CHIMERIC CHAINS CODING FOR PROTEINS INDUCING EFFECTS AGAINST VIRUS

Field of the Invention

The present invention is related with the field of the biotechnology and the pharmaceutical industry, in particular with the obtaining of chimeric nucleotide chains which, when introduced into an expression vector, give rise to proteins able to elicit a serotype-specific humoral immune response and protection against the Dengue virus infection; quoted from now on as DEN, avoiding the effects of the serotype-specific viral immunoamplification, causing hemorrhages and clinical complications described in this type of pathology.

Previous Technique

The Dengue virus (DEN), is a coated virus whose lipid membrane contains two of its three structural proteins: the envelope protein (E) and the membrane protein (M). The E protein covers an icosaedric nucleocapsid composed by the third of its structural proteins, the core protein. This virus belongs to the *Flaviviridae* family and four different serotypes exist. Its transmission to the man is carried out through the mosquito *Aedes aegypti* that belongs to the *Stegomia* family. The disease produced in the human for this virus was considered as benign and was described as Dengue Fever or Classical Dengue (DF) until the appearance of a more serious modality and sometimes lethal, characterized by hemorrhagic fever and shock, denominated: Hemorrhagic Dengue Fever and Dengue Shock Syndrome (HDF/DSS) (Hammon WMc. New hemorrhagic fever in children in the Philippines and Thailand. Trans Assoc Physicians 1960; 73: 140-155). Several epidemiological studies have been carried out evidencing as a risk factor the sequential infection of two different viral serotypes (Kourí GP, Guzmán MG, Brave JR. Why dengue hemorrhagic fever in Cuba) 2. An integral analysis. Trans Roy Soc Trop Med Hyg 1987; 72: 821-823). This phenomenon is explained by the immuno-enhancement, which is based on an increase of the viral infection by increasing the entrance of the virus-antibody complex to the cell through the Fc receptors of the target cell (monocytes) (halstead SB. Pathogenesis of dengue: challenges to molecular biology. Science 1988; 239: 476-481).

Different technologies have been developed to produce live attenuated vaccines, but at present there exist multiple unsolved issues on the possible benefits of these vaccines, since they could revert to the virulence, viral interference and inter-genomic recombination. Alternately, recombinant antigens can be obtained as possible components of a subunit vaccine (Feighny, R., Borrous, J. and Putnak R. Dengue type-2 virus envelope protein made using recombinant baculovirus protects mice against virus challenge. Am. J. Trop. Med. Hyg. 1994. 50(3). 322-328; Deubel, V., Staropoli, I., Megret,

F., *et al.* Affinity-purified dengue-2 virus envelope glycoprotein induces neutralizing antibodies and protective immunity in mice. Vaccine. 1997. 15, 1946-1954).

The main antigen of the virus is the envelope protein DENe. This protein is the major component of the viral surface and is thought to mediate the binding of the virus to the cellular receptor (A Heinz FX, Berge R, Tuma W et al. A topological and functional model of epitopes on the structural glycoprotein of tick-borne encephalitis virus defined by monoclonal antibodies. Virology. 1983; 126: 525). This protein has structural homology with that of the tick borne encephalitis virus (TBE) (Rey, F.A., Heinz, F.X., Mandl, C., *et al.* The envelope glycoprotein from tick borne encephalitis virus at 2 A° resolution. Nature 1995; 375: 291-298) and it is also structurally conserved among serotypes.

The insect cells constitute one of the systems most used for the expression of diverse heterologous genes that employ the baculovirus system as vectors. These vectors have been used for the expression of several combinations of structural and nonstructural proteins of the Encephalitis Japanese virus (JEV), DEN-1, DEN-2 and DEN-4, (Matsuura Y, Miyamoto M, Soto T et al. Characterization of japanese encephalitis virus envelope protein expressed by recombinant baculoviruses. Virology 1989; 173: 677-682; Deubel V, Bordier M, Megret F *et al.* Processing, secretion and immunoreactivity of carboxy terminally truncated dengue-2 envelope proteins expressed in insect cell by recombinant baculoviruses. Virology 1991; 180: 440-447; Putnak R, Feighny R, Burrous J *et al.* Dengue 1 virus envelope glycoprotein gene expressed in recombinant baculovirus elicit virus neutralization antibodies in mice and protects them from virus challenge. Am J Trop Med Hyg 1991; 45: 159-167; Feighny R, Burrous J, Putnak R. Dengue type 2 virus envelope protein made using recombinant baculovirus protects mice against virus challenge. Am J Trop Med Hyg 1994; 50: 322-328). Another system used has been the cells of *Drosophila melanogaster* expressing different variants of the E protein (PCT/US96/07627). In spite of obtaining an appropriate functional response, upon using the proteins expressed in these systems, they imply a high cost for the development of scale-up production processes; therefore, the expression in yeast has been an alternative to produce recombinant structural proteins of flavivirus. However, in the case of the DENe protein, expressed in *Pichia pastoris* (PCT/US96/07627; Sugrue R.J., Fu H., Howe J., Chan Y. Expression of the Dengue virus structural proteins in *Pichia pastoris* leads to the generation of virus-like particles. J Virol. 1997. 78, 1861-1866), the levels of expression are low, either secreted or intracellularly, hindering the purification process.

In parallel, several variants of the DENe protein have been obtained in bacteria. One of them was the C-terminal portion of the E protein of the JEV fused to a protein of the Tryptophan metabolism (TrpE) of *E. coli.* This protein was produced as inclusion bodies and was recognized by neutralizing monoclonal antibodies (Mabs) using

immunodetection techniques. However, pure preparations of this protein were unable to develop neutralizing antibodies and to protect against viral challenge (Mason P.W., Zogel M.V., Semproni A.R., et al. The antigenic structure of dengue type 1 virus envelope and NS1 protein expressed in *E. coli*. J Gen Virol. 1990. 71: 2107-2114). In addition, another construction was made (Srivastava A.K., Morita K., Matsuo S., et al. Japanese encephalitis virus fusion protein with protein A expressed in *E. coli* confers protection in mice. Microbiol Immunol. 1991. 35: 863-870), that contains the protein A of *Staphylococcus aurius* fused to the C-terminal fragment of the E protein, followed by the N-terminal segment of the nonstructural protein of the JEV, the NS1. In this case the fused protein was soluble, facilitating its purification by affinity chromatography. Upon immunizing mice with this pure protein high neutralizing antibodies titers were obtained, which also inhibited haemagglutination and protected against the viral challenge with the JEV. Similar results were obtained using the DENe region of the DEN-2 fused to the protein A of *S. aureus* (Srivastava A. K., Putnak R. J., Warren R. L., Hoke C. H. Mice immunized with a dengue type 2 virus E and NS1 fusion protein made in *Escherichia coli* are protected against lethal dengue virus infection. Vaccine.1995. 13: 1251-1258); however, it is not possible to use these preparations in humans due to the presence of the protein A, which has shown a high affinity for the human immunoglobulin G (IgG).

Finally, it has been reported a fusion protein that contains the B domain of the DENe protein of DEN-2 and the maltose binding protein (MBP) of *E. coli* (Simmons M., Nelson W.M., Wu S.J., Hayes C.G. Evaluation of the protective efficacy of a recombinant dengue envelope B domain fusion protein against dengue 2 virus infection in mice. Am J Trop Med Hyg. 1998. 58: 655-662) denominated MBP-DomB. This protein variant was immunogenic in mice and elicited neutralizing antibodies.

In our case, the subject of this invention relies on the obtaining of chimeric sequences, as for instance, in the first case, the sequence coding for a region of the DENe protein linked to the N-terminal fragment of a mutated protein with dehydrogenase activity (MDH) from *Neisseria meningitidis*; in the second case, the sequence coding for a region of the DENe protein linked to the entire gene of the MDH protein in two different positions, and in the third case, the chimeric sequences are formed by two fragments of the DENe protein from two different viral serotypes fused to the same gene coding for the MDH protein. These chimeric chains when inserted into a suitable vector, give rise to insoluble chimeric proteins within the bacterium's cytoplasm. These proteins are then capable to elicit high levels of neutralizing antibodies against DEN, inhibitors of the viral hemagglutination and to protect immunized mice against viral challenge.

With regards to the insolubility of the aforementioned proteins, an easy scale-up folding process was achieved *in vitro*, as well as the expression and purification processes which

were superior to those used by Simmons et al, 1998. On the other hand, the serotype specificity of the antibodies is demonstrated, generated by immunization of mice with these proteins, at the level of neutralization, inhibition of hemaglutination and ELISA, using doses lower than those employed by Simmons et al, 1998. This fact constitutes the first report on the expression of insoluble DENe proteins in *E. coli* capable of stimulating a functionl immune response.

In addition, considering the results obtained with the dimeric variant, it is possible to generate serotype-specific antibodies with the same molecule for two different viral serotypes, capable of neutralizing viral infection and protect mice against viral challenge.

Concerning the MDH protein, a search for homology with other sequences was done in the EMBL data base, revealing that the first 110 amino acids are highly similar to the lipoic binding domain region and the flexible hinge of the dihydrolipoamide acetyltransferase (E2 enzyme of the pyruvate dehydrogenase complex and α-cetoglutarate dehydrogenase), and the rest of the protein is highly similar to the lipoamide dehydrogenase (LPDH), enzyme E3 of said complexes (Stephens, P. E; H. M. Darlinson,andJ. R. Guest.,1983. The Pyruvate dehydrogenase complex of *E. coli.* Eur. J. Biochem. 133: 155-162.

On the other hand, it was also found that patients with Primary Biliary Cirrhosis (PBC) produced anti-mitochondrial autoantibodies, specific for the lipoic acid binding site, common among these proteins (Gershwin ME, Mackay IR, Sturgess A, Coppel RL. Identification and specificity of a cDNA encoding the 70 KDa mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987;138:3525-31). Therefore, we decided to mutate this region within the protein to avoid any autoimmune response when immunized in humans as chimeric proteins. The mutated MDH protein of our invention was used in a Phase I clinical trial and showed to be safe and immunogenic in humans, and also was not recognized by sera of patients with PBC (Pérez, A., F. Dickinson, Z. Cinza, A. Ruíz, T. Serrano, J. Sosa, S. González, Y. Gutiérrez, C. Nazábal, O. Gutiérrez, D. Guzmán, M. Díaz, M. Delgado, E. Caballero, G. Sardiñas, A. Alvarez, A. Martín, G. Guillén, R. Silva. Safety and preliminary immunogenicity of the recombinant outer membrane protein of *Neisseria meningitidis* in human volunteers. Biotech. Appl. Biochem. 34: 121-125). However, the possible use of the MBP in humans has not been demonstrated yet (Simmons M., Nelson W.M., Wu S.J., Hayes C.G. Evaluation of the protective efficacy of a recombinant dengue envelope B domain fusion protein against dengue 2 virus infection in mice. Am J Trop Med Hyg. 1998. 58: 655-662).

Detailed description of the invention

In this invention is described the obtaining of chimeric nucleotide chains that when introduced into an expression vector, give rise to chimeric proteins capable of inducing a

serotype-specific humoral immune response and protecting against the infection by Dengue virus, as for instance, the sequence coding for a region of the DENe protein from each one of the viral serotypes of the Dengue virus, linked to the N-terminal fragment of a mutated protein with dehydrogenase activity (MDH) from *Neisseria meningitidis*; in the second case, the sequence coding for a region of the DENe protein linked to the entire gene of the MDH protein in two different positions: within one site of the sequence coding for the structural domain of the MDH protein (lipoic acid binding domain and the 3' end of the gen), and in the third case, the chimeric sequences are formed by two fragments of the DENe protein from two different viral serotypes, DEN-2 and DEN-4, in two different positions of the MDH gen: one within a particular site of the sequence coding for the lipoic acid binding domain (serotype 4) and the other in the 3' end of the MDH gen (serotype 2). This was called a dimeric construct.

This chimeric proteins were obtained insoluble within the bacterium's cytoplasm. A purification process by immobilazed metal affinity chromatography (IMAC) was done , which led to obtain pure proteins for immunogenicity studies.

When antigenicity results were analyzed a strong recognition of all the recombinant chimeric proteins for the hyperimmune ascitic liquids (HMAF) anti-DEN was demonstrated, being higher for the case of the fusion to the entire MDH gene, which evidences a positive effect on the folding of the region from the DENe protein given by the MDH. In the cases where the serotype 2 was used, all recombinant proteins obtained were recognized by a serotype-specific neutralizing antibody (3H5), being also higher for the case of the fusion to the entire MDH gene, as well as in the dimeric protein. It was also observed that the recognition for the HMAF from the homologous serotype in each case was significantly higher than the recognition for the HMAF from the heterologous serotypes, evidencing the exposure of serotype-specific epitopes and permitting thus its use as a diagnostic mean for Dengue and serotyping.

When all the recombinant chimeric proteins were immunized in mice a neutralizing and protective response was obtained. Highest neutralizing titers were obtained with the sequences fused to the entire gene of the MDH and with the dimeric protein, independently of the position of the fragment from the DENe protein. This showed an immunopotentiator effect of the immune response mediated by the MDH that can be explained by the influence in the folding of the DENe protein reflected in the antigenicity results obtained. It was also demonstrated for the first time, and contrary to the previous state of the art, that the insolubility of these proteins do not affect the capacity of generating a suitable immune response.

The immune response produced in all the cases was serotype-specific (antibodies against the immunized homologous serotype) in the viral neutralization, the hemoagglutination inhibition and ELISA. The generation of serotype-specific antibodies means that they are not capable to recognize antigenic determinants from virus of heterologous serotypes that favour the immunoenhacement phenomenum. This characteristic is of great importance for the development of a vaccine candidate against the Dengue virus since the recognition of antibodies to heterologous serotypes could be one of the causes for the Hemorrhagic Dengue Fever (HDF).

Besides it was showed the induction of antibodies against two viral serotypes after immunization with just one of the chimeric proteins, which permits the formulation of a vaccine candidate against the four serotypes, using only two of our available recombinant chimeric proteins.

The obtaining of the mutant MDH protein consisted of the elimination of the lipoic acid binding site in the sequence ETDKAT, based on the covalent binding of this fatty acid with the epsilon-amine groups of lysine (K) (Tuaillon N, Andre C, Briand JP et al. A lipoyl synthetic octadecapeptide of dihydrolipoamide acetyltransferase specifically recognized by anti-M2 autoantibodies in Primary Biliary Cirrhosis. J Immunol 1992; 148:445-50).

The mutagenesis was done by using PCR with a pair of primers to amplify the N-terminal region (from the start codon of the lpdA gene until the lipoic acid binding site, 135 bp) and the C-terminal of the protein (from the lipoic acid binding site until the 3' end of the gene); thus, being eliminated the possibility to generate autoimmune reactions, as demonstrated in the human clinical trials.

Deposit of the biological material

Plasmids PLL1, PLL2, PLL3, PLH1, PLH2, PLH3,PAZ1, PAZ2, PAZ3, PID1, PID2 and PID3 were deposited according to the Budapest Treaty in the Belgian Coordinated collection of Microorganism – BCCM™ , LMBP-COLLECTION, and with the date and under the access numbers (pending).

Description of Figures

Figure 1. Cloning strategy of the E2 fragment to obtain PLL1.

DENe2: Fragment of the envelope protein of DEN-2.

N-term: Nucleotide sequence that codifies for the first 45 amino acids of the MDH protein.

