Val Gly Leu Gly Leu Glu Thr Arg Ala Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys Gln Ile Gln Lys Val Glu Thr Trp
225                 230                 235                 240

Ala Leu Arg His Pro Gly Phe Thr Val Ile Ala Leu Phe Leu Ala His
                245                 250                 255

Ala Ile Gly Thr Ser Ile Thr Gln Lys Gly Ile Ile Phe Ile Leu Leu
            260                 265                 270

Met Leu Val Thr Pro Ser Met Ala Met Arg Cys Val Gly Ile Gly Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
    290                 295                 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Ile Glu Leu Leu Lys Thr Glu Val Thr Asn Pro Ala Val Leu
                325                 330                 335

Arg Lys Leu Cys Ile Glu Ala Lys Ile Ser Asn Thr Thr Thr Asp Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Thr Leu Val Glu Glu Gln Asp Ala
        355                 360                 365

Asn Phe Val Cys Arg Arg Thr Phe Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Ile Thr Cys Ala Lys Phe Lys
385                 390                 395                 400

Cys Val Thr Lys Leu Glu Gly Lys Ile Val Gln Tyr Glu Asn Leu Lys
                405                 410                 415

Tyr Ser Val Ile Val Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Thr Glu His Gly Thr Thr Ala Thr Ile Thr Pro Gln Ala
        435                 440                 445
```

-continued

```
Pro Thr Ser Glu Ile Gln Leu Thr Asp Tyr Gly Thr Leu Thr Leu Asp
    450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Thr
465                 470                 475                 480

Met Lys Glu Arg Ser Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Thr Ser Gly Ala Ser Thr Ser Gln Glu Thr Trp Asn
            500                 505                 510

Arg Gln Asp Leu Leu Val Thr Phe Lys Thr Ala His Ala Lys Lys Gln
        515                 520                 525

Glu Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Thr Ser Gly Thr Thr Thr Ile Phe Ala
545                 550                 555                 560

Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Thr Leu Lys Gly
                565                 570                 575

Met Ser Tyr Val Met Cys Thr Gly Ser Phe Lys Leu Glu Lys Glu Val
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val Lys Tyr Glu Gly
        595                 600                 605

Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly
    610                 615                 620

Ala Thr Gln Asn Gly Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp
625                 630                 635                 640

Lys Glu Lys Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser
                645                 650                 655

Tyr Ile Val Val Gly Ala Gly Glu Lys Ala Leu Lys Leu Ser Trp Phe
            660                 665                 670

Lys Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly
        675                 680                 685

Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Ile Gly Gly Val Phe Thr Ser Met Gly Lys Leu Val His Gln Val Phe
705                 710                 715                 720

Gly Thr Ala Tyr Gly Val Leu Phe Ser Gly Val Ser Trp Thr Met Lys
                725                 730                 735

Ile Gly Ile Gly Ile Leu Leu Thr Trp Leu Gly Leu Asn Ser Arg Asn
            740                 745                 750

Thr Ser Leu Ser Met Met Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
        755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
```

-continued

```
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Asp Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Lys Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Leu Pro Glu  Thr Ile Leu
    1235                1240                1245
```

-continued

```
Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                 1255                 1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                 1270                 1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                 1285                 1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                 1300                 1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                 1315                 1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                 1330                 1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                 1345                 1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                 1360                 1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                 1375                 1380

Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                 1390                 1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                 1405                 1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                 1420                 1425

Met Ser  Ile Lys Asn Glu Glu  Glu Asp Gln Thr Leu  Thr Ile Leu
    1430                 1435                 1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                 1450                 1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                 1465                 1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                 1480                 1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                 1495                 1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                 1510                 1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                 1525                 1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                 1540                 1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                 1555                 1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                 1570                 1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                 1585                 1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                 1600                 1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                 1615                 1620
```

-continued

```
Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                1690                1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                1720                1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
```

-continued

```
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                2110                2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                2125                2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                2140                2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                2155                2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                2170                2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                2185                2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                2200                2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                2215                2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                2230                2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                2245                2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                2260                2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                2275                2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                2290                2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                2305                2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                2320                2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                2335                2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Leu Phe  Leu Leu Val
    2345                2350                2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                2365                2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                2380                2385
```

-continued

```
Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                2395                2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                2410                2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                2425                2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                2440                2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                2455                2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                2470                2475

Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                2485                2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                2500                2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                2515                2520

Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                2530                2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                2545                2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                2560                2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                2575                2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                2590                2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                2605                2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                2620                2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                2635                2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                2650                2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                2665                2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                2680                2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                2695                2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                2710                2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                2725                2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                2740                2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                2755                2760
```

-continued

```
Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                 2770                 2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                 2785                 2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                 2800                 2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                 2815                 2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                 2830                 2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                 2845                 2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                 2860                 2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                 2875                 2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                 2890                 2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                 2905                 2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                 2920                 2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                 2935                 2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                 2950                 2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                 2965                 2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                 2980                 2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                 2995                 3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                 3010                 3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                 3025                 3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                 3040                 3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                 3055                 3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                 3070                 3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                 3085                 3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                 3100                 3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
```

-continued

```
    3140                3145                3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                3160                3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                3175                3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                3190                3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                3205                3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                3220                3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                3235                3240

Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                3250                3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                3265                3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                3280                3285

His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                3295                3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                3310                3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                3325                3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                3340                3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                3355                3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                3370                3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                3385                3390
```

<210> SEQ ID NO 4
<211> LENGTH: 10723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 2, BVS

<400> SEQUENCE: 4

```
agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta     60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg    120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag    180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg    240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga    300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt    360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg    420

attccaacag tgatggcgtt ccatttaacc acacgtaacg gagaaccaca catgatcgtc    480
```

-continued

agcagacaag agaaagggaa aagtcttctg tttaaaacag aggttggcgt gaacatgtgt 540

accctcatgg ccatggacct tggtgaattg tgtgaagaca caatcacgta cgagtgtccc 600

cttctcaggc agaatgagcc agaagacata gactgttggt gcaactctac gtccacgtgg 660

gtaacttatg ggacgtgtac caccatggga gaacatagaa gagaaaaaag atcagtggca 720

ctcgttccac atgtgggaat gggactggag acacgaactg aaacatggat gtcatcagaa 780

ggggcctgga aacatgtcca gagaattgaa acttggatct tgagacatcc aggcttcacc 840

atgatggcag caatcctggc atacaccata ggaacgacac atttccaaag agccctgatc 900

ttcatcttac tgacagctgt cactccttca atgacaatgc gttgcatagg aatgtcaaat 960

agagactttg tggaaggggt ttcaggagga agctgggttg acatagtctt agaacatgga 1020

agctgtgtga cgacgatggc aaaaaacaaa ccaacattgg attttgaact gataaaaaca 1080

gaagccaaac agcctgccac cctaaggaag tactgtatag aggcaaagct aaccaacaca 1140

acaacagaat ctcgctgccc aacacaaggg gaacccagcc taaatgaaga gcaggacaaa 1200

aggttcgtct gcaaacactc catggtagac agaggatggg gaaatggatg tggactattt 1260

ggaaagggag gcattgtgac ctgtgctatg ttcagatgca aaaagaacat ggaaggaaaa 1320

gttgtgcaac cagaaaactt ggaatacacc attgtgataa cacctcactc aggggaagag 1380

catgcagtcg gaaatgacac aggaaaacat ggcaaggaaa tcaaaataac accacagagt 1440

tccatcacag aagcagaatt gacaggttat ggcactgtca caatggagtg ctctccaaga 1500

acgggcctcg acttcaatga gatggtgttg ctgcagatgg aaaataaagc ttggctggtg 1560

cacaggcaat ggttcctaga cctgccgtta ccatggttgc ccggagcgga cacacaaggg 1620

tcaaattgga tacagaaaga gacattggtc actttcaaaa atccccatgc gaagaaacag 1680

gatgttgttg ttttaggatc ccaagaaggg gccatgcaca cagcacttac aggggccaca 1740

gaaatccaaa tgtcatcagg aaacttactc ttcacaggac atctcaagtg caggctgaga 1800

atggacaagc tacagctcaa aggaatgtca tactctatgt gcacaggaaa gtttaaagtt 1860

gtgaaggaaa tagcagaaac acaacatgga acaatagtta tcagagtgca atatgaaggg 1920

gacggctctc catgcaagat ccctttttgag ataatggatt tggaaaaaag acatgtctta 1980

ggtcgcctga ttacagtcaa cccaattgtg acagaaaaag atagcccagt caacatagaa 2040

gcagaacctc catttggaga cagctacatc atcataggag tagagccggg acaactgaag 2100

ctcaactggt ttaagaaagg aagttctatc ggccaaatgt ttgagacaac aatgaggggg 2160

gcgaagagaa tggccatttt aggtgacaca gcctgggatt ttggatcctt gggaggagtg 2220

tttacatcta taggaaaggc tctccaccaa gtctttggag caatctatgg agctgccttc 2280

agtggggttt catggactat gaaaatcctc ataggagtca ttatcacatg gataggaatg 2340

aattcacgca gcacctcact gtctgtgaca ctagtattgg tgggaattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

-continued

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

-continued

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctcccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

ccaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctttttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

-continued

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccgtattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

-continued

```
ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca   9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc   9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga   9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct   9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat   9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata   9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg  10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca  10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc  10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa  10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga  10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc  10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca  10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg  10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc  10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga  10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag  10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca  10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct                    10723
```

<210> SEQ ID NO 5
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 2, BVS

<400> SEQUENCE: 5

```
Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1               5                   10                  15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
            20                  25                  30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
        35                  40                  45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65                  70                  75                  80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
                85                  90                  95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
            100                 105                 110

Met Ala Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val
        115                 120                 125

Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Val Gly
    130                 135                 140

Val Asn Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu
```

-continued

```
145                 150                 155                 160

Asp Thr Ile Thr Tyr Glu Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu
                165                 170                 175

Asp Ile Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly
            180                 185                 190

Thr Cys Thr Thr Met Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala
        195                 200                 205

Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp
225                 230                 235                 240

Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile Leu Ala Tyr
                245                 250                 255

Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu
            260                 265                 270

Thr Ala Val Thr Pro Ser Met Thr Met Arg Cys Ile Gly Met Ser Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val
    290                 295                 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu
                325                 330                 335

Arg Lys Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys
        355                 360                 365

Arg Phe Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Arg
385                 390                 395                 400

Cys Lys Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu
                405                 410                 415

Tyr Thr Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly
            420                 425                 430

Asn Asp Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser
        435                 440                 445

Ser Ile Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu
    450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln
465                 470                 475                 480

Met Glu Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile
            500                 505                 510

Gln Lys Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln
        515                 520                 525

Asp Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr
545                 550                 555                 560
```

-continued

```
Gly His Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly
               565                 570                 575

Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly
        595                 600                 605

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys
    610                 615                 620

Arg His Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu
625                 630                 635                 640

Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser
                645                 650                 655

Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe
            660                 665                 670

Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly
        675                 680                 685

Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Leu Gly Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe
705                 710                 715                 720

Gly Ala Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys
                725                 730                 735

Ile Leu Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser
            740                 745                 750

Thr Ser Leu Ser Val Thr Leu Val Leu Val Gly Ile Val Thr Leu Tyr
        755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960
```

-continued

```
Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Asp Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Lys Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                1240                1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                1255                1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                1270                1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                1285                1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                1300                1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                1315                1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                1330                1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
```

-continued

```
    1340                1345                1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                1360                1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                1375                1380

Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                1390                1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                1405                1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                1420                1425

Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
    1430                1435                1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                1450                1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                1465                1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                1480                1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                1495                1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                1510                1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                1525                1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                1540                1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                1555                1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                1570                1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                1585                1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                1615                1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                1690                1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                1720                1725
```

-continued

```
Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100
```

-continued

```
Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                 2110                 2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Pro Gly  Gly Arg Ala
    2120                 2125                 2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                 2140                 2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                 2155                 2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                 2170                 2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                 2185                 2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                 2200                 2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                 2215                 2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                 2230                 2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                 2245                 2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                 2260                 2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                 2275                 2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                 2290                 2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                 2305                 2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                 2320                 2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                 2335                 2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Phe Phe  Leu Leu Val
    2345                 2350                 2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                 2365                 2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                 2380                 2385

Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                 2395                 2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                 2410                 2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                 2425                 2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                 2440                 2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                 2455                 2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                 2470                 2475

Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
```

-continued

```
    2480                2485                2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                2500                2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                2515                2520

Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                2530                2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                2545                2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                2560                2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                2575                2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                2590                2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                2605                2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                2620                2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                2635                2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                2650                2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                2665                2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                2680                2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                2695                2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                2710                2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                2725                2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                2740                2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                2755                2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                2770                2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                2785                2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                2800                2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                2815                2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                2830                2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                2845                2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                2860                2865
```

-continued

```
Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                2875                2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                2890                2895

Ala Leu  Gly Ala Val Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                2905                2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                2920                2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                2935                2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                2950                2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                2965                2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                2980                2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                2995                3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                3010                3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                3025                3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                3040                3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                3055                3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                3070                3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                3085                3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                3100                3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                3115                3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                3130                3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                3145                3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                3160                3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                3175                3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                3190                3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                3205                3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                3220                3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                3235                3240
```

-continued

```
Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                 3250                 3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                 3265                 3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                 3280                 3285

His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                 3295                 3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                 3310                 3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                 3325                 3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                 3340                 3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                 3355                 3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                 3370                 3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                 3385                 3390


<210> SEQ ID NO 6
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 2, MVS

<400> SEQUENCE: 6

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1               5                   10                  15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
            20                  25                  30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
        35                  40                  45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65                  70                  75                  80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
                85                  90                  95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
            100                 105                 110

Met Ala Phe His Leu Thr Thr Arg Asn Gly Glu Pro His Met Ile Val
        115                 120                 125

Ser Arg Gln Glu Lys Gly Lys Ser Leu Leu Phe Lys Thr Glu Val Gly
    130                 135                 140

Val Asn Met Cys Thr Leu Met Ala Met Asp Leu Gly Glu Leu Cys Glu
145                 150                 155                 160

Asp Thr Ile Thr Tyr Glu Cys Pro Leu Leu Arg Gln Asn Glu Pro Glu
                165                 170                 175

Asp Ile Asp Cys Trp Cys Asn Ser Thr Ser Thr Trp Val Thr Tyr Gly
            180                 185                 190
```

-continued

```
Thr Cys Thr Thr Met Gly Glu His Arg Arg Glu Lys Arg Ser Val Ala
        195                 200                 205

Leu Val Pro His Val Gly Met Gly Leu Glu Thr Arg Thr Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys His Val Gln Arg Ile Glu Thr Trp
225                 230                 235                 240

Ile Leu Arg His Pro Gly Phe Thr Met Met Ala Ala Ile Leu Ala Tyr
                245                 250                 255

Thr Ile Gly Thr Thr His Phe Gln Arg Ala Leu Ile Phe Ile Leu Leu
            260                 265                 270

Thr Ala Val Thr Pro Ser Met Thr Met Arg Cys Ile Gly Met Ser Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ser Trp Val Asp Ile Val
    290                 295                 300

Leu Glu His Gly Ser Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Phe Glu Leu Ile Lys Thr Glu Ala Lys Gln Pro Ala Thr Leu
                325                 330                 335

Arg Lys Tyr Cys Ile Glu Ala Lys Leu Thr Asn Thr Thr Thr Glu Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Pro Ser Leu Asn Glu Glu Gln Asp Lys
        355                 360                 365

Arg Phe Val Cys Lys His Ser Met Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Gly Ile Val Thr Cys Ala Met Phe Arg
385                 390                 395                 400

Cys Lys Lys Asn Met Glu Gly Lys Val Val Gln Pro Glu Asn Leu Glu
                405                 410                 415

Tyr Thr Ile Val Ile Thr Pro His Ser Gly Glu Glu His Ala Val Gly
            420                 425                 430

Asn Asp Thr Gly Lys His Gly Lys Glu Ile Lys Ile Thr Pro Gln Ser
        435                 440                 445

Ser Ile Thr Glu Ala Glu Leu Thr Gly Tyr Gly Thr Val Thr Met Glu
    450                 455                 460

Cys Ser Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Val Leu Leu Gln
465                 470                 475                 480

Met Glu Asn Lys Ala Trp Leu Val His Arg Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Leu Pro Gly Ala Asp Thr Gln Gly Ser Asn Trp Ile
            500                 505                 510

Gln Lys Glu Thr Leu Val Thr Phe Lys Asn Pro His Ala Lys Lys Gln
        515                 520                 525

Asp Val Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu
    530                 535                 540

Thr Gly Ala Thr Glu Ile Gln Met Ser Ser Gly Asn Leu Leu Phe Thr
545                 550                 555                 560

Gly His Leu Lys Cys Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly
                565                 570                 575

Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Val Val Lys Glu Ile
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly
```

-continued

```
        595                 600                 605

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys
    610                 615                 620

Arg His Val Leu Gly Arg Leu Ile Thr Val Asn Pro Ile Val Thr Glu
625                 630                 635                 640

Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser
                645                 650                 655

Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe
            660                 665                 670

Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly
        675                 680                 685

Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Leu Gly Gly Val Phe Thr Ser Ile Gly Lys Ala Leu His Gln Val Phe
705                 710                 715                 720

Gly Ala Ile Tyr Gly Ala Ala Phe Ser Gly Val Ser Trp Thr Met Lys
                725                 730                 735

Ile Leu Ile Gly Val Ile Ile Thr Trp Ile Gly Met Asn Ser Arg Ser
            740                 745                 750

Thr Ser Leu Ser Val Thr Leu Val Leu Val Gly Ile Val Thr Leu Tyr
        755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005
```

-continued

```
Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Asp Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Lys Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                1240                1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                1255                1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                1270                1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                1285                1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                1300                1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                1315                1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                1330                1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                1345                1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                1360                1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                1375                1380
```

-continued

```
Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                 1390                 1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                 1405                 1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                 1420                 1425

Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
    1430                 1435                 1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                 1450                 1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                 1465                 1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                 1480                 1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                 1495                 1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                 1510                 1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                 1525                 1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                 1540                 1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                 1555                 1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                 1570                 1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                 1585                 1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                 1600                 1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                 1615                 1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                 1630                 1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                 1645                 1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                 1660                 1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                 1675                 1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                 1690                 1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                 1705                 1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                 1720                 1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                 1735                 1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                 1750                 1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
```

-continued

```
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Ala Arg  Asp Ala Leu
    2105                2110                2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                2125                2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                2140                2145
```

-continued

```
Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                 2155                 2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                 2170                 2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                 2185                 2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                 2200                 2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                 2215                 2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                 2230                 2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                 2245                 2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                 2260                 2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                 2275                 2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                 2290                 2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                 2305                 2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                 2320                 2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                 2335                 2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Leu Phe  Leu Leu Val
    2345                 2350                 2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                 2365                 2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                 2380                 2385

Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                 2395                 2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                 2410                 2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                 2425                 2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                 2440                 2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                 2455                 2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                 2470                 2475

Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                 2485                 2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                 2500                 2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                 2515                 2520
```

-continued

```
Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                 2530                 2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                 2545                 2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                 2560                 2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                 2575                 2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                 2590                 2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                 2605                 2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                 2620                 2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                 2635                 2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                 2650                 2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                 2665                 2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                 2680                 2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                 2695                 2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                 2710                 2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                 2725                 2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                 2740                 2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                 2755                 2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                 2770                 2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                 2785                 2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                 2800                 2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                 2815                 2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                 2830                 2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                 2845                 2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                 2860                 2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                 2875                 2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                 2890                 2895

Ala Leu  Gly Ala Val Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
```

-continued

```
    2900                2905                2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                2920                2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                2935                2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                2950                2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                2965                2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                2980                2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                2995                3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                3010                3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                3025                3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                3040                3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                3055                3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                3070                3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                3085                3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                3100                3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                3115                3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                3130                3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                3145                3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                3160                3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                3175                3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                3190                3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                3205                3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                3220                3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                3235                3240

Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                3250                3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                3265                3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                3280                3285
```

-continued

```
His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                 3295                 3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                 3310                 3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                 3325                 3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                 3340                 3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                 3355                 3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                 3370                 3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                 3385                 3390


<210> SEQ ID NO 7
<211> LENGTH: 10717
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 3, BVS

<400> SEQUENCE: 7

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta     60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg    120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag    180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg    240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga    300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt    360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg    420

attccaacag tgatggcgtt ccatttaacc acgcgtgatg gagagccgcg catgattgtg    480

gggaagaatg aaagaggaaa atccctactt ttcaagacag cctctggaat caacatgtgc    540

acactcatag ctatggatct gggagagatg tgtgatgaca cggtcactta caaatgcccc    600

cacattaccg aagtggagcc tgaagacatt gactgctggt gcaaccttac atcgacatgg    660

gtgacttatg gaacatgcaa tcaagctgga gagcatagac gcgataagag atcagtggcg    720

ttagctcccc atgttggcat gggactggac acacgcactc aaacctggat gtcggctgaa    780

ggagcttgga gacaagtcga gaaggtagag acatgggccc ttaggcaccc agggtttacc    840

atactagccc tatttcttgc ccattacata ggcacttcct tgacccagaa agtggttatt    900

tttatactat taatgctggt taccccatcc atgacaatga gatgtgtagg agtaggaaac    960

agagattttg tggaaggcct atcgggagct acgtgggttg acgtggtgct cgagcacggt   1020

gggtgtgtga ctaccatggc taagaacaag cccacgctgg acatagagct tcagaagacc   1080

gaggccaccc aactggcgac cctaaggaag ctatgcattg agggaaaaat taccaacata   1140

acaaccgact caagatgtcc cacccaaggg gaagcgattt tacctgagga gcaggaccag   1200

aactacgtgt gtaagcatac atacgtggac agaggctggg gaaacggttg tggtttgttt   1260

ggcaagggaa gcttggtgac atgcgcgaaa tttcaatgtt tagaatcaat agagggaaaa   1320
```

-continued

gtggtgcaac atgagaacct caaatacacc gtcatcatca cagtgcacac aggagaccaa 1380

caccaggtgg gaaatgaaac gcagggagtc acggctgaga taacacccca ggcatcaacc 1440

gctgaagcca ttttacctga atatggaacc ctcgggctag aatgctcacc acggacaggt 1500

ttggatttca atgaaatgat ctcattgaca atgaagaaca aagcatggat ggtacataga 1560

caatggttct ttgacttacc cctaccatgg acatcaggag cttcagcaga aacaccaact 1620

tggaacagga aagagcttct tgtgacattt aaaaatgcac atgcaaaaaa gcaagaagta 1680

gttgttcttg gatcacaaga gggagcaatg catacagcac tgacaggagc tacagagatc 1740

caaacctcag gaggcacaag tatctttgcg gggcacttaa aatgtagact caagatggac 1800

aaattggaac tcaaggggat gagctatgca atgtgcttga gtagctttgt gttgaagaaa 1860

gaagtctccg aaacgcagca tgggacaata ctcattaagg ttgagtacaa aggggaagat 1920

gcaccctgca agattccttt ctccacggag gatggacaag gaaaagctct caatggcaga 1980

ctgatcacag ccaatccagt ggtgaccaag aaggaggagc ctgtcaacat tgaggctgaa 2040

cctccttttg gagaaagtaa catagtaatt ggaattggag acaaagccct gaaaatcaac 2100

tggtacaaga agggaagctc gattgggaag atgttcgagg ccactgccag aggtgcaagg 2160

cgcatggcca tcttgggaga cacagcctgg gactttggat cagtgggtgg tgttttgaat 2220

tcattaggga aaatggtcca ccaaatattt gggagtgctt acacagccct atttggtgga 2280

gtctcctgga tgatgaaaat tggaataggt gtcctcttaa cctggatagg gttgaactca 2340

aaaaatactt ctatgtcatt ttcatgcatc gcggccggca ttgtgacact gtatttggga 2400

gtcatggtgc aggccgatag tggttgcgtt gtgagctgga aaaacaaaga actgaaatgt 2460

ggcagtggga ttttcatcac agacaacgtg cacacatgga cagaacaata caagttccaa 2520

ccagaatccc cttcaaaact agcttcagct atccagaaag cccatgaaga ggacatttgt 2580

ggaatccgct cagtaacaag actggagaat ctgatgtgga aacaaataac accagaattg 2640

aatcacattc tatcagaaaa tgaggtgaag ttaactatta tgacaggaga catcaaagga 2700

atcatgcagg caggaaaacg atctctgcgg cctcagccca ctgagctgaa gtattcatgg 2760

aaaacatggg gcaaagcaaa aatgctctct acagagtctc ataaccagac ctttctcatt 2820

gatggccccg aaacagcaga atgccccaac acaaatagag cttggaattc gttggaagtt 2880

gaagactatg gctttggagt attcaccacc aatatatggc taaaattgaa agaaaaacag 2940

gatgtattct gcgactcaaa actcatgtca gcggccataa aagacaacag agccgtccat 3000

gccgatatgg gttattggat agaaagtgca ctcaatgaca catggaagat agagaaagcc 3060

tctttcattg aagttaaaaa ctgccactgg ccaaaatcac acaccctctg gagcaatgga 3120

gtgctagaaa gtgagatgat aattccaaag aatctcgctg gaccagtgtc tcaacacaac 3180

tatagaccag gctaccatac acaaataaca ggaccatggc atctaggtaa gcttgagatg 3240

gactttgatt tctgtgatgg aacaacagtg gtagtgactg aggactgcgg aaatagagga 3300

ccctctttga gaacaaccac tgcctctgga aaactcataa cagaatggtg ctgccgatct 3360

tgcacattac caccgctaag atacagaggt gaggatgggt gctggtacgg gatggaaatc 3420

agaccattga aggagaaaga agagaatttg gtcaactcct tggtcacagc tggacatggg 3480

caggtcgaca acttttcact aggagtcttg ggaatggcat tgttcctgga ggaaatgctt 3540

aggacccgag taggaacgaa acatgcaata ctactagttg cagtttcttt tgtgacattg 3600

-continued

atcacaggga acatgtcctt tagagacctg ggaagagtga tggttatggt aggcgccact 3660

atgacggatg acataggtat gggcgtgact tatcttgccc tactagcagc cttcaaagtc 3720

agaccaactt ttgcagctgg actactcttg agaaagctga cctccaagga attgatgatg 3780

actactatag gaattgtact cctctcccag agcaccatac cagagaccat tcttgagttg 3840

actgatgcgt tagccttagg catgatggtc ctcaaaatgg tgagaaatat ggaaaagtat 3900

caattggcag tgactatcat ggctatcttg tgcgtcccaa acgcagtgat attacaaaac 3960

gcatggaaag tgagttgcac aatattggca gtggtgtccg tttccccact gttcttaaca 4020

tcctcacagc aaaaaacaga ttggatacca ttagcattga cgatcaaagg tctcaatcca 4080

acagctattt ttctaacaac cctctcaaga accagcaaga aaaggagctg gccattaaat 4140

gaggctatca tggcagtcgg gatggtgagc attttagcca gttctctcct aaaaaatgat 4200

attcccatga caggaccatt agtggctgga gggctcctca ctgtgtgcta cgtgctcact 4260

ggacgatcgg ccgatttgga actggagaga gcagccgatg tcaaatggga agaccaggca 4320

gagatatcag gaagcagtcc aatcctgtca ataacaatat cagaagatgg tagcatgtcg 4380

ataaaaaatg aagaggaaga acaaacactg accatactca ttagaacagg attgctggtg 4440

atctcaggac tttttcctgt atcaatacca atcacggcag cagcatggta cctgtgggaa 4500

gtgaagaaac aacgggccgg agtattgtgg gatgttcctt cacccccacc catgggaaag 4560

gctgaactgg aagatggagc ctatagaatt aagcaaaaag ggattcttgg atattcccag 4620

atcggagccg gagtttacaa agaaggaaca ttccatacaa tgtggcatgt cacacgtggc 4680

gctgttctaa tgcataaagg aaagaggatt gaaccatcat gggcggacgt caagaaagac 4740

ctaatatcat atggaggagg ctggaagtta gaaggagaat ggaaggaagg agaagaagtc 4800

caggtattgg cactggagcc tggaaaaaat ccaagagccg tccaaacgaa acctggtctt 4860

ttcaaaacca acgccggaac aataggtgct gtatctctgg acttttctcc tggaacgtca 4920

ggatctccaa ttatcgacaa aaaaggaaaa gttgtgggtc tttatggtaa tggtgttgtt 4980

acaaggagtg gagcatatgt gagtgctata gcccagactg aaaaaagcat tgaagacaac 5040

ccagagatcg aagatgacat tttccgaaag agaagactga ccatcatgga cctccaccca 5100

ggagcgggaa agacgaagag ataccttccg gccatagtca gagaagctat aaaacggggt 5160

ttgagaacat taatcttggc ccccactaga gttgtggcag ctgaaatgga ggaagccctt 5220

agaggacttc caataagata ccagacccca gccatcagag ctgtgcacac cgggcgggag 5280

attgtggacc taatgtgtca tgccacattt accatgaggc tgctatcacc agttagagtg 5340

ccaaactaca acctgattat catggacgaa gcccatttca cagacccagc aagtatagca 5400

gctagaggat acatctcaac tcgagtggag atgggtgagg cagctgggat ttttatgaca 5460

gccactcccc cgggaagcag agacccattt cctcagagca atgcaccaat catagatgaa 5520

gaaagagaaa tccctgaacg ctcgtggaat tccggacatg aatgggtcac ggattttaaa 5580

gggaagactg tttggttcgt tccaagtata aaagcaggaa atgatatagc agcttgcctg 5640

aggaaaaatg gaaagaaagt gatacaactc agtaggaaga cctttgattc tgagtatgtc 5700

aagactagaa ccaatgattg ggacttcgtg gttacaactg acatttcaga aatgggtgcc 5760

aatttcaagg ctgagagggt tatagacccc agacgctgca tgaaaccagt catactaaca 5820

gatggtgaag agcgggtgat tctggcagga cctatgccag tgacccactc tagtgcagca 5880

-continued

caaagaagag ggagaatagg aagaaatcca aaaaatgaga atgaccagta catatacatg 5940

ggggaacctc tggaaaatga tgaagactgt gcacactgga aagaagctaa aatgctccta 6000

gataacatca acacgccaga aggaatcatt cctagcatgt tcgaaccaga gcgtgaaaag 6060

gtggatgcca ttgatggcga ataccgcttg agaggagaag caaggaaaac ctttgtagac 6120

ttaatgagaa gaggagacct accagtctgg ttggcctaca gagtggcagc tgaaggcatc 6180

aactacgcag acagaaggtg gtgttttgat ggagtcaaga acaaccaaat cctagaagaa 6240

aacgtggaag ttgaaatctg gacaaaagaa ggggaaagga agaaattgaa acccagatgg 6300

ttggatgcta ggatctattc tgacccactg gcgctaaaag aatttaagga atttgcagcc 6360

ggaagaaagt ctctgaccct gaacctaatc acagaaatgg gtaggctccc aaccttcatg 6420

actcagaagg caagaaacgc actggacaac ttagcagtgc tgcacacggc tgaggcaggt 6480

ggaagggcgt acaaccatgc tctcagtgaa ctgccggaga ccctggagac attgctttta 6540

ctgacacttc tggctacagt cacgggaggg atctttttat tcttgatgag cgcaaggggc 6600

atagggaaga tgaccctggg aatgtgctgc ataatcacgg ctagcatcct cctatggtac 6660

gcacaaatac agccacactg gatagcagct tcaataatac tggagttttt tctcatagtt 6720

ttgcttattc cagaacctga aaaacagaga acaccccaag acaaccaact gacctacgtt 6780

gtcatagcca tcctcacagt ggtggccgca accatggcaa acgagatggg tttcctagaa 6840

aaaacgaaga aagatctcgg attgggaagc attgcaaccc agcaacccga gagcaacatc 6900

ctggacatag atctacgtcc tgcatcagca tggacgctgt atgccgtggc cacaacattt 6960

gttacaccaa tgttgagaca tagcattgaa aattcctcag tgaatgtgtc cctaacagct 7020

atagccaacc aagccacagt gttaatgggt ctcgggaaag gatggccatt gtcaaagatg 7080

gacatcggag ttccccttct cgccattgga tgctactcac aagtcaaccc cataactctc 7140

acagcagctc ttttcttatt ggtagcacat tatgccatca tagggccagg actccaagca 7200

aaagcaacca gagaagctca gaaaagagca gcggcgggca tcatgaaaaa cccaactgtc 7260

gatggaataa cagtgattga cctagatcca ataccttatg atccaaagtt tgaaaagcag 7320

ttgggacaag taatgctcct agtcctctgc gtgactcaag tattgatgat gaggactaca 7380

tgggctctgt gtgaggcttt aaccttagct accgggccca tctccacatt gtgggaagga 7440

aatccaggga ggttttggaa cactaccatt gcggtgtcaa tggctaacat ttttagaggg 7500

agttacttgg ccggagctgg acttctcttt tctattatga agaacacaac caacacaaga 7560

aggggaactg gcaacatagg agagacgctt ggagagaaat ggaaaagccg attgaacgcg 7620

ttgggaaaaa gtgaattcca gatctacaag aaaagtggaa tccaggaagt ggatagaacc 7680

ttagcaaaag aaggcattaa aagaggagaa acggaccatc acgctgtgtc gcgaggctca 7740

gcaaaactga gatggttcgt tgagagaaac atggtcacac cagaagggaa agtagtggac 7800

ctcggttgtg gcagaggagg ctggtcatac tattgtggag gactaaagaa tgtaagagaa 7860

gtcaaaggcc taacaaaagg aggaccagga cacgaagaac ccatccccat gtcaacatat 7920

gggtggaatc tagtgcgtct tcaaagtgga gttgacgttt tcttcatccc gccagaaaag 7980

tgtgacacat tattgtgtga cataggggag tcatcaccaa atcccacagt ggaagcagga 8040

cgaacactca gagtccttaa cttagtagaa aattggttga acaacaacac tcaattttgc 8100

ataaaggttc tcaacccata tatgccctca gtcatagaaa aaatggaagc actacaaagg 8160

-continued

aaatatggag gagccttagt gaggaatcca ctctcacgaa actccacaca tgagatgtac 8220

tgggtatcca atgcttccgg gaacatagtg tcatcagtga acatgatttc aaggatgttg 8280

atcaacagat ttacaatgag atacaagaaa gccacttacg agccggatgt tgacctcgga 8340

agcggaaccc gtaacatcgg gattgaaagt gagataccaa acctagatat aattgggaaa 8400

agaatagaaa aaataaagca agagcatgaa acatcatggc actatgacca agaccaccca 8460

tacaaaacgt gggcatacca tggtagctat gaaacaaaac agactggatc agcatcatcc 8520

atggtcaacg gagtggtcag gctgctgaca aaaccttggg acgtcgtccc catggtgaca 8580

cagatggcaa tgacagacac gactccattt ggacaacagc gcgtttttaa agagaaagtg 8640

gacacgagaa cccaagaacc gaaagaaggc acgaagaaac taatgaaaat aacagcagag 8700

tggctttgga aagaattagg gaagaaaaag acacccagga tgtgcaccag agaagaattc 8760

acaagaaagg tgagaagcaa tgcagccttg ggggccatat tcactgatga gaacaagtgg 8820

aagtcggcac gtgaggctgt tgaagatagt aggttttggg agctggttga caaggaaagg 8880

aatctccatc ttgaaggaaa gtgtgaaaca tgtgtgtaca acatgatggg aaaaagagag 8940

aagaagctag gggaattcgg caaggcaaaa ggcagcagag ccatatggta catgtggctt 9000

ggagcacgct tcttagagtt tgaagcccta ggattcttaa atgaagatca ctggttctcc 9060

agagagaact ccctgagtgg agtggaagga gaagggctgc acaagctagg ttacattcta 9120

agagacgtga gcaagaaaga gggaggagca atgtatgccg atgacaccgc aggatgggat 9180

acaagaatca cactagaaga cctaaaaaat gaagaaatgg taacaaacca catggaagga 9240

gaacacaaga aactagccga ggccattttc aaactaacgt accaaaacaa ggtggtgcgt 9300

gtgcaaagac caacaccaag aggcacagta atggacatca tatcgagaag agaccaaaga 9360

ggtagtggac aagttggcac ctatggactc aatactttca ccaatatgga agcccaacta 9420

atcagacaga tggagggaga aggagtcttt aaaagcattc agcacctaac aatcacagaa 9480

gaaatcgctg tgcaaaactg gttagcaaga gtggggcgcg aaaggttatc aagaatggcc 9540

atcagtggag atgattgtgt tgtgaaacct ttagatgaca ggttcgcaag cgctttaaca 9600

gctctaaatg acatgggaaa gattaggaaa gacatacaac aatgggaacc ttcaagagga 9660

tggaatgatt ggacacaagt gcccttctgt tcacaccatt tccatgagtt aatcatgaaa 9720

gacggtcgcg tactcgttgt tccatgtaga aaccaagatg aactgattgg cagagcccga 9780

atctcccaag gagcagggtg gtctttgcgg gagacggcct gtttggggaa gtcttacgcc 9840

caaatgtgga gcttgatgta cttccacaga cgcgacctca ggctggcggc aaatgctatt 9900

tgctcggcag taccatcaca ttgggttcca acaagtcgaa caacctggtc catacatgct 9960

aaacatgaat ggatgacaac ggaagacatg ctgacagtct ggaacagggt gtggattcaa 10020

gaaaacccat ggatggaaga caaaactcca gtggaatcat gggaggaaat cccatacttg 10080

gggaaaagag aagaccaatg gtgcggctca ttgattgggt taacaagcag ggccacctgg 10140

gcaaagaaca tccaagcagc aataaatcaa gttagatccc ttataggcaa tgaagaatac 10200

acagattaca tgccatccat gaaaagattc agaagagaag aggaagaagc aggagttctg 10260

tggtagaaag caaaactaac atgaaacaag gctagaagtc aggtcggatt aagccatagt 10320

acggaaaaaa ctatgctacc tgtgagcccc gtccaaggac gttaaaagaa gtcaggccat 10380

cataaatgcc atagcttgag taaactatgc agcctgtagc tccacctgag aaggtgtaaa 10440

-continued

aaatccggga ggccacaaac catggaagct gtacgcatgg cgtagtggac tagcggttag 10500

aggagacccc tcccttacaa atcgcagcaa caatgggggc ccaaggcgag atgaagctgt 10560

agtctcgctg gaaggactag aggttagagg agacccccc gaaacaaaaa acagcatatt 10620

gacgctggga aagaccagag atcctgctgt ctcctcagca tcattccagg cacagaacgc 10680

cagaaaatgg aatggtgctg ttgaatcaac aggttct 10717

<210> SEQ ID NO 8
<211> LENGTH: 3389
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 3, BVS

<400> SEQUENCE: 8

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Arg Met Ile Val
115 120 125

Gly Lys Asn Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ala Ser Gly
130 135 140

Ile Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Asp
145 150 155 160

Asp Thr Val Thr Tyr Lys Cys Pro His Ile Thr Glu Val Glu Pro Glu
165 170 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Thr Tyr Gly
180 185 190

Thr Cys Asn Gln Ala Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
195 200 205

Leu Ala Pro His Val Gly Met Gly Leu Asp Thr Arg Thr Gln Thr Trp
210 215 220

Met Ser Ala Glu Gly Ala Trp Arg Gln Val Glu Lys Val Glu Thr Trp
225 230 235 240

Ala Leu Arg His Pro Gly Phe Thr Ile Leu Ala Leu Phe Leu Ala His
245 250 255

Tyr Ile Gly Thr Ser Leu Thr Gln Lys Val Val Ile Phe Ile Leu Leu
260 265 270

Met Leu Val Thr Pro Ser Met Thr Met Arg Cys Val Gly Val Gly Asn
275 280 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
290 295 300

-continued

```
Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305                 310                 315                 320

Leu Asp Ile Glu Leu Gln Lys Thr Glu Ala Thr Gln Leu Ala Thr Leu
                325                 330                 335

Arg Lys Leu Cys Ile Glu Gly Lys Ile Thr Asn Ile Thr Thr Asp Ser
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Ile Leu Pro Glu Glu Gln Asp Gln
        355                 360                 365

Asn Tyr Val Cys Lys His Thr Tyr Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Gln
385                 390                 395                 400

Cys Leu Glu Ser Ile Glu Gly Lys Val Val Gln His Glu Asn Leu Lys
                405                 410                 415

Tyr Thr Val Ile Ile Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Gln Gly Val Thr Ala Glu Ile Thr Pro Gln Ala Ser Thr
        435                 440                 445

Ala Glu Ala Ile Leu Pro Glu Tyr Gly Thr Leu Gly Leu Glu Cys Ser
    450                 455                 460

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Ile Ser Leu Thr Met Lys
465                 470                 475                 480

Asn Lys Ala Trp Met Val His Arg Gln Trp Phe Phe Asp Leu Pro Leu
                485                 490                 495

Pro Trp Thr Ser Gly Ala Ser Ala Glu Thr Pro Thr Trp Asn Arg Lys
            500                 505                 510

Glu Leu Leu Val Thr Phe Lys Asn Ala His Ala Lys Lys Gln Glu Val
        515                 520                 525

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
    530                 535                 540

Ala Thr Glu Ile Gln Thr Ser Gly Gly Thr Ser Ile Phe Ala Gly His
545                 550                 555                 560

Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Glu Leu Lys Gly Met Ser
                565                 570                 575

Tyr Ala Met Cys Leu Ser Ser Phe Val Leu Lys Lys Glu Val Ser Glu
            580                 585                 590

Thr Gln His Gly Thr Ile Leu Ile Lys Val Glu Tyr Lys Gly Glu Asp
        595                 600                 605

Ala Pro Cys Lys Ile Pro Phe Ser Thr Glu Asp Gly Gln Gly Lys Ala
    610                 615                 620

Leu Asn Gly Arg Leu Ile Thr Ala Asn Pro Val Val Thr Lys Lys Glu
625                 630                 635                 640

Glu Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser Asn Ile
                645                 650                 655

Val Ile Gly Ile Gly Asp Lys Ala Leu Lys Ile Asn Trp Tyr Lys Lys
            660                 665                 670

Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala Arg
        675                 680                 685

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly
    690                 695                 700
```

-continued

```
Gly Val Leu Asn Ser Leu Gly Lys Met Val His Gln Ile Phe Gly Ser
705                 710                 715                 720

Ala Tyr Thr Ala Leu Phe Gly Gly Val Ser Trp Met Met Lys Ile Gly
                725                 730                 735

Ile Gly Val Leu Leu Thr Trp Ile Gly Leu Asn Ser Lys Asn Thr Ser
            740                 745                 750

Met Ser Phe Ser Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr Leu Gly
        755                 760                 765

Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys Asn Lys
    770                 775                 780

Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val His Thr
785                 790                 795                 800

Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys Leu Ala
                805                 810                 815

Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile Arg Ser
            820                 825                 830

Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro Glu Leu
        835                 840                 845

Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met Thr Gly
    850                 855                 860

Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg Pro Gln
865                 870                 875                 880

Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala Lys Met
                885                 890                 895

Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly Pro Glu
            900                 905                 910

Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu Glu Val
        915                 920                 925

Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu Lys Leu
    930                 935                 940

Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser Ala Ala
945                 950                 955                 960

Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp Ile Glu
                965                 970                 975

Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe Ile Glu
            980                 985                 990

Val Lys Asn Cys His Trp Pro Lys  Ser His Thr Leu Trp  Ser Asn Gly
        995                 1000                1005

Val Leu  Glu Ser Glu Met Ile  Ile Pro Lys Asn Leu  Ala Gly Pro
    1010                1015                1020

Val Ser  Gln His Asn Tyr Arg  Pro Gly Tyr His Thr  Gln Ile Thr
    1025                1030                1035

Gly Pro  Trp His Leu Gly Lys  Leu Glu Met Asp Phe  Asp Phe Cys
    1040                1045                1050

Asp Gly  Thr Thr Val Val Val  Thr Glu Asp Cys Gly  Asn Arg Gly
    1055                1060                1065

Pro Ser  Leu Arg Thr Thr Thr  Ala Ser Gly Lys Leu  Ile Thr Glu
    1070                1075                1080

Trp Cys  Cys Arg Ser Cys Thr  Leu Pro Pro Leu Arg  Tyr Arg Gly
    1085                1090                1095

Glu Asp  Gly Cys Trp Tyr Gly  Met Glu Ile Arg Pro  Leu Lys Glu
```

-continued

```
    1100                1105                1110

Lys Glu  Glu Asn Leu Val Asn  Ser Leu Val Thr Ala  Gly His Gly
    1115                1120                1125

Gln Val  Asp Asn Phe Ser Leu  Gly Val Leu Gly Met  Ala Leu Phe
    1130                1135                1140

Leu Glu  Glu Met Leu Arg Thr  Arg Val Gly Thr Lys  His Ala Ile
    1145                1150                1155

Leu Leu  Val Ala Val Ser Phe  Val Thr Leu Ile Thr  Gly Asn Met
    1160                1165                1170

Ser Phe  Arg Asp Leu Gly Arg  Val Met Val Met Val  Gly Ala Thr
    1175                1180                1185

Met Thr  Asp Asp Ile Gly Met  Gly Val Thr Tyr Leu  Ala Leu Leu
    1190                1195                1200

Ala Ala  Phe Lys Val Arg Pro  Thr Phe Ala Ala Gly  Leu Leu Leu
    1205                1210                1215

Arg Lys  Leu Thr Ser Lys Glu  Leu Met Met Thr Thr  Ile Gly Ile
    1220                1225                1230

Val Leu  Leu Ser Gln Ser Thr  Ile Pro Glu Thr Ile  Leu Glu Leu
    1235                1240                1245

Thr Asp  Ala Leu Ala Leu Gly  Met Met Val Leu Lys  Met Val Arg
    1250                1255                1260

Asn Met  Glu Lys Tyr Gln Leu  Ala Val Thr Ile Met  Ala Ile Leu
    1265                1270                1275

Cys Val  Pro Asn Ala Val Ile  Leu Gln Asn Ala Trp  Lys Val Ser
    1280                1285                1290

Cys Thr  Ile Leu Ala Val Val  Ser Val Ser Pro Leu  Phe Leu Thr
    1295                1300                1305

Ser Ser  Gln Gln Lys Thr Asp  Trp Ile Pro Leu Ala  Leu Thr Ile
    1310                1315                1320

Lys Gly  Leu Asn Pro Thr Ala  Ile Phe Leu Thr Thr  Leu Ser Arg
    1325                1330                1335

Thr Ser  Lys Lys Arg Ser Trp  Pro Leu Asn Glu Ala  Ile Met Ala
    1340                1345                1350

Val Gly  Met Val Ser Ile Leu  Ala Ser Ser Leu Leu  Lys Asn Asp
    1355                1360                1365

Ile Pro  Met Thr Gly Pro Leu  Val Ala Gly Gly Leu  Leu Thr Val
    1370                1375                1380

Cys Tyr  Val Leu Thr Gly Arg  Ser Ala Asp Leu Glu  Leu Glu Arg
    1385                1390                1395

Ala Ala  Asp Val Lys Trp Glu  Asp Gln Ala Glu Ile  Ser Gly Ser
    1400                1405                1410

Ser Pro  Ile Leu Ser Ile Thr  Ile Ser Glu Asp Gly  Ser Met Ser
    1415                1420                1425

Ile Lys  Asn Glu Glu Glu Glu  Gln Thr Leu Thr Ile  Leu Ile Arg
    1430                1435                1440

Thr Gly  Leu Leu Val Ile Ser  Gly Leu Phe Pro Val  Ser Ile Pro
    1445                1450                1455

Ile Thr  Ala Ala Ala Trp Tyr  Leu Trp Glu Val Lys  Lys Gln Arg
    1460                1465                1470

Ala Gly  Val Leu Trp Asp Val  Pro Ser Pro Pro Pro  Met Gly Lys
    1475                1480                1485
```

-continued

```
Ala Glu  Leu Glu Asp Gly Ala  Tyr Arg Ile Lys Gln  Lys Gly Ile
    1490                 1495                 1500

Leu Gly  Tyr Ser Gln Ile Gly  Ala Gly Val Tyr Lys  Glu Gly Thr
    1505                 1510                 1515

Phe His  Thr Met Trp His Val  Thr Arg Gly Ala Val  Leu Met His
    1520                 1525                 1530

Lys Gly  Lys Arg Ile Glu Pro  Ser Trp Ala Asp Val  Lys Lys Asp
    1535                 1540                 1545

Leu Ile  Ser Tyr Gly Gly Gly  Trp Lys Leu Glu Gly  Glu Trp Lys
    1550                 1555                 1560

Glu Gly  Glu Glu Val Gln Val  Leu Ala Leu Glu Pro  Gly Lys Asn
    1565                 1570                 1575

Pro Arg  Ala Val Gln Thr Lys  Pro Gly Leu Phe Lys  Thr Asn Ala
    1580                 1585                 1590