Figure 2. Cloning strategy of the E2 fragment to obtain PLL2.

DENe2: Fragment of the envelope protein of DEN-2.

MDH: dehydrogenase mutant.

Figure 3. Cloning strategy of the E2 fragment to obtain PLL3.

DENe2: Fragment of the envelope protein of DEN-2.

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-2 virus (Sec. Id. No. 22) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.1 and Sequence No. 2 from the DEN-2 virus strain genotype Jamaica (Deubel V., Kinney R.M., Trent D.W. Nucleotide sequence and deduced amino acid sequence of the nonstructural proteins of Dengue type 2 virus, Jamaica genotype: Comparative analysis of the full-length genome.Virology 1988.165:234-244).

The vector was created by digestion of the pM108 His plasmid with *Xba I/Bam HI*, which contains the nucleotide sequence that codifies for the N-terminal region of the MDH and for a sequence of 6 histidines (Sequence No. 23). Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 (Hill C.W., Harnish B.W. 1982. Transposition of a chromosomal segment bounded by redundant rRNA genes in *Escherichia coli.* J Bacteriology. 149:449-457) were transformed with the selected clone, called pLL1 (Fig.1 and Sequence No. 24). Upon growing the colony in Luria Bertani (LB) medium, a SDS-PAGE of the cellular lysate was done. As a result a 25 kDA band was obtained, which accounted for 10% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the N-terminal region from the MDH protein and the DENe protein fragment from the DEN-2 virus. The protein was recognized in Immunoblotting by polyclonal antibodies (PA) anti-DEN-2 contained in the HMAF. This protein was denominated PLL1 (Sequence No. 25).

EXAMPLE 2. Purification of the PLL1 protein

The biomass obtained from the *E. coli* strain transformed with pLL1 and grown at 37°C was disrupted by French press. The recombinant protein was obtained preponderantly as insoluble form associated to the pellet of the cellular disruption. From the pellet the protein was extracted with urea 6 M and the supernatant, containing the PLL1 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The protein was eluted with Imidazole 50 mM and the obtained volume was loaded onto a G-25 column to finally obtain the protein in the formulation buffer NaCl 100 mM, KCl_2 2 mM, Na_2HPO_4 10 mM, pH 7.2, KH_2PO_4 1 mM (PBS). This preparation was used for immunological studies.

MDH: dehydrogenase mutant.

Figure 4. Cloning strategy of the E1 fragment to obtain PLH1.

DENe1: Fragment of the envelope protein of DEN-1.

N-term: Nucleotide sequence that codifies for the first 45 amino acids of the MDH protein.

Figure 5. Cloning strategy of the E1 fragment to obtain PLH2.

DENe1: Fragment of the envelope protein of DEN-1.

N-term: Nucleotide sequence that codifies for the first 45 amino acids of the MDH protein.

Figure 6. Cloning strategy of the E1 fragment to obtain PLH3.

DENe1: Fragment of the envelope protein of DEN-1.

MDH: dehydrogenase mutant.

Figure 7. Cloning strategy of the E3 fragment to obtain PAZ1.

DENe3: Fragment of the envelope protein of DEN-3.

N-term: Nucleotide sequence that codifies for the first 45 amino acids of the MDH protein.

Figure 8. Cloning strategy of the E3 fragment to obtain PAZ2.

DENe3: Fragment of the envelope protein of DEN-3.

MDH: dehydrogenase mutant.

Figure 9. Cloning strategy of the E3 fragment to obtain PAZ3.

DENe3: Fragment of the envelope protein of DEN-3.

MDH: dehydrogenase mutant.

Figure 10. Cloning strategy of the E4 fragment to obtain PID1.

DENe4: Fragment of the envelope protein of DEN-4.

N-term: Nucleotide sequence that codifies for the first 45 amino acids of the MDH protein.

Figure 11. Cloning strategy of the E4 fragment to obtain PID2.

DENe4: Fragment of the envelope protein of DEN-4.

MDH: dehydrogenase mutant.

Figure 12. Cloning strategy of the E4 fragment to obtain PID3.

DENe4: Fragment of the envelope protein of DEN-4.

MDH: dehydrogenase mutant.

Figura 13. Cloning strategy to obtain PD4D2.

DENe4: Fragment of the envelope protein of DEN-4.

DENe2: Fragment of the envelope protein of DEN-2.

MDH: dehydrogenase mutant.

EXAMPLES OF PERFORMANCE

EXAMPLE 1. Obtaining of PLL1

EXAMPLE 3. Antigenic characterization of PLL1

The purified fraction of PLL1 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 1).

Table 1. Reactivity of PLL1 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PLL1**
HMAF	DEN-1	+
HMAF	DEN-2	++
HMAF	DEN-3	-
HMAF	DEN-4	-
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	+

* A total of 10 μg of purified PLL1 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-2. This result coincides with the fact that the cloned region belongs to serotype 2. The recognition by HMAF against the other serotypes was less than in the case of serotype 2, in decreasing order: DEN-1, DEN-3 and DEN-4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. On the other hand, the Mab 3H5 had reactivity indeed. This recognition by Western blotting relied on disulphide bond since when the sample was reduced the signal was lost. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-2 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting.

EXAMPLE 4. Characterization of the antibody response generated by PLL1

A total of 25 Balb/c mice were i.p immunized with 35 ug of purified PLL1 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-2 were obtained while, no reactivity was obtained against the rest of the serotypes (table 2 and table 5). In addition, the hemagglutination inhibition assay (HIA) was done and only positive titers were found against DEN-2 (table 3 and table 5). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1:320 against DEN-2 were obtained. However, no neutralization of the viral infection was found against the rest of the serotypes (table 4 and table 5). These results indicate the high serotype-specificity of the antibodies elicited by PLL1.

Table 2. Antibody titers against DEN-2 from the sera obtained upon immunization of mice with PLL1.

Mouse	Titers anti-DEN-2 PLL1	Titers anti-DEN-2 PBSControl (-)
1	1/ 128 000	< 1: 100
2	1/ 64 000	< 1: 100
3	1/ 64 000	< 1: 100
4	1/ 128 000	< 1: 100
5	1/ 32 000	< 1: 100
6	1/ 32 000	< 1: 100
7	1/ 64 000	< 1: 100
8	1/ 32 000	< 1: 100
9	1/ 128 000	< 1: 100
10	1/ 512 000	< 1: 100

Table 3. Titers by HI of the sera from the animals immunized with PLL1.

Mouse	Titers by HI* anti-DEN-2 PLL1	Titers by HI anti-DEN-2 PBS C(-)
1	< 1: 5	< 1: 5
2	>1: 640	< 1: 5
3	1: 320	< 1: 5
4	1: 320	< 1: 5
5	>1: 640	< 1: 5
6	>1: 640	< 1: 5
7	>1: 640	< 1: 5
8	1: 320	< 1: 5
9	>1: 640	< 1: 5
10	< 1: 5	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 4. Viral neutralization assay with the sera of animals immunized with PLL1.

Mouse	Neutralizing titers* anti-DEN-2 PLL1	Neutralizing titers anti-DEN-2 PBS C(-)
1	1: 320	< 1: 5
2	1: 320	< 1: 5
3	1: 320	< 1: 5
4	1: 320	< 1: 5
5	1: 80	< 1: 5
6	1: 160	< 1: 5
7	1: 320	< 1: 5
8	1: 40	< 1: 5
9	1: 160	< 1: 5
10	1: 320	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 5. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PLL1.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1 (PLL1)	< 1/100	> 1: 128 000	< 1/100	< 1/100
2 (PLL1)	< 1/100	1: 128 000	< 1/100	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PLL1	< 1/ 5	>1/ 320	< 1/5	< 1/5

Mixture of sera *	Neutralizing titers *** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1 (PLL1)	< 1: 5	1: 320	< 1: 5	< 1: 5
2 (PLL1)	< 1: 5	1: 160	< 1: 5	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 5. Obtaining of PLL2

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-2 virus (Sec. Id. No. 22) was amplified with the

oligonucleotides identified in the list of sequences as Sequence No.1 and Sequence No. 3 from the DEN-2 virus strain genotype Jamaica (Deubel V., Kinney R.M., Trent D.W. Nucleotide sequence and deduced amino acid sequence of the nonstructural proteins of Dengue type 2 virus, Jamaica genotype: Comparative analysis of the full-length genome.Virology 1988.165:234-244).

The vector was created by digestion of the pM84 His plasmid with *Xba I/ Eco RI*, which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines (Sequence No. 26). This digestion permits the insertion of the amplified fragment by PCR within the coding region for a structural domain of the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells MM294 (Hanahan D. 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166:557-580) were transformed with the selected clone, called pLL2 (Fig. 2 and Sequence No. 27). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 10% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-2 virus. The protein was recognized in Immunoblotting by a HMAF anti-DEN-2 and was denominated PLL2 (Sequence No. 28).

EXAMPLE 6. Purification of the PLL2 protein

The biomass obtained from the *E. coli* strain transformed with pLL2 and grown at 37^0C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. From the soluble fraction a metal ions-affinity chromatography was done using theChelating-sepharose FF column previously coupled with Cu^{++} ions. The column was washed using Imidazolee 15 mM and theprotein was eluted with Imidazolee 100 mM. On the other hand, the protein associated to the insoluble fraction was extracted using Urea 8 M, and the supernatant, containing the PLL2 protein, was loaded onto a G-25 column to eliminate the chaotropic agent . The fraction obtained was then loaded onto theChelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The protein was eluted with Imidazolee 100 mM. Finally, the pure fraction of each form of the protein were loaded onto a G-25 column to obtain the protein in the formulation buffer NaCl 100 mM, KCl_2 2 mM, Na_2HPO_4 10 mM, pH 7.2, KH_2PO_4 1 mM (PBS). This preparations were used for immunological studies.

EXAMPLE 7. Antigenic characterization of PLL2

The purified fraction of PLL2 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 6).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-2. The recognition by HMAF against the other serotypes was less than in the case of serotype 2, in decreasing order: DEN-1, DEN-3 and DEN-4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Nevertheless, related to the Mab 3H5, a great reactivity was observed (even higher than those obtained with the PLL1) either by Dot blot and Western blot. Contrariously to the PLL1 results, the recognition with the Mab 3H5 was the same when the reducing agents are present in the sample, indicating a possible conformational difference between both proteins. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-2 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting.

Table 6. Reactivity of PLL2 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PLL2 sol, ins**
HMAF	DEN-1	++
HMAF	DEN-2	+++
HMAF	DEN-3	-
HMAF	DEN-4	-
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	+++

*A total of 10 μg of purified PLL2 was applied. The intensity of the signal obtained was evaluated from + to ++.

** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.

*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 8. Characterization of the antibody response generated by PLL2

A total of 25 Balb/c mice were i.p immunized with 35 ug of purified PLL2 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-2 were obtained while, no reactivity was obtained against the rest of the serotypes (table 7 and table 10). In addition, the hemagglutination inhibition assay (HI) was done and only positive titers were found against DEN-2 (table 8 and table 10). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-2 were obtained . Similarly to the results obtained with PLL1, no neutralization of the viral infection was found against the rest of the serotypes (table 9 and table 10). On the other hand, the results obtained with both variants of PLL2 were similar, indicating that the solubility status of the protein do not influece in the capacity of generating functional antibodies.

Table 7. Antibody titers against DEN-2 from the sera obtained upon immunization of mice with PLL2 soluble and insoluble.

Mouse	**Titers anti-DEN-2 (PLL2)**		**Titers anti-DEN-2 PBS C(-)**
	PLL2 s	**PLL2 ins**	
1	>1: 128 000	1: 64000	< 1: 100
2	1: 128 000	>1: 128 000	< 1: 100
3	>1: 128 000	1: 128 000	< 1: 100
4	>1: 128 000	>1: 128 000	< 1: 100
5	1:64 000	>1: 128 000	< 1: 100
6	>1: 128 000	1: 128 000	< 1: 100
7	1: 64000	>1: 128 000	< 1: 100
8	>1: 128 000	1: 64000	< 1: 100
9	>1: 128 000	1: 64000	< 1: 100
10	1: 128 000	>1: 128 000	< 1: 100

Table 8. Titers by HI of the sera from the animals immunized with PLL2 soluble and insoluble.

Mouse	Titers by HI* anti-DEN-2 (PLL2)		Titers by HI anti-DEN-2 PBS C(-)
	PLL2 s	PLL2 ins	
1	>1: 640	>1: 640	< 1: 5
2	>1: 640	>1: 640	< 1: 5
3	1: 320	>1: 640	< 1: 5
4	>1: 640	1: 320	< 1: 5
5	1: 320	< 1: 5	< 1: 5
6	>1: 640	>1: 640	< 1: 5
7	>1: 640	1: 320	< 1: 5
8	< 1: 5	1: 320	< 1: 5
9	1: 320	>1: 640	< 1: 5
10	>1: 640	>1: 640	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 9. Viral neutralization assay with the sera of animals immunized with PLL2 soluble and insoluble.

Mouse	Neutralizing titers* anti-DEN-2 (PLL2)		Neutralizing titers anti-DEN-2 PBS C(-)
	PLL2 s	PLL2 ins	
1	>1: 1280	>1: 1280	>1: 1280
2	>1: 1280	>1: 1280	< 1: 5
3	>1: 1280	1: 640	< 1: 5
4	1: 640	>1: 1280	< 1: 5
5	1: 640	1: 640	< 1: 5
6	>1: 1280	>1: 1280	< 1: 5
7	>1: 1280	>1:1280	< 1: 5
8	>1: 1280	>1: 1280	< 1: 5
9	>1: 1280	>1: 1280	< 1: 5
10	>1: 1280	>1: 1280	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 10. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PLL2 soluble and insoluble.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1 (PLL2 sol.)	< 1/100	> 1: 128 000	< 1/100	< 1/100
2 (PLL2 sol.)	< 1/100	1: 64000	< 1/100	< 1/100
1 (PLL2 ins.)	< 1/100	1: 64000	< 1/100	< 1/100
2 (PLL2 ins.)	< 1/100	> 1: 128 000	< 1/100	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PLL2 sol.	< 1/ 5	>1/ 320	< 1/5	< 1/5
PLL2 ins.	< 1/ 5	>1/ 320	< 1/5	< 1/5

Mixture of sera *	Neutralizing titers *** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1 (PLL2)	< 1: 5	1: 320	< 1: 5	< 1: 5
2 (PLL2)	< 1: 5	1: 160	< 1: 5	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 9. Obtaining of pLL3

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-2 virus (Sec. Id. No. 22) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.4 and Sequence No.5 from the DEN-2 virus strain genotype Jamaica (Deubel V., Kinney R.M., Trent D.W. Nucleotide sequence and deduced amino acid sequence of the nonstructural proteins of Dengue type 2 virus, Jamaica genotype: Comparative analysis of the full-length genome.Virology 1988.165:234-244).

The vector was created by digestion of the pD4 plasmid with *Bam HI/Bam HI* which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines without stop codon (Sequence No. 29). This digestion permits to the fusion of the amplified fragment by PCR after the C- terminal region for the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pLL3 (Fig. 3 and Sequence No. 30). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-2 virus. The protein was recognized in Immunoblotting by a HMAFI anti-DEN-2 and was denominated PLL3 (Sequence No. 31).