Gly Thr  Ile Gly Ala Val Ser  Leu Asp Phe Ser Pro  Gly Thr Ser
    1595                 1600                 1605

Gly Ser  Pro Ile Ile Asp Lys  Lys Gly Lys Val Val  Gly Leu Tyr
    1610                 1615                 1620

Gly Asn  Gly Val Val Thr Arg  Ser Gly Ala Tyr Val  Ser Ala Ile
    1625                 1630                 1635

Ala Gln  Thr Glu Lys Ser Ile  Glu Asp Asn Pro Glu  Ile Glu Asp
    1640                 1645                 1650

Asp Ile  Phe Arg Lys Arg Arg  Leu Thr Ile Met Asp  Leu His Pro
    1655                 1660                 1665

Gly Ala  Gly Lys Thr Lys Arg  Tyr Leu Pro Ala Ile  Val Arg Glu
    1670                 1675                 1680

Ala Ile  Lys Arg Gly Leu Arg  Thr Leu Ile Leu Ala  Pro Thr Arg
    1685                 1690                 1695

Val Val  Ala Ala Glu Met Glu  Glu Ala Leu Arg Gly  Leu Pro Ile
    1700                 1705                 1710

Arg Tyr  Gln Thr Pro Ala Ile  Arg Ala Val His Thr  Gly Arg Glu
    1715                 1720                 1725

Ile Val  Asp Leu Met Cys His  Ala Thr Phe Thr Met  Arg Leu Leu
    1730                 1735                 1740

Ser Pro  Val Arg Val Pro Asn  Tyr Asn Leu Ile Ile  Met Asp Glu
    1745                 1750                 1755

Ala His  Phe Thr Asp Pro Ala  Ser Ile Ala Ala Arg  Gly Tyr Ile
    1760                 1765                 1770

Ser Thr  Arg Val Glu Met Gly  Glu Ala Ala Gly Ile  Phe Met Thr
    1775                 1780                 1785

Ala Thr  Pro Pro Gly Ser Arg  Asp Pro Phe Pro Gln  Ser Asn Ala
    1790                 1795                 1800

Pro Ile  Ile Asp Glu Glu Arg  Glu Ile Pro Glu Arg  Ser Trp Asn
    1805                 1810                 1815

Ser Gly  His Glu Trp Val Thr  Asp Phe Lys Gly Lys  Thr Val Trp
    1820                 1825                 1830

Phe Val  Pro Ser Ile Lys Ala  Gly Asn Asp Ile Ala  Ala Cys Leu
    1835                 1840                 1845

Arg Lys  Asn Gly Lys Lys Val  Ile Gln Leu Ser Arg  Lys Thr Phe
    1850                 1855                 1860
```

-continued

```
Asp Ser  Glu Tyr Val Lys Thr  Arg Thr Asn Asp Trp  Asp Phe Val
    1865                1870                1875

Val Thr  Thr Asp Ile Ser Glu  Met Gly Ala Asn Phe  Lys Ala Glu
    1880                1885                1890

Arg Val  Ile Asp Pro Arg Arg  Cys Met Lys Pro Val  Ile Leu Thr
    1895                1900                1905

Asp Gly  Glu Glu Arg Val Ile  Leu Ala Gly Pro Met  Pro Val Thr
    1910                1915                1920

His Ser  Ser Ala Ala Gln Arg  Arg Gly Arg Ile Gly  Arg Asn Pro
    1925                1930                1935

Lys Asn  Glu Asn Asp Gln Tyr  Ile Tyr Met Gly Glu  Pro Leu Glu
    1940                1945                1950

Asn Asp  Glu Asp Cys Ala His  Trp Lys Glu Ala Lys  Met Leu Leu
    1955                1960                1965

Asp Asn  Ile Asn Thr Pro Glu  Gly Ile Ile Pro Ser  Met Phe Glu
    1970                1975                1980

Pro Glu  Arg Glu Lys Val Asp  Ala Ile Asp Gly Glu  Tyr Arg Leu
    1985                1990                1995

Arg Gly  Glu Ala Arg Lys Thr  Phe Val Asp Leu Met  Arg Arg Gly
    2000                2005                2010

Asp Leu  Pro Val Trp Leu Ala  Tyr Arg Val Ala Ala  Glu Gly Ile
    2015                2020                2025

Asn Tyr  Ala Asp Arg Arg Trp  Cys Phe Asp Gly Val  Lys Asn Asn
    2030                2035                2040

Gln Ile  Leu Glu Glu Asn Val  Glu Val Glu Ile Trp  Thr Lys Glu
    2045                2050                2055

Gly Glu  Arg Lys Lys Leu Lys  Pro Arg Trp Leu Asp  Ala Arg Ile
    2060                2065                2070

Tyr Ser  Asp Pro Leu Ala Leu  Lys Glu Phe Lys Glu  Phe Ala Ala
    2075                2080                2085

Gly Arg  Lys Ser Leu Thr Leu  Asn Leu Ile Thr Glu  Met Gly Arg
    2090                2095                2100

Leu Pro  Thr Phe Met Thr Gln  Lys Ala Arg Asn Ala  Leu Asp Asn
    2105                2110                2115

Leu Ala  Val Leu His Thr Ala  Glu Ala Gly Gly Arg  Ala Tyr Asn
    2120                2125                2130

His Ala  Leu Ser Glu Leu Pro  Glu Thr Leu Glu Thr  Leu Leu Leu
    2135                2140                2145

Leu Thr  Leu Leu Ala Thr Val  Thr Gly Gly Ile Phe  Leu Phe Leu
    2150                2155                2160

Met Ser  Ala Arg Gly Ile Gly  Lys Met Thr Leu Gly  Met Cys Cys
    2165                2170                2175

Ile Ile  Thr Ala Ser Ile Leu  Leu Trp Tyr Ala Gln  Ile Gln Pro
    2180                2185                2190

His Trp  Ile Ala Ala Ser Ile  Ile Leu Glu Phe Phe  Leu Ile Val
    2195                2200                2205

Leu Leu  Ile Pro Glu Pro Glu  Lys Gln Arg Thr Pro  Gln Asp Asn
    2210                2215                2220

Gln Leu  Thr Tyr Val Val Ile  Ala Ile Leu Thr Val  Val Ala Ala
    2225                2230                2235

Thr Met  Ala Asn Glu Met Gly  Phe Leu Glu Lys Thr  Lys Lys Asp
```

-continued

```
    2240                2245                2250

Leu Gly  Leu Gly Ser Ile Ala  Thr Gln Gln Pro Glu  Ser Asn Ile
    2255                2260                2265

Leu Asp  Ile Asp Leu Arg Pro  Ala Ser Ala Trp Thr  Leu Tyr Ala
    2270                2275                2280

Val Ala  Thr Thr Phe Val Thr  Pro Met Leu Arg His  Ser Ile Glu
    2285                2290                2295

Asn Ser  Ser Val Asn Val Ser  Leu Thr Ala Ile Ala  Asn Gln Ala
    2300                2305                2310

Thr Val  Leu Met Gly Leu Gly  Lys Gly Trp Pro Leu  Ser Lys Met
    2315                2320                2325

Asp Ile  Gly Val Pro Leu Leu  Ala Ile Gly Cys Tyr  Ser Gln Val
    2330                2335                2340

Asn Pro  Ile Thr Leu Thr Ala  Ala Leu Phe Leu Leu  Val Ala His
    2345                2350                2355

Tyr Ala  Ile Ile Gly Pro Gly  Leu Gln Ala Lys Ala  Thr Arg Glu
    2360                2365                2370

Ala Gln  Lys Arg Ala Ala Ala  Gly Ile Met Lys Asn  Pro Thr Val
    2375                2380                2385

Asp Gly  Ile Thr Val Ile Asp  Leu Asp Pro Ile Pro  Tyr Asp Pro
    2390                2395                2400

Lys Phe  Glu Lys Gln Leu Gly  Gln Val Met Leu Leu  Val Leu Cys
    2405                2410                2415

Val Thr  Gln Val Leu Met Met  Arg Thr Thr Trp Ala  Leu Cys Glu
    2420                2425                2430

Ala Leu  Thr Leu Ala Thr Gly  Pro Ile Ser Thr Leu  Trp Glu Gly
    2435                2440                2445

Asn Pro  Gly Arg Phe Trp Asn  Thr Thr Ile Ala Val  Ser Met Ala
    2450                2455                2460

Asn Ile  Phe Arg Gly Ser Tyr  Leu Ala Gly Ala Gly  Leu Leu Phe
    2465                2470                2475

Ser Ile  Met Lys Asn Thr Thr  Asn Thr Arg Arg Gly  Thr Gly Asn
    2480                2485                2490

Ile Gly  Glu Thr Leu Gly Glu  Lys Trp Lys Ser Arg  Leu Asn Ala
    2495                2500                2505

Leu Gly  Lys Ser Glu Phe Gln  Ile Tyr Lys Lys Ser  Gly Ile Gln
    2510                2515                2520

Glu Val  Asp Arg Thr Leu Ala  Lys Glu Gly Ile Lys  Arg Gly Glu
    2525                2530                2535

Thr Asp  His His Ala Val Ser  Arg Gly Ser Ala Lys  Leu Arg Trp
    2540                2545                2550

Phe Val  Glu Arg Asn Met Val  Thr Pro Glu Gly Lys  Val Val Asp
    2555                2560                2565

Leu Gly  Cys Gly Arg Gly Gly  Trp Ser Tyr Tyr Cys  Gly Gly Leu
    2570                2575                2580

Lys Asn  Val Arg Glu Val Lys  Gly Leu Thr Lys Gly  Gly Pro Gly
    2585                2590                2595

His Glu  Glu Pro Ile Pro Met  Ser Thr Tyr Gly Trp  Asn Leu Val
    2600                2605                2610

Arg Leu  Gln Ser Gly Val Asp  Val Phe Phe Ile Pro  Pro Glu Lys
    2615                2620                2625
```

-continued

```
Cys Asp  Thr Leu Leu Cys Asp  Ile Gly Glu Ser Ser  Pro Asn Pro
    2630                 2635                 2640

Thr Val  Glu Ala Gly Arg Thr  Leu Arg Val Leu Asn  Leu Val Glu
    2645                 2650                 2655

Asn Trp  Leu Asn Asn Asn Thr  Gln Phe Cys Ile Lys  Val Leu Asn
    2660                 2665                 2670

Pro Tyr  Met Pro Ser Val Ile  Glu Lys Met Glu Ala  Leu Gln Arg
    2675                 2680                 2685

Lys Tyr  Gly Gly Ala Leu Val  Arg Asn Pro Leu Ser  Arg Asn Ser
    2690                 2695                 2700

Thr His  Glu Met Tyr Trp Val  Ser Asn Ala Ser Gly  Asn Ile Val
    2705                 2710                 2715

Ser Ser  Val Asn Met Ile Ser  Arg Met Leu Ile Asn  Arg Phe Thr
    2720                 2725                 2730

Met Arg  Tyr Lys Lys Ala Thr  Tyr Glu Pro Asp Val  Asp Leu Gly
    2735                 2740                 2745

Ser Gly  Thr Arg Asn Ile Gly  Ile Glu Ser Glu Ile  Pro Asn Leu
    2750                 2755                 2760

Asp Ile  Ile Gly Lys Arg Ile  Glu Lys Ile Lys Gln  Glu His Glu
    2765                 2770                 2775

Thr Ser  Trp His Tyr Asp Gln  Asp His Pro Tyr Lys  Thr Trp Ala
    2780                 2785                 2790

Tyr His  Gly Ser Tyr Glu Thr  Lys Gln Thr Gly Ser  Ala Ser Ser
    2795                 2800                 2805

Met Val  Asn Gly Val Val Arg  Leu Leu Thr Lys Pro  Trp Asp Val
    2810                 2815                 2820

Val Pro  Met Val Thr Gln Met  Ala Met Thr Asp Thr  Thr Pro Phe
    2825                 2830                 2835

Gly Gln  Gln Arg Val Phe Lys  Glu Lys Val Asp Thr  Arg Thr Gln
    2840                 2845                 2850

Glu Pro  Lys Glu Gly Thr Lys  Lys Leu Met Lys Ile  Thr Ala Glu
    2855                 2860                 2865

Trp Leu  Trp Lys Glu Leu Gly  Lys Lys Lys Thr Pro  Arg Met Cys
    2870                 2875                 2880

Thr Arg  Glu Glu Phe Thr Arg  Lys Val Arg Ser Asn  Ala Ala Leu
    2885                 2890                 2895

Gly Ala  Ile Phe Thr Asp Glu  Asn Lys Trp Lys Ser  Ala Arg Glu
    2900                 2905                 2910

Ala Val  Glu Asp Ser Arg Phe  Trp Glu Leu Val Asp  Lys Glu Arg
    2915                 2920                 2925

Asn Leu  His Leu Glu Gly Lys  Cys Glu Thr Cys Val  Tyr Asn Met
    2930                 2935                 2940

Met Gly  Lys Arg Glu Lys Lys  Leu Gly Glu Phe Gly  Lys Ala Lys
    2945                 2950                 2955

Gly Ser  Arg Ala Ile Trp Tyr  Met Trp Leu Gly Ala  Arg Phe Leu
    2960                 2965                 2970

Glu Phe  Glu Ala Leu Gly Phe  Leu Asn Glu Asp His  Trp Phe Ser
    2975                 2980                 2985

Arg Glu  Asn Ser Leu Ser Gly  Val Glu Gly Glu Gly  Leu His Lys
    2990                 2995                 3000
```

-continued

```
Leu Gly  Tyr Ile Leu Arg Asp  Val Ser Lys Lys Glu  Gly Gly Ala
    3005                 3010                 3015

Met Tyr  Ala Asp Asp Thr Ala  Gly Trp Asp Thr Arg  Ile Thr Leu
    3020                 3025                 3030

Glu Asp  Leu Lys Asn Glu Glu  Met Val Thr Asn His  Met Glu Gly
    3035                 3040                 3045

Glu His  Lys Lys Leu Ala Glu  Ala Ile Phe Lys Leu  Thr Tyr Gln
    3050                 3055                 3060

Asn Lys  Val Val Arg Val Gln  Arg Pro Thr Pro Arg  Gly Thr Val
    3065                 3070                 3075

Met Asp  Ile Ile Ser Arg Arg  Asp Gln Arg Gly Ser  Gly Gln Val
    3080                 3085                 3090

Gly Thr  Tyr Gly Leu Asn Thr  Phe Thr Asn Met Glu  Ala Gln Leu
    3095                 3100                 3105

Ile Arg  Gln Met Glu Gly Glu  Gly Val Phe Lys Ser  Ile Gln His
    3110                 3115                 3120

Leu Thr  Ile Thr Glu Glu Ile  Ala Val Gln Asn Trp  Leu Ala Arg
    3125                 3130                 3135

Val Gly  Arg Glu Arg Leu Ser  Arg Met Ala Ile Ser  Gly Asp Asp
    3140                 3145                 3150

Cys Val  Val Lys Pro Leu Asp  Asp Arg Phe Ala Ser  Ala Leu Thr
    3155                 3160                 3165

Ala Leu  Asn Asp Met Gly Lys  Ile Arg Lys Asp Ile  Gln Gln Trp
    3170                 3175                 3180

Glu Pro  Ser Arg Gly Trp Asn  Asp Trp Thr Gln Val  Pro Phe Cys
    3185                 3190                 3195

Ser His  His Phe His Glu Leu  Ile Met Lys Asp Gly  Arg Val Leu
    3200                 3205                 3210

Val Val  Pro Cys Arg Asn Gln  Asp Glu Leu Ile Gly  Arg Ala Arg
    3215                 3220                 3225

Ile Ser  Gln Gly Ala Gly Trp  Ser Leu Arg Glu Thr  Ala Cys Leu
    3230                 3235                 3240

Gly Lys  Ser Tyr Ala Gln Met  Trp Ser Leu Met Tyr  Phe His Arg
    3245                 3250                 3255

Arg Asp  Leu Arg Leu Ala Ala  Asn Ala Ile Cys Ser  Ala Val Pro
    3260                 3265                 3270

Ser His  Trp Val Pro Thr Ser  Arg Thr Thr Trp Ser  Ile His Ala
    3275                 3280                 3285

Lys His  Glu Trp Met Thr Thr  Glu Asp Met Leu Thr  Val Trp Asn
    3290                 3295                 3300

Arg Val  Trp Ile Gln Glu Asn  Pro Trp Met Glu Asp  Lys Thr Pro
    3305                 3310                 3315

Val Glu  Ser Trp Glu Glu Ile  Pro Tyr Leu Gly Lys  Arg Glu Asp
    3320                 3325                 3330

Gln Trp  Cys Gly Ser Leu Ile  Gly Leu Thr Ser Arg  Ala Thr Trp
    3335                 3340                 3345

Ala Lys  Asn Ile Gln Ala Ala  Ile Asn Gln Val Arg  Ser Leu Ile
    3350                 3355                 3360

Gly Asn  Glu Glu Tyr Thr Asp  Tyr Met Pro Ser Met  Lys Arg Phe
    3365                 3370                 3375

Arg Arg  Glu Glu Glu Glu Ala  Gly Val Leu Trp
```

-continued

3380 3385

<210> SEQ ID NO 9
<211> LENGTH: 3389
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 3, MVS

<400> SEQUENCE: 9

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Arg Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Arg Met Ile Val
115 120 125

Gly Lys Asn Glu Arg Gly Lys Ser Leu Leu Phe Lys Thr Ala Ser Gly
130 135 140

Ile Asn Met Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Asp
145 150 155 160

Asp Thr Val Thr Tyr Lys Cys Pro His Ile Thr Glu Val Glu Pro Glu
165 170 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Thr Tyr Gly
180 185 190

Thr Cys Asn Gln Ala Gly Glu His Arg Arg Asp Lys Arg Ser Val Ala
195 200 205

Leu Ala Pro His Val Gly Met Gly Leu Asp Thr Arg Thr Gln Thr Trp
210 215 220

Met Ser Ala Glu Gly Ala Trp Arg Gln Val Glu Lys Val Glu Thr Trp
225 230 235 240

Ala Leu Arg His Pro Gly Phe Thr Ile Leu Ala Leu Phe Leu Ala His
245 250 255

Tyr Ile Gly Thr Ser Leu Thr Gln Lys Val Val Ile Phe Ile Leu Leu
260 265 270

Met Leu Val Thr Pro Ser Met Thr Met Arg Cys Val Gly Val Gly Asn
275 280 285

Arg Asp Phe Val Glu Gly Leu Ser Gly Ala Thr Trp Val Asp Val Val
290 295 300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Lys Asn Lys Pro Thr
305 310 315 320

Leu Asp Ile Glu Leu Gln Lys Thr Glu Ala Thr Gln Leu Ala Thr Leu
325 330 335

Arg Lys Leu Cys Ile Glu Gly Lys Ile Thr Asn Ile Thr Thr Asp Ser

-continued

```
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Ala Ile Leu Pro Glu Glu Gln Asp Gln
        355                 360                 365

Asn Tyr Val Cys Lys His Thr Tyr Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Ser Leu Val Thr Cys Ala Lys Phe Gln
385                 390                 395                 400

Cys Leu Glu Ser Ile Glu Gly Lys Val Val Gln His Glu Asn Leu Lys
                405                 410                 415

Tyr Thr Val Ile Ile Thr Val His Thr Gly Asp Gln His Gln Val Gly
            420                 425                 430

Asn Glu Thr Gln Gly Val Thr Ala Glu Ile Thr Pro Gln Ala Ser Thr
        435                 440                 445

Ala Glu Ala Ile Leu Pro Glu Tyr Gly Thr Leu Gly Leu Glu Cys Ser
    450                 455                 460

Pro Arg Thr Gly Leu Asp Phe Asn Glu Met Ile Ser Leu Thr Met Lys
465                 470                 475                 480

Asn Lys Ala Trp Met Val His Arg Gln Trp Phe Phe Asp Leu Pro Leu
                485                 490                 495

Pro Trp Thr Ser Gly Ala Ser Ala Glu Thr Pro Thr Trp Asn Arg Lys
            500                 505                 510

Glu Leu Leu Val Thr Phe Lys Asn Ala His Ala Lys Lys Gln Glu Val
        515                 520                 525

Val Val Leu Gly Ser Gln Glu Gly Ala Met His Thr Ala Leu Thr Gly
    530                 535                 540

Ala Thr Glu Ile Gln Thr Ser Gly Gly Thr Ser Ile Phe Ala Gly His
545                 550                 555                 560

Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Glu Leu Lys Gly Met Ser
                565                 570                 575

Tyr Ala Met Cys Leu Ser Ser Phe Val Leu Lys Lys Glu Val Ser Glu
            580                 585                 590

Thr Gln His Gly Thr Ile Leu Ile Lys Val Glu Tyr Lys Gly Glu Asp
        595                 600                 605

Ala Pro Cys Lys Ile Pro Phe Ser Thr Glu Asp Gly Gln Gly Lys Ala
    610                 615                 620

Leu Asn Gly Arg Leu Ile Thr Ala Asn Pro Val Val Thr Lys Lys Glu
625                 630                 635                 640

Glu Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser Asn Ile
                645                 650                 655

Val Ile Gly Ile Gly Asp Lys Ala Leu Lys Ile Asn Trp Tyr Lys Lys
            660                 665                 670

Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala Arg
        675                 680                 685

Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly
    690                 695                 700

Gly Val Leu Asn Ser Leu Gly Lys Met Val His Gln Ile Phe Gly Ser
705                 710                 715                 720

Ala Tyr Thr Ala Leu Phe Gly Gly Val Ser Trp Met Met Lys Ile Gly
                725                 730                 735

Ile Gly Val Leu Leu Thr Trp Ile Gly Leu Asn Ser Lys Asn Thr Ser
            740                 745                 750
```

-continued

```
Met Ser Phe Ser Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr Leu Gly
        755                 760                 765

Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys Asn Lys
    770                 775                 780

Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val His Thr
785                 790                 795                 800

Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys Leu Ala
                805                 810                 815

Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile Arg Ser
            820                 825                 830

Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro Glu Leu
        835                 840                 845

Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met Thr Gly
    850                 855                 860

Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg Pro Gln
865                 870                 875                 880

Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala Lys Met
                885                 890                 895

Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly Pro Glu
            900                 905                 910

Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu Glu Val
        915                 920                 925

Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu Lys Leu
    930                 935                 940

Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser Ala Ala
945                 950                 955                 960

Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp Ile Glu
                965                 970                 975

Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe Ile Glu
            980                 985                 990

Val Lys Asn Cys His Trp Pro Lys  Ser His Thr Leu Trp  Ser Asn Gly
        995                 1000                1005

Val Leu  Glu Ser Glu Met Ile  Ile Pro Lys Asn Leu  Ala Gly Pro
    1010                1015                1020

Val Ser  Gln His Asn Tyr Arg  Pro Gly Tyr His Thr  Gln Ile Thr
    1025                1030                1035

Gly Pro  Trp His Leu Gly Lys  Leu Glu Met Asp Phe  Asp Phe Cys
    1040                1045                1050

Asp Gly  Thr Thr Val Val Val  Thr Glu Asp Cys Gly  Asn Arg Gly
    1055                1060                1065

Pro Ser  Leu Arg Thr Thr Thr  Ala Ser Gly Lys Leu  Ile Thr Glu
    1070                1075                1080

Trp Cys  Cys Arg Ser Cys Thr  Leu Pro Pro Leu Arg  Tyr Arg Gly
    1085                1090                1095

Glu Asp  Gly Cys Trp Tyr Gly  Met Glu Ile Arg Pro  Leu Lys Glu
    1100                1105                1110

Lys Glu  Glu Asn Leu Val Asn  Ser Leu Val Thr Ala  Gly His Gly
    1115                1120                1125

Gln Val  Asp Asn Phe Ser Leu  Gly Val Leu Gly Met  Ala Leu Phe
    1130                1135                1140
```

-continued

```
Leu Glu  Glu Met Leu Arg Thr  Arg Val Gly Thr Lys  His Ala Ile
    1145                1150                1155

Leu Leu  Val Ala Val Ser Phe  Val Thr Leu Ile Thr  Gly Asn Met
    1160                1165                1170

Ser Phe  Arg Asp Leu Gly Arg  Val Met Val Met Val  Gly Ala Thr
    1175                1180                1185

Met Thr  Asp Asp Ile Gly Met  Gly Val Thr Tyr Leu  Ala Leu Leu
    1190                1195                1200

Ala Ala  Phe Lys Val Arg Pro  Thr Phe Ala Ala Gly  Leu Leu Leu
    1205                1210                1215