EXAMPLE 10. Purification of the PLL3 protein

The biomass obtained from the *E. coli* strain transformed with pLL2 and grown at 37^{0}C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. From the insoluble fraction, the protein was extracted using Urea 6 M, and the supernatant, containing the PLL3 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-Sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The colum was washed with Imidazolee 30 mM and the protein was eluted with Imidazole 100 mM. Finally, the pure fraction of the protein was loaded onto a G-25 column to obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 11. Antigenic characterization of PLL3

The purified fraction of PLL3 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 11).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-2. The recognition by HMAF against the other serotypes was lower than in the case of serotype 2, in decreasing order: DEN-1, DEN-3 and DEN-4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Nevertheless, related to the Mab 3H5, a great reactivity was observed (similar to those obtained with the PLL2) either by Dot blot and Western blot. Contrariously to the PLL1 results, the recognition with the Mab 3H5 was the same when the reducing agents are present in the sample, indicating a possible conformational difference between both proteins. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-2 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting. These results were similar to those obtained with PLL2.

Table 11. Reactivity of PLL3 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PLL3**

HMAF	DEN-1	++
HMAF	DEN-2	+++
HMAF	DEN-3	-
HMAF	DEN-4	-
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	+++

*A total of 10 μg of purified PLL3 was applied. The intensity of the signal obtained was evaluated from + to ++.

** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.

*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 12. Characterization of the antibody response generated by PLL3

A total of 25 Balb/c mice were i.p immunized with 35 ug of purified PLL3 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-2 were obtained while, no reactivity was obtained against the rest of the serotypes (table 12 and table 15). In addition, the hemagglutination inhibition (HI) assay was done and only positive titers were found against DEN-2 (table 13 and table 15). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-2 were obtained (table 14). No neutralization of the viral infection was found against the rest of the serotypes (table 15). Using the three tests, high levels of serotype-specific antibodies were detected , similar of those obtained after the immunization with the PLL2 protein.

Table 12. Antibody titers against DEN-2 from the sera obtained upon immunization of mice with PLL3.

Mouse	**Titers anti-DEN-2 (PLL3)**	**Titers anti-DEN-2 PBS C(-)**
1	1: 128 000	< 1: 100
2	>1: 128 000	< 1: 100
3	>1: 128 000	< 1: 100
4	1:64 000	< 1: 100
5	>1: 128 000	< 1: 100
6	1: 64 000	< 1: 100
7	>1: 128 000	< 1: 100

8	>1: 128 000	< 1: 100
9	1: 128 000	< 1: 100
10	>1: 128 000	< 1: 100

Table 13. Titers by HI of the sera from the animals immunized with PLL3.

Mouse	Titers by HI* anti-DEN-2 (PLL3)	Titers by HI anti-DEN-2 PBS C(-)
1	>1: 640	< 1: 5
2	1: 320	< 1: 5
3	1: 320	< 1: 5
4	>1: 640	< 1: 5
5	1: 320	< 1: 5
6	>1: 640	< 1: 5
7	>1: 640	< 1: 5
8	>1: 640	< 1: 5
9	1: 320	< 1: 5
10	>1: 640	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 14. Viral neutralization assay with the sera of animals immunized with PLL3.

Mouse	Neutralizing titers anti- DEN-2 PLL3	Neutralizing titers anti- DEN-2 PBS C(-)
1	>1: 1280	< 1: 5
2	>1: 1280	< 1: 5
3	>1: 1280	< 1: 5
4	1: 640	< 1: 5
5	>1: 1280	< 1: 5
6	>1: 1280	< 1: 5
7	>1: 1280	< 1: 5
8	>1: 1280	< 1: 5
9	1: 640	< 1: 5
10	>1: 1280	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 15. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PLL3.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)

1(PLL3)	< 1/100	> 1: 128 000	< 1/100	< 1/100
2(PLL3)	< 1/100	> 1: 128 000	< 1/100	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PLL3	< 1/ 5	>1/ 320	< 1/ 5	< 1/ 5

Mixture of sera *	Neutralizing titers *** anti-DEN-1	Neutralizing titers *** anti-DEN-2	Neutralizing titers *** anti-DEN-3	Neutralizing titers *** anti-DEN-4
1(PLL3)	< 1: 5	>1: 1280	< 1: 5	< 1: 5
2(PLL3)	< 1: 5	>1: 1280	< 1: 5	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 13. Obtaining of pLH1

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-1 virus (Sec. Id. No. 32) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.6 and Sequence No.7 from the DEN-1 virus strain genotype (Chu M.C., O'Rourke E.J., Trent D.W.Genetic relatedness among structural protein genes of dengue 1 virus strains.J. Gen. Virol.1989. 70:1701-1712).

The vector was created by digestion of the pM108 His plasmid with *Xba I/Bam HI*, which contains the nucleotide sequence that codifies for the N-terminal region of the MDH and for a sequence of 6 histidines (Sequence No. 23). Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pLH1 (Fig. 4 and Sequence No.33). Upon growing the colony in Luria Bertani (LB) medium, a SDS-PAGE of the cellular lysate was done. As a result a 25 kDA band was obtained, which accounted for 10% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the N-terminal region from the MDH protein and the DENe protein fragment from the DEN-1 virus. The protein was recognized in Immunoblotting by polyclonal antibodies (PA) anti-DEN-1 contained in the HMAF. This protein was denominated PLH1 (Sequence No.34).

EXAMPLE 14. Purification of the protein PLH1

The biomass obtained from the *E. coli* strain transformed with pLH1 and grown at 37°C was disrupted by French press. The recombinant protein was obtained preponderantly as insoluble form associated to the pellet of the cellular disruption. From the pellet, the protein was extracted with urea 7 M and the supernatant, containing the PLH1 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The protein was eluted with Imidazolee 60 mM and the obtained volume was loaded onto a G-25 column to finally obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 15. Antigenic characterization of PLH1

The purified fraction of PLH1 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 16).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-1. The recognition by HMAF against the other serotypes was lower than in the case of serotype 1. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-1 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting.

Table 16. Reactivity of PLH1 protein to monoclonal and polyclonal antibodies

Abs**	*Specificity****	*PLH1*
HMAF	DEN-1	++
HMAF	DEN-2	+
HMAF	DEN-3	-
HMAF	DEN-4	-
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

*A total of 10 μg of purified PLH1 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 16. Characterization of the antibody response generated by PLH1

A total of 25 Balb/c mice were i.p immunized with 35 ug of purified PLH1 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-1 were obtained while, no reactivity was obtained against the rest of the serotypes (table 17 and table 20). In addition, the HI assay was done and only positive titers were found against DEN-1 (table 18 and table 20). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1:320 against DEN-1 were obtained. However, no neutralization of the viral infection was found against the rest of the serotypes (table 19 and table 20). These results indicate the high serotype-specificity of the antibodies elicited by PLH1.

Table 17. Antibody titers against DEN-1 from the sera obtained upon immunization of mice with PLH1.

Mouse	Titers anti-DEN-1 (PLH1)	Titers anti-DEN-1 PBS C(-)
1	1/ 64 000	< 1: 100
2	1/ 128 000	< 1: 100
3	1/ 64 000	< 1: 100
4	1/ 32 000	< 1: 100
5	1/ 32 000	< 1: 100
6	1/ 64 000	< 1: 100
7	1/ 128 000	< 1: 100
8	1/ 64 000	< 1: 100
9	1/ 128 000	< 1: 100
10	1/ 128 000	< 1: 100

Table 18. Titers by HI of the sera from the animals immunized with PLH1.

Mouse	Titers by HI* anti-DEN-1 (PLH1)	Titers by HI* anti-DEN-1 PBS C(-)
1	>1: 640	< 1: 5
2	1: 320	< 1: 5
3	>1: 640	< 1: 5
4	>1: 640	< 1: 5
5	>1: 640	< 1: 5
6	1:320	< 1: 5

7	1: 40	< 1: 5
8	1:320	< 1: 5
9	>1: 640	< 1: 5
10	< 1: 5	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 19. Viral neutralization assay with the sera of animals immunized with PLH1.

Mouse	Neutralizing titers* anti-DEN-1 (PLH1)	Neutralizing titers* anti-DEN-1 PBS C(-)
1	1: 80	< 1: 5
2	1: 320	< 1: 5
3	1: 40	< 1: 5
4	1: 320	< 1: 5
5	1: 80	< 1: 5
6	1: 160	< 1: 5
7	1: 320	< 1: 5
8	1: 320	< 1: 5
9	1: 320	< 1: 5
10	1: 320	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 20. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PLH1.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1 (PLH1)	1: 128 000	< 1/100	< 1/100	< 1/100
2 (PLH1)	> 1: 128 000	< 1/100	< 1/100	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PLH1	>1: 320	< 1/ 5	< 1/ 5	< 1/ 5

Mixture of sera *	Neutralizing titers *** anti-DEN-1	Neutralizing titers *** anti-DEN-2	Neutralizing titers *** anti-DEN-3	Neutralizing titers *** anti-DEN-4
1(PLH1)	1: 160	< 1: 5	< 1: 5	< 1: 5
2(PLH1)	1: 320	< 1: 5	< 1: 5	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 17. Obtaining of pLH2

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-1 virus (Sequence No. 32) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.6 and Sequence No.8 from a DEN-1 viral strain (Chu M.C., O'Rourke E.J., Trent D.W.Genetic relatedness among structural protein genes of dengue 1 virus strains. J. Gen. Virol.1989. 70:1701-1712).

The vector was created by digestion of the pM84 His plasmid with *Xba I/ Eco RI*, which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines (Sequence No. 26). This digestion permits the insertion of the amplified fragment by PCR within the coding region for a structural domain of the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells MM294 were transformed with the selected clone, called pLH2 (Fig. 5 and Sequence No.35). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-1 virus. The protein was recognized in Immunoblotting by a HMAF anti-DEN-1 and was denominated PLH2 (Sequence No.36).

EXAMPLE 18. Purification of the PLH2 protein

The biomass obtained from the *E. coli* strain transformed with pLH2 and grown at 37^0C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. The protein associated to the insoluble fraction was extracted using Urea 7 M, and the supernatant, containing the PLH2 protein, was loaded onto a G-25 column to eliminate the chaotropic agent . The fraction obtained was then loaded onto theChelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The column was washed with Imidazolee 40 mM and the protein was eluted with Imidazole 100 mM. Finally, the pure fraction was loaded onto a G-25 column to obtain the protein in the formulation (PBS). This preparation was used for immunological studies.

EXAMPLE 19. Antigenic characterization of PLH2

The purified fraction of PLH2 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 21).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-1 (higher than those obtained with PLH1). The recognition by HMAF against the other serotypes was lower than in the case of serotype 1. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against five human sera of high titers and three of low titers against DEN-1 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting.

Table 21. Reactivity of PLH2 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PLH2*
HMAF	DEN-1	+++
HMAF	DEN-2	+
HMAF	DEN-3	-
HMAF	DEN-4	-
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

*A total of 10 μg of purified PLH2 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 20. Characterization of the antibody response generated by PLH2

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PLH2 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-1 were obtained while, no reactivity was obtained against the rest of the serotypes (table 22 and table 25). In addition, the HI assay was done and only positive titers were found against DEN-1 (table 23 and table 25). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280

against DEN-1 were obtained (table 24). No neutralization of the viral infection was found against the rest of the serotypes. (table 25).

Table 22. Antibody titers against DEN-1 from the sera obtained upon immunization of mice with PLH2.

Mouse	Titers anti-DEN-1 (PLH2)	Titers anti-DEN-1 PBS C(-)
1	1: 128 000	< 1: 100
2	>1: 128 000	< 1: 100
3	1: 64000	< 1: 100
4	>1: 128 000	< 1: 100
5	1: 128 000	< 1: 100
6	1: 64000	< 1: 100
7	>1: 128 000	< 1: 100
8	1: 128 000	< 1: 100
9	>1: 128 000	< 1: 100
10	>1: 128 000	< 1: 100

Table 23. Titers by HI of the sera from the animals immunized with PLH2.

Mouse	Titers by HI* anti-DEN-1 (PLH2)	Titers by HI anti-DEN -1 PBS C(-)
1	< 1: 5	< 1: 5
2	>1: 640	< 1: 5
3	1: 320	< 1: 5
4	1: 320	< 1: 5
5	>1: 640	< 1: 5
6	>1: 640	< 1: 5
7	>1: 640	< 1: 5
8	1: 320	< 1: 5
9	>1: 640	< 1: 5
10	< 1: 5	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 24 . Viral neutralization assay with the sera of animals immunized with PLH2.

Mouse	Neutralizing titers* anti-DEN-1 (PLH2)	Neutralizing titers* anti-DEN-1. C(-)
1	>1: 1280	< 1: 5
2	>1: 1280	< 1: 5
3	>1: 1280	< 1: 5
4	>1: 1280	< 1: 5

5	>1: 1280	< 1: 5
6	>1: 1280	< 1: 5
7	>1: 1280	< 1: 5
8	>1: 1280	< 1: 5
9	1: 640	< 1: 5
10	>1: 1280	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 25. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PLH2.

Mixture of sera *	**ELISA (anti-DEN-1)**	**ELISA (anti DEN-2)**	**ELISA(anti-DEN-3)**	**ELISA(anti-DEN- 4)**
1 (PLH2)	1: 64 000	< 1/100	< 1/100	< 1/100
2 (PLH2)	> 1: 128 000	< 1/100	< 1/100	< 1/100

Mixture of sera *	**HI**anti-DEN-1**	**HI anti-DEN-2**	**HI anti-DEN-3**	**HI anti-DEN-4**
PLH2	>1/ 320	< 1/ 5	< 1/ 5	< 1/ 5

Mixture of sera *	**Neutralizing titers*** anti-DEN-1**	**Neutralizing titers anti-DEN-2**	**Neutralizing titers anti-DEN-3**	**Neutralizing titers anti-DEN-4**
1(PLH2)	>1: 1280	< 1: 5	< 1: 5	< 1: 5
2(PLH2)	>1: 1280	< 1: 5	< 1: 5	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 21. Obtaining of pLH3

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-1 virus (Sec. Id. No. 32) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.9 and Sequence No.10 from the DEN-1 virus strain (Chu M.C., O'Rourke E.J., Trent D.W.Genetic relatedness among structural protein genes of dengue 1 virus strain. J. Gen. Virol.1989. 70:1701-1712).

The vector was created by digestion of the pD4 plasmid with *Bam HI/Bam HI* which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines without stop codon (Sequence No.29). This digestion permits the fusion of the amplified fragment by PCR after the C- terminal region for the MDH protein. Upon

ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pLH3 (Fig. 6 and Sequence No.37). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-1 virus. The protein was recognized in Immunoblotting by a HMAF anti-DEN-1 and was denominated PLH3 (Sequence No.38).

EXAMPLE 22. Purification of the PLH3 protein

The biomass obtained from the *E. coli* strain transformed with pLH3 and grown at 37^0C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. From the insoluble fraction, the protein was extracted using Urea 6 M, and the supernatant, containing the PLH3 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-Sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The colum was washed with Imidazolee 30 mM and the protein was eluted with Imidazole 250 mM. Finally, the pure fraction of the protein was loaded onto a G-25 column to obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 23. Antigenic characterization of PLH3

The purified fraction of PLH3 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 26).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-1. The recognition by HMAF against the other serotypes was lower than in the case of serotype 1. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-1 was measured, achieving a substantial signal in both cases by Dot blotting and Western blotting.

Table 26. Reactivity of PLH3 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PLH3*
HMAF	DEN-1	+++
HMAF	DEN-2	+
HMAF	DEN-3	-
HMAF	DEN-4	-
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PLH3 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 24. Characterization of the antibody response generated by PLH3

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PLH3 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-1 were obtained while, no reactivity was obtained against the rest of the serotypes (table 27 and table 30). In addition, the HI assay was done and only positive titers were found against DEN-1 (table 28andtable 30). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-1 were obtained (table 29). No neutralization of the viral infection was found against the rest of the serotypes (table 30).

Table 27. Antibody titers against DEN-1 from the sera obtained upon immunization of mice with PLH3.

Mouse	**Titers anti-DEN-1 PLH3**	**Titers anti-DEN-1 PBSControl (-)**
1	1: 64 000	< 1: 100
2	>1: 128 000	< 1: 100
3	1: 64 000	< 1: 100
4	>1: 128 000	< 1: 100
5	1: 128 000	< 1: 100
6	1: 128 000	< 1: 100
7	1: 128 000	< 1: 100
8	>1: 128 000	< 1: 100

9	1: 64 000	< 1: 100
10	>1: 128 000	< 1: 100

Table 28 . Titers by HI of the sera from the animals immunized with PLH3.

Mouse	Titers by HI* anti-DEN-1 PLH3	Titers by HI anti-DEN-1 PBS C(-)
1	1: 320	< 1: 5
2	>1: 640	< 1: 5
3	>1: 640	< 1: 5
4	1: 320	< 1: 5
5	1: 320	< 1: 5
6	>1: 640	< 1: 5
7	1: 320	< 1: 5
8	< 1: 5	< 1: 5
9	>1: 640	< 1: 5
10	>1: 640	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 29. Viral neutralization assay with the sera of animals immunized with PLH3.

Mouse	Neutralizing titers* anti- DEN-1 PLH3	Neutralizing titers* anti- DEN-1 PBS C(-)
1	>1: 1280	< 1: 5
2	>1: 1280	< 1: 5
3	>1: 1280	< 1: 5
4	1: 640	< 1: 5
5	1: 640	< 1: 5
6	>1: 1280	< 1: 5
7	>1: 1280	< 1: 5
8	>1: 1280	< 1: 5
9	>1: 1280	< 1: 5
10	>1: 1280	< 1: 5

*The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 30. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PLH3.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1(PLH3)	1: 128 000	< 1/100	< 1/100	< 1/100
2(PLH3)	> 1: 128 000	< 1/100	< 1/100	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PLH3	>1/ 320	< 1/ 5	< 1/ 5	< 1/ 5

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PLH3)	>1: 1280	< 1: 5	< 1: 5	< 1: 5
2(PLH3)	>1: 1280	< 1: 5	< 1: 5	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 25. Obtaining of pAZ1

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-3 virus (Seq. 39) was amplified with the oligonucleotides identified in the list of sequences as Sequence No. 11 and Sequence No.12 from the DEN-3 virus strain genotype (Osatomi K., Sumiyoshi H. Complete nucleotide sequence of dengue type 3 virus genome RNA. Virology.1990. 176(2):643-647).

The vector was created by digestion of the pM108 His plasmid with *Xba I/Bam HI*, which contains the nucleotide sequence that codifies for the N-terminal region of the MDH and for a sequence of 6 histidines (Sequence No. 23). Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pAZ1 (Fig. 7 and Sequence No.40). Upon growing the colony in Luria Bertani (LB) medium, a SDS-PAGE of the cellular lysate was done. As a result a 25 kDA band was obtained, which accounted for 10% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the N-terminal region from the MDH protein and the DENe protein fragment from the DEN-3 virus. The protein was recognized in Immunoblotting by polyclonal antibodies (PA) anti-DEN-3 contained in the HMAF. This protein was denominated PAZ1 (Sequence No.41).

EXAMPLE 26. Purification of the protein PAZ1

The biomass obtained from the *E. coli* strain transformed with pAZ1 and grown at 37^{0}C was disrupted by French press. The recombinant protein was obtained preponderantly as insoluble form associated to the pellet of the cellular disruption. From the pellet, the protein was extracted with urea 7 M and the supernatant, containing the PLH1 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The protein was eluted with Imidazolee 60 mM and the obtained volume was loaded onto a G-25 column to finally obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 27. Antigenic characterization of PAZ1

The purified fraction of PAZ1 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 31).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-3. The recognition by HMAF against the other serotypes was lower than in the case of serotype 3. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-3 was measured, achieving a substantial signal in both cases by Dot blotting Western blotting.

Table 31. Reactivity of PAZ1 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PAZ1*
HMAF	DEN-1	-
HMAF	DEN-2	+
HMAF	DEN-3	++
HMAF	DEN-4	+
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-

Mab 3H5	NT	-

* A total of 10 μg of purified PAZ1 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 28. Characterization of the antibody response generated by PAZ1

A total of 25 Balb/c mice were i.p immunized with 35 ug of purified PAZ1 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-1 were obtained while, no reactivity was obtained against the rest of the serotypes (table 32 and table 35). In addition, the HI assay was done and only positive titers were found against DEN-3 (table 33 and table 35). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1:320 against DEN- 3 were obtained. However, no neutralization of the viral infection was found against the rest of the serotypes (table 34 and table 35). These results indicate the high serotype-specificity of the antibodies elicited by PAZ1.

Table 32. Antibody titers against DEN-3 from the sera obtained upon immunization of mice with PAZ1.

Mouse	Titers anti-DEN-3 PAZ1	Titers anti-DEN-3 PBSControl (-)
1	1/ 64 000	< 1: 100
2	1/ 128 000	< 1: 100
3	1/ 32 000	< 1: 100
4	1/ 64 000	< 1: 100
5	1/ 64 000	< 1: 100
6	1/ 128 000	< 1: 100
7	1/ 64 000	< 1: 100
8	1/ 64 000	< 1: 100
9	1/ 128 000	< 1: 100
10	1/ 128 000	< 1: 100

Table 33. Titers by HI of the sera from the animals immunized with PAZ1.

Mouse	Titers by HI* anti-DEN-3 PAZ1	Titers by HI anti-DEN-3 PBS C(-)
1	>1: 640	< 1: 5
2	1: 320	< 1: 5
3	1: 320	< 1: 5
4	1: 640	< 1: 5
5	< 1/ 5	< 1: 5
6	1: 320	< 1: 5
7	< 1/ 5	< 1: 5

8	1: 320	< 1: 5
9	>1: 640	< 1: 5
10	>1: 640	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 34. Viral neutralization assay with the sera of animals immunized with PAZ1.

Mouse	Neutralizing titers anti- DEN-3 PAZ1	Neutralizing titers anti-DEN-3 PBS C(-)
1	1: 160	< 1: 5
2	1: 320	< 1: 5
3	1: 320	< 1: 5
4	1: 320	< 1: 5
5	1: 40	< 1: 5
6	1: 40	< 1: 5
7	1: 320	< 1: 5
8	1: 320	< 1: 5
9	1: 160	< 1: 5
10	1: 320	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 35. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PAZ1.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti-DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1 (PAZ1)	< 1/100	< 1/100	1: 64 000	< 1/100
2 (PAZ1)	< 1/100	< 1/100	> 1: 128 000	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PAZ1	< 1/ 5	< 1/ 5	>1: 320	< 1/ 5

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PAZ1)	< 1: 5	< 1: 5	1: 320	< 1: 5
2(PAZ1)	< 1: 5	< 1: 5	1: 320	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 29. Obtaining of pAZ2

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-3 virus (Sequence No.39) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.11 and Sequence No.13 from a DEN-3 viral strain (Osatomi K., Sumiyoshi H. Complete nucleotide sequence of dengue type 3 virus genome RNA. Virology.1990. 176(2):643-647).

The vector was created by digestion of the pM84 His plasmid with *Xba I/ Eco RI*, which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines (Sequence No. 26). This digestion permits the insertion of the amplified fragment by PCR within the coding region for a structural domain of the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells MM294 were transformed with the selected clone, called pAZ2 (Fig. 8 and Sequence No.42). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-3 virus. The protein was recognized in Immunoblotting by a HMAF anti-DEN-3 and was denominated PAZ2 (Sequence No.43).

EXAMPLE 30. Purification of the PAZ2 protein

The biomass obtained from the *E. coli* strain transformed with pAZ2 and grown at 37^0C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. The protein associated to the insoluble fraction was extracted using Urea 7 M, and the supernatant, containing the PAZ2 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto theChelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The column was washed with Imidazolee 40 mM and the protein was eluted with Imidazole 100 mM. Finally, the pure fraction was loaded onto a G-25 column to obtain the protein in the formulation (PBS). This preparation was used for immunological studies.

EXAMPLE 31. Antigenic characterization of PAZ2

The purified fraction of PAZ2 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 36).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-3 (higher than those obtained with PAZ1). The recognition by HMAF against the other serotypes was lower than in the case of serotype 3. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against five human sera of high titers and three of low titers against DEN-3 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting.

Table 36. Reactivity of PAZ2 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PAZ2*
HMAF	DEN-1	-
HMAF	DEN-2	-
HMAF	DEN-3	+++
HMAF	DEN-4	+
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PAZ2 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype

EXAMPLE 32. Characterization of the antibody response generated by PAZ2

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PAZ2 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-3 were obtained while, no reactivity was obtained against the rest of the serotypes (table 37 and table 40). In addition, the HI assay was done and only positive titers were found against DEN-3 (table 38 and table 40). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-3 were obtained (table 39). No neutralization of the viral infection was found against the rest of the serotypes (table 40).

Table 37. Antibody titers against DEN-3 from the sera obtained upon immunization of mice with PAZ2.

Mouse	Titers anti-DEN-3 PAZ2	Titers anti-DEN-3 PBS Control (-)
1	>1: 128 000	< 1: 100
2	1: 128 000	< 1: 100
3	>1: 128 000	< 1: 100
4	>1: 128 000	< 1: 100
5	1: 128 000	< 1: 100
6	>1: 128 000	< 1: 100
7	1: 64000	< 1: 100
8	>1: 128 000	< 1: 100
9	1: 64000	< 1: 100
10	>1: 128 000	< 1: 100

Table 38. Titers by HI of the sera from the animals immunized with PAZ2.

Mouse	Titers anti-DEN-3 PAZ2	Titers anti-DEN-3 PBS
1	>1: 640	< 1: 5
2	1: 320	< 1: 5
3	>1: 640	< 1: 5
4	>1: 640	< 1: 5
5	>1: 640	< 1: 5
6	1: 320	< 1: 5
7	<1: 5	< 1: 5
8	1: 320	< 1: 5
9	>1: 640	< 1: 5
10	>1: 640	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 39. Viral neutralization assay with the sera of animals immunized with PAZ2.

Mouse	Neutralizing titers anti- DEN-3 PAZ2	Neutralizing titers anti-DEN-3 PBS C(-)
1	>1: 1280	< 1: 5
2	>1: 1280	< 1: 5
3	>1: 1280	< 1: 5
4	>1: 1280	< 1: 5
5	>1: 1280	< 1: 5
6	1: 640	< 1: 5
7	>1: 1280	< 1: 5
8	1: 640	< 1: 5
9	>1: 1280	< 1: 5
10	1: 640	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 40. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PAZ2.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1 (PAZ2)	< 1/100	< 1/100	> 1: 128 000	< 1/100
2(PAZ2)	< 1/100	< 1/100	> 1: 128 000	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PAZ2	< 1/ 5	< 1/ 5	>1/ 320	< 1/ 5

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PAZ2)	< 1: 5	< 1: 5	>1: 1280	< 1: 5
2(PAZ2)	< 1: 5	< 1: 5	>1: 1280	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 33. Obtaining of pAZ3

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-3 virus (Seq. 39) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.14 and Sequence No.15 from the DEN-3 viral strain (Osatomi K., Sumiyoshi H. Complete nucleotide sequence of dengue type 3 virus genome RNA. Virology.1990. 176(2):643-647).

The vector was created by digestion of the pD4 plasmid with *Bam HI/Bam HI* which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines without stop codon (Sequence No.29). This digestion permits the fusion of the amplified fragment by PCR after the C- terminal region for the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pAZ3 (Fig. 9 and Sequence No.44). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-3 virus. The protein was recognized

in Immunoblotting by a HMAF anti-DEN-3 and was denominated PAZ3 (Sequence No.45).

EXAMPLE 34. Purification of the PAZ3 protein

The biomass obtained from the *E. coli* strain transformed with pAZ3 and grown at 37°C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. From the insoluble fraction, the protein was extracted using Urea 7 M, and the supernatant, containing the PAZ3 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-Sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The colum was washed with Imidazolee 45 mM and the protein was eluted with Imidazole 230 mM. Finally, the pure fraction of the protein was loaded onto a G-25 column to obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 35. Antigenic characterization of PAZ3

The purified fraction of PAZ3 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 26).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-3. The recognition by HMAF against the other serotypes was lower than in the case of serotype 3. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-3 was measured, achieving a substantial signal in both cases by Dot blotting and Western blotting.

Table 41. Reactivity of PAZ3 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PAZ3*
HMAF	DEN-1	-
HMAF	DEN-2	-
HMAF	DEN-3	+++
HMAF	DEN-4	+
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PAZ3 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 36. Characterization of the antibody response generated by PAZ3

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PAZ3 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-3 were obtained while, no reactivity was obtained against the rest of the serotypes (table 42 and table 45). In addition, the HI assay was done and only positive titers were found against DEN-3 (table 43 and table 45). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-3 were obtained (table 44). No neutralization of the viral infection was found against the rest of the serotypes (table 45).

Table 42. Antibody titers against DEN-3 from the sera obtained upon immunization of mice with PAZ3.

Mouse	Titers anti-DEN-3 PAZ3	Titers anti-DEN-3 PBSControl (-)
1	>1: 128 000	< 1: 100
2	1: 128 000	< 1: 100
3	>1: 128 000	< 1: 100
4	1: 128 000	< 1: 100
5	1: 128 000	< 1: 100
6	>1: 128 000	< 1: 100
7	>1: 128 000	< 1: 100
8	1: 128 000	< 1: 100
9	1: 128 000	< 1: 100
10	>1: 128 000	< 1: 100

Table 43. Titers by HI of the sera from the animals immunized with PAZ3.

Mouse	Titers by HI* anti-DEN-3 PAZ3	Titers by HI anti-DEN-3 PBS C(-)
1	>1: 640	< 1: 5
2	>1: 640	< 1: 5
3	1: 320	< 1: 5
4	< 1: 5	< 1: 5
5	>1: 640	< 1: 5
6	< 1: 5	< 1: 5
7	1: 320	< 1: 5
8	>1: 640	< 1: 5
9	>1: 640	< 1: 5
10	1: 320	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 44. Viral neutralization assay with the sera of animals immunized with PAZ3.