Arg Lys  Leu Thr Ser Lys Glu  Leu Met Met Thr Thr  Ile Gly Ile
    1220                1225                1230

Val Leu  Leu Ser Gln Ser Thr  Ile Pro Glu Thr Ile  Leu Glu Leu
    1235                1240                1245

Thr Asp  Ala Leu Ala Leu Gly  Met Met Val Leu Lys  Met Val Arg
    1250                1255                1260

Asn Met  Glu Lys Tyr Gln Leu  Ala Val Thr Ile Met  Ala Ile Leu
    1265                1270                1275

Cys Val  Pro Asn Ala Val Ile  Leu Gln Asn Ala Trp  Lys Val Ser
    1280                1285                1290

Cys Thr  Ile Leu Ala Val Val  Ser Val Ser Pro Leu  Phe Leu Thr
    1295                1300                1305

Ser Ser  Gln Gln Lys Thr Asp  Trp Ile Pro Leu Ala  Leu Thr Ile
    1310                1315                1320

Lys Gly  Leu Asn Pro Thr Ala  Ile Phe Leu Thr Thr  Leu Ser Arg
    1325                1330                1335

Thr Ser  Lys Lys Arg Ser Trp  Pro Leu Asn Glu Ala  Ile Met Ala
    1340                1345                1350

Val Gly  Met Val Ser Ile Leu  Ala Ser Ser Leu Leu  Lys Asn Asp
    1355                1360                1365

Ile Pro  Met Thr Gly Pro Leu  Val Ala Gly Gly Leu  Leu Thr Val
    1370                1375                1380

Cys Tyr  Val Leu Thr Gly Arg  Ser Ala Asp Leu Glu  Leu Glu Arg
    1385                1390                1395

Ala Ala  Asp Val Lys Trp Glu  Asp Gln Ala Glu Ile  Ser Gly Ser
    1400                1405                1410

Ser Pro  Ile Leu Ser Ile Thr  Ile Ser Glu Asp Gly  Ser Met Ser
    1415                1420                1425

Ile Lys  Asn Glu Glu Glu Glu  Gln Thr Leu Thr Ile  Leu Ile Arg
    1430                1435                1440

Thr Gly  Leu Leu Val Ile Ser  Gly Leu Phe Pro Val  Ser Ile Pro
    1445                1450                1455

Ile Thr  Ala Ala Ala Trp Tyr  Leu Trp Glu Val Lys  Lys Gln Arg
    1460                1465                1470

Ala Gly  Val Leu Trp Asp Val  Pro Ser Pro Pro Pro  Met Gly Lys
    1475                1480                1485

Ala Glu  Leu Glu Asp Gly Ala  Tyr Arg Ile Lys Gln  Lys Gly Ile
    1490                1495                1500

Leu Gly  Tyr Ser Gln Ile Gly  Ala Gly Val Tyr Lys  Glu Gly Thr
    1505                1510                1515

Phe His  Thr Met Trp His Val  Thr Arg Gly Ala Val  Leu Met His
```

-continued

```
    1520                1525                1530

Lys Gly  Lys Arg Ile Glu Pro  Ser Trp Ala Asp Val  Lys Lys Asp
    1535                1540                1545

Leu Ile  Ser Tyr Gly Gly Gly  Trp Lys Leu Glu Gly  Glu Trp Lys
    1550                1555                1560

Glu Gly  Glu Glu Val Gln Val  Leu Ala Leu Glu Pro  Gly Lys Asn
    1565                1570                1575

Pro Arg  Ala Val Gln Thr Lys  Pro Gly Leu Phe Lys  Thr Asn Ala
    1580                1585                1590

Gly Thr  Ile Gly Ala Val Ser  Leu Asp Phe Ser Pro  Gly Thr Ser
    1595                1600                1605

Gly Ser  Pro Ile Ile Asp Lys  Lys Gly Lys Val Val  Gly Leu Tyr
    1610                1615                1620

Gly Asn  Gly Val Val Thr Arg  Ser Gly Ala Tyr Val  Ser Ala Ile
    1625                1630                1635

Ala Gln  Thr Glu Lys Ser Ile  Glu Asp Asn Pro Glu  Ile Glu Asp
    1640                1645                1650

Asp Ile  Phe Arg Lys Arg Arg  Leu Thr Ile Met Asp  Leu His Pro
    1655                1660                1665

Gly Ala  Gly Lys Thr Lys Arg  Tyr Leu Pro Ala Ile  Val Arg Glu
    1670                1675                1680

Ala Ile  Lys Arg Gly Leu Arg  Thr Leu Ile Leu Ala  Pro Thr Arg
    1685                1690                1695

Val Val  Ala Ala Glu Met Glu  Glu Ala Leu Arg Gly  Leu Pro Ile
    1700                1705                1710

Arg Tyr  Gln Thr Pro Ala Ile  Arg Ala Val His Thr  Gly Arg Glu
    1715                1720                1725

Ile Val  Asp Leu Met Cys His  Ala Thr Phe Thr Met  Arg Leu Leu
    1730                1735                1740

Ser Pro  Val Arg Val Pro Asn  Tyr Asn Leu Ile Ile  Met Asp Glu
    1745                1750                1755

Ala His  Phe Thr Asp Pro Ala  Ser Ile Ala Ala Arg  Gly Tyr Ile
    1760                1765                1770

Ser Thr  Arg Val Glu Met Gly  Glu Ala Ala Gly Ile  Phe Met Thr
    1775                1780                1785

Ala Thr  Pro Pro Gly Ser Arg  Asp Pro Phe Pro Gln  Ser Asn Ala
    1790                1795                1800

Pro Ile  Ile Asp Glu Glu Arg  Glu Ile Pro Glu Arg  Ser Trp Asn
    1805                1810                1815

Ser Gly  His Glu Trp Val Thr  Asp Phe Lys Gly Lys  Thr Val Trp
    1820                1825                1830

Phe Val  Pro Ser Ile Lys Ala  Gly Asn Asp Ile Ala  Ala Cys Leu
    1835                1840                1845

Arg Lys  Asn Gly Lys Lys Val  Ile Gln Leu Ser Arg  Lys Thr Phe
    1850                1855                1860

Asp Ser  Glu Tyr Val Lys Thr  Arg Thr Asn Asp Trp  Asp Phe Val
    1865                1870                1875

Val Thr  Thr Asp Ile Ser Glu  Met Gly Ala Asn Phe  Lys Ala Glu
    1880                1885                1890

Arg Val  Ile Asp Pro Arg Arg  Cys Met Lys Pro Val  Ile Leu Thr
    1895                1900                1905
```

-continued

```
Asp Gly  Glu Glu Arg Val Ile  Leu Ala Gly Pro Met  Pro Val Thr
    1910                1915                1920

His Ser  Ser Ala Ala Gln Arg  Arg Gly Arg Ile Gly  Arg Asn Pro
    1925                1930                1935

Lys Asn  Glu Asn Asp Gln Tyr  Ile Tyr Met Gly Glu  Pro Leu Glu
    1940                1945                1950

Asn Asp  Glu Asp Cys Ala His  Trp Lys Glu Ala Lys  Met Leu Leu
    1955                1960                1965

Asp Asn  Ile Asn Thr Pro Glu  Gly Ile Ile Pro Ser  Met Phe Glu
    1970                1975                1980

Pro Glu  Arg Glu Lys Val Asp  Ala Ile Asp Gly Glu  Tyr Arg Leu
    1985                1990                1995

Arg Gly  Glu Ala Arg Lys Thr  Phe Val Asp Leu Met  Arg Arg Gly
    2000                2005                2010

Asp Leu  Pro Val Trp Leu Ala  Tyr Arg Val Ala Ala  Glu Gly Ile
    2015                2020                2025

Asn Tyr  Ala Asp Arg Arg Trp  Cys Phe Asp Gly Val  Lys Asn Asn
    2030                2035                2040

Gln Ile  Leu Glu Glu Asn Val  Glu Val Glu Ile Trp  Thr Lys Glu
    2045                2050                2055

Gly Glu  Arg Lys Lys Leu Lys  Pro Arg Trp Leu Asp  Ala Arg Ile
    2060                2065                2070

Tyr Ser  Asp Pro Leu Ala Leu  Lys Glu Phe Lys Glu  Phe Ala Ala
    2075                2080                2085

Gly Arg  Lys Ser Leu Thr Leu  Asn Leu Ile Thr Glu  Met Gly Arg
    2090                2095                2100

Leu Pro  Thr Phe Met Thr Gln  Lys Ala Arg Asp Ala  Leu Asp Asn
    2105                2110                2115

Leu Ala  Val Leu His Thr Ala  Glu Ala Gly Gly Arg  Ala Tyr Asn
    2120                2125                2130

His Ala  Leu Ser Glu Leu Pro  Glu Thr Leu Glu Thr  Leu Leu Leu
    2135                2140                2145

Leu Thr  Leu Leu Ala Thr Val  Thr Gly Gly Ile Phe  Leu Phe Leu
    2150                2155                2160

Met Ser  Ala Arg Gly Ile Gly  Lys Met Thr Leu Gly  Met Cys Cys
    2165                2170                2175

Ile Ile  Thr Ala Ser Ile Leu  Leu Trp Tyr Ala Gln  Ile Gln Pro
    2180                2185                2190

His Trp  Ile Ala Ala Ser Ile  Ile Leu Glu Phe Phe  Leu Ile Val
    2195                2200                2205

Leu Leu  Ile Pro Glu Pro Glu  Lys Gln Arg Thr Pro  Gln Asp Asn
    2210                2215                2220

Gln Leu  Thr Tyr Val Val Ile  Ala Ile Leu Thr Val  Val Ala Ala
    2225                2230                2235

Thr Met  Ala Asn Glu Met Gly  Phe Leu Glu Lys Thr  Lys Lys Asp
    2240                2245                2250

Leu Gly  Leu Gly Ser Ile Ala  Thr Gln Gln Pro Glu  Ser Asn Ile
    2255                2260                2265

Leu Asp  Ile Asp Leu Arg Pro  Ala Ser Ala Trp Thr  Leu Tyr Ala
    2270                2275                2280
```

-continued

```
Val Ala  Thr Thr Phe Val Thr  Pro Met Leu Arg His  Ser Ile Glu
    2285                 2290                 2295

Asn Ser  Ser Val Asn Val Ser  Leu Thr Ala Ile Ala  Asn Gln Ala
    2300                 2305                 2310

Thr Val  Leu Met Gly Leu Gly  Lys Gly Trp Pro Leu  Ser Lys Met
    2315                 2320                 2325

Asp Ile  Gly Val Pro Leu Leu  Ala Ile Gly Cys Tyr  Ser Gln Val
    2330                 2335                 2340

Asn Pro  Ile Thr Leu Thr Ala  Ala Leu Phe Leu Leu  Val Ala His
    2345                 2350                 2355

Tyr Ala  Ile Ile Gly Pro Gly  Leu Gln Ala Lys Ala  Thr Arg Glu
    2360                 2365                 2370

Ala Gln  Lys Arg Ala Ala Ala  Gly Ile Met Lys Asn  Pro Thr Val
    2375                 2380                 2385

Asp Gly  Ile Thr Val Ile Asp  Leu Asp Pro Ile Pro  Tyr Asp Pro
    2390                 2395                 2400

Lys Phe  Glu Lys Gln Leu Gly  Gln Val Met Leu Leu  Val Leu Cys
    2405                 2410                 2415

Val Thr  Gln Val Leu Met Met  Arg Thr Thr Trp Ala  Leu Cys Glu
    2420                 2425                 2430

Ala Leu  Thr Leu Ala Thr Gly  Pro Ile Ser Thr Leu  Trp Glu Gly
    2435                 2440                 2445

Asn Pro  Gly Arg Phe Trp Asn  Thr Thr Ile Ala Val  Ser Met Ala
    2450                 2455                 2460

Asn Ile  Phe Arg Gly Ser Tyr  Leu Ala Gly Ala Gly  Leu Leu Phe
    2465                 2470                 2475

Ser Ile  Met Lys Asn Thr Thr  Asn Thr Arg Arg Gly  Thr Gly Asn
    2480                 2485                 2490

Ile Gly  Glu Thr Leu Gly Glu  Lys Trp Lys Ser Arg  Leu Asn Ala
    2495                 2500                 2505

Leu Gly  Lys Ser Glu Phe Gln  Ile Tyr Lys Lys Ser  Gly Ile Gln
    2510                 2515                 2520

Glu Val  Asp Arg Thr Leu Ala  Lys Glu Gly Ile Lys  Arg Gly Glu
    2525                 2530                 2535

Thr Asp  His His Ala Val Ser  Arg Gly Ser Ala Lys  Leu Arg Trp
    2540                 2545                 2550

Phe Val  Glu Arg Asn Met Val  Thr Pro Glu Gly Lys  Val Val Asp
    2555                 2560                 2565

Leu Gly  Cys Gly Arg Gly Gly  Trp Ser Tyr Tyr Cys  Gly Gly Leu
    2570                 2575                 2580

Lys Asn  Val Arg Glu Val Lys  Gly Leu Thr Lys Gly  Gly Pro Gly
    2585                 2590                 2595

His Glu  Glu Pro Ile Pro Met  Ser Thr Tyr Gly Trp  Asn Leu Val
    2600                 2605                 2610

Arg Leu  Gln Ser Gly Val Asp  Val Phe Phe Ile Pro  Pro Glu Lys
    2615                 2620                 2625

Cys Asp  Thr Leu Leu Cys Asp  Ile Gly Glu Ser Ser  Pro Asn Pro
    2630                 2635                 2640

Thr Val  Glu Ala Gly Arg Thr  Leu Arg Val Leu Asn  Leu Val Glu
    2645                 2650                 2655

Asn Trp  Leu Asn Asn Asn Thr  Gln Phe Cys Ile Lys  Val Leu Asn
```

-continued

```
    2660                2665                2670

Pro Tyr  Met Pro Ser Val Ile  Glu Lys Met Glu Ala  Leu Gln Arg
    2675                2680                2685

Lys Tyr  Gly Gly Ala Leu Val  Arg Asn Pro Leu Ser  Arg Asn Ser
    2690                2695                2700

Thr His  Glu Met Tyr Trp Val  Ser Asn Ala Ser Gly  Asn Ile Val
    2705                2710                2715

Ser Ser  Val Asn Met Ile Ser  Arg Met Leu Ile Asn  Arg Phe Thr
    2720                2725                2730

Met Arg  Tyr Lys Lys Ala Thr  Tyr Glu Pro Asp Val  Asp Leu Gly
    2735                2740                2745

Ser Gly  Thr Arg Asn Ile Gly  Ile Glu Ser Glu Ile  Pro Asn Leu
    2750                2755                2760

Asp Ile  Ile Gly Lys Arg Ile  Glu Lys Ile Lys Gln  Glu His Glu
    2765                2770                2775

Thr Ser  Trp His Tyr Asp Gln  Asp His Pro Tyr Lys  Thr Trp Ala
    2780                2785                2790

Tyr His  Gly Ser Tyr Glu Thr  Lys Gln Thr Gly Ser  Ala Ser Ser
    2795                2800                2805

Met Val  Asn Gly Val Val Arg  Leu Leu Thr Lys Pro  Trp Asp Val
    2810                2815                2820

Val Pro  Met Val Thr Gln Met  Ala Met Thr Asp Thr  Thr Pro Phe
    2825                2830                2835

Gly Gln  Gln Arg Val Phe Lys  Glu Lys Val Asp Thr  Arg Thr Gln
    2840                2845                2850

Glu Pro  Lys Glu Gly Thr Lys  Lys Leu Met Lys Ile  Thr Ala Glu
    2855                2860                2865

Trp Leu  Trp Lys Glu Leu Gly  Lys Lys Lys Thr Pro  Arg Met Cys
    2870                2875                2880

Thr Arg  Glu Glu Phe Thr Arg  Lys Val Arg Ser Asn  Ala Ala Leu
    2885                2890                2895

Gly Ala  Ile Phe Thr Asp Glu  Asn Lys Trp Lys Ser  Ala Arg Glu
    2900                2905                2910

Ala Val  Glu Asp Ser Arg Phe  Trp Glu Leu Val Asp  Lys Glu Arg
    2915                2920                2925

Asn Leu  His Leu Glu Gly Lys  Cys Glu Thr Cys Val  Tyr Asn Met
    2930                2935                2940

Met Gly  Lys Arg Glu Lys Lys  Leu Gly Glu Phe Gly  Lys Ala Lys
    2945                2950                2955

Gly Ser  Arg Ala Ile Trp Tyr  Met Trp Leu Gly Ala  Arg Phe Leu
    2960                2965                2970

Glu Phe  Glu Ala Leu Gly Phe  Leu Asn Glu Asp His  Trp Phe Ser
    2975                2980                2985

Arg Glu  Asn Ser Leu Ser Gly  Val Glu Gly Glu Gly  Leu His Lys
    2990                2995                3000

Leu Gly  Tyr Ile Leu Arg Asp  Val Ser Lys Lys Glu  Gly Gly Ala
    3005                3010                3015

Met Tyr  Ala Asp Asp Thr Ala  Gly Trp Asp Thr Arg  Ile Thr Leu
    3020                3025                3030

Glu Asp  Leu Lys Asn Glu Glu  Met Val Thr Asn His  Met Glu Gly
    3035                3040                3045
```

-continued

```
Glu His  Lys Lys Leu Ala Glu  Ala Ile Phe Lys Leu  Thr Tyr Gln
    3050                3055                3060

Asn Lys  Val Val Arg Val Gln  Arg Pro Thr Pro Arg  Gly Thr Val
    3065                3070                3075

Met Asp  Ile Ile Ser Arg Arg  Asp Gln Arg Gly Ser  Gly Gln Val
    3080                3085                3090

Gly Thr  Tyr Gly Leu Asn Thr  Phe Thr Asn Met Glu  Ala Gln Leu
    3095                3100                3105

Ile Arg  Gln Met Glu Gly Glu  Gly Val Phe Lys Ser  Ile Gln His
    3110                3115                3120

Leu Thr  Ile Thr Glu Glu Ile  Ala Val Gln Asn Trp  Leu Ala Arg
    3125                3130                3135

Val Gly  Arg Glu Arg Leu Ser  Arg Met Ala Ile Ser  Gly Asp Asp
    3140                3145                3150

Cys Val  Val Lys Pro Leu Asp  Asp Arg Phe Ala Ser  Ala Leu Thr
    3155                3160                3165

Ala Leu  Asn Asp Met Gly Lys  Ile Arg Lys Asp Ile  Gln Gln Trp
    3170                3175                3180

Glu Pro  Ser Arg Gly Trp Asn  Asp Trp Thr Gln Val  Pro Phe Cys
    3185                3190                3195

Ser His  His Phe His Glu Leu  Ile Met Lys Asp Gly  Arg Val Leu
    3200                3205                3210

Val Val  Pro Cys Arg Asn Gln  Asp Glu Leu Ile Gly  Arg Ala Arg
    3215                3220                3225

Ile Ser  Gln Gly Ala Gly Trp  Ser Leu Arg Glu Thr  Ala Cys Leu
    3230                3235                3240

Gly Lys  Ser Tyr Ala Gln Met  Trp Ser Leu Met Tyr  Phe His Arg
    3245                3250                3255

Arg Asp  Leu Arg Leu Ala Ala  Asn Ala Ile Cys Ser  Ala Val Pro
    3260                3265                3270

Ser His  Trp Val Pro Thr Ser  Arg Thr Thr Trp Ser  Ile His Ala
    3275                3280                3285

Lys His  Glu Trp Met Thr Thr  Glu Asp Met Leu Thr  Val Trp Asn
    3290                3295                3300

Arg Val  Trp Ile Gln Glu Asn  Pro Trp Met Glu Asp  Lys Thr Pro
    3305                3310                3315

Val Glu  Ser Trp Glu Glu Ile  Pro Tyr Leu Gly Lys  Arg Glu Asp
    3320                3325                3330

Gln Trp  Cys Gly Ser Leu Ile  Gly Leu Thr Ser Arg  Ala Thr Trp
    3335                3340                3345

Ala Lys  Asn Ile Gln Ala Ala  Ile Asn Gln Val Arg  Ser Leu Ile
    3350                3355                3360

Gly Asn  Glu Glu Tyr Thr Asp  Tyr Met Pro Ser Met  Lys Arg Phe
    3365                3370                3375

Arg Arg  Glu Glu Glu Glu Ala  Gly Val Leu Trp
    3380                3385


<210> SEQ ID NO 10
<211> LENGTH: 10723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Dengue virus serotype 4, BVS
<220> FEATURE:
<221> NAME/KEY: n
<222> LOCATION: (3773)..(3773)
<223> OTHER INFORMATION: a or g
<220> FEATURE:
<221> NAME/KEY: n
<222> LOCATION: (7026)..(7026)
<223> OTHER INFORMATION: t or c
<220> FEATURE:
<221> NAME/KEY: n
<222> LOCATION: (7538)..(7538)
<223> OTHER INFORMATION: t or c

<400> SEQUENCE: 10

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gacctttaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagctctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgatg gcgaacccct catgatagtg 480

gcaaaacatg aaaggggggag acctctcttg tttaagacaa cagaggggat caacaaatgc 540

actctcattg ccatggactt gggtgaaatg tgtgaggaca ctgtcacgta taaatgcccc 600

ttactggtca ataccgaacc tgaagacatt gattgctggt gcaatctcac gtctacctgg 660

gtcatgtatg ggacatgcac ccagagcgga gaacggagac gagagaagcg ctcagtagct 720

ttaacaccac attcaggaat gggattggaa acaagagctg agacatggat gtcatcggaa 780

ggggcttgga agcatgctca gagagtagag agctggatac tcagaaaccc aggattcgcg 840

ctcttggcag gatttatggc ttatatgatt gggcaaacag gaatccagcg aactgtcttc 900

tttgtcctaa tgatgctggt cgccccatcc tacggaatgc gatgcgtagg agtaggaaac 960

agagactttg tggaaggagt ctcaggtgga gcatgggtcg atctggtgct agaacatgga 1020

ggatgcgtca caaccatggc ccagggaaaa ccaaccttgg attttgaact gactaagaca 1080

acagccaagg aagtggctct gttaagaacc tattgcattg aagcctcaat atcaaacata 1140

accacggcaa caagatgtcc aacgcaagga gagccttatc taaaagagga acaagaccaa 1200

cagtacattt gccggagaga tgtggtagac agagggtggg gcaatggctg tggcttgttt 1260

ggaaaaggag gagttgtgac atgtgcgaag ttttcatgtt cggggaagat aacaggcaat 1320

ttggtccaaa ttgagaacct tgaatacaca gtggttgtaa cagtccacaa tggagacacc 1380

catgcagtag gaaatgacac gtccaatcat ggagttacag ccacgataac tcccaggtca 1440

ccatcggtgg aagtcaaatt gccggactat ggagaactaa cactcgattg tgaacccagg 1500

tctggaattg actttaatga gatgattctg atgaaaatga aaaagaaaac atggcttgtg 1560

cataagcaat ggtttttgga tctacctcta ccatggacag caggagcaga cacatcagag 1620

gttcactgga attacaaaga gagaatggtg acatttaagg ttcctcatgc caagagacag 1680

gatgtgacag tgctgggatc tcaggaagga gccatgcatt ctgccctcgc tggagccaca 1740

gaagtggact ccggtgatgg aaatcacatg tttgcaggac atctcaagtg caaagtccgt 1800

-continued

atggagaaat tgagaatcaa gggaatgtca tacacgatgt gttcaggaaa gttctcaatt 1860

gacaaagaga tggcagaaac acagcatggg acaacagtgg tgaaagtcaa gtatgaaggt 1920

gctggagctc cgtgtaaagt ccccatagag ataagagatg tgaacaagga aaaagtggtt 1980

gggcgtatca tctcatccac ccctttggct gagaatacca acagtgtaac caacatagag 2040

ttagaacccc cctttgggga cagctacata gtgataggtg ttggaaacag tgcattaaca 2100

ctccattggt tcaggaaagg gagttccatt ggcaagatgt ttgagtccac atacagaggt 2160

gcaaaacgaa tggccattct aggtgaaaca gcttgggatt ttggttccgt tggtggactg 2220

ttcacatcat tgggaaaggc tgtgcaccag gtttttggaa gtgtgtatac aaccctgttt 2280

ggaggagtct catggatgat tagaatccta attgggttcc tagtgttgtg gattggcacg 2340

aactcaagga acacttcaat ggctatgacg tgcatagctg ccggcattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cgggtgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc canggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

-continued

```
aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca   4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa   4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg   4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac   4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc   4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg   4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg   4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg   4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat   4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca   4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag   4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa   4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct   4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga   4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt   4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa   5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc   5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa   5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa   5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg   5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt   5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga tccagcaagt   5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt   5460

atgacagcca ctccccggg aagcagagac ccatttcctc agagcaatgc accaatcata   5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat   5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct   5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag   5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg   5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata   5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt   5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata   5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg   6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt   6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt   6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa   6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta   6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc   6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt   6360
```

-continued

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggtaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagcnatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttnta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

-continued

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttccc tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> SEQ ID NO 11
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Dengue virus serotype 4, BVS
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (1226)..(1226)
<223> OTHER INFORMATION: Lys or Arg
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (2481)..(2481)
<223> OTHER INFORMATION: Ser or Phe

<400> SEQUENCE: 11

```
Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1               5                   10                  15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
            20                  25                  30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
        35                  40                  45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
    50                  55                  60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65                  70                  75                  80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
                85                  90                  95

Arg Arg Arg Ser Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
            100                 105                 110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Leu Met Ile Val
        115                 120                 125

Ala Lys His Glu Arg Gly Arg Pro Leu Leu Phe Lys Thr Thr Glu Gly
    130                 135                 140

Ile Asn Lys Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Glu
145                 150                 155                 160

Asp Thr Val Thr Tyr Lys Cys Pro Leu Leu Val Asn Thr Glu Pro Glu
                165                 170                 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Met Tyr Gly
            180                 185                 190

Thr Cys Thr Gln Ser Gly Glu Arg Arg Arg Glu Lys Arg Ser Val Ala
        195                 200                 205

Leu Thr Pro His Ser Gly Met Gly Leu Glu Thr Arg Ala Glu Thr Trp
    210                 215                 220

Met Ser Ser Glu Gly Ala Trp Lys His Ala Gln Arg Val Glu Ser Trp
225                 230                 235                 240

Ile Leu Arg Asn Pro Gly Phe Ala Leu Leu Ala Gly Phe Met Ala Tyr
                245                 250                 255

Met Ile Gly Gln Thr Gly Ile Gln Arg Thr Val Phe Phe Val Leu Met
            260                 265                 270

Met Leu Val Ala Pro Ser Tyr Gly Met Arg Cys Val Gly Val Gly Asn
        275                 280                 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ala Trp Val Asp Leu Val
    290                 295                 300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Gln Gly Lys Pro Thr
305                 310                 315                 320

Leu Asp Phe Glu Leu Thr Lys Thr Thr Ala Lys Glu Val Ala Leu Leu
                325                 330                 335

Arg Thr Tyr Cys Ile Glu Ala Ser Ile Ser Asn Ile Thr Thr Ala Thr
```

-continued

```
            340                 345                 350

Arg Cys Pro Thr Gln Gly Glu Pro Tyr Leu Lys Glu Glu Gln Asp Gln
        355                 360                 365

Gln Tyr Ile Cys Arg Arg Asp Val Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Gly Val Val Thr Cys Ala Lys Phe Ser
385                 390                 395                 400

Cys Ser Gly Lys Ile Thr Gly Asn Leu Val Gln Ile Glu Asn Leu Glu
                405                 410                 415

Tyr Thr Val Val Val Thr Val His Asn Gly Asp Thr His Ala Val Gly
            420                 425                 430

Asn Asp Thr Ser Asn His Gly Val Thr Ala Thr Ile Thr Pro Arg Ser
        435                 440                 445

Pro Ser Val Glu Val Lys Leu Pro Asp Tyr Gly Glu Leu Thr Leu Asp
    450                 455                 460

Cys Glu Pro Arg Ser Gly Ile Asp Phe Asn Glu Met Ile Leu Met Lys
465                 470                 475                 480

Met Lys Lys Lys Thr Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Thr Ala Gly Ala Asp Thr Ser Glu Val His Trp Asn
            500                 505                 510

Tyr Lys Glu Arg Met Val Thr Phe Lys Val Pro His Ala Lys Arg Gln
        515                 520                 525

Asp Val Thr Val Leu Gly Ser Gln Glu Gly Ala Met His Ser Ala Leu
    530                 535                 540

Ala Gly Ala Thr Glu Val Asp Ser Gly Asp Gly Asn His Met Phe Ala
545                 550                 555                 560

Gly His Leu Lys Cys Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly
                565                 570                 575

Met Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly
        595                 600                 605

Ala Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys
    610                 615                 620

Glu Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn
625                 630                 635                 640

Thr Asn Ser Val Thr Asn Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser
                645                 650                 655

Tyr Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe
            660                 665                 670

Arg Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly
        675                 680                 685

Ala Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Val Gly Gly Leu Phe Thr Ser Leu Gly Lys Ala Val His Gln Val Phe
705                 710                 715                 720

Gly Ser Val Tyr Thr Thr Leu Phe Gly Gly Val Ser Trp Met Ile Arg
                725                 730                 735

Ile Leu Ile Gly Phe Leu Val Leu Trp Ile Gly Thr Asn Ser Arg Asn
            740                 745                 750
```

-continued

```
Thr Ser Met Ala Met Thr Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
        755                 760                 765

Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140
```

-continued

```
Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Gly Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Xaa Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                1240                1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                1255                1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                1270                1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                1285                1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                1300                1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                1315                1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                1330                1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                1345                1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                1360                1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                1375                1380

Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                1390                1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                1405                1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                1420                1425

Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
    1430                1435                1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                1450                1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                1465                1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                1480                1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                1495                1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                1510                1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
```

-continued

```
    1520                1525                1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                1540                1545

Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                1555                1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                1570                1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                1585                1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                1615                1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                1690                1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                1720                1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905
```

-continued

```
Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920

Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Val Arg  Asp Ala Leu
    2105                2110                2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                2125                2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                2140                2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                2155                2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                2170                2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                2185                2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                2200                2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                2215                2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                2230                2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                2245                2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                2260                2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                2275                2280
```

-continued

```
Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                2290                2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
    2300                2305                2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                2320                2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                2335                2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Leu Phe  Leu Leu Val
    2345                2350                2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                2365                2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                2380                2385

Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                2395                2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                2410                2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                2425                2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                2440                2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                2455                2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                2470                2475

Leu Phe  Xaa Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                2485                2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                2500                2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                2515                2520

Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                2530                2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                2545                2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                2560                2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                2575                2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                2590                2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                2605                2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                2620                2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                2635                2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                2650                2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
```

-continued

```
    2660                2665                2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                2680                2685

Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                2695                2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                2710                2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                2725                2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                2740                2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                2755                2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                2770                2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                2785                2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                2800                2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                2815                2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                2830                2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                2845                2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                2860                2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                2875                2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                2890                2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                2905                2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                2920                2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                2935                2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                2950                2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                2965                2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                2980                2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                2995                3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                3010                3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                3025                3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                3040                3045
```

-continued

```
Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                3055                3060

Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                3070                3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                3085                3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                3100                3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                3115                3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                3130                3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                3145                3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                3160                3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                3175                3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                3190                3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                3205                3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                3220                3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                3235                3240

Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                3250                3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                3265                3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                3280                3285

His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                3295                3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                3310                3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                3325                3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                3340                3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                3355                3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                3370                3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                3385                3390
```

<210> SEQ ID NO 12
<211> LENGTH: 3391
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Dengue virus serotype 4, MVS
<220> FEATURE:
<221> NAME/KEY: Xaa
<222> LOCATION: (1226)..(1226)
<223> OTHER INFORMATION: Lys or Arg

<400> SEQUENCE: 12

Met Asn Asn Gln Arg Lys Lys Ala Lys Asn Thr Pro Phe Asn Met Leu
1 5 10 15

Lys Arg Glu Arg Asn Arg Val Ser Thr Val Gln Gln Leu Thr Lys Arg
20 25 30

Phe Ser Leu Gly Met Leu Gln Gly Arg Gly Pro Leu Lys Leu Phe Met
35 40 45

Ala Leu Val Ala Phe Leu Arg Phe Leu Thr Ile Pro Pro Thr Ala Gly
50 55 60

Ile Leu Lys Arg Trp Gly Thr Ile Lys Lys Ser Lys Ala Ile Asn Val
65 70 75 80

Leu Arg Gly Phe Arg Lys Glu Ile Gly Arg Met Leu Asn Ile Leu Asn
85 90 95

Arg Arg Arg Ser Ser Ala Gly Met Ile Ile Met Leu Ile Pro Thr Val
100 105 110

Met Ala Phe His Leu Thr Thr Arg Asp Gly Glu Pro Leu Met Ile Val
115 120 125

Ala Lys His Glu Arg Gly Arg Pro Leu Leu Phe Lys Thr Thr Glu Gly
130 135 140

Ile Asn Lys Cys Thr Leu Ile Ala Met Asp Leu Gly Glu Met Cys Glu
145 150 155 160

Asp Thr Val Thr Tyr Lys Cys Pro Leu Leu Val Asn Thr Glu Pro Glu
165 170 175

Asp Ile Asp Cys Trp Cys Asn Leu Thr Ser Thr Trp Val Met Tyr Gly
180 185 190

Thr Cys Thr Gln Ser Gly Glu Arg Arg Arg Glu Lys Arg Ser Val Ala
195 200 205

Leu Thr Pro His Ser Gly Met Gly Leu Glu Thr Arg Ala Glu Thr Trp
210 215 220

Met Ser Ser Glu Gly Ala Trp Lys His Ala Gln Arg Val Glu Ser Trp
225 230 235 240

Ile Leu Arg Asn Pro Gly Phe Ala Leu Leu Ala Gly Phe Met Ala Tyr
245 250 255

Met Ile Gly Gln Thr Gly Ile Gln Arg Thr Val Phe Phe Val Leu Met
260 265 270

Met Leu Val Ala Pro Ser Tyr Gly Met Arg Cys Val Gly Val Gly Asn
275 280 285

Arg Asp Phe Val Glu Gly Val Ser Gly Gly Ala Trp Val Asp Leu Val
290 295 300

Leu Glu His Gly Gly Cys Val Thr Thr Met Ala Gln Gly Lys Pro Thr
305 310 315 320

Leu Asp Phe Glu Leu Thr Lys Thr Thr Ala Lys Glu Val Ala Leu Leu
325 330 335

Arg Thr Tyr Cys Ile Glu Ala Ser Ile Ser Asn Ile Thr Thr Ala Thr
340 345 350

Arg Cys Pro Thr Gln Gly Glu Pro Tyr Leu Lys Glu Glu Gln Asp Gln
355 360 365

-continued

```
Gln Tyr Ile Cys Arg Arg Asp Val Val Asp Arg Gly Trp Gly Asn Gly
    370                 375                 380

Cys Gly Leu Phe Gly Lys Gly Gly Val Val Thr Cys Ala Lys Phe Ser
385                 390                 395                 400

Cys Ser Gly Lys Ile Thr Gly Asn Leu Val Gln Ile Glu Asn Leu Glu
                405                 410                 415

Tyr Thr Val Val Val Thr Val His Asn Gly Asp Thr His Ala Val Gly
            420                 425                 430

Asn Asp Thr Ser Asn His Gly Val Thr Ala Thr Ile Thr Pro Arg Ser
        435                 440                 445

Pro Ser Val Glu Val Lys Leu Pro Asp Tyr Gly Glu Leu Thr Leu Asp
    450                 455                 460

Cys Glu Pro Arg Ser Gly Ile Asp Phe Asn Glu Met Ile Leu Met Lys
465                 470                 475                 480

Met Lys Lys Lys Thr Trp Leu Val His Lys Gln Trp Phe Leu Asp Leu
                485                 490                 495

Pro Leu Pro Trp Thr Ala Gly Ala Asp Thr Ser Glu Val His Trp Asn
            500                 505                 510

Tyr Lys Glu Arg Met Val Thr Phe Lys Val Pro His Ala Lys Arg Gln
        515                 520                 525

Asp Val Thr Val Leu Gly Ser Gln Glu Gly Ala Met His Ser Ala Leu
    530                 535                 540

Ala Gly Ala Thr Glu Val Asp Ser Gly Asp Gly Asn His Met Phe Ala
545                 550                 555                 560

Gly His Leu Lys Cys Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly
                565                 570                 575

Met Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met
            580                 585                 590

Ala Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly
        595                 600                 605

Ala Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys
    610                 615                 620

Glu Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn
625                 630                 635                 640

Thr Asn Ser Val Thr Asn Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser
                645                 650                 655

Tyr Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe
            660                 665                 670

Arg Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly
        675                 680                 685

Ala Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser
    690                 695                 700

Val Gly Gly Leu Phe Thr Ser Leu Gly Lys Ala Val His Gln Val Phe
705                 710                 715                 720

Gly Ser Val Tyr Thr Thr Leu Phe Gly Gly Val Ser Trp Met Ile Arg
                725                 730                 735

Ile Leu Ile Gly Phe Leu Val Leu Trp Ile Gly Thr Asn Ser Arg Asn
            740                 745                 750

Thr Ser Met Ala Met Thr Cys Ile Ala Ala Gly Ile Val Thr Leu Tyr
        755                 760                 765
```

-continued

```
Leu Gly Val Met Val Gln Ala Asp Ser Gly Cys Val Val Ser Trp Lys
    770                 775                 780

Asn Lys Glu Leu Lys Cys Gly Ser Gly Ile Phe Ile Thr Asp Asn Val
785                 790                 795                 800

His Thr Trp Thr Glu Gln Tyr Lys Phe Gln Pro Glu Ser Pro Ser Lys
                805                 810                 815

Leu Ala Ser Ala Ile Gln Lys Ala His Glu Glu Asp Ile Cys Gly Ile
            820                 825                 830

Arg Ser Val Thr Arg Leu Glu Asn Leu Met Trp Lys Gln Ile Thr Pro
        835                 840                 845

Glu Leu Asn His Ile Leu Ser Glu Asn Glu Val Lys Leu Thr Ile Met
    850                 855                 860

Thr Gly Asp Ile Lys Gly Ile Met Gln Ala Gly Lys Arg Ser Leu Arg
865                 870                 875                 880

Pro Gln Pro Thr Glu Leu Lys Tyr Ser Trp Lys Thr Trp Gly Lys Ala
                885                 890                 895

Lys Met Leu Ser Thr Glu Ser His Asn Gln Thr Phe Leu Ile Asp Gly
            900                 905                 910

Pro Glu Thr Ala Glu Cys Pro Asn Thr Asn Arg Ala Trp Asn Ser Leu
        915                 920                 925

Glu Val Glu Asp Tyr Gly Phe Gly Val Phe Thr Thr Asn Ile Trp Leu
    930                 935                 940

Lys Leu Lys Glu Lys Gln Asp Val Phe Cys Asp Ser Lys Leu Met Ser
945                 950                 955                 960

Ala Ala Ile Lys Asp Asn Arg Ala Val His Ala Asp Met Gly Tyr Trp
                965                 970                 975

Ile Glu Ser Ala Leu Asn Asp Thr Trp Lys Ile Glu Lys Ala Ser Phe
            980                 985                 990

Ile Glu Val Lys Asn Cys His Trp  Pro Lys Ser His Thr  Leu Trp Ser
        995                 1000                1005

Asn Gly  Val Leu Glu Ser Glu  Met Ile Ile Pro Lys  Asn Leu Ala
    1010                1015                1020

Gly Pro  Val Ser Gln His Asn  Tyr Arg Pro Gly Tyr  His Thr Gln
    1025                1030                1035

Ile Thr  Gly Pro Trp His Leu  Gly Lys Leu Glu Met  Asp Phe Asp
    1040                1045                1050

Phe Cys  Asp Gly Thr Thr Val  Val Val Thr Glu Asp  Cys Gly Asn
    1055                1060                1065

Arg Gly  Pro Ser Leu Arg Thr  Thr Thr Ala Ser Gly  Lys Leu Ile
    1070                1075                1080

Thr Glu  Trp Cys Cys Arg Ser  Cys Thr Leu Pro Pro  Leu Arg Tyr
    1085                1090                1095

Arg Gly  Glu Asp Gly Cys Trp  Tyr Gly Met Glu Ile  Arg Pro Leu
    1100                1105                1110

Lys Glu  Lys Glu Glu Asn Leu  Val Asn Ser Leu Val  Thr Ala Gly
    1115                1120                1125

His Gly  Gln Val Asp Asn Phe  Ser Leu Gly Val Leu  Gly Met Ala
    1130                1135                1140

Leu Phe  Leu Glu Glu Met Leu  Arg Thr Arg Val Gly  Thr Lys His
    1145                1150                1155

Ala Ile  Leu Leu Val Ala Val  Ser Phe Val Thr Leu  Ile Thr Gly
```

-continued

```
    1160                1165                1170

Asn Met  Ser Phe Arg Asp Leu  Gly Arg Val Met Val  Met Val Gly
    1175                1180                1185

Ala Thr  Met Thr Gly Asp Ile  Gly Met Gly Val Thr  Tyr Leu Ala
    1190                1195                1200

Leu Leu  Ala Ala Phe Lys Val  Arg Pro Thr Phe Ala  Ala Gly Leu
    1205                1210                1215

Leu Leu  Arg Lys Leu Thr Ser  Xaa Glu Leu Met Met  Thr Thr Ile
    1220                1225                1230

Gly Ile  Val Leu Leu Ser Gln  Ser Thr Ile Pro Glu  Thr Ile Leu
    1235                1240                1245

Glu Leu  Thr Asp Ala Leu Ala  Leu Gly Met Met Val  Leu Lys Met
    1250                1255                1260

Val Arg  Asn Met Glu Lys Tyr  Gln Leu Ala Val Thr  Ile Met Ala
    1265                1270                1275

Ile Leu  Cys Val Pro Asn Ala  Val Ile Leu Gln Asn  Ala Trp Lys
    1280                1285                1290

Val Ser  Cys Thr Ile Leu Ala  Val Val Ser Val Ser  Pro Leu Phe
    1295                1300                1305

Leu Thr  Ser Ser Gln Gln Lys  Thr Asp Trp Ile Pro  Leu Ala Leu
    1310                1315                1320

Thr Ile  Lys Gly Leu Asn Pro  Thr Ala Ile Phe Leu  Thr Thr Leu
    1325                1330                1335

Ser Arg  Thr Ser Lys Lys Arg  Ser Trp Pro Leu Asn  Glu Ala Ile
    1340                1345                1350

Met Ala  Val Gly Met Val Ser  Ile Leu Ala Ser Ser  Leu Leu Lys
    1355                1360                1365

Asn Asp  Ile Pro Met Thr Gly  Pro Leu Val Ala Gly  Gly Leu Leu
    1370                1375                1380

Thr Val  Cys Tyr Val Leu Thr  Gly Arg Ser Ala Asp  Leu Glu Leu
    1385                1390                1395

Glu Arg  Ala Ala Asp Val Lys  Trp Glu Asp Gln Ala  Glu Ile Ser
    1400                1405                1410

Gly Ser  Ser Pro Ile Leu Ser  Ile Thr Ile Ser Glu  Asp Gly Ser
    1415                1420                1425

Met Ser  Ile Lys Asn Glu Glu  Glu Glu Gln Thr Leu  Thr Ile Leu
    1430                1435                1440

Ile Arg  Thr Gly Leu Leu Val  Ile Ser Gly Leu Phe  Pro Val Ser
    1445                1450                1455

Ile Pro  Ile Thr Ala Ala Ala  Trp Tyr Leu Trp Glu  Val Lys Lys
    1460                1465                1470

Gln Arg  Ala Gly Val Leu Trp  Asp Val Pro Ser Pro  Pro Pro Met
    1475                1480                1485

Gly Lys  Ala Glu Leu Glu Asp  Gly Ala Tyr Arg Ile  Lys Gln Lys
    1490                1495                1500

Gly Ile  Leu Gly Tyr Ser Gln  Ile Gly Ala Gly Val  Tyr Lys Glu
    1505                1510                1515

Gly Thr  Phe His Thr Met Trp  His Val Thr Arg Gly  Ala Val Leu
    1520                1525                1530

Met His  Lys Gly Lys Arg Ile  Glu Pro Ser Trp Ala  Asp Val Lys
    1535                1540                1545
```

-continued

```
Lys Asp  Leu Ile Ser Tyr Gly  Gly Gly Trp Lys Leu  Glu Gly Glu
    1550                1555                1560

Trp Lys  Glu Gly Glu Glu Val  Gln Val Leu Ala Leu  Glu Pro Gly
    1565                1570                1575

Lys Asn  Pro Arg Ala Val Gln  Thr Lys Pro Gly Leu  Phe Lys Thr
    1580                1585                1590

Asn Ala  Gly Thr Ile Gly Ala  Val Ser Leu Asp Phe  Ser Pro Gly
    1595                1600                1605

Thr Ser  Gly Ser Pro Ile Ile  Asp Lys Lys Gly Lys  Val Val Gly
    1610                1615                1620

Leu Tyr  Gly Asn Gly Val Val  Thr Arg Ser Gly Ala  Tyr Val Ser
    1625                1630                1635

Ala Ile  Ala Gln Thr Glu Lys  Ser Ile Glu Asp Asn  Pro Glu Ile
    1640                1645                1650

Glu Asp  Asp Ile Phe Arg Lys  Arg Arg Leu Thr Ile  Met Asp Leu
    1655                1660                1665

His Pro  Gly Ala Gly Lys Thr  Lys Arg Tyr Leu Pro  Ala Ile Val
    1670                1675                1680

Arg Glu  Ala Ile Lys Arg Gly  Leu Arg Thr Leu Ile  Leu Ala Pro
    1685                1690                1695

Thr Arg  Val Val Ala Ala Glu  Met Glu Glu Ala Leu  Arg Gly Leu
    1700                1705                1710

Pro Ile  Arg Tyr Gln Thr Pro  Ala Ile Arg Ala Val  His Thr Gly
    1715                1720                1725

Arg Glu  Ile Val Asp Leu Met  Cys His Ala Thr Phe  Thr Met Arg
    1730                1735                1740

Leu Leu  Ser Pro Val Arg Val  Pro Asn Tyr Asn Leu  Ile Ile Met
    1745                1750                1755

Asp Glu  Ala His Phe Thr Asp  Pro Ala Ser Ile Ala  Ala Arg Gly
    1760                1765                1770

Tyr Ile  Ser Thr Arg Val Glu  Met Gly Glu Ala Ala  Gly Ile Phe
    1775                1780                1785

Met Thr  Ala Thr Pro Pro Gly  Ser Arg Asp Pro Phe  Pro Gln Ser
    1790                1795                1800

Asn Ala  Pro Ile Ile Asp Glu  Glu Arg Glu Ile Pro  Glu Arg Ser
    1805                1810                1815

Trp Asn  Ser Gly His Glu Trp  Val Thr Asp Phe Lys  Gly Lys Thr
    1820                1825                1830

Val Trp  Phe Val Pro Ser Ile  Lys Ala Gly Asn Asp  Ile Ala Ala
    1835                1840                1845

Cys Leu  Arg Lys Asn Gly Lys  Lys Val Ile Gln Leu  Ser Arg Lys
    1850                1855                1860

Thr Phe  Asp Ser Glu Tyr Val  Lys Thr Arg Thr Asn  Asp Trp Asp
    1865                1870                1875

Phe Val  Val Thr Thr Asp Ile  Ser Glu Met Gly Ala  Asn Phe Lys
    1880                1885                1890

Ala Glu  Arg Val Ile Asp Pro  Arg Arg Cys Met Lys  Pro Val Ile
    1895                1900                1905

Leu Thr  Asp Gly Glu Glu Arg  Val Ile Leu Ala Gly  Pro Met Pro
    1910                1915                1920
```