Mouse	Neutralizing titers anti- DEN-3 PAZ3	Neutralizing titers anti- DEN-3 PBS C(-)
1	>1: 1280	< 1: 5
2	1: 640	< 1: 5
3	>1: 1280	< 1: 5
4	>1: 1280	< 1: 5
5	>1: 1280	< 1: 5
6	>1: 1280	< 1: 5
7	>1: 1280	< 1: 5
8	>1: 1280	< 1: 5
9	>1: 1280	< 1: 5
10	>1: 1280	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 45. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PAZ3.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1(PAZ3)	< 1/100	< 1/100	> 1: 128 000	< 1/100
2(PAZ3)	< 1/100	< 1/100	1: 128 000	< 1/100

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PAZ3	< 1/ 5	< 1/ 5	>1/ 320	< 1/ 5

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PAZ3)	< 1: 5	< 1: 5	>1: 1280	< 1: 5
2(PAZ3)	< 1: 5	< 1: 5	>1: 1280	< 1: 5

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 37. Obtaining of pID1

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-4 virus (Sequence No.46) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.17 and Sequence

No.18 from the DEN-4 virus strain genotype (Zhao B., Mackow E.R., Buckler-White A.J., Markoff L., Chancock R.M., Lai C.-J., Makino Y. Cloning full-length Dengue type 4 viral DNA sequences: Analysis of genes coding for structural proteins. Virology 1986. 155:77-88).

The vector was created by digestion of the pM108 His plasmid with *Xba I/Bam HI*, which contains the nucleotide sequence that codifies for the N-terminal region of the MDH and for a sequence of 6 histidines (Sequence No. 23). Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pID1 (Fig.10 and Sequence No.47). Upon growing the colony in Luria Bertani (LB) medium, a SDS-PAGE of the cellular lysate was done. As a result a 25 kDA band was obtained, which accounted for 10% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the N-terminal region from the MDH protein and the DENe protein fragment from the DEN-4 virus. The protein was recognized in Immunoblotting by polyclonal antibodies (PA) anti-DEN-4 contained in the HMAF. This protein was denominated PID1 (Sequence No.48).

EXAMPLE 38. Purification of the protein PID1

The biomass obtained from the *E. coli* strain transformed with pID1 and grown at $37^{0}C$ was disrupted by French press. The recombinant protein was obtained preponderantly as insoluble form associated to the pellet of the cellular disruption. From the pellet, the protein was extracted with urea 6 M and the supernatant, containing the PID1 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The protein was eluted with Imidazolee 60 mM and the obtained volume was loaded onto a G-25 column to finally obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 39. Antigenic characterization of PID1

The purified fraction of PID1 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 46).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-4. The recognition by HMAF against the other serotypes was lower than in the case of serotype 4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-4 was measured, achieving a substantial signal in both cases by Dot blotting Western blotting.

Table 46. Reactivity of PID1 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PID1*
HMAF	DEN-1	-
HMAF	DEN-2	-
HMAF	DEN-3	+
HMAF	DEN-4	++
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PID1 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 40. Characterization of the antibody response generated by PID1

A total of 25 Balb/c mice were i.p immunized with 35 ug of purified PID1 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-1 were obtained while, no reactivity was obtained against the rest of the serotypes (table 47andtable 50). In addition, the HI assay was done and only positive titers were found against DEN-4 (table 48andtable 50). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1:320 against DEN- 4 were obtained. However, no neutralization of the viral infection was found against the rest of the serotypes (table 49andtable 50). These results indicate the high serotype-specificity of the antibodies elicited by PID1.

Table 47. Antibody titers against DEN-4 from the sera obtained upon immunization of mice with PID1.

Mouse	**Titers anti-DEN-4 PID1**	**Titers anti-DEN-4 PBS Control (-)**
1	1/ 128 000	< 1: 100
2	1/ 128 000	< 1: 100
3	1/ 64 000	< 1: 100

4	1/ 64 000	< 1: 100
5	1/ 128 000	< 1: 100
6	1/ 32 000	< 1: 100
7	1/ 128 000	< 1: 100
8	1/ 32 000	< 1: 100
9	1/ 128 000	< 1: 100
10	1/ 128 000	< 1: 100

Table 48. Titers by HI of the sera from the animals immunized with PID1.

Mouse	Titers by HI* anti-DEN-4 PID1	Titers by HI anti-DEN-4 PBS C(-)
1	1: 320	< 1: 5
2	1: 320	< 1: 5
3	1: 640	< 1: 5
4	1:40	< 1: 5
5	< 1/ 5	< 1: 5
6	1: 320	< 1: 5
7	1: 640	< 1: 5
8	1: 640	< 1: 5
9	1:40	< 1: 5
10	1: 320	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 49. Viral neutralization assay with the sera of animals immunized with PID1.

Mouse	Neutralizing titers anti- DEN-4 PID1	Neutralizing titers anti-DEN-4 PBS C(-)
1	1: 320	< 1: 5
2	1: 80	< 1: 5
3	1: 320	< 1: 5
4	1: 320	< 1: 5
5	1: 160	< 1: 5
6	1: 320	< 1: 5
7	1: 320	< 1: 5
8	1: 320	< 1: 5
9	1: 160	< 1: 5
10	1: 40	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 50. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PID1.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1 (PID1)	< 1/100	< 1/100	< 1/100	1: 64 000
2(PID1)	< 1/100	< 1/100	< 1/100	> 1: 128 000

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PID1	< 1/ 5	< 1/ 5	< 1/ 5	>1:320

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PID1)	< 1: 5	< 1: 5	< 1: 5	1: 160
2(PID1)	< 1: 5	< 1: 5	< 1: 5	1: 320

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 41. Obtención de pID2

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-4 virus (Sequence No.46) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.16 and Sequence No.18 from a DEN-4 viral strain (Zhao B., Mackow E.R., Buckler-White A.J., Markoff L., Chancock R.M., Lai C.-J., Makino Y. Cloning full-length Dengue type 4 viral DNA sequences: Analysis of genes coding for structural proteins. Virology 1986. 155:77-88.

The vector was created by digestion of the pM84 His plasmid with *Xba I/ Eco RI*, which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines (Sequence No. 26). This digestion permits the insertion of the amplified fragment by PCR within the coding region for a structural domain of the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells MM294 were transformed with the selected clone, called pID2 (Fig. 11 and Sequence No.49). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH

protein and the DENe protein fragment from the DEN-4 virus. The protein was recognized in Immunoblotting by a HMAF anti-DEN-4 and was denominated PID2 (Sequence No.50).

EXAMPLE 42. Purification of the PID2 protein

The biomass obtained from the *E. coli* strain transformed with pID2 and grown at 37°C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. The protein associated to the insoluble fraction was extracted using Urea 6 M, and the supernatant, containing the PID2 protein, was loaded onto a G-25 column to eliminate the chaotropic agent . The fraction obtained was then loaded onto theChelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The column was washed with Imidazolee 30 mM and the protein was eluted with Imidazole 250 mM. Finally, the pure fraction was loaded onto a G-25 column to obtain the protein in the formulation (PBS). This preparation was used for immunological studies.

EXAMPLE 43. Caracterización antigénica de PID2

The purified fraction of PID2 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 51).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-4 (higher than those obtained with PID1). The recognition by HMAF against the other serotypes was lower than in the case of serotype 4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against five human sera of high titers and three of low titers against DEN-3 was measured, achieving a substantial signal in both cases by Western blotting and Dot blotting.

Table 51. Reactivity of PID2 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PID2*
HMAF	DEN-1	-
HMAF	DEN-2	-
HMAF	DEN-3	+
HMAF	DEN-4	+++
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PID2 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 44. Characterization of the antibody response generated by PID2

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PID2 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-4 were obtained while, no reactivity was obtained against the rest of the serotypes (table 52 and table 55). In addition, the HI assay was done and only positive titers were found against DEN-4 (table 53 and table 55). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-4 were obtained (table 54). No neutralization of the viral infection was found against the rest of the serotypes (table 55).

Table 52. Antibody titers against DEN-4 from the sera obtained upon immunization of mice with PID2.

Mouse	Titers anti-DEN-4 PID2	Titers anti-DEN-4 PBSControl (-)
1	1: 64000	< 1: 100
2	>1: 128 000	< 1: 100
3	1: 128 000	< 1: 100
4	>1: 128 000	< 1: 100
5	1: 64000	< 1: 100
6	>1: 128 000	< 1: 100
7	>1: 128 000	< 1: 100
8	>1: 128 000	< 1: 100
9	>1: 128 000	< 1: 100
10	1: 128 000	< 1: 100

Table 53. Titers by HI of the sera from the animals immunized with PID2.

Mouse	Titers by HI* anti-DEN-4 (PID2)	Titers by HI anti-DEN-4 PBS C(-)
1	1: 320	< 1: 5
2	1: 320	< 1: 5
3	1: 640	< 1: 5
4	1:40	< 1: 5
5	< 1/ 5	< 1: 5
6	1: 320	< 1: 5
7	1: 640	< 1: 5
8	1: 640	< 1: 5
9	1:40	< 1: 5
10	1: 320	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 54. Viral neutralization assay with the sera of animals immunized with PID2.

Mouse	Neutralizing titers anti- DEN-4 PID2	Neutralizing titers anti-DEN-4 PBS C(-)
1	>1: 1280	< 1: 5
2	>1: 1280	< 1: 5
3	>1: 1280	< 1: 5
4	>1: 1280	< 1: 5
5	1: 640	< 1: 5
6	>1: 1280	< 1: 5
7	>1: 1280	< 1: 5
8	>1: 1280	< 1: 5
9	1: 640	< 1: 5
10	>1: 1280	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 55. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PID2.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1(PID2)	< 1/100	< 1/100	< 1/100	> 1: 128 000
2(PID2)	< 1/100	< 1/100	< 1/100	1: 64 000

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PID2	< 1/ 5	< 1/ 5	< 1/ 5	>1/ 320

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PID2)	< 1: 5	< 1: 5	< 1: 5	>1: 1280
2(PID2)	< 1: 5	< 1: 5	< 1: 5	>1: 1280

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 45. Obtaining of pID3

The nucleotide sequence that codifies for the amino acids from 286 to 426 of the envelope protein from the DEN-4 virus (Sequence No.46) was amplified with the oligonucleotides identified in the list of sequences as Sequence No.19 and Sequence No.20 from the DEN-4 viral strain (Zhao B., Mackow E.R., Buckler-White A.J., Markoff L., Chancock R.M., Lai C.-J., Makino Y. Cloning full-length Dengue type 4 viral DNA sequences: Analysis of genes coding for structural proteins. Virology 1986. 155:77-88).

The vector was created by digestion of the pD4 plasmid with *Bam HI/Bam HI* which contains the nucleotide sequence that codifies for the MDH protein and for a sequence of 6 histidines without stop codon (Sequence No.29). This digestion permits the fusion of the amplified fragment by PCR after the C- terminal region for the MDH protein. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells W3110 were transformed with the selected clone, called pID3 (fig. 12 and Sequence No.51). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 80 kDA band was obtained, which accounted for 20% of the total cellular proteins. The size of the protein obtained corresponded to the sum of the size of the MDH protein and the DENe protein fragment from the DEN-4 virus. The protein was recognized in Immunoblotting by a HMAF anti-DEN-4 and was denominated PID3 (Sequence No.52).

EXAMPLE 46. Purificación de la proteína PID3

The biomass obtained from the *E. coli* strain transformed with pID3 and grown at 37^0C was disrupted by French press. The recombinant protein was obtained in both forms: soluble and insoluble. From the insoluble fraction, the protein was extracted using Urea 6 M, and the supernatant, containing the PID3 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-Sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. The colum was washed with Imidazolee 45 mM and the protein was eluted with Imidazole 200 mM. Finally, the pure fraction of the protein was loaded onto a G-25 column to obtain the protein in the formulation buffer (PBS). This preparation was used for immunological studies.

EXAMPLE 47. Antigenic characterization of PID3

The purified fraction of PID3 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 56).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-4. The recognition by HMAF against the other serotypes was lower than in the case of serotype 4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint

Louis Encephalitis virus had not any recognition at all. Finally, the reactivity against three human sera of high titers and three of low titers against DEN-4 was measured, achieving a substantial signal in both cases by Dot blotting and Western blotting.

Table 56. Reactivity of PID3 protein to monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PID3*
HMAF	DEN-1	-
HMAF	DEN-2	-
HMAF	DEN-3	+
HMAF	DEN-4	+++
HMAF	EEE	-
HMAF	YFV	-
HMAF	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PID3 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 48. Characterization of the antibody response generated by PID3

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PAZ3 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-4 were obtained while, no reactivity was obtained against the rest of the serotypes (table 57 and table 60). In addition, the HI assay was done and only positive titers were found against DEN-4 (table 58 and table 60). Finally, the *in vitro* neutralization assay was done and neutralization titers of 1: 1280 against DEN-4 were obtained (table 59). No neutralization of the viral infection was found against the rest of the serotypes (table 60).

Table 57. Antibody titers against DEN-4 from the sera obtained upon immunization of mice with PID3.

Mouse	Titers anti-DEN-4 (PID3)	Titers anti-DEN-4 PBSControl (-)
1	>1: 128 000	< 1: 100
2	>1: 128 000	< 1: 100
3	>1: 128 000	< 1: 100
4	1: 64 000	< 1: 100
5	1: 64 000	< 1: 100
6	>1: 128 000	< 1: 100
7	1: 128 000	< 1: 100
8	>1: 128 000	< 1: 100
9	1: 128 000	< 1: 100
10	>1: 128 000	< 1: 100

Table 58. Titers by HI of the sera from the animals immunized with PID3.

Mouse	Titers by HI* anti-DEN-4 PID3	Titers by HI anti-DEN-4 PBS C(-)
1	>1: 640	< 1: 5
2	1: 320	< 1: 5
3	>1: 640	< 1: 5
4	>1: 640	< 1: 5
5	< 1: 5	< 1: 5
6	>1: 640	< 1: 5
7	1: 320	< 1: 5
8	>1: 640	< 1: 5
9	1: 320	< 1: 5
10	>1: 640	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 59. Viral neutralization assay with the sera of animals immunized with PID3.

Mouse	Neutralizing titers* anti- DEN-4 PID3	Neutralizing titers anti-DEN-4 PBS C(-)
1	>1: 1280	< 1: 5
2	>1: 1280	< 1: 5
3	>1: 1280	< 1: 5
4	>1: 1280	< 1: 5
5	>1: 1280	< 1: 5
6	>1: 1280	< 1: 5
7	>1: 1280	< 1: 5
8	1: 640	< 1: 5
9	>1: 1280	< 1: 5
10	>1: 1280	< 1: 5

* The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 60. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PID3.

Mixture of sera *	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1(PID3)	< 1/100	< 1/100	< 1/100	> 1: 128 000
2(PID3)	< 1/100	< 1/100	< 1/100	1: 128 000

Mixture of sera *	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PID3	< 1/ 5	< 1/ 5	< 1/ 5	>1/ 320

Mixture of sera *	Neutralizing titers*** anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PID3)	< 1: 5	< 1: 5	< 1: 5	>1: 1280
2(PID3)	< 1: 5	< 1: 5	< 1: 5	>1/1280

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 49. Obtaining of pD4D2

The nucleotide sequence that codifies for the amino acids 286 to 426 of the E protein from the DEN-4 virus (Sequence No.46) was amplified with the oligonuclotides identified in the sequencing list as Sequence No.16 and Sequence No.21 from the viral strain of DEN-4 (Zhao B., Mackow E.R., Buckler-White A.J., Markoff L., Chancock R.M., Lai C.-J., Makino Y. Cloning full-length Dengue type 4 viral DNA sequences: Analysis of genes coding for structural proteins. Virology 1986. 155:77-88).