-continued

```
Val Thr  His Ser Ser Ala Ala  Gln Arg Arg Gly Arg  Ile Gly Arg
    1925                1930                1935

Asn Pro  Lys Asn Glu Asn Asp  Gln Tyr Ile Tyr Met  Gly Glu Pro
    1940                1945                1950

Leu Glu  Asn Asp Glu Asp Cys  Ala His Trp Lys Glu  Ala Lys Met
    1955                1960                1965

Leu Leu  Asp Asn Ile Asn Thr  Pro Glu Gly Ile Ile  Pro Ser Met
    1970                1975                1980

Phe Glu  Pro Glu Arg Glu Lys  Val Asp Ala Ile Asp  Gly Glu Tyr
    1985                1990                1995

Arg Leu  Arg Gly Glu Ala Arg  Lys Thr Phe Val Asp  Leu Met Arg
    2000                2005                2010

Arg Gly  Asp Leu Pro Val Trp  Leu Ala Tyr Arg Val  Ala Ala Glu
    2015                2020                2025

Gly Ile  Asn Tyr Ala Asp Arg  Arg Trp Cys Phe Asp  Gly Val Lys
    2030                2035                2040

Asn Asn  Gln Ile Leu Glu Glu  Asn Val Glu Val Glu  Ile Trp Thr
    2045                2050                2055

Lys Glu  Gly Glu Arg Lys Lys  Leu Lys Pro Arg Trp  Leu Asp Ala
    2060                2065                2070

Arg Ile  Tyr Ser Asp Pro Leu  Ala Leu Lys Glu Phe  Lys Glu Phe
    2075                2080                2085

Ala Ala  Gly Arg Lys Ser Leu  Thr Leu Asn Leu Ile  Thr Glu Met
    2090                2095                2100

Gly Arg  Leu Pro Thr Phe Met  Thr Gln Lys Val Arg  Asp Ala Leu
    2105                2110                2115

Asp Asn  Leu Ala Val Leu His  Thr Ala Glu Ala Gly  Gly Arg Ala
    2120                2125                2130

Tyr Asn  His Ala Leu Ser Glu  Leu Pro Glu Thr Leu  Glu Thr Leu
    2135                2140                2145

Leu Leu  Leu Thr Leu Leu Ala  Thr Val Thr Gly Gly  Ile Phe Leu
    2150                2155                2160

Phe Leu  Met Ser Ala Arg Gly  Ile Gly Lys Met Thr  Leu Gly Met
    2165                2170                2175

Cys Cys  Ile Ile Thr Ala Ser  Ile Leu Leu Trp Tyr  Ala Gln Ile
    2180                2185                2190

Gln Pro  His Trp Ile Ala Ala  Ser Ile Ile Leu Glu  Phe Phe Leu
    2195                2200                2205

Ile Val  Leu Leu Ile Pro Glu  Pro Glu Lys Gln Arg  Thr Pro Gln
    2210                2215                2220

Asp Asn  Gln Leu Thr Tyr Val  Val Ile Ala Ile Leu  Thr Val Val
    2225                2230                2235

Ala Ala  Thr Met Ala Asn Glu  Met Gly Phe Leu Glu  Lys Thr Lys
    2240                2245                2250

Lys Asp  Leu Gly Leu Gly Ser  Ile Ala Thr Gln Gln  Pro Glu Ser
    2255                2260                2265

Asn Ile  Leu Asp Ile Asp Leu  Arg Pro Ala Ser Ala  Trp Thr Leu
    2270                2275                2280

Tyr Ala  Val Ala Thr Thr Phe  Val Thr Pro Met Leu  Arg His Ser
    2285                2290                2295

Ile Glu  Asn Ser Ser Val Asn  Val Ser Leu Thr Ala  Ile Ala Asn
```

-continued

```
    2300                2305                2310

Gln Ala  Thr Val Leu Met Gly  Leu Gly Lys Gly Trp  Pro Leu Ser
    2315                2320                2325

Lys Met  Asp Ile Gly Val Pro  Leu Leu Ala Ile Gly  Cys Tyr Ser
    2330                2335                2340

Gln Val  Asn Pro Ile Thr Leu  Thr Ala Ala Leu Phe  Leu Leu Val
    2345                2350                2355

Ala His  Tyr Ala Ile Ile Gly  Pro Gly Leu Gln Ala  Lys Ala Thr
    2360                2365                2370

Arg Glu  Ala Gln Lys Arg Ala  Ala Ala Gly Ile Met  Lys Asn Pro
    2375                2380                2385

Thr Val  Asp Gly Ile Thr Val  Ile Asp Leu Asp Pro  Ile Pro Tyr
    2390                2395                2400

Asp Pro  Lys Phe Glu Lys Gln  Leu Gly Gln Val Met  Leu Leu Val
    2405                2410                2415

Leu Cys  Val Thr Gln Val Leu  Met Met Arg Thr Thr  Trp Ala Leu
    2420                2425                2430

Cys Glu  Ala Leu Thr Leu Ala  Thr Gly Pro Ile Ser  Thr Leu Trp
    2435                2440                2445

Glu Gly  Asn Pro Gly Arg Phe  Trp Asn Thr Thr Ile  Ala Val Ser
    2450                2455                2460

Met Ala  Asn Ile Phe Arg Gly  Ser Tyr Leu Ala Gly  Ala Gly Leu
    2465                2470                2475

Leu Phe  Ser Ile Met Lys Asn  Thr Thr Asn Thr Arg  Arg Gly Thr
    2480                2485                2490

Gly Asn  Ile Gly Glu Thr Leu  Gly Glu Lys Trp Lys  Ser Arg Leu
    2495                2500                2505

Asn Ala  Leu Gly Lys Ser Glu  Phe Gln Ile Tyr Lys  Lys Ser Gly
    2510                2515                2520

Ile Gln  Glu Val Asp Arg Thr  Leu Ala Lys Glu Gly  Ile Lys Arg
    2525                2530                2535

Gly Glu  Thr Asp His His Ala  Val Ser Arg Gly Ser  Ala Lys Leu
    2540                2545                2550

Arg Trp  Phe Val Glu Arg Asn  Met Val Thr Pro Glu  Gly Lys Val
    2555                2560                2565

Val Asp  Leu Gly Cys Gly Arg  Gly Gly Trp Ser Tyr  Tyr Cys Gly
    2570                2575                2580

Gly Leu  Lys Asn Val Arg Glu  Val Lys Gly Leu Thr  Lys Gly Gly
    2585                2590                2595

Pro Gly  His Glu Glu Pro Ile  Pro Met Ser Thr Tyr  Gly Trp Asn
    2600                2605                2610

Leu Val  Arg Leu Gln Ser Gly  Val Asp Val Phe Phe  Ile Pro Pro
    2615                2620                2625

Glu Lys  Cys Asp Thr Leu Leu  Cys Asp Ile Gly Glu  Ser Ser Pro
    2630                2635                2640

Asn Pro  Thr Val Glu Ala Gly  Arg Thr Leu Arg Val  Leu Asn Leu
    2645                2650                2655

Val Glu  Asn Trp Leu Asn Asn  Asn Thr Gln Phe Cys  Ile Lys Val
    2660                2665                2670

Leu Asn  Pro Tyr Met Pro Ser  Val Ile Glu Lys Met  Glu Ala Leu
    2675                2680                2685
```

-continued

```
Gln Arg  Lys Tyr Gly Gly Ala  Leu Val Arg Asn Pro  Leu Ser Arg
    2690                 2695                 2700

Asn Ser  Thr His Glu Met Tyr  Trp Val Ser Asn Ala  Ser Gly Asn
    2705                 2710                 2715

Ile Val  Ser Ser Val Asn Met  Ile Ser Arg Met Leu  Ile Asn Arg
    2720                 2725                 2730

Phe Thr  Met Arg Tyr Lys Lys  Ala Thr Tyr Glu Pro  Asp Val Asp
    2735                 2740                 2745

Leu Gly  Ser Gly Thr Arg Asn  Ile Gly Ile Glu Ser  Glu Ile Pro
    2750                 2755                 2760

Asn Leu  Asp Ile Ile Gly Lys  Arg Ile Glu Lys Ile  Lys Gln Glu
    2765                 2770                 2775

His Glu  Thr Ser Trp His Tyr  Asp Gln Asp His Pro  Tyr Lys Thr
    2780                 2785                 2790

Trp Ala  Tyr His Gly Ser Tyr  Glu Thr Lys Gln Thr  Gly Ser Ala
    2795                 2800                 2805

Ser Ser  Met Val Asn Gly Val  Val Arg Leu Leu Thr  Lys Pro Trp
    2810                 2815                 2820

Asp Val  Val Pro Met Val Thr  Gln Met Ala Met Thr  Asp Thr Thr
    2825                 2830                 2835

Pro Phe  Gly Gln Gln Arg Val  Phe Lys Glu Lys Val  Asp Thr Arg
    2840                 2845                 2850

Thr Gln  Glu Pro Lys Glu Gly  Thr Lys Lys Leu Met  Lys Ile Thr
    2855                 2860                 2865

Ala Glu  Trp Leu Trp Lys Glu  Leu Gly Lys Lys Lys  Thr Pro Arg
    2870                 2875                 2880

Met Cys  Thr Arg Glu Glu Phe  Thr Arg Lys Val Arg  Ser Asn Ala
    2885                 2890                 2895

Ala Leu  Gly Ala Ile Phe Thr  Asp Glu Asn Lys Trp  Lys Ser Ala
    2900                 2905                 2910

Arg Glu  Ala Val Glu Asp Ser  Arg Phe Trp Glu Leu  Val Asp Lys
    2915                 2920                 2925

Glu Arg  Asn Leu His Leu Glu  Gly Lys Cys Glu Thr  Cys Val Tyr
    2930                 2935                 2940

Asn Met  Met Gly Lys Arg Glu  Lys Lys Leu Gly Glu  Phe Gly Lys
    2945                 2950                 2955

Ala Lys  Gly Ser Arg Ala Ile  Trp Tyr Met Trp Leu  Gly Ala Arg
    2960                 2965                 2970

Phe Leu  Glu Phe Glu Ala Leu  Gly Phe Leu Asn Glu  Asp His Trp
    2975                 2980                 2985

Phe Ser  Arg Glu Asn Ser Leu  Ser Gly Val Glu Gly  Glu Gly Leu
    2990                 2995                 3000

His Lys  Leu Gly Tyr Ile Leu  Arg Asp Val Ser Lys  Lys Glu Gly
    3005                 3010                 3015

Gly Ala  Met Tyr Ala Asp Asp  Thr Ala Gly Trp Asp  Thr Arg Ile
    3020                 3025                 3030

Thr Leu  Glu Asp Leu Lys Asn  Glu Glu Met Val Thr  Asn His Met
    3035                 3040                 3045

Glu Gly  Glu His Lys Lys Leu  Ala Glu Ala Ile Phe  Lys Leu Thr
    3050                 3055                 3060
```

-continued

```
Tyr Gln  Asn Lys Val Val Arg  Val Gln Arg Pro Thr  Pro Arg Gly
    3065                 3070                 3075

Thr Val  Met Asp Ile Ile Ser  Arg Arg Asp Gln Arg  Gly Ser Gly
    3080                 3085                 3090

Gln Val  Gly Thr Tyr Gly Leu  Asn Thr Phe Thr Asn  Met Glu Ala
    3095                 3100                 3105

Gln Leu  Ile Arg Gln Met Glu  Gly Glu Gly Val Phe  Lys Ser Ile
    3110                 3115                 3120

Gln His  Leu Thr Ile Thr Glu  Glu Ile Ala Val Gln  Asn Trp Leu
    3125                 3130                 3135

Ala Arg  Val Gly Arg Glu Arg  Leu Ser Arg Met Ala  Ile Ser Gly
    3140                 3145                 3150

Asp Asp  Cys Val Val Lys Pro  Leu Asp Asp Arg Phe  Ala Ser Ala
    3155                 3160                 3165

Leu Thr  Ala Leu Asn Asp Met  Gly Lys Ile Arg Lys  Asp Ile Gln
    3170                 3175                 3180

Gln Trp  Glu Pro Ser Arg Gly  Trp Asn Asp Trp Thr  Gln Val Pro
    3185                 3190                 3195

Phe Cys  Ser His His Phe His  Glu Leu Ile Met Lys  Asp Gly Arg
    3200                 3205                 3210

Val Leu  Val Val Pro Cys Arg  Asn Gln Asp Glu Leu  Ile Gly Arg
    3215                 3220                 3225

Ala Arg  Ile Ser Gln Gly Ala  Gly Trp Ser Leu Arg  Glu Thr Ala
    3230                 3235                 3240

Cys Leu  Gly Lys Ser Tyr Ala  Gln Met Trp Ser Leu  Met Tyr Phe
    3245                 3250                 3255

His Arg  Arg Asp Leu Arg Leu  Ala Ala Asn Ala Ile  Cys Ser Ala
    3260                 3265                 3270

Val Pro  Ser His Trp Val Pro  Thr Ser Arg Thr Thr  Trp Ser Ile
    3275                 3280                 3285

His Ala  Lys His Glu Trp Met  Thr Thr Glu Asp Met  Leu Thr Val
    3290                 3295                 3300

Trp Asn  Arg Val Trp Ile Gln  Glu Asn Pro Trp Met  Glu Asp Lys
    3305                 3310                 3315

Thr Pro  Val Glu Ser Trp Glu  Glu Ile Pro Tyr Leu  Gly Lys Arg
    3320                 3325                 3330

Glu Asp  Gln Trp Cys Gly Ser  Leu Ile Gly Leu Thr  Ser Arg Ala
    3335                 3340                 3345

Thr Trp  Ala Lys Asn Ile Gln  Ala Ala Ile Asn Gln  Val Arg Ser
    3350                 3355                 3360

Leu Ile  Gly Asn Glu Glu Tyr  Thr Asp Tyr Met Pro  Ser Met Lys
    3365                 3370                 3375

Arg Phe  Arg Arg Glu Glu Glu  Glu Ala Gly Val Leu  Trp
    3380                 3385                 3390


<210> SEQ ID NO 13
<211> LENGTH: 10723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus seroytpe 1, MVS

<400> SEQUENCE: 13
```

-continued

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgggg gagagccgca tatgatagtt 480

agcaagcagg aaagaggaaa gtcacttttg ttcaagacct ctgcaggtgt caacatgtgc 540

accctcattg cgatggattt gggagagttg tgtgaggaca cgatgaccta caaatgcccc 600

cggatcactg aggcggaacc agatgacgtt gactgttggt gcaatgccac ggacacatgg 660

gtgacctatg gaacgtgctc tcaaactggc gaacaccgac gagacaaacg ttccgtcgca 720

ttggccccac acgtggggct tggcctagaa acaagagccg aaacgtggat gtcctctgaa 780

ggtgcttgga aacagataca aaaagtagag acttgggctc tgagacatcc aggattcacg 840

gtgatagccc tttttctagc acatgccata ggaacatcca tcacccagaa agggatcatt 900

ttcattttgc tgatgctggt aacaccatct atggccatgc gatgcgtggg aataggcaac 960

agagacttcg tggaaggact gtcaggagca acatgggtgg atgtggtact ggagcatgga 1020

agttgcgtca ccaccatggc aaaaaacaaa ccaacactgg acattgaact cttgaagacg 1080

gaggtcacaa accctgcagt tctgcgtaaa ttgtgcattg aagctaaaat atcaaacacc 1140

accaccgatt cgagatgtcc aacacaagga gaagccacac tggtggaaga acaagacgcg 1200

aactttgtgt gccgacgaac gttcgtggac agaggctggg gcaatggctg tgggctattc 1260

ggaaaaggta gtctaataac gtgtgccaag tttaagtgtg tgacaaaact agaaggaaag 1320

atagttcaat atgaaaacct aaaatattca gtgatagtca ccgtccacac tggagatcag 1380

caccaggtgg gaaatgagac tacagaacat ggaacaactg caaccataac acctcaagct 1440

cctacgtcgg aaatacagct gaccgactac ggaaccctta cattagattg ttcacctagg 1500

acagggctag attttaacga gatggtgttg ctgacaatga aagaaagatc atggcttgtc 1560

cacaaacaat ggttcctaga cttaccactg ccttggacct ctggggcttc aacatcccaa 1620

gagacttgga acagacaaga tttactggtc acatttaaga cagctcatgc aaagaagcag 1680

gaagtagtcg tactaggatc acaagaagga gcaatgcaca ctgcgctgac tggagcgaca 1740

gaaatccaaa cgtcaggaac gacaacaatt ttcgcaggac acctaaaatg cagactaaaa 1800

atggacaaac taactttaaa agggatgtca tatgtgatgt gcacaggctc attcaagtta 1860

gagaaagaag tggctgagac ccagcatgga actgttctgg tgcaggttaa atatgaagga 1920

acagacgcac catgcaagat tccctttttcg acccaagatg agaaaggagc aacccagaat 1980

gggagattaa taacagccaa ccccatagtc actgacaaag aaaaaccagt caatattgag 2040

gcagaaccac cctttggtga gagctacatc gtggtaggag caggtgaaaa agctttgaaa 2100

ctaagctggt tcaagaaagg aagcagcata gggaaaatgt ttgaagcaac tgcccgagga 2160

gcacgaagga tggccattct gggagacacc gcatgggact tcggttctat aggaggagtg 2220

ttcacgtcta tgggaaaact ggtacaccag gtttttggaa ctgcatatgg agttttgttt 2280

-continued

agcggagttt cttggaccat gaaaatagga atagggattc tgctgacatg gctaggatta 2340

aattcaagga acacgtccct ttcgatgatg tgcatcgcag ccggcattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccctaccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagatcaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

-continued

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctcccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

-continued

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc gaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

-continued

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> SEQ ID NO 14
<211> LENGTH: 10723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 2, MVS

<400> SEQUENCE: 14

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

-continued

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acacgtaacg gagaaccaca catgatcgtc 480

agcagacaag agaaagggaa aagtcttctg tttaaaacag aggttggcgt gaacatgtgt 540

accctcatgg ccatggacct tggtgaattg tgtgaagaca caatcacgta cgagtgtccc 600

cttctcaggc agaatgagcc agaagacata gactgttggt gcaactctac gtccacgtgg 660

gtaacttatg ggacgtgtac caccatggga gaacatagaa gagaaaaaag atcagtggca 720

ctcgttccac atgtgggaat gggactggag acacgaactg aaacatggat gtcatcagaa 780

ggggcctgga aacatgtcca gagaattgaa acttggatct tgagacatcc aggcttcacc 840

atgatggcag caatcctggc atacaccata ggaacgacac atttccaaag agccctgatc 900

ttcatcttac tgacagctgt cactccttca atgacaatgc gttgcatagg aatgtcaaat 960

agagactttg tggaaggggt ttcaggagga agctgggttg acatagtctt agaacatgga 1020

agctgtgtga cgacgatggc aaaaaacaaa ccaacattgg attttgaact gataaaaaca 1080

gaagccaaac agcctgccac cctaaggaag tactgtatag aggcaaagct aaccaacaca 1140

acaacagaat ctcgctgccc aacacaaggg gaacccagcc taaatgaaga gcaggacaaa 1200

aggttcgtct gcaaacactc catggtagac agaggatggg gaaatggatg tggactattt 1260

ggaaagggag gcattgtgac ctgtgctatg ttcagatgca aaaagaacat ggaaggaaaa 1320

gttgtgcaac cagaaaactt ggaatacacc attgtgataa cacctcactc aggggaagag 1380

catgcagtcg gaaatgacac aggaaaacat ggcaaggaaa tcaaaataac accacagagt 1440

tccatcacag aagcagaatt gacaggttat ggcactgtca caatggagtg ctctccaaga 1500

acgggcctcg acttcaatga gatggtgttg ctgcagatgg aaaataaagc ttggctggtg 1560

cacaggcaat ggttcctaga cctgccgtta ccatggttgc ccggagcgga cacacaaggg 1620

tcaaattgga tacagaaaga gacattggtc actttcaaaa atccccatgc gaagaaacag 1680

gatgttgttg ttttaggatc ccaagaaggg gccatgcaca cagcacttac aggggccaca 1740

gaaatccaaa tgtcatcagg aaacttactc ttcacaggac atctcaagtg caggctgaga 1800

atggacaagc tacagctcaa aggaatgtca tactctatgt gcacaggaaa gtttaaagtt 1860

gtgaaggaaa tagcagaaac acaacatgga acaatagtta tcagagtgca atatgaaggg 1920

gacggctctc catgcaagat ccctttttgag ataatggatt tggaaaaaag acatgtctta 1980

ggtcgcctga ttacagtcaa cccaattgtg acagaaaaag atagcccagt caacatagaa 2040

gcagaacctc catttggaga cagctacatc atcataggag tagagccggg acaactgaag 2100

ctcaactggt ttaagaaagg aagttctatc ggccaaatgt ttgagacaac aatgaggggg 2160

gcgaagagaa tggccatttt aggtgacaca gcctgggatt ttggatcctt gggaggagtg 2220

tttacatcta taggaaaggc tctccaccaa gtctttggag caatctatgg agctgccttc 2280

agtggggttt catggactat gaaaatcctc ataggagtca ttatcacatg gataggaatg 2340

aattcacgca gcacctcact gtctgtgaca ctagtattgg tgggaattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

-continued

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cggatgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc caaggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

-continued

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga cccagcaagt 5400

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctccccggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagctatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

-continued

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccgtattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

-continued

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttcca tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg 10020

attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca 10080

tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc 10140

acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa 10200

gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga 10260

gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc 10320

catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca 10380

ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg 10440

tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc 10500

ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga 10560

agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag 10620

catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca 10680

gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct 10723

<210> SEQ ID NO 15
<211> LENGTH: 10717
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 3, MVS

<400> SEQUENCE: 15

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gaccattaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagatctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgatg gagagccgcg catgattgtg 480

gggaagaatg aaagaggaaa atccctactt ttcaagacag cctctggaat caacatgtgc 540

acactcatag ctatggatct gggagagatg tgtgatgaca cggtcactta caaatgcccc 600

cacattaccg aagtggagcc tgaagacatt gactgctggt gcaaccttac atcgacatgg 660

gtgacttatg gaacatgcaa tcaagctgga gagcatagac gcgataagag atcagtggcg 720

-continued

ttagctcccc atgttggcat gggactggac acacgcactc aaacctggat gtcggctgaa 780

ggagcttgga gacaagtcga gaaggtagag acatgggccc ttaggcaccc agggtttacc 840

atactagccc tatttcttgc ccattacata ggcacttcct tgacccagaa agtggttatt 900

tttatactat taatgctggt taccccatcc atgacaatga gatgtgtagg agtaggaaac 960

agagattttg tggaaggcct atcgggagct acgtgggttg acgtggtgct cgagcacggt 1020

gggtgtgtga ctaccatggc taagaacaag cccacgctgg acatagagct tcagaagacc 1080

gaggccaccc aactggcgac cctaaggaag ctatgcattg agggaaaaat taccaacata 1140

acaaccgact caagatgtcc cacccaaggg gaagcgattt tacctgagga gcaggaccag 1200

aactacgtgt gtaagcatac atacgtggac agaggctggg gaaacggttg tggtttgttt 1260

ggcaagggaa gcttggtgac atgcgcgaaa tttcaatgtt tagaatcaat agagggaaaa 1320

gtggtgcaac atgagaacct caaatacacc gtcatcatca cagtgcacac aggagaccaa 1380

caccaggtgg gaaatgaaac gcagggagtc acggctgaga taacacccca ggcatcaacc 1440

gctgaagcca ttttacctga atatggaacc ctcgggctag aatgctcacc acggacaggt 1500

ttggatttca atgaaatgat ctcattgaca atgaagaaca aagcatggat ggtacataga 1560

caatggttct ttgacttacc cctaccatgg acatcaggag cttcagcaga aacaccaact 1620

tggaacagga aagagcttct tgtgacattt aaaaatgcac atgcaaaaaa gcaagaagta 1680

gttgttcttg gatcacaaga gggagcaatg catacagcac tgacaggagc tacagagatc 1740

caaacctcag gaggcacaag tatctttgcg gggcacttaa aatgtagact caagatggac 1800

aaattggaac tcaaggggat gagctatgca atgtgcttga gtagctttgt gttgaagaaa 1860

gaagtctccg aaacgcagca tgggacaata ctcattaagg ttgagtacaa aggggaagat 1920

gcaccctgca agattccttt ctccacggag gatggacaag gaaaagctct caatggcaga 1980

ctgatcacag ccaatccagt ggtgaccaag aaggaggagc ctgtcaacat tgaggctgaa 2040

cctccttttg gagaaagtaa catagtaatt ggaattggag acaaagccct gaaaatcaac 2100

tggtacaaga agggaagctc gattgggaag atgttcgagg ccactgccag aggtgcaagg 2160

cgcatggcca tcttgggaga cacagcctgg gactttggat cagtgggtgg tgttttgaat 2220

tcattaggga aaatggtcca ccaaatattt gggagtgctt acacagccct atttggtgga 2280

gtctcctgga tgatgaaaat tggaataggt gtcctcttaa cctggatagg gttgaactca 2340

aaaaatactt ctatgtcatt ttcatgcatc gcggccggca ttgtgacact gtatttggga 2400

gtcatggtgc aggccgatag tggttgcgtt gtgagctgga aaaacaaaga actgaaatgt 2460

ggcagtggga ttttcatcac agacaacgtg cacacatgga cagaacaata caagttccaa 2520

ccagaatccc cttcaaaact agcttcagct atccagaaag cccatgaaga ggacatttgt 2580

ggaatccgct cagtaacaag actggagaat ctgatgtgga aacaaataac accagaattg 2640

aatcacattc tatcagaaaa tgaggtgaag ttaactatta tgacaggaga catcaaagga 2700

atcatgcagg caggaaaacg atctctgcgg cctcagccca ctgagctgaa gtattcatgg 2760

aaaacatggg gcaaagcaaa aatgctctct acagagtctc ataaccagac ctttctcatt 2820

gatggccccg aaacagcaga atgccccaac acaaatagag cttggaattc gttggaagtt 2880

gaagactatg gctttggagt attcaccacc aatatatggc taaaattgaa agaaaaacag 2940

gatgtattct gcgactcaaa actcatgtca gcggccataa aagacaacag agccgtccat 3000

-continued

gccgatatgg gttattggat agaaagtgca ctcaatgaca catggaagat agagaaagcc 3060

tctttcattg aagttaaaaa ctgccactgg ccaaaatcac acaccctctg gagcaatgga 3120

gtgctagaaa gtgagatgat aattccaaag aatctcgctg gaccagtgtc tcaacacaac 3180

tatagaccag gctaccatac acaaataaca ggaccatggc atctaggtaa gcttgagatg 3240

gactttgatt tctgtgatgg aacaacagtg gtagtgactg aggactgcgg aaatagagga 3300

ccctctttga gaacaaccac tgcctctgga aaactcataa cagaatggtg ctgccgatct 3360

tgcacattac caccgctaag atacagaggt gaggatgggt gctggtacgg gatggaaatc 3420

agaccattga aggagaaaga agagaatttg gtcaactcct tggtcacagc tggacatggg 3480

caggtcgaca acttttcact aggagtcttg ggaatggcat tgttcctgga ggaaatgctt 3540

aggacccgag taggaacgaa acatgcaata ctactagttg cagtttcttt tgtgacattg 3600

atcacaggga acatgtcctt tagagacctg ggaagagtga tggttatggt aggcgccact 3660

atgacggatg acataggtat gggcgtgact tatcttgccc tactagcagc cttcaaagtc 3720

agaccaactt ttgcagctgg actactcttg agaaagctga cctccaagga attgatgatg 3780

actactatag gaattgtact cctctcccag agcaccatac cagagaccat tcttgagttg 3840

actgatgcgt tagccttagg catgatggtc ctcaaaatgg tgagaaatat ggaaaagtat 3900

caattggcag tgactatcat ggctatcttg tgcgtcccaa acgcagtgat attacaaaac 3960

gcatggaaag tgagttgcac aatattggca gtggtgtccg tttccccact gttcttaaca 4020

tcctcacagc aaaaaacaga ttggatacca ttagcattga cgatcaaagg tctcaatcca 4080

acagctattt ttctaacaac cctctcaaga accagcaaga aaaggagctg gccattaaat 4140

gaggctatca tggcagtcgg gatggtgagc attttagcca gttctctcct aaaaaatgat 4200

attcccatga caggaccatt agtggctgga gggctcctca ctgtgtgcta cgtgctcact 4260

ggacgatcgg ccgatttgga actggagaga gcagccgatg tcaaatggga agaccaggca 4320

gagatatcag gaagcagtcc aatcctgtca ataacaatat cagaagatgg tagcatgtcg 4380

ataaaaaatg aagaggaaga acaaacactg accatactca ttagaacagg attgctggtg 4440

atctcaggac tttttcctgt atcaatacca atcacggcag cagcatggta cctgtgggaa 4500

gtgaagaaac aacgggccgg agtattgtgg gatgttcctt caccccacc catgggaaag 4560

gctgaactgg aagatggagc ctatagaatt aagcaaaaag ggattcttgg atattcccag 4620

atcggagccg gagtttacaa agaaggaaca ttccatacaa tgtggcatgt cacacgtggc 4680

gctgttctaa tgcataaagg aaagaggatt gaaccatcat gggcggacgt caagaaagac 4740

ctaatatcat atggaggagg ctggaagtta gaaggagaat ggaaggaagg agaagaagtc 4800

caggtattgg cactggagcc tggaaaaaat ccaagagccg tccaaacgaa acctggtctt 4860

ttcaaaacca acgccggaac aataggtgct gtatctctgg acttttctcc tggaacgtca 4920

ggatctccaa ttatcgacaa aaaaggaaaa gttgtgggtc tttatggtaa tggtgttgtt 4980

acaaggagtg gagcatatgt gagtgctata gcccagactg aaaaaagcat tgaagacaac 5040

ccagagatcg aagatgacat tttccgaaag agaagactga ccatcatgga cctccaccca 5100

ggagcgggaa agacgaagag ataccttccg gccatagtca gagaagctat aaaacggggt 5160

ttgagaacat taatcttggc ccccactaga gttgtggcag ctgaaatgga ggaagccctt 5220

agaggacttc caataagata ccagacccca gccatcagag ctgtgcacac cgggcgggag 5280

-continued

attgtggacc taatgtgtca tgccacattt accatgaggc tgctatcacc agttagagtg 5340

ccaaactaca acctgattat catggacgaa gcccatttca cagacccagc aagtatagca 5400

gctagaggat acatctcaac tcgagtggag atgggtgagg cagctgggat ttttatgaca 5460

gccactcccc cgggaagcag agacccattt cctcagagca atgcaccaat catagatgaa 5520

gaaagagaaa tccctgaacg ctcgtggaat tccggacatg aatgggtcac ggattttaaa 5580

gggaagactg tttggttcgt tccaagtata aaagcaggaa atgatatagc agcttgcctg 5640

aggaaaaatg gaaagaaagt gatacaactc agtaggaaga cctttgattc tgagtatgtc 5700

aagactagaa ccaatgattg ggacttcgtg gttacaactg acatttcaga aatgggtgcc 5760

aatttcaagg ctgagagggt tatagacccc agacgctgca tgaaaccagt catactaaca 5820

gatggtgaag agcgggtgat tctggcagga cctatgccag tgacccactc tagtgcagca 5880

caaagaagag ggagaatagg aagaaatcca aaaaatgaga atgaccagta catatacatg 5940

ggggaacctc tggaaaatga tgaagactgt gcacactgga aagaagctaa aatgctccta 6000

gataacatca acacgccaga aggaatcatt cctagcatgt tcgaaccaga gcgtgaaaag 6060

gtggatgcca ttgatggcga ataccgcttg agaggagaag caaggaaaac ctttgtagac 6120

ttaatgagaa gaggagacct accagtctgg ttggcctaca gagtggcagc tgaaggcatc 6180

aactacgcag acagaaggtg gtgttttgat ggagtcaaga acaaccaaat cctagaagaa 6240

aacgtggaag ttgaaatctg gacaaaagaa ggggaaagga agaaattgaa acccagatgg 6300

ttggatgcta ggatctattc tgacccactg gcgctaaaag aatttaagga atttgcagcc 6360

ggaagaaagt ctctgaccct gaacctaatc acagaaatgg gtaggctccc aaccttcatg 6420

actcagaagg caagagacgc actggacaac ttagcagtgc tgcacacggc tgaggcaggt 6480

ggaagggcgt acaaccatgc tctcagtgaa ctgccggaga ccctggagac attgctttta 6540

ctgacacttc tggctacagt cacgggaggg atctttttat tcttgatgag cgcaaggggc 6600

atagggaaga tgaccctggg aatgtgctgc ataatcacgg ctagcatcct cctatggtac 6660

gcacaaatac agccacactg gatagcagct tcaataatac tggagttttt tctcatagtt 6720

ttgcttattc cagaacctga aaaacagaga acaccccaag acaaccaact gacctacgtt 6780

gtcatagcca tcctcacagt ggtggccgca accatggcaa acgagatggg tttcctagaa 6840

aaaacgaaga aagatctcgg attgggaagc attgcaaccc agcaacccga gagcaacatc 6900

ctggacatag atctacgtcc tgcatcagca tggacgctgt atgccgtggc cacaacattt 6960

gttacaccaa tgttgagaca tagcattgaa aattcctcag tgaatgtgtc cctaacagct 7020

atagccaacc aagccacagt gttaatgggt ctcgggaaag gatggccatt gtcaaagatg 7080

gacatcggag ttccccttct cgccattgga tgctactcac aagtcaaccc cataactctc 7140

acagcagctc ttttcttatt ggtagcacat tatgccatca tagggccagg actccaagca 7200

aaagcaacca gagaagctca gaaaagagca gcggcgggca tcatgaaaaa cccaactgtc 7260

gatggaataa cagtgattga cctagatcca ataccttatg atccaaagtt tgaaaagcag 7320

ttgggacaag taatgctcct agtcctctgc gtgactcaag tattgatgat gaggactaca 7380

tgggctctgt gtgaggcttt aaccttagct accgggccca tctccacatt gtgggaagga 7440

aatccaggga ggttttggaa cactaccatt gcggtgtcaa tggctaacat ttttagaggg 7500

agttacttgg ccggagctgg acttctcttt tctattatga agaacacaac caacacaaga 7560

-continued

aggggaactg gcaacatagg agagacgctt ggagagaaat ggaaaagccg attgaacgcg 7620

ttgggaaaaa gtgaattcca gatctacaag aaaagtggaa tccaggaagt ggatagaacc 7680

ttagcaaaag aaggcattaa aagaggagaa acggaccatc acgctgtgtc gcgaggctca 7740

gcaaaactga gatggttcgt tgagagaaac atggtcacac cagaagggaa agtagtggac 7800

ctcggttgtg gcagaggagg ctggtcatac tattgtggag gactaaagaa tgtaagagaa 7860

gtcaaaggcc taacaaaagg aggaccagga cacgaagaac ccatccccat gtcaacatat 7920

gggtggaatc tagtgcgtct tcaaagtgga gttgacgttt tcttcatccc gccagaaaag 7980

tgtgacacat tattgtgtga cataggggag tcatcaccaa atcccacagt ggaagcagga 8040

cgaacactca gagtccttaa cttagtagaa aattggttga acaacaacac tcaattttgc 8100

ataaaggttc tcaacccata tatgccctca gtcatagaaa aaatggaagc actacaaagg 8160

aaatatggag gagccttagt gaggaatcca ctctcacgaa actccacaca tgagatgtac 8220

tgggtatcca atgcttccgg gaacatagtg tcatcagtga acatgatttc aaggatgttg 8280

atcaacagat ttacaatgag atacaagaaa gccacttacg agccggatgt tgacctcgga 8340

agcggaaccc gtaacatcgg gattgaaagt gagataccaa acctagatat aattgggaaa 8400

agaatagaaa aaataaagca agagcatgaa acatcatggc actatgacca agaccaccca 8460

tacaaaacgt gggcatacca tggtagctat gaaacaaaac agactggatc agcatcatcc 8520

atggtcaacg gagtggtcag gctgctgaca aaaccttggg acgtcgtccc catggtgaca 8580

cagatggcaa tgacagacac gactccattt ggacaacagc gcgtttttaa agagaaagtg 8640

gacacgagaa cccaagaacc gaaagaaggc acgaagaaac taatgaaaat aacagcagag 8700

tggctttgga aagaattagg gaagaaaaag acacccagga tgtgcaccag agaagaattc 8760

acaagaaagg tgagaagcaa tgcagccttg ggggccatat tcactgatga gaacaagtgg 8820

aagtcggcac gtgaggctgt tgaagatagt aggttttggg agctggttga caaggaaagg 8880

aatctccatc ttgaaggaaa gtgtgaaaca tgtgtgtaca acatgatggg aaaaagagag 8940

aagaagctag gggaattcgg caaggcaaaa ggcagcagag ccatatggta catgtggctt 9000

ggagcacgct tcttagagtt tgaagcccta ggattcttaa atgaagatca ctggttctcc 9060

agagagaact ccctgagtgg agtggaagga gaagggctgc acaagctagg ttacattcta 9120

agagacgtga gcaagaaaga gggaggagca atgtatgccg atgacaccgc aggatgggat 9180

acaagaatca cactagaaga cctaaaaaat gaagaaatgg taacaaacca catggaagga 9240

gaacacaaga aactagccga ggccattttc aaactaacgt accaaaacaa ggtggtgcgt 9300

gtgcaaagac caacaccaag aggcacagta atggacatca tatcgagaag agaccaaaga 9360

ggtagtggac aagttggcac ctatggactc aatactttca ccaatatgga agcccaacta 9420

atcagacaga tggagggaga aggagtcttt aaaagcattc agcacctaac aatcacagaa 9480

gaaatcgctg tgcaaaactg gttagcaaga gtggggcgcg aaaggttatc aagaatggcc 9540

atcagtggag atgattgtgt tgtgaaacct ttagatgaca ggttcgcaag cgctttaaca 9600

gctctaaatg acatgggaaa gattaggaaa gacatacaac aatgggaacc ttcaagagga 9660

tggaatgatt ggacacaagt gcccttctgt tcacaccatt tccatgagtt aatcatgaaa 9720

gacggtcgcg tactcgttgt tccatgtaga aaccaagatg aactgattgg cagagcccga 9780

atctcccaag gagcagggtg gtctttgcgg gagacggcct gtttggggaa gtcttacgcc 9840

-continued

caaatgtgga gcttgatgta cttccacaga cgcgacctca ggctggcggc aaatgctatt 9900

tgctcggcag taccatcaca ttgggttcca acaagtcgaa caacctggtc catacatgct 9960

aaacatgaat ggatgacaac ggaagacatg ctgacagtct ggaacagggt gtggattcaa 10020

gaaaacccat ggatggaaga caaaactcca gtggaatcat gggaggaaat cccatacttg 10080

gggaaaagag aagaccaatg gtgcggctca ttgattgggt taacaagcag ggccacctgg 10140

gcaaagaaca tccaagcagc aataaatcaa gttagatccc ttataggcaa tgaagaatac 10200

acagattaca tgccatccat gaaaagattc agaagagaag aggaagaagc aggagttctg 10260

tggtagaaag caaaactaac atgaaacaag gctagaagtc aggtcggatt aagccatagt 10320

acggaaaaaa ctatgctacc tgtgagcccc gtccaaggac gttaaaagaa gtcaggccat 10380

cataaatgcc atagcttgag taaactatgc agcctgtagc tccacctgag aaggtgtaaa 10440

aaatccggga ggccacaaac catggaagct gtacgcatgg cgtagtggac tagcggttag 10500

aggagacccc tcccttacaa atcgcagcaa caatgggggc ccaaggcgag atgaagctgt 10560

agtctcgctg gaaggactag aggttagagg agacccccccc gaaacaaaaa acagcatatt 10620

gacgctggga aagaccagag atcctgctgt ctcctcagca tcattccagg cacagaacgc 10680

cagaaaatgg aatggtgctg ttgaatcaac aggttct 10717

<210> SEQ ID NO 16
<211> LENGTH: 10723
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Dengue virus serotype 4, MVS
<220> FEATURE:
<221> NAME/KEY: n
<222> LOCATION: (3773)..(3773)
<223> OTHER INFORMATION: a or g
<220> FEATURE:
<221> NAME/KEY: n
<222> LOCATION: (7026)..(7026)
<223> OTHER INFORMATION: t or c