The vector was created by digestion *Xba /Xba I* of the pLL3 plasmid, which contains the MDH gene plus a sequence of 6 histidines in the 3' region of the gene and the sequence of the E fragment from DEN-2 in the 3' end. As result, two regions of the E protein from serotypes 2 and 4 were obtained, fused to the same MDH gene. Upon ligation, the potential recombinants were analyzed by restriction enzyme digestion and positive clones were sequenced to check up the junctions. Competent cells MM294 were transformed with the selected clone, called pD4D2 (Fig.13 and Sequence No.53). Upon growing the colony in LB medium, a SDS-PAGE of the cellular lysate was done. As a result a 110 kDa band was obtained, which accounted for 20% of the total cellular proteins. The size of the

protein obtained corresponded to the sum of the MDH protein and the two fragments of the DENe protein from Dengue virus. This protein was recognized by polyclonal antibodies anti-DEN-2 and anti-DEN-4 contained in the HMAF. This protein was denominated PD4D2 (Sequence No.54).

EXAMPLE 50. Purification of the PD4D2 protein

The biomass obtained from the *E. coli* strain transformed with pD4D2 and grown at 37^0C was disrupted by French press. The recombinant protein was obtained either soluble or insoluble. From the pellet the protein was extracted with urea 6 M and the supernatant, containing the PD4D2 protein, was loaded onto a G-25 column to eliminate the chaotropic agent. The fraction obtained was then loaded onto the Chelating-sepharose FF column (*Pharmacia, UK*), in the presence of Cu^{++} ions. A washing step was done with Imidazolee 30 mM and the protein was eluted with Imidazole 250 mM. Finally, the pure preparation was loaded onto a G-25 column to obtain the protein in the formulation buffer and use it for immunological studies.

EXAMPLE 51. Antigenic characterization of PD4D2

The purified fraction of PD4D2 was characterized either by its recognition by different polyclonal sera and/or murine monoclonal antibodies, as well as by positive human sera to Dengue (table 61).

The highest recognitions in Dot blotting were obtained with the HMAF anti-DEN-2 and anti-DEN-4. The recognition of the two other serotypes was less than for the case of serotypes 2 and 4. The antibodies generated by other flavivirus like the Yellow Fever virus and the Saint Louis Encephalitis virus had not any recognition at all. On the other hand, the Mab 3H5 had reactivity indeed, similar to that obtained for PLL2andPLL3. Finally, the reactivity against human sera of high and low titers against DEN-2 and DEN-4 was measured, achieving a substantial signal for both cases by Western blotting and Dot blotting.

Table 61. Reactivity of PD4D2 protein against monoclonal and polyclonal antibodies.

Abs**	*Specificity****	*PD4D2*
HMAF	DEN-1	-
HMAF	DEN-2	+++
HMAF	DEN-3	-
HMAF	DEN-4	+++
HMAF	EEE	-
HMAF		

HMAF	YFV	-
	SLV	-
Mab 3H5	NT	-

* A total of 10 μg of purified PD4D2 was applied. The intensity of the signal obtained was evaluated from + to ++.
** HMAF were used 1:100 while the Mab 3H5 was used in dilution 1:1000.
*** EEE: Equine Encephalitis virus. YFV: Yellow Fever virus. SLV: Saint Louis Encephalitis virus. NT: Neutralizing specific-serotype.

EXAMPLE 52. Characterization of the antibody response generated by PD4D2

A total of 25 Balb/c mice were i.p immunized with 20 ug of purified PD4D2 in Freund adjuvant; 10 animals were bled after four doses and the antibodies anti-DEN were evaluated by ELISA. High antibody titers against DEN-2 and DEN-4 were obtained while, no reactivity was obtained against the rest of the serotypes (table 62 and table 65). In addition, the hemagglutination inhibition assay (HI) was done and only positive titers were found against DEN-2 and DEN-4 (table 63 and table 65). Finally, the *in vitro* neutralization assay was done and neutralization titers of >1:1280 against DEN-2 and >1:1280 against DEN-4 were obtained (table 64). No neutralization of the viral infection was found against the rest of the serotypes (table 65).

Table 62. Antibody titers against DEN-2 and DEN-4 from the sera obtained upon immunization of mice with PD4D2.

Mouse	Titers by ELISA (PD4D2)		Titers by ELISA PBS C(-)	
	Anti-DEN-4	Anti-DEN-2	Anti-DEN-4	Anti-DEN-2
1	>1: 128 000	>1: 128 000	< 1: 100	< 1: 100
2	1: 128 000	1: 128 000	< 1: 100	< 1: 100
3	>1: 128 000	>1: 128 000	< 1: 100	< 1: 100
4	>1: 128 000	>1: 128 000	< 1: 100	< 1: 100
5	1:64 000	>1: 128 000	< 1: 100	< 1: 100
6	>1: 128 000	1: 128 000	< 1: 100	< 1: 100
7	1: 64 000	>1: 128 000	< 1: 100	< 1: 100
8	>1: 128 000	>1: 128 000	< 1: 100	< 1: 100
9	>1: 128 000	1: 128 000	< 1: 100	< 1: 100
10	1: 128 000	>1: 128 000	< 1: 100	< 1: 100

Table 63. Titers by HI of the sera from the animals immunized with PD4D2.

Mouse	Titers by HI (PD4D2)		Titers by HI PBS C(-)	
	Anti-DEN-4	Anti-DEN-2	Anti-DEN-4	Anti-DEN-2
1	>1: 640	>1: 640	< 1: 5	< 1: 5
2	>1: 640	>1: 640	< 1: 5	< 1: 5
3	>1: 640	1: 320	< 1: 5	< 1: 5
4	>1: 640	>1: 640	< 1: 5	< 1: 5
5	1: 320	1: 640	< 1: 5	< 1: 5
6	>1: 640	>1: 640	< 1: 5	< 1: 5
7	>1: 640	>1: 640	< 1: 5	< 1: 5
8	1: 320	1: 320	< 1: 5	< 1: 5
9	1: 320	1: 320	< 1: 5	< 1: 5
10	>1: 640	>1: 640	< 1: 5	< 1: 5

*The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.

Table 64. Viral neutralization assay with the sera of animals immunized with PD4D2.

Mouse	Neutralizing titers* (PD4D2)		Neutralizing titers PBS C(-)	
	Anti-DEN-4	Anti-DEN-2	Anti-DEN-4	Anti-DEN-2
1	>1: 1280	>1: 1280	< 1: 5	< 1: 5
2	>1: 1280	>1: 1280	< 1: 5	< 1: 5
3	1: 1280	1: 1280	< 1: 5	< 1: 5
4	>1: 1280	>1: 1280	< 1: 5	< 1: 5
5	1: 640	1: 1280	< 1: 5	< 1: 5
6	>1: 1280	>1: 1280	< 1: 5	< 1: 5
7	>1:1280	>1:1280	< 1: 5	< 1: 5
8	1: 640	>1: 1280	< 1: 5	< 1: 5
9	>1: 1280	>1: 1280	< 1: 5	< 1: 5
10	>1: 1280	>1: 1280	< 1: 5	< 1: 5

*The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

Table 65. Cross-reactivity assay against all the viral serotypes by ELISA, HI and viral neutralization with the sera of animals immunized with PD4D2.

Mixture of sera*	ELISA (anti-DEN-1)	ELISA (anti DEN-2)	ELISA (anti-DEN-3)	ELISA (anti-DEN- 4)
1(PD4D2)	< 1/100	> 1: 128 000	< 1/100	1: 64 000
2(PD4D2)	< 1/100	> 1: 128 000	< 1/100	> 1: 128 000

Mixture of sera*	HI**anti-DEN-1	HI anti-DEN-2	HI anti-DEN-3	HI anti-DEN-4
PD4D2	< 1: 5	> 1: 320	< 1: 5	> 1: 320

Mixture of sera *	Neutralizing titers ***anti-DEN-1	Neutralizing titers anti-DEN-2	Neutralizing titers anti-DEN-3	Neutralizing titers anti-DEN-4
1(PD4D2)	< 1: 5	>1: 1280	< 1: 5	>1: 1280
2(PD4D2)	< 1: 5	>1: 1280	< 1: 5	>1: 1280

* Each mixture was formed by three sera.
** The HI titers were defined as the highest dilution capable of inhibiting the hemagglutination of goose's erythrocytes against 8 hemagglutination viral units.
*** The neutralizing titers were defined as the highest dilution of serum having 50% reduction of the number of viral plaques.

EXAMPLE 53. Protection assay

For the evaluation of the protection conferred to mice immunized with all the assayed variants upon challenge with homologous lethal DEN, 15 mice of each group were used. Each animal received one dose of 100 LD_{50} of lethal DEN by intracraneal inoculation and they were observed during 21 days to study the percentage of lethality. As positive controls, groups of 15 mice immunized with the four viral preparations (DEN-1, DEN-2, DEN-3 and DEN-4) were used. All the mice of these control groups survived while mice from the negative control group become sick between 7-11 days after challenge; therefore, achieving a 100% of mortality. Finally, groups immunized with the fusion proteins under study had between an 80% and 100% of protection and in all the cases, significant differences with respect to the control group were found (table 66).

Table 66. Percentage of survival in mice immunized with the protein variants assayed upon challenge with the homologous lethal Dengue virus.

Immunogen	Percentage of survival*
PBS	0
DEN-1	100
DEN-2	100
DEN-3	100
DEN-4	100
PLL1	86
PLL2	100
PLL3	100
PLH1	80
PLH2	100
PLH3	100

PAZ1	80
PAZ2	100
PAZ3	100
PID1	86
PID2	100
PID3	100
PD4D2	100 (DEN-4) 100 (DEN-2)

* It was calculated: (# of mice survived)/ (# total of mice). Data of survivors were taken 21 after challenge. In the case of mice immunized with pD4D2, 15 were challenged with DEN-4 and 15 with DEN-2.

EXAMPLE 54. Lymphoproliferative response

Animals from different groups immunized with the chimeric proteins containing the E fragment from DEN-2 (PLL1, PLL2 and PLL3), and a placebo group, were sacrified 15 days after the last dose. Then, the spleen of the animals was harvested and the lymphoproliferative response against the four serotypes of Dengue virus was studied. Table 67 shows the results of the stimulation indexes obtained, which demonstrate that a serotype specific response was achieved.

Table 67. Stimulation indexes, against the four Dengue viral serotypes, of lymphocytes from mice immunized with PLL1, PLL2 and PLL3.

	PLL1	**PLL2**	**PLL3**	**C(-)**
DEN-1	1.3 *	1.0	0.8	1.2
DEN-2	**12.5**	**10.3**	**8.9**	1.4
DEN-3	1.0	1.6	1.8	1.4
DEN-4	1.7	1.5	1.7	1.1
Control Antigen	1.1	1.0	1.3	0.9
PHA**	**13.3**	**16.5**	**11.1**	**12.0**

*Stimulation index: cocient of the counts per minute of the samples between the counts per minute of the control of the spontaneous synthesis of DNA.
** mitogen: Phytohemagglutinin.

SEQUENCE LIST

<110> CENTER FOR GENETIC ENGINEERING AND BIOTECNOLOGY

<120> CHIMERIC CHAINS CODING FOR PROTEINS INDUCING EFECTS AGAINST VIRUSES.

<130> Dengue

<140>
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<160> 54

<170> PatentIn Ver. 2.1

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<211> 25
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<213> Escherichia coli

<220>
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<223> Sequence of the Xba-I primer for the amplification of DENe-2 fragment

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<210> 2
<211> 29
<212> DNA
<213> Escherichia coli

<220>
<221> primer_bind
<222> (1)..(29)
<223> Sequence of the Bam-HI primer for the amplification of DENe-2 fragment

<400> 2
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<210> 3
<211> 25
<212> DNA
<213> Escherichia coli

<220>
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<222> (1)..(25)
<223> Sequence of the Eco-RI primer for the amplification of DENe-2 fragment

<400> 3
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<210> 4
<211> 21
<212> DNA
<213> Escherichia coli

<220>
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<223> Sequence of the Bam-HI primer for the amplification of DENe-2 fragment

<400> 4
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<210> 5
<211> 31
<212> DNA
<213> Escherichia coli

<220>
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<222> (1)..(31)
<223> Sequence of the Bam-HI primer for the amplification of DENe-2 fragment

<400> 5
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<210> 6
<211> 25
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<220>
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<223> Sequence of the Xba-I primer for the amplification of DENe-1 fragment

<400> 6
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<210> 7
<211> 28
<212> DNA
<213> Escherichia coli

<220>
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<223> Sequence of the Bam-HI primer for the amplification of DENe-1 fragment

<400> 7
gaggatcctt acccgccaat agaaccga 28

<210> 8
<211> 25
<212> DNA
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<220>
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<400> 8
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<210> 9
<211> 24
<212> DNA
<213> Escherichia coli

<220>
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<223> Sequence of the Bam-HI primer for the amplification of DENe-1 fragment

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<210> 10
<211> 26
<212> DNA
<213> Escherichia coli

<220>
<221> primer_bind
<222> (1)..(26)
<223> Sequence of the Bam-HI primer for the amplification of DENe-1 fragment

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<210> 11
<211> 29
<212> DNA
<213> Escherichia coli

<220>
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<223> Sequence of the Xba-I primer for the amplification of DENe-3 fragment

<400> 11
tttctagata gactcaagat ggacaaatt 29

<210> 12

<211> 28
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<220>
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<210> 13
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<220>
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<220>
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<210> 15
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<223> Sequence of the Bam-HI primer for the amplification of DENe-3 fragment

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<210> 16
<211> 30

<212> DNA
<213> Escherichia coli

<220>
<221> primer_bind
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<223> Sequence of the Xba-I primer for the amplification of DENe-4 fragment

<400> 16
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<210> 17
<211> 28
<212> DNA
<213> Escherichia coli

<220>
<221> primer_bind
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<223> Sequence of the Bam-HI primer for the amplification of DENe-4 fragment

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<210> 18
<211> 25
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<220>
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<400> 18
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<210> 19
<211> 27
<212> DNA
<213> Escherichia coli

<220>
<221> primer_bind
<222> (1)..(27)
<223> Sequence of the Bam-HI primer for the amplification of DENe-4 fragment

<400> 19
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<210> 20
<211> 28
<212> DNA

<213> Escherichia coli

<220>
<221> primer_bind
<222> (1)..(28)
<223> Sequence of the Bam-HI primer for the amplification of DENe-4 fragment

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<210> 21
<211> 26
<212> DNA
<213> Escherichia coli

<220>
<221> primer_bind
<222> (1)..(26)
<223> Sequence of the primer Xba-I for the amplification of DENe-4 fragment

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<210> 22
<211> 429
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(426)
<223> Nucleotidic sequence coding for the aminoacids 286 to 426 of DEN-2 envelope protein

<400> 22
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aagtttaaaa ttgtgaagga aatagcagaa acacaacatg gaacaatagt tatcagagta 120
caatatgaag gggacggctc tccatgtaag atccctttg agataatgga tttggaaaaa 180
agacacgtct taggtcgcct gattacagtt aacccgatcg taacagaaaa agatagccca 240
gtcaacatag aagcagaacc tccattcgga gacagctaca tcatcatagg agtagagccg 300
ggacaattga aactcaactg gtttaagaaa ggaagttcca tcggccaaat gtttgagaca 360
acaatgagag gagcgaagag aatggccatt ttaggtgaca cagcctggga ttttggatcc 420
ctgggagga 429

<210> 23
<211> 168
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(168)
<223> Nucleotidic sequence coding for the first 45 aminoacids of the MDH.