<400> SEQUENCE: 16

agttgttagt ctacgtggac cgacaaagac agattctttg agggagctaa gctcaatgta 60

gttctaacag ttttttaatt agagagcaga tctctgatga ataaccaacg gaaaaaggcg 120

aaaaacacgc ctttcaatat gctgaaacgc gagagaaacc gcgtgtcgac tgtgcaacag 180

ctgacaaaga gattctcact tggaatgctg cagggacgag gacctttaaa actgttcatg 240

gccctggtgg cgttccttcg tttcctaaca atcccaccaa cagcagggat attgaagaga 300

tggggaacaa ttaaaaaatc aaaagctatt aatgttttga gagggttcag gaaagagatt 360

ggaaggatgc tgaacatctt gaataggaga cgcagctctg caggcatgat cattatgctg 420

attccaacag tgatggcgtt ccatttaacc acgcgtgatg gcgaacccct catgatagtg 480

gcaaaacatg aaaggggggag acctctcttg tttaagacaa cagaggggat caacaaatgc 540

actctcattg ccatggactt gggtgaaatg tgtgaggaca ctgtcacgta taaatgcccc 600

ttactggtca ataccgaacc tgaagacatt gattgctggt gcaatctcac gtctacctgg 660

gtcatgtatg ggacatgcac ccagagcgga gaacggagac gagagaagcg ctcagtagct 720

ttaacaccac attcaggaat gggattggaa acaagagctg agacatggat gtcatcggaa 780

ggggcttgga agcatgctca gagagtagag agctggatac tcagaaaccc aggattcgcg 840

-continued

ctcttggcag gatttatggc ttatatgatt gggcaaacag gaatccagcg aactgtcttc 900

tttgtcctaa tgatgctggt cgccccatcc tacggaatgc gatgcgtagg agtaggaaac 960

agagactttg tggaaggagt ctcaggtgga gcatgggtcg atctggtgct agaacatgga 1020

ggatgcgtca caaccatggc ccagggaaaa ccaaccttgg attttgaact gactaagaca 1080

acagccaagg aagtggctct gttaagaacc tattgcattg aagcctcaat atcaaacata 1140

accacggcaa caagatgtcc aacgcaagga gagcctttatc taaaagagga acaagaccaa 1200

cagtacattt gccggagaga tgtggtagac agagggtggg gcaatggctg tggcttgttt 1260

ggaaaaggag gagttgtgac atgtgcgaag ttttcatgtt cggggaagat aacaggcaat 1320

ttggtccaaa ttgagaacct tgaatacaca gtggttgtaa cagtccacaa tggagacacc 1380

catgcagtag gaaatgacac gtccaatcat ggagttacag ccacgataac tcccaggtca 1440

ccatcggtgg aagtcaaatt gccggactat ggagaactaa cactcgattg tgaacccagg 1500

tctggaattg actttaatga gatgattctg atgaaaatga aaaagaaaac atggcttgtg 1560

cataagcaat ggtttttgga tctacctcta ccatggacag caggagcaga cacatcagag 1620

gttcactgga attacaaaga gagaatggtg acatttaagg ttcctcatgc caagagacag 1680

gatgtgacag tgctgggatc tcaggaagga gccatgcatt ctgccctcgc tggagccaca 1740

gaagtggact ccggtgatgg aaatcacatg tttgcaggac atctcaagtg caaagtccgt 1800

atggagaaat tgagaatcaa gggaatgtca tacacgatgt gttcaggaaa gttctcaatt 1860

gacaaagaga tggcagaaac acagcatggg acaacagtgg tgaaagtcaa gtatgaaggt 1920

gctggagctc cgtgtaaagt ccccatagag ataagagatg tgaacaagga aaaagtggtt 1980

gggcgtatca tctcatccac ccctttggct gagaatacca acagtgtaac caacatagag 2040

ttagaacccc cctttgggga cagctacata gtgataggtg ttggaaacag tgcattaaca 2100

ctccattggt tcaggaaagg gagttccatt ggcaagatgt ttgagtccac atacagaggt 2160

gcaaaacgaa tggccattct aggtgaaaca gcttgggatt ttggttccgt tggtggactg 2220

ttcacatcat tgggaaaggc tgtgcaccag gtttttggaa gtgtgtatac aaccctgttt 2280

ggaggagtct catggatgat tagaatccta attgggttcc tagtgttgtg gattggcacg 2340

aactcaagga acacttcaat ggctatgacg tgcatagctg ccggcattgt gacactgtat 2400

ttgggagtca tggtgcaggc cgatagtggt tgcgttgtga gctggaaaaa caaagaactg 2460

aaatgtggca gtgggatttt catcacagac aacgtgcaca catggacaga acaatacaag 2520

ttccaaccag aatccccttc aaaactagct tcagctatcc agaaagccca tgaagaggac 2580

atttgtggaa tccgctcagt aacaagactg gagaatctga tgtggaaaca aataacacca 2640

gaattgaatc acattctatc agaaaatgag gtgaagttaa ctattatgac aggagacatc 2700

aaaggaatca tgcaggcagg aaaacgatct ctgcggcctc agcccactga gctgaagtat 2760

tcatggaaaa catggggcaa agcaaaaatg ctctctacag agtctcataa ccagaccttt 2820

ctcattgatg gccccgaaac agcagaatgc cccaacacaa atagagcttg gaattcgttg 2880

gaagttgaag actatggctt tggagtattc accaccaata tatggctaaa attgaaagaa 2940

aaacaggatg tattctgcga ctcaaaactc atgtcagcgg ccataaaaga caacagagcc 3000

gtccatgccg atatgggtta ttggatagaa agtgcactca atgacacatg gaagatagag 3060

aaagcctctt tcattgaagt taaaaactgc cactggccaa aatcacacac cctctggagc 3120

-continued

aatggagtgc tagaaagtga gatgataatt ccaaagaatc tcgctggacc agtgtctcaa 3180

cacaactata gaccaggcta ccatacacaa ataacaggac catggcatct aggtaagctt 3240

gagatggact ttgatttctg tgatggaaca acagtggtag tgactgagga ctgcggaaat 3300

agaggaccct ctttgagaac aaccactgcc tctggaaaac tcataacaga atggtgctgc 3360

cgatcttgca cattaccacc gctaagatac agaggtgagg atgggtgctg gtacgggatg 3420

gaaatcagac cattgaagga gaaagaagag aatttggtca actccttggt cacagctgga 3480

catgggcagg tcgacaactt ttcactagga gtcttgggaa tggcattgtt cctggaggaa 3540

atgcttagga cccgagtagg aacgaaacat gcaatactac tagttgcagt ttcttttgtg 3600

acattgatca cagggaacat gtcctttaga gacctgggaa gagtgatggt tatggtaggc 3660

gccactatga cgggtgacat aggtatgggc gtgacttatc ttgccctact agcagccttc 3720

aaagtcagac caacttttgc agctggacta ctcttgagaa agctgacctc canggaattg 3780

atgatgacta ctataggaat tgtactcctc tcccagagca ccataccaga gaccattctt 3840

gagttgactg atgcgttagc cttaggcatg atggtcctca aaatggtgag aaatatggaa 3900

aagtatcaat tggcagtgac tatcatggct atcttgtgcg tcccaaacgc agtgatatta 3960

caaaacgcat ggaaagtgag ttgcacaata ttggcagtgg tgtccgtttc cccactgttc 4020

ttaacatcct cacagcaaaa aacagattgg ataccattag cattgacgat caaaggtctc 4080

aatccaacag ctatttttct aacaaccctc tcaagaacca gcaagaaaag gagctggcca 4140

ttaaatgagg ctatcatggc agtcgggatg gtgagcattt tagccagttc tctcctaaaa 4200

aatgatattc ccatgacagg accattagtg gctggagggc tcctcactgt gtgctacgtg 4260

ctcactggac gatcggccga tttggaactg gagagagcag ccgatgtcaa atgggaagac 4320

caggcagaga tatcaggaag cagtccaatc ctgtcaataa caatatcaga agatggtagc 4380

atgtcgataa aaaatgaaga ggaagaacaa acactgacca tactcattag aacaggattg 4440

ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg 4500

tgggaagtga agaaacaacg ggccggagta ttgtgggatg ttccttcacc cccacccatg 4560

ggaaaggctg aactggaaga tggagcctat agaattaagc aaaaagggat tcttggatat 4620

tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca 4680

cgtggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgtcaag 4740

aaagacctaa tatcatatgg aggaggctgg aagttagaag gagaatggaa ggaaggagaa 4800

gaagtccagg tattggcact ggagcctgga aaaaatccaa gagccgtcca aacgaaacct 4860

ggtcttttca aaaccaacgc cggaacaata ggtgctgtat ctctggactt ttctcctgga 4920

acgtcaggat ctccaattat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt 4980

gttgttacaa ggagtggagc atatgtgagt gctatagccc agactgaaaa aagcattgaa 5040

gacaacccag agatcgaaga tgacattttc cgaaagagaa gactgaccat catggacctc 5100

cacccaggag cgggaaagac gaagagatac cttccggcca tagtcagaga agctataaaa 5160

cggggtttga gaacattaat cttggccccc actagagttg tggcagctga aatggaggaa 5220

gcccttagag gacttccaat aagataccag accccagcca tcagagctgt gcacaccggg 5280

cgggagattg tggacctaat gtgtcatgcc acatttacca tgaggctgct atcaccagtt 5340

agagtgccaa actacaacct gattatcatg gacgaagccc atttcacaga tccagcaagt 5400

-continued

atagcagcta gaggatacat ctcaactcga gtggagatgg gtgaggcagc tgggattttt 5460

atgacagcca ctccccgggg aagcagagac ccatttcctc agagcaatgc accaatcata 5520

gatgaagaaa gagaaatccc tgaacgctcg tggaattccg gacatgaatg ggtcacggat 5580

tttaaaggga agactgtttg gttcgttcca agtataaaag caggaaatga tatagcagct 5640

tgcctgagga aaaatggaaa gaaagtgata caactcagta ggaagacctt tgattctgag 5700

tatgtcaaga ctagaaccaa tgattgggac ttcgtggtta caactgacat ttcagaaatg 5760

ggtgccaatt tcaaggctga gagggttata gaccccagac gctgcatgaa accagtcata 5820

ctaacagatg gtgaagagcg ggtgattctg gcaggaccta tgccagtgac ccactctagt 5880

gcagcacaaa gaagagggag aataggaaga aatccaaaaa atgagaatga ccagtacata 5940

tacatggggg aacctctgga aaatgatgaa gactgtgcac actggaaaga agctaaaatg 6000

ctcctagata acatcaacac gccagaagga atcattccta gcatgttcga accagagcgt 6060

gaaaaggtgg atgccattga tggcgaatac cgcttgagag gagaagcaag gaaaaccttt 6120

gtagacttaa tgagaagagg agacctacca gtctggttgg cctacagagt ggcagctgaa 6180

ggcatcaact acgcagacag aaggtggtgt tttgatggag tcaagaacaa ccaaatccta 6240

gaagaaaacg tggaagttga aatctggaca aaagaagggg aaaggaagaa attgaaaccc 6300

agatggttgg atgctaggat ctattctgac ccactggcgc taaaagaatt taaggaattt 6360

gcagccggaa gaaagtctct gaccctgaac ctaatcacag aaatgggtag gctcccaacc 6420

ttcatgactc agaaggtaag agacgcactg gacaacttag cagtgctgca cacggctgag 6480

gcaggtggaa gggcgtacaa ccatgctctc agtgaactgc cggagaccct ggagacattg 6540

cttttactga cacttctggc tacagtcacg ggagggatct ttttattctt gatgagcgca 6600

aggggcatag ggaagatgac cctgggaatg tgctgcataa tcacggctag catcctccta 6660

tggtacgcac aaatacagcc acactggata gcagcttcaa taatactgga gtttttttctc 6720

atagttttgc ttattccaga acctgaaaaa cagagaacac cccaagacaa ccaactgacc 6780

tacgttgtca tagccatcct cacagtggtg gccgcaacca tggcaaacga gatgggtttc 6840

ctagaaaaaa cgaagaaaga tctcggattg ggaagcattg caacccagca acccgagagc 6900

aacatcctgg acatagatct acgtcctgca tcagcatgga cgctgtatgc cgtggccaca 6960

acatttgtta caccaatgtt gagacatagc attgaaaatt cctcagtgaa tgtgtcccta 7020

acagcnatag ccaaccaagc cacagtgtta atgggtctcg ggaaaggatg gccattgtca 7080

aagatggaca tcggagttcc ccttctcgcc attggatgct actcacaagt caaccccata 7140

actctcacag cagctctttt cttattggta gcacattatg ccatcatagg gccaggactc 7200

caagcaaaag caaccagaga agctcagaaa agagcagcgg cgggcatcat gaaaaaccca 7260

actgtcgatg gaataacagt gattgaccta gatccaatac cttatgatcc aaagtttgaa 7320

aagcagttgg gacaagtaat gctcctagtc ctctgcgtga ctcaagtatt gatgatgagg 7380

actacatggg ctctgtgtga ggctttaacc ttagctaccg ggcccatctc cacattgtgg 7440

gaaggaaatc cagggaggtt ttggaacact accattgcgg tgtcaatggc taacattttt 7500

agagggagtt acttggccgg agctggactt ctcttttcta ttatgaagaa cacaaccaac 7560

acaagaaggg gaactggcaa cataggagag acgcttggag agaaatggaa aagccgattg 7620

aacgcattgg gaaaaagtga attccagatc tacaagaaaa gtggaatcca ggaagtggat 7680

-continued

agaaccttag caaaagaagg cattaaaaga ggagaaacgg accatcacgc tgtgtcgcga 7740

ggctcagcaa aactgagatg gttcgttgag agaaacatgg tcacaccaga agggaaagta 7800

gtggacctcg gttgtggcag aggaggctgg tcatactatt gtggaggact aaagaatgta 7860

agagaagtca aaggcctaac aaaaggagga ccaggacacg aagaacccat ccccatgtca 7920

acatatgggt ggaatctagt gcgtcttcaa agtggagttg acgttttctt catcccgcca 7980

gaaaagtgtg acacattatt gtgtgacata ggggagtcat caccaaatcc cacagtggaa 8040

gcaggacgaa cactcagagt ccttaactta gtagaaaatt ggttgaacaa caacactcaa 8100

ttttgcataa aggttctcaa cccatatatg ccctcagtca tagaaaaaat ggaagcacta 8160

caaaggaaat atggaggagc cttagtgagg aatccactct cacgaaactc cacacatgag 8220

atgtactggg tatccaatgc ttccgggaac atagtgtcat cagtgaacat gatttcaagg 8280

atgttgatca acagatttac aatgagatac aagaaagcca cttacgagcc ggatgttgac 8340

ctcggaagcg gaacccgtaa catcgggatt gaaagtgaga taccaaacct agatataatt 8400

gggaaaagaa tagaaaaaat aaagcaagag catgaaacat catggcacta tgaccaagac 8460

cacccataca aaacgtgggc ataccatggt agctatgaaa caaaacagac tggatcagca 8520

tcatccatgg tcaacggagt ggtcaggctg ctgacaaaac cttgggacgt cgtccccatg 8580

gtgacacaga tggcaatgac agacacgact ccatttggac aacagcgcgt ttttaaagag 8640

aaagtggaca cgagaaccca agaaccgaaa gaaggcacga agaaactaat gaaaataaca 8700

gcagagtggc tttggaaaga attagggaag aaaaagacac ccaggatgtg caccagagaa 8760

gaattcacaa gaaaggtgag aagcaatgca gccttggggg ccatattcac tgatgagaac 8820

aagtggaagt cggcacgtga ggctgttgaa gatagtaggt tttgggagct ggttgacaag 8880

gaaaggaatc tccatcttga aggaaagtgt gaaacatgtg tgtacaacat gatgggaaaa 8940

agagagaaga agctagggga attcggcaag gcaaaaggca gcagagccat atggtacatg 9000

tggcttggag cacgcttctt agagtttgaa gccctaggat tcttaaatga agatcactgg 9060

ttctccagag agaactccct gagtggagtg gaaggagaag ggctgcacaa gctaggttac 9120

attctaagag acgtgagcaa gaaagaggga ggagcaatgt atgccgatga caccgcagga 9180

tgggatacaa gaatcacact agaagaccta aaaaatgaag aaatggtaac aaaccacatg 9240

gaaggagaac acaagaaact agccgaggcc attttcaaac taacgtacca aaacaaggtg 9300

gtgcgtgtgc aaagaccaac accaagaggc acagtaatgg acatcatatc gagaagagac 9360

caaagaggta gtggacaagt tggcacctat ggactcaata ctttcaccaa tatggaagcc 9420

caactaatca gacagatgga gggagaagga gtctttaaaa gcattcagca cctaacaatc 9480

acagaagaaa tcgctgtgca aaactggtta gcaagagtgg ggcgcgaaag gttatcaaga 9540

atggccatca gtggagatga ttgtgttgtg aaacctttag atgacaggtt cgcaagcgct 9600

ttaacagctc taaatgacat gggaaagatt aggaaagaca tacaacaatg ggaaccttca 9660

agaggatgga atgattggac acaagtgccc ttctgttcac accatttcca tgagttaatc 9720

atgaaagacg gtcgcgtact cgttgttccc tgtagaaacc aagatgaact gattggcaga 9780

gcccgaatct cccaaggagc agggtggtct ttgcgggaga cggcctgttt ggggaagtct 9840

tacgcccaaa tgtggagctt gatgtacttc cacagacgcg acctcaggct ggcggcaaat 9900

gctatttgct cggcagtacc atcacattgg gttccaacaa gtcgaacaac ctggtccata 9960

-continued

catgctaaac atgaatggat gacaacggaa gacatgctga cagtctggaa cagggtgtgg	10020
attcaagaaa acccatggat ggaagacaaa actccagtgg aatcatggga ggaaatccca	10080
tacttgggga aaagagaaga ccaatggtgc ggctcattga ttgggttaac aagcagggcc	10140
acctgggcaa agaacatcca agcagcaata aatcaagtta gatcccttat aggcaatgaa	10200
gaatacacag attacatgcc atccatgaaa agattcagaa gagaagagga agaagcagga	10260
gttctgtggt agaaagcaaa actaacatga aacaaggcta gaagtcaggt cggattaagc	10320
catagtacgg aaaaaactat gctacctgtg agccccgtcc aaggacgtta aaagaagtca	10380
ggccatcata aatgccatag cttgagtaaa ctatgcagcc tgtagctcca cctgagaagg	10440
tgtaaaaaat ccgggaggcc acaaaccatg gaagctgtac gcatggcgta gtggactagc	10500
ggttagagga gacccctccc ttacaaatcg cagcaacaat gggggcccaa ggcgagatga	10560
agctgtagtc tcgctggaag gactagaggt tagaggagac ccccccgaaa caaaaaacag	10620
catattgacg ctgggaaaga ccagagatcc tgctgtctcc tcagcatcat tccaggcaca	10680
gaacgccaga aaatggaatg gtgctgttga atcaacaggt tct	10723

1. A modified live, attenuated dengue-2 virus strain PDK-53, wherein the modified live, attenuated dengue-2 virus strain PDK-53

is represented by a polynucleotide molecule encoding a modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule, or

is obtainable by a method for producing a modified live, attenuated dengue-2 virus strain PDK-53, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule, and

b) introducing the RNA transcribed in step a) into cells for production of the modified live, attenuated dengue-2 virus strain PDK-53,

wherein the polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule comprises at least one mutation, wherein the at least one mutation comprises:

an adenine to guanine mutation at position 592 encoding a glutamic acid instead of a lysine in the polypeptide molecule at amino acid position 166 corresponding to prM-52, and

an adenine to guanine mutation at position 8803 encoding a valine instead of an isoleucine in the polypeptide molecule at amino acid position 2903 corresponding to NS5-412.

2. The modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1**, wherein the polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule further comprises at least one additional mutation of:

a guanine to cytosine mutation at nucleic acid position 6481 encoding a proline instead of an alanine in the polypeptide molecule at amino acid position 2129 corresponding to NS4A-36, and

a cytosine to thymine mutation at position 7156 encoding a phenylalanine instead of a leucine in the polypeptide molecule at amino acid position 2354 corresponding to NS4B-111.

3. The modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1**, wherein the polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule is represented by SEQ ID NO: 4 or SEQ ID NO: 14.

4. The modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1**, wherein the modified, live attenuated dengue-2 virus strain PDK-53 polypeptide molecule is represented by SEQ ID NO: 5 or SEQ ID NO: 6.

5. A pharmaceutical composition comprising the modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1**, and a pharmaceutically acceptable excipient.

6. A method for inducing an immune response against dengue virus in a subject, the method comprising administering to the subject a pharmaceutically acceptable amount of the composition of claim **5**.

7. A vector comprising a polynucleotide molecule encoding a modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule, wherein the polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule comprises at least one mutation, wherein the at least one mutation comprises:

an adenine to guanine mutation at position 592 encoding a glutamic acid instead of a lysine in the polypeptide molecule at amino acid position 166 corresponding to prM-52, and

an adenine to guanine mutation at position 8803 encoding a valine instead of an isoleucine in the polypeptide molecule at amino acid position 2903 corresponding to NS5-412.

8. The vector according to claim 7, wherein the vector is a plasmid vector.

9. The vector according to claim 7, wherein the vector is a cDNA infectious clone.

10. An isolated cell comprising

the modified live, attenuated dengue-2 virus strain PDK-53 according to claim 1, or

a vector comprising a polynucleotide molecule encoding a modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule, wherein the polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule comprises at least one mutation, wherein the at least one mutation comprises:

an adenine to guanine mutation at position 592 encoding a glutamic acid instead of a lysine in the polypeptide molecule at amino acid position 166 corresponding to prM-52, and

an adenine to guanine mutation at position 8803 encoding a valine instead of an isoleucine in the polypeptide molecule at amino acid position 2903 corresponding to NS5-412.

11. A method for producing a modified live, attenuated dengue-2 virus strain PDK-53, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding a modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule, wherein the polynucleotide molecule encoding the modified live, attenuated dengue-2 virus strain PDK-53 polypeptide molecule comprises at least one mutation, wherein the at least one mutation comprises:

an adenine to guanine mutation at position 592 encoding a glutamic acid instead of a lysine in the polypeptide molecule at amino acid position 166 corresponding to prM-52, and

an adenine to guanine mutation at position 8803 encoding a valine instead of an isoleucine in the polypeptide molecule at amino acid position 2903 corresponding to NS5-412;

and

b) introducing the RNA transcribed in step a) into cells for production of the modified live, attenuated dengue-2 virus strain PDK-53.

12. The method of claim 11, further comprising the following step:

c) passaging the modified live, attenuated dengue-2 virus strain PDK-53 produced in step b) up to 10 times in cells.

13. The method of claim 11, wherein the cells are Vero cells.

14. An immunogenic composition comprising the modified live, attenuated dengue-2 virus strain PDK-53 according to claim 1 and a pharmaceutically acceptable carrier.

15. The immunogenic composition of claim 14, further comprising a dengue-1/dengue-2 chimera, wherein the dengue-1/dengue-2 chimera

is represented by a polynucleotide molecule encoding a dengue-1/dengue-2 polypeptide chimera,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera, or

is obtainable by a method for producing a dengue-1/dengue-2 chimera, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera, and

b) introducing the RNA transcribed in step a) into cells for production of the dengue-1/dengue-2 chimera,

wherein the polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera comprises a first nucleotide sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus strain PDK-53, a second nucleotide sequence encoding at least one structural protein from dengue-1, and at least one mutation, wherein the at least one mutation comprises one or more of:

an adenine to cytosine mutation at position 3823 encoding a leucine instead of an isoleucine in the dengue-1/dengue-2 polypeptide chimera at amino acid position 1243 corresponding to NS2A-116;

an adenine to thymine mutation at position 4407 encoding an aspartic acid instead of a glutamic acid in the dengue-1/dengue-2 polypeptide chimera at amino acid position 1437 corresponding to NS2B-92; and

an adenine to guanine mutation at position 7311.

16. The immunogenic composition according to claim 15, wherein the polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera further comprises at least one additional mutation of:

a cytosine to thymine mutation at position 7148 encoding an isoleucine instead of a threonine in the dengue-1/dengue-2 polypeptide chimera at amino acid position 2351 corresponding to NS4B-108; and

a guanine to cytosine mutation at position 2384 encoding an alanine instead of glycine in the dengue-1/dengue-2 polypeptide chimera at amino acid position 763 corresponding to E-483.

17. The immunogenic composition according to claim 15, wherein the polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera is represented by SEQ ID NO: 1 or SEQ ID NO: 13.

18. The immunogenic composition according to claim 15, wherein the dengue-1/dengue-2 polypeptide chimera is represented by SEQ ID NO: 2 or SEQ ID NO: 3.

19. The immunogenic composition of claim 15, wherein the nonstructural proteins from the modified live, attenuated dengue-2 virus strain PDK-53 are selected from the group consisting of NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

20. The immunogenic composition of claim 15, wherein the at least one structural protein from dengue-1 is selected from the group consisting of capsid protein (C), premembrane/membrane protein (prM) and envelope protein (E).

21. The immunogenic composition of claim 14, further comprising a dengue-3/dengue-2 chimera, wherein the dengue-3/dengue-2 chimera

is represented by a polynucleotide molecule encoding a dengue-3/dengue-2 polypeptide chimera,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera, or

is obtainable by a method for producing a dengue-3/dengue-2 chimera, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera, and

b) introducing the RNA transcribed in step a) into cells for production of the dengue-3/dengue-2 chimera,

wherein the polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera comprises a first nucleotide sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus strain PDK-53, a second nucleotide sequence encoding at least one structural protein from dengue-3, and at least one mutation, wherein the at least one mutation comprises one or more of:

an adenine to thymine mutation at position 1603 encoding a serine instead of a threonine in the dengue-3/dengue-2 polypeptide chimera at amino acid position 503 corresponding to E-223; and

an adenine to guanine mutation at position 7620.

22. The immunogenic composition of claim **21**, wherein the polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera further comprises a guanine to adenine mutation at position 6436 encoding an asparagine instead of an aspartic acid in the dengue-3/dengue-2 polypeptide chimera at amino acid position 2114 corresponding to NS4A-23.

23. The immunogenic composition of claim **21**, wherein the polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera is represented by SEQ ID NO: 7 or SEQ ID NO: 15.

24. The immunogenic composition of claim **21**, wherein the dengue-3/dengue-2 polypeptide chimera is represented by SEQ ID NO: 8 or SEQ ID NO: 9.

25. The immunogenic composition of claim **21**, wherein the nonstructural proteins from the modified live, attenuated dengue-2 virus strain PDK-53 are selected from the group consisting of NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

26. The immunogenic composition of claim **21**, wherein the at least one structural protein from dengue-3 is selected from the group consisting of capsid protein (C), premembrane/membrane protein (prM) and envelope protein (E).

27. The immunogenic composition of claim **14**, further comprising a dengue-4/dengue-2 chimera, wherein the dengue-4/dengue-2 chimera

is represented by a polynucleotide molecule encoding a dengue-4/dengue-2 polypeptide chimera,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera, or

is obtainable by a method for producing a dengue-4/dengue-2 chimera, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera, and

b) introducing the RNA transcribed in step a) into cells for production of the dengue-4/dengue-2 chimera,

wherein the polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera comprises a first nucleotide sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus strain PDK-53, a second nucleotide sequence encoding at least one structural protein from dengue-4, and at least one mutation, wherein the at least one mutation comprises one or more of:

an adenine to thymine mutation at position 225;

an adenine to guanine mutation at position 3674 encoding a glycine instead of an aspartic acid in the dengue-4/dengue-2 polypeptide chimera at amino acid position 1193 corresponding to NS2A-66;

a cytosine to thymine mutation at position 5391;

a cytosine to thymine mutation at position 6437 encoding a valine instead of an alanine in the dengue-4/dengue-2 polypeptide chimera at amino acid position 2114 corresponding to NS4A-21, and

an adenine to cytosine mutation at position 9750.

28. The immunogenic composition of claim **27**, wherein the polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera further comprises a thymine to cytosine mutation at position 7026.

29. The immunogenic composition of claim **27**, wherein the polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera further comprises an adenine to guanine mutation at position 3773 encoding an arginine instead of a lysine in the dengue-4/dengue-2 polypeptide chimera at amino acid position 1226 corresponding to NS2A-99.

30. The immunogenic composition of claim **27**, wherein the polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera further comprises a cytosine to thymine mutation at position 7538 encoding a phenylalanine instead of a serine in the dengue-4/dengue-2 polypeptide chimera at amino acid position 2481 corresponding to NS4B-238.

31. The immunogenic composition of claim **27**, wherein the polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera is represented by SEQ ID NO: 10 or SEQ ID NO: 16.

32. The immunogenic composition of claim **27**, wherein the dengue-4/dengue-2 polypeptide chimera is represented by SEQ ID NO: 11 or SEQ ID NO: 12.

33. The immunogenic composition of claim **27**, wherein the nonstructural proteins from the modified live, attenuated dengue-2 virus strain PDK-53 are selected from the group consisting of NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

34. The immunogenic composition of claim **27**, wherein the at least one structural protein from dengue-4 is selected from the group consisting of capsid protein (C), premembrane/membrane protein (prM) and envelope protein (E).

35. An immunogenic composition comprising the modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1** and a pharmaceutically acceptable carrier, wherein the immunogenic composition is a tetravalent composition.

36. The immunogenic composition of claim **35**, further comprising one or more of:

i) a dengue-1/dengue-2 chimera, wherein the dengue-1/dengue-2 chimera

is represented by a polynucleotide molecule encoding a dengue-1/dengue-2 polypeptide chimera,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera, or

is obtainable by a method for producing a dengue-1/dengue-2 chimera, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera, and

b) introducing the RNA transcribed in step a) into cells for production of the dengue-1/dengue-2 chimera,

wherein the polynucleotide molecule encoding the dengue-1/dengue-2 polypeptide chimera comprises a first nucleotide sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus strain PDK-53, a second nucleotide sequence encoding at least one structural protein from dengue-1, and at least one mutation, wherein the at least one mutation comprises one or more of:

an adenine to cytosine mutation at position 3823 encoding a leucine instead of an isoleucine in the dengue-1/dengue-2 polypeptide chimera at amino acid position 1243 corresponding to NS2A-116;

an adenine to thymine mutation at position 4407 encoding an aspartic acid instead of a glutamic acid in the dengue-1/dengue-2 polypeptide chimera at amino acid position 1437 corresponding to NS2B-92; and

an adenine to guanine mutation at position 7311;

ii) a dengue-3/dengue-2 chimera, wherein the dengue-3/dengue-2 chimera

is represented by a polynucleotide molecule encoding a dengue-3/dengue-2 polypeptide chimera,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera, or

is obtainable by a method for producing a dengue-3/dengue-2 chimera, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera, and

b) introducing the RNA transcribed in step a) into cells for production of the dengue-3/dengue-2 chimera,

wherein the polynucleotide molecule encoding the dengue-3/dengue-2 polypeptide chimera comprises a first nucleotide sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus strain PDK-53, a second nucleotide sequence encoding at least one structural protein from dengue-3, and at least one mutation, wherein the at least one mutation comprises one or more of:

an adenine to thymine mutation at position 1603 encoding a serine instead of a threonine in the dengue-3/dengue-2 polypeptide chimera at amino acid position 503 corresponding to E-223; and

an adenine to guanine mutation at position 7620; and

iii) a dengue-4/dengue-2 chimera, wherein the dengue-4/dengue-2 chimera

is represented by a polynucleotide molecule encoding a dengue-4/dengue-2 polypeptide chimera,

comprises an RNA transcribed from a cDNA comprising a polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera,

comprises one or more polypeptide molecules encoded by a polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera, or

is obtainable by a method for producing a dengue-4/dengue-2 chimera, the method comprising the following steps:

a) transcribing an RNA from a cDNA comprising a polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera, and

b) introducing the RNA transcribed in step a) into cells for production of the dengue-4/dengue-2 chimera,

wherein the polynucleotide molecule encoding the dengue-4/dengue-2 polypeptide chimera comprises a first nucleotide sequence encoding nonstructural proteins from a modified live, attenuated dengue-2 virus strain PDK-53, a second nucleotide sequence encoding at least one structural protein from dengue-4, and at least one mutation, wherein the at least one mutation comprises one or more of:

an adenine to thymine mutation at position 225;

an adenine to guanine mutation at position 3674 encoding a glycine instead of an aspartic acid in the dengue-4/dengue-2 polypeptide chimera at amino acid position 1193 corresponding to NS2A-66;

a cytosine to thymine mutation at position 5391;

a cytosine to thymine mutation at position 6437 encoding a valine instead of an alanine in the dengue-4/dengue-2 polypeptide chimera at amino acid position 2114 corresponding to NS4A-21, and

an adenine to cytosine mutation at position 9750.

37. The immunogenic composition of claim **35**, in combination with an immunogenic composition for a flavivirus selected from the group consisting of yellow fever virus, tick-borne encephalitis virus, Japanese encephalitis virus, West Nile virus, hepatitis C virus, and a combination of two or more thereof.

38. A method for inducing an immune response against all four dengue virus serotypes in a subject, the method comprising administering to the subject a pharmaceutically acceptable amount of the immunogenic composition of claim **35**.

39. A method for inducing an immune response against all four dengue virus serotypes in a subject, the method comprising administering to the subject a pharmaceutically acceptable amount of the immunogenic composition of claim **36**.

40. A kit comprising

at least one modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1**; or

at least one composition comprising a modified live, attenuated dengue-2 virus strain PDK-53 according to claim **1** and a pharmaceutically acceptable excipient;

and a container.

* * * * *