<400> 23
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
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ggcgacacta ttgctgtgga cgataccctg attactttgg atctagac 168

<210> 24
<211> 603
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(603)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pLL1

<400> 24
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aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagacag gctgcgcatg 180
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<210> 25
<211> 195
<212> PRT
<213> Escherichia coli

<220>
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<222> (1)..(195)
<223> Aminoacidic sequence of the PLL1 protein

<400> 25
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Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
20 25 30

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35 40 45

Thr Leu Asp Leu Asp Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly
50 55 60

Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Ile Val Lys Glu Ile
65 70 75 80

Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly
85 90 95

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys

100 105 110

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

Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser
130 135 140

Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe
145 150 155 160

Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly
165 170 175

Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
180 185 190

Leu Gly Gly
195

<210> 26
<211> 1851
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(1851)
<223> Nucleotidic sequence of the MDH in the plasmid pM84 His.

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gctgcatttg ccgctgccga tgaaggcttg aaagtcgcca tcgtcgaacg ttacaaaact 540
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gaaatgggct gcgacgcggc agacatcggc aaaaccatcc acccgcaccc gggcgaatcc 1800
atcggtatgg cggcggaagt ggcattgggt acttgtaccg acaaaaaaaa a 1851

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aggctgcgca tggacaaact acagctcaaa ggaatgtcat actctatgtg tacaggaaag 240
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ggaggcgtga attcgatgaa ttcgatggac gtacctgctg aagttgcagg cgtagtcaaa 660
gaagttaaag ttaaagtcgg cgacaaaatc tctgaaggtg gtttgattgt cgtcgttgaa 720
gctgaaggca cggcagccgc tcctaaagcc gaagcggctg ccgccccggc gcaagaagcc 780
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ccgaacggtg gcgatatgat cggcgaagtc tgccttgcca tcgaaatggg ctgcgacgcg 2160
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gtggcattgg gtacttgtac cgacaaaaaa aaa 2253

<210> 28
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<212> PRT
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<220>

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<223> Aminoacidic sequence of PLL2.

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Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
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Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
        35                  40                  45

Thr Leu Asp Leu Asp Arg Leu Arg Met Asp Lys Leu Gln Leu Lys Gly
    50                  55                  60

Met Ser Tyr Ser Met Cys Thr Gly Lys Phe Lys Ile Val Lys Glu Ile
65                  70                  75                  80

Ala Glu Thr Gln His Gly Thr Ile Val Ile Arg Val Gln Tyr Glu Gly
                85                  90                  95

Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu Ile Met Asp Leu Glu Lys
            100                 105                 110

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

Lys Asp Ser Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Asp Ser
    130                 135                 140

Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln Leu Lys Leu Asn Trp Phe
145                 150                 155                 160

Lys Lys Gly Ser Ser Ile Gly Gln Met Phe Glu Thr Thr Met Arg Gly
                165                 170                 175

Ala Lys Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
            180                 185                 190

Leu Gly Gly Val Asn Ser Met Asn Ser Met Asp Val Pro Ala Glu Val
        195                 200                 205

Ala Gly Val Val Lys Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser
    210                 215                 220

Glu Gly Gly Leu Ile Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala
225                 230                 235                 240

Pro Lys Ala Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala
                245                 250                 255

Ala Ala Pro Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala
            260                 265                 270

Glu Tyr Asp Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala
        275                 280                 285

Ala Phe Ala Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg
    290                 295                 300
```

Tyr Lys Thr Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser
305 310 315 320

Lys Ala Leu Leu His Asn Ala Ala Val Ile Asp Glu Val Arg His Leu
325 330 335

Ala Ala Asn Gly Ile Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met
340 345 350

Leu Arg Ala Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu
355 360 365

Ala Gly Met Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly
370 375 380

Gln Phe Leu Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp
385 390 395 400

Ala Tyr Glu Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe
405 410 415

Lys Asn Cys Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe
420 425 430

Ile Pro Glu Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu
435 440 445

Lys Glu Val Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly
450 455 460

Leu Glu Met Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val
465 470 475 480

Val Glu Met Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val
485 490 495

Lys Val Trp Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val
500 505 510

Asn Thr Lys Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val
515 520 525

Thr Phe Glu Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala
530 535 540

Val Leu Val Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala
545 550 555 560

Glu Lys Ala Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp
565 570 575

Lys Gln Met Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile
580 585 590

Val Gly Gln Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val
595 600 605

Ala Ala Glu Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val
610 615 620

```
Ile Pro Gly Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu
625                 630                 635                 640

Thr Glu Leu Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn
                645                 650                 655

Phe Pro Trp Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys
            660                 665                 670

Pro Phe Thr Lys Leu Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly
        675                 680                 685

Gly Gly Ile Val Gly Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys
    690                 695                 700

Leu Ala Ile Glu Met Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile
705                 710                 715                 720

His Pro His Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu
                725                 730                 735

Gly Thr Cys Thr Asp Leu Pro Pro Gln Lys Lys Lys
            740                 745
```

<210> 29
<211> 1821
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(1821)
<223> Nucleotidic sequence of the MDH in the plasmid pD4

<400> 29

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atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg acacgaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagaaat ggacgtacct 180
gctgaagttg caggcgtagt caaagaagtt aaagttaaag tcggcgacaa aatctctgaa 240
ggtggtttga ttgtcgtcgt tgaagctgaa ggcacggcag ccgctcctaa agccgaagcg 300
gctgccgccc cggcgcaaga agcccctaaa gctgccgctc ctgctccgca agccgcgcaa 360
ttcggcggtt ctgccgatgc cgagtacgac gtggtcgtat tgggtggcgg tcccggcggt 420
tactccgctg catttgccgc tgccgatgaa ggcttgaaag tcgccatcgt cgaacgttac 480
aaaactttgg gcggcgtttg cctgaacgtc ggctgtatcc cttccaaagc cttgttgcac 540
aatgccgccg ttatcgacga agtgcgccac ttggctgcca acggtatcaa ataccccgag 600
ccggaactcg acatcgatat gcttcgcgcc tacaaagacg gcgtagtttc ccgcctcacg 660
ggcggtttgg caggtatggc gaaaagccgt aaagtggacg ttatccaagg cgacgggcaa 720
ttcttagatc cgcaccactt ggaagtgtcg ctgactgccg gcgacgcgta cgaacaggca 780
gcccctaccg gcgagaaaaa aatcgttgcc ttcaaaaact gtatcattgc agcaggcagc 840
cgcgtaacca aactgccttt cattcctgaa gatccgcgca tcatcgattc cagcggcgca 900
ttggctctga aagaagtacc gggcaaactg ctgattatcg gcggcggcat tatcggcctc 960
gagatgggta cggtttacag cacgctgggt tcgcgtttgg atgtggttga aatgatggac 1020
ggcctgatgc aaggcgcaga ccgcgatttg gtaaaagtat ggcaaaaaca aaacgaatac 1080
cgttttgaca acattatggt caacaccaaa accgttgcag ttgagccgaa agaagacggc 1140
gtttacgtta cctttgaagg cgcgaacgcg cctaaagagc cgcaacgcta cgatgccgta 1200
ttggttgccg ccggccgcgc gcccaacggc aaactcatca gcgcggaaaa agcaggcgtt 1260
gccgtaaccg atcgcggctt catcgaagtg gacaaacaaa tgcgtaccaa tgtgccgcac 1320
atctacgcca tcggcgacat cgtcggtcag ccgatgttgg cgcacaaagc cgttcacgaa 1380
ggccacgttg ccgccgaaaa ctgcgccggc cacaaagcct acttcgacgc acgcgtgatt 1440
```

ccgggcgttg cctacacttc ccccgaagtg gcgtgggtgg gcgaaaccga actgtccgcc 1500
aaagcctccg gccgcaaaat caccaaagcc aacttcccgt gggcggcttc cggccgtgcg 1560
attgccaacg gttgcgacaa gccgtttacc aagctgattt ttgatgccga aaccggccgc 1620
atcatcggcg gcggcattgt cggtccgaac ggtggcgata tgatcggcga agtctgcctt 1680
gccatcgaaa tgggctgcga cgcggcagac atcggcaaaa ccatccaccc gcacccgacc 1740
ttgggcgaat ccatcggtat ggcggcggaa gtggcattgg gtacttgtac cgacctgcct 1800
ccgcaaaaga aaaaaggatc c 1821

<210> 30
<211> 2259
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2259)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pLL3

<400> 30
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg acacgaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagaaat ggacgtacct 180
gctgaagttg caggcgtagt caaagaagtt aaagttaaag tcggcgacaa aatctctgaa 240
ggtggtttga ttgtcgtcgt tgaagctgaa ggcacggcag ccgctcctaa agccgaagcg 300
gctgccgccc cggcgcaaga agcccctaaa gctgccgctc ctgctccgca agccgcgcaa 360
ttcggcggtt ctgccgatgc cgagtacgac gtggtcgtat tgggtggcgg tcccggcggt 420
tactccgctg catttgccgc tgccgatgaa ggcttgaaag tcgccatcgt cgaacgttac 480
aaaactttgg gcggcgtttg cctgaacgtc ggctgtatcc cttccaaagc cttgttgcac 540
aatgccgccg ttatcgacga agtgcgccac ttggctgcca acggtatcaa ataccccgag 600
ccggaactcg acatcgatat gcttcgcgcc tacaaagacg gcgtagtttc ccgcctcacg 660
ggcggtttgg caggtatggc gaaaagccgt aaagtggacg ttatccaagg cgacgggcaa 720
ttcttagatc cgcaccactt ggaagtgtcg ctgactgccg gcgacgcgta cgaacaggca 780
gcccctaccg gcgagaaaaa aatcgttgcc ttcaaaaact gtatcattgc agcaggcagc 840
cgcgtaacca aactgccttt cattcctgaa gatccgcgca tcatcgattc cagcggcgca 900
ttggctctga aagaagtacc gggcaaactg ctgattatcg gcggcggcat tatcggcctc 960
gagatgggta cggtttacag cacgctgggt tcgcgtttgg atgtggttga aatgatggac 1020
ggcctgatgc aaggcgcaga ccgcgatttg gtaaaagtat ggcaaaaaca aaacgaatac 1080
cgttttgaca acattatggt caacaccaaa accgttgcag ttgagccgaa agaagacggc 1140
gtttacgtta cctttgaagg cgcgaacgcg cctaaagagc cgcaacgcta cgatgccgta 1200
ttggttgccg ccggccgcgc gcccaacggc aaactcatca gcgcggaaaa agcaggcgtt 1260
gccgtaaccg atcgcggctt catcgaagtg gacaaacaaa tgcgtaccaa tgtgccgcac 1320
atctacgcca tcggcgacat cgtcggtcag ccgatgttgg cgcacaaagc cgttcacgaa 1380
ggccacgttg ccgccgaaaa ctgcgccggc cacaaagcct acttcgacgc acgcgtgatt 1440
ccgggcgttg cctacacttc ccccgaagtg gcgtgggtgg gcgaaaccga actgtccgcc 1500
aaagcctccg gccgcaaaat caccaaagcc aacttcccgt gggcggcttc cggccgtgcg 1560
attgccaacg gttgcgacaa gccgtttacc aagctgattt ttgatgccga aaccggccgc 1620
atcatcggcg gcggcattgt cggtccgaac ggtggcgata tgatcggcga agtctgcctt 1680
gccatcgaaa tgggctgcga cgcggcagac atcggcaaaa ccatccaccc gcacccgacc 1740
ttgggcgaat ccatcggtat ggcggcggaa gtggcattgg gtacttgtac cgacctgcct 1800
ccgcaaaaga aaaaaggatc cgacaggctg agaatggaca aactacagct caaaggaatg 1860
tcatactcta tgtgtacagg aaagtttaaa attgtgaagg aaatagcaga aacacaacat 1920
ggaacaatag ttatcagagt acaatatgaa ggggacggct ctccatgtaa gatccctttt 1980
gagataatgg atttggaaaa aagacacgtc ttaggtcgcc tgattacagt taacccgatc 2040
gtaacagaaa aagatagccc agtcaacata gaagcagaac ctccattcgg agacagctac 2100
atcatcatag gagtagagcc gggacaattg aaactcaact ggtttaagaa aggaagttcc 2160
atcggccaaa tgtttgagac aacaatgaga ggagcgaaga gaatggccat tttaggtgac 2220
acagcctggg attttgggtc tctgggtggt taaggatcc 2259

<210> 31
<211> 745
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(745)
<223> Aminoacidic sequence of PLL3

<400> 31

```
His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
  1               5                  10                  15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
             20                  25                  30

Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
         35                  40                  45

Thr Leu Asp Met Asn Ser Met Asp Val Pro Ala Glu Val Ala Gly Val
     50                  55                  60

Val Lys Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser Glu Gly Gly
 65                  70                  75                  80

Leu Ile Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala Pro Lys Ala
                 85                  90                  95

Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala Ala Pro
            100                 105                 110

Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu Tyr Asp
        115                 120                 125

Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala Phe Ala
    130                 135                 140

Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr Lys Thr
145                 150                 155                 160

Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys Ala Leu
                165                 170                 175

Leu His Asn Ala Ala Val Ile Asp Glu Val Arg His Leu Ala Ala Asn
            180                 185                 190

Gly Ile Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met Leu Arg Ala
        195                 200                 205

Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala Gly Met
    210                 215                 220

Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln Phe Leu
225                 230                 235                 240

Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala Tyr Glu
                245                 250                 255

Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys Asn Cys
            260                 265                 270
```

Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile Pro Glu
275 280 285

Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys Glu Val
290 295 300

Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu Glu Met
305 310 315 320

Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val Glu Met
325 330 335

Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys Val Trp
340 345 350

Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn Thr Lys
355 360 365

Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr Phe Glu
370 375 380

Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val Leu Val
385 390 395 400

Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu Lys Ala
405 410 415

Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys Gln Met
420 425 430

Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val Gly Gln
435 440 445

Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala Ala Glu
450 455 460

Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile Pro Gly
465 470 475 480

Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr Glu Leu
485 490 495

Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe Pro Trp
500 505 510

Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro Phe Thr
515 520 525

Lys Leu Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly Gly Gly Ile
530 535 540

Val Gly Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys Leu Ala Ile
545 550 555 560

Glu Met Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile His Pro His
565 570 575

Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly Thr Cys
580 585 590

Thr Asp Leu Pro Pro Gln Lys Lys Lys Gly Ser Arg Leu Arg Met Asp
595 600 605

Lys Leu Gln Leu Lys Gly Met Ser Tyr Ser Met Cys Thr Gly Lys Phe
610 615 620

Lys Ile Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile Val Ile
625 630 635 640

Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro Phe Glu
645 650 655

Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile Thr Val
660 665 670

Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu Ala Glu
675 680 685

Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro Gly Gln
690 695 700

Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln Met Phe
705 710 715 720

Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly Asp Thr
725 730 735

Ala Trp Asp Phe Gly Ser Leu Gly Gly
740 745

<210> 32
<211> 429
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(429)
<223> Nucleotidic sequence coding for the aminoacids 286 to 426 of the DEN-1 envelope protein

<400> 32
agactaaaaa tggataaact gactttaaaa ggggtatcat atgtaatgtg cacagggtca 60
ttcaagttag agaaggaagt ggctgagacc cagcatggaa ctgttctagt gcaggttaaa 120
tacgaaggaa cagatgcacc atgcaagatc cccttctcgt cccaagatga gaaaggagta 180
acccagaatg ggagattgat aacagccaac cccatagtca ttgacaaaga aaaaccagtc 240
aacattgaag cggagccacc ttttggtgag agctatattg tggtaggagc aggtgaaaaa 300
gctttgaaac taagctggtt caagaaggga agcagtatag ggaaaatgtt tgaagcaact 360
gcccgtggag cacgaaggat ggccatcctg ggagacaccg catgggactt cggttctata 420
ggagggtaa 429

<210> 33
<211> 615
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(615)

<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pLH1

<400> 33

atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagattt ggatctagac 180
aggctcaaaa tggataaact gactttaaaa ggggtatcat atgtaatgtg cacagggtca 240
ttcaagttag agaaggaagt ggctgagacc cagcatggaa ctgttctagt gcaggttaaa 300
tacgaaggaa cagatgcacc atgcaagatc cccttctcgt cccaagatga gaaaggagta 360
acccagaatg ggagattgat aacagccaac cccatagtca ttgacaaaga aaaaccagtc 420
aacattgaag cggagccacc ttttggtgag agctatattg tggtaggagc aggtgaaaaa 480
gctttgaaac taagctggtt caagaaggga agcagtatag ggaaaatgtt tgaagcaact 540
gcccgtggag cacgaaggat ggccatcctg ggagacaccg catgggactt cggttctatt 600
ggcgggtaag gatcc 615

<210> 34
<211> 174
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(174)
<223> Aminoacidic sequence of the PLH1

<400> 34

His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
1 5 10 15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
20 25 30

Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
35 40 45

Thr Leu Asp Leu Asp Phe Lys Leu Glu Lys Glu Val Ala Glu Thr Gln
50 55 60

His Gly Thr Val Leu Val Gln Val Lys Tyr Gln Gly Thr Asp Ala Pro
65 70 75 80

Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly Val Thr Gln Asn
85 90 95

Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp Lys Glu Lys Pro Val
100 105 110

Asn Ile Glu Thr Glu Pro Pro Phe Gly Glu Ser Tyr Ile Val Val Gly
115 120 125

Ala Gly Glu Lys Ala Leu Lys Gln Cys Trp Phe Lys Lys Gly Ser Ser
130 135 140

Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala Arg Arg Met Ala
145 150 155 160

Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Ile Gly Gly
165 170

<210> 35
<211> 2253
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2253)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pLH2

<400> 35
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aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagattt ggatctagac 180
aggctcaaaa tggataaact gactttaaaa ggggtatcat atgtaatgtg cacagggtca 240
ttcaagttag agaaggaagt ggctgagacc cagcatggaa ctgttctagt gcaggttaaa 300
tacgaaggaa cagatgcacc atgcaagatc cccttctcgt cccaagatga gaaaggagta 360
acccagaatg ggagattgat aacagccaac cccatagtca ttgacaaaga aaaaccagtc 420
aacattgaag cggagccacc ttttggtgag agctatattg tggtaggagc aggtgaaaaa 480
gctttgaaac taagctggtt caagaaggga agcagtatag ggaaaatgtt tgaagcaact 540
gcccgtggag cacgaaggat ggccatcctg ggagacaccg catgggactt cggatctata 600
ggaggggtga attcgatgaa ttcgatggac gtacctgctg aagttgcagg cgtagtcaaa 660
gaagttaaag ttaaagtcgg cgacaaaatc tctgaaggtg gtttgattgt cgtcgttgaa 720
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gatgaaggct tgaaagtcgc catcgtcgaa cgttacaaaa ctttgggcgg cgtttgcctg 960
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cgccacttgg ctgccaacgg tatcaaatac cccgagccgg aactcgacat cgatatgctt 1080
cgcgcctaca aagacggcgt agtttcccgc ctcacgggcg gtttggcagg tatggcgaaa 1140
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cctgaagatc cgcgcatcat cgattccagc ggcgcattgg ctctgaaaga agtaccgggc 1380
aaactgctga ttatcggcgg cggcattatc ggcctcgaga tgggtacggt ttacagcacg 1440
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gatttggtaa aagtatggca aaaacaaaac gaataccgtt ttgacaacat tatggtcaac 1560
accaaaaccg ttgcagttga gccgaaagaa gacggcgttt acgttacctt tgaaggcgcg 1620
aacgcgccta aagagccgca acgctacgat gccgtattgg ttgccgccgg ccgcgcgccc 1680
aacggcaaac tcatcagcgc ggaaaaagca ggcgttgccg taaccgatcg cggcttcatc 1740
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gccggccaca aagcctactt cgacgcacgc gtgattccgg gcgttgccta cacttccccc 1920
gaagtggcgt gggtgggcga aaccgaactg tccgccaaag cctccggccg caaaatcacc 1980
aaagccaact tcccgtgggc ggcttccggc cgtgcgattg ccaacggttg cgacaagccg 2040
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<210> 36
<211> 727
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(727)

<223> Aminoacidic sequence of the PLH2

<400> 36

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His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
 1               5                  10                  15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
            20                  25                  30

Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
        35                  40                  45

Thr Leu Asp Leu Asp Phe Lys Leu Glu Lys Glu Val Ala Glu Thr Gln
    50                  55                  60

His Gly Thr Val Leu Val Gln Val Lys Tyr Gln Gly Thr Asp Ala Pro
65                  70                  75                  80

Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu Lys Gly Val Thr Gln Asn
                85                  90                  95

Arg Leu Ile Thr Ala Asn Pro Ile Val Thr Asp Lys Glu Lys Pro Val
            100                 105                 110

Asn Ile Glu Thr Glu Pro Pro Phe Gly Glu Ser Tyr Ile Val Val Gly
        115                 120                 125

Ala Gly Glu Lys Ala Leu Lys Gln Cys Trp Phe Lys Lys Gly Ser Ser
    130                 135                 140

Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala Arg Arg Met Ala
145                 150                 155                 160

Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Ile Gly Gly Val Asn
                165                 170                 175

Ser Met Asn Ser Met Asp Val Pro Ala Glu Val Ala Gly Val Val Lys
            180                 185                 190

Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser Glu Gly Gly Leu Ile
        195                 200                 205

Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala Pro Lys Ala Glu Ala
    210                 215                 220

Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala Ala Pro Ala Pro
225                 230                 235                 240

Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu Tyr Asp Val Val
                245                 250                 255

Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala Phe Ala Ala Ala
            260                 265                 270

Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr Lys Thr Leu Gly
        275                 280                 285

Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys Ala Leu Leu His
    290                 295                 300

Asn Ala Ala Val Ile Asp Glu Val Arg His Leu Ala Ala Asn Gly Ile
```

```
305                 310                 315                 320

Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met Leu Arg Ala Tyr Lys
                325                 330                 335

Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala Gly Met Ala Lys
            340                 345                 350

Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln Phe Leu Asp Pro
        355                 360                 365

His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala Tyr Glu Gln Ala
    370                 375                 380

Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys Asn Cys Ile Ile
385                 390                 395                 400

Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile Pro Glu Asp Pro
                405                 410                 415

Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys Glu Val Pro Gly
            420                 425                 430

Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu Glu Met Gly Thr
        435                 440                 445

Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val Glu Met Met Asp
    450                 455                 460

Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys Val Trp Gln Lys
465                 470                 475                 480

Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn Thr Lys Thr Val
                485                 490                 495

Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr Phe Glu Gly Ala
            500                 505                 510

Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val Leu Val Ala Ala
        515                 520                 525

Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu Lys Ala Gly Val
    530                 535                 540

Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys Gln Met Arg Thr
545                 550                 555                 560

Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val Gly Gln Pro Met
                565                 570                 575

Leu Ala His Lys Ala Val His Glu Gly His Val Ala Ala Glu Asn Cys
            580                 585                 590

Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile Pro Gly Val Ala
        595                 600                 605

Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr Glu Leu Ser Ala
    610                 615                 620

Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe Pro Trp Ala Ala
625                 630                 635                 640
```

Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro Phe Thr Lys Leu
645 650 655

Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly Gly Gly Ile Val Gly
660 665 670

Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys Leu Ala Ile Glu Met
675 680 685

Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile His Pro His Pro Gly
690 695 700

Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly Thr Cys Thr Asp
705 710 715 720

Leu Pro Pro Gln Lys Lys Lys
725

<210> 37
<211> 2250
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2250)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pLH3

<400> 37
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg acacgaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagaaat ggacgtacct 180
gctgaagttg caggcgtagt caaagaagtt aaagttaaag tcggcgacaa aatctctgaa 240
ggtggtttga ttgtcgtcgt tgaagctgaa ggcacggcag ccgctcctaa agccgaagcg 300
gctgccgccc cggcgcaaga agcccctaaa gctgccgctc ctgctccgca agccgcgcaa 360
ttcggcggtt ctgccgatgc cgagtacgac gtggtcgtat tgggtggcgg tcccggcggt 420
tactccgctg catttgccgc tgccgatgaa ggcttgaaag tcgccatcgt cgaacgttac 480
aaaactttgg gcggcgtttg cctgaacgtc ggctgtatcc cttccaaagc cttgttgcac 540
aatgccgccg ttatcgacga agtgcgccac ttggctgcca acggtatcaa ataccccgag 600
ccggaactcg acatcgatat gcttcgcgcc tacaaagacg gcgtagtttc ccgcctcacg 660
ggcggtttgg caggtatggc gaaaagccgt aaagtggacg ttatccaagg cgacgggcaa 720
ttcttagatc cgcaccactt ggaagtgtcg ctgactgccg gcgacgcgta cgaacaggca 780
gcccctaccg gcgagaaaaa aatcgttgcc ttcaaaaact gtatcattgc agcaggcagc 840
cgcgtaacca aactgccttt cattcctgaa gatccgcgca tcatcgattc cagcggcgca 900
ttggctctga aagaagtacc gggcaaactg ctgattatcg gcggcggcat tatcggcctc 960
gagatgggta cggtttacag cacgctgggt tcgcgtttgg atgtggttga aatgatggac 1020
ggcctgatgc aaggcgcaga ccgcgatttg gtaaaagtat ggcaaaaaca aaacgaatac 1080
cgttttgaca acattatggt caacaccaaa accgttgcag ttgagccgaa agaagacggc 1140
gtttacgtta cctttgaagg cgcgaacgcg cctaaagagc cgcaacgcta cgatgccgta 1200
ttggttgccg ccggccgcgc gcccaacggc aaactcatca gcgcggaaaa agcaggcgtt 1260
gccgtaaccg atcgcggctt catcgaagtg gacaaacaaa tgcgtaccaa tgtgccgcac 1320
atctacgcca tcggcgacat cgtcggtcag ccgatgttgg cgcacaaagc cgttcacgaa 1380
ggccacgttg ccgccgaaaa ctgcgccggc cacaaagcct acttcgacgc acgcgtgatt 1440
ccgggcgttg cctacacttc ccccgaagtg gcgtgggtgg gcgaaaccga actgtccgcc 1500
aaagcctccg gccgcaaaat caccaaagcc aacttcccgt gggcggcttc cggccgtgcg 1560
attgccaacg gttgcgacaa gccgtttacc aagctgattt ttgatgccga aaccggccgc 1620
atcatcggcg gcggcattgt cggtccgaac ggtggcgata tgatcggcga agtctgcctt 1680
gccatcgaaa tgggctgcga cgcggcagac atcggcaaaa ccatccaccc gcacccgacc 1740

ttgggcgaat ccatcggtat ggcggcggaa gtggcattgg gtacttgtac cgacctgcct 1800
ccgcaaaaga aaaaaggatc cagactaaaa atggataaac tgactttaaa aggggtatca 1860
tatgtaatgt gcacagggtc attcaagtta gagaaggaag tggctgagac ccagcatgga 1920
actgttctag tgcaggttaa atacgaagga acagatgcac catgcaagat ccccttctcg 1980
tcccaagatg agaaaggagt aacccagaat gggagattga taacagccaa ccccatagtc 2040
attgacaaag aaaaaccagt caacattgaa gcggagccac cttttggtga gagctatatt 2100
gtggtaggag caggtgaaaa agctttgaaa ctaagctggt tcaagaaggg aagcagtata 2160
gggaaaatgt ttgaagcaac tgcccgtgga gcacgaagga tggccatcct gggagacacc 2220
gcatgggact tcggttctat aggtgggtaa 2250

<210> 38
<211> 724
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(724)
<223> Aminoacidic sequence of the PLH3

<400> 38

His	His	His	His	His	His	Met	Val	Asp	Lys	Arg	Met	Ala	Leu	Val	Glu
1				5				10					15		
Leu	Lys	Val	Pro	Asp	Ile	Gly	Gly	His	Glu	Asn	Val	Asp	Ile	Ile	Ala
			20					25				30			
Val	Glu	Val	Asn	Val	Gly	Asp	Thr	Ile	Ala	Val	Asp	Asp	Thr	Leu	Ile
	35					40					45				
Thr	Leu	Asp	Met	Asn	Ser	Met	Asp	Val	Pro	Ala	Glu	Val	Ala	Gly	Val
50					55					60					
Val	Lys	Glu	Val	Lys	Val	Lys	Val	Gly	Asp	Lys	Ile	Ser	Glu	Gly	Gly
65				70					75					80	
Leu	Ile	Val	Val	Val	Glu	Ala	Glu	Gly	Thr	Ala	Ala	Ala	Pro	Lys	Ala
			85					90					95		
Glu	Ala	Ala	Ala	Ala	Pro	Ala	Gln	Glu	Ala	Pro	Lys	Ala	Ala	Ala	Pro
		100					105					110			
Ala	Pro	Gln	Ala	Ala	Gln	Phe	Gly	Gly	Ser	Ala	Asp	Ala	Glu	Tyr	Asp
	115					120					125				
Val	Val	Val	Leu	Gly	Gly	Gly	Pro	Gly	Gly	Tyr	Ser	Ala	Ala	Phe	Ala
130					135					140					
Ala	Ala	Asp	Glu	Gly	Leu	Lys	Val	Ala	Ile	Val	Glu	Arg	Tyr	Lys	Thr
145				150					155					160	
Leu	Gly	Gly	Val	Cys	Leu	Asn	Val	Gly	Cys	Ile	Pro	Ser	Lys	Ala	Leu
			165					170					175		
Leu	His	Asn	Ala	Ala	Val	Ile	Asp	Glu	Val	Arg	His	Leu	Ala	Ala	Asn
		180					185					190			
Gly	Ile	Lys	Tyr	Pro	Glu	Pro	Glu	Leu	Asp	Ile	Asp	Met	Leu	Arg	Ala
	195					200					205				

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Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala Gly Met
    210                 215                 220

Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln Phe Leu
225                 230                 235                 240

Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala Tyr Glu
                245                 250                 255

Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys Asn Cys
            260                 265                 270

Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile Pro Glu
        275                 280                 285

Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys Glu Val
    290                 295                 300

Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu Glu Met
305                 310                 315                 320

Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val Glu Met
                325                 330                 335

Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys Val Trp
            340                 345                 350

Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn Thr Lys
        355                 360                 365

Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr Phe Glu
    370                 375                 380

Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val Leu Val
385                 390                 395                 400

Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu Lys Ala
                405                 410                 415

Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys Gln Met
            420                 425                 430

Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val Gly Gln
        435                 440                 445

Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala Ala Glu
    450                 455                 460

Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile Pro Gly
465                 470                 475                 480

Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr Glu Leu
                485                 490                 495

Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe Pro Trp
            500                 505                 510

Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro Phe Thr
        515                 520                 525

Lys Leu Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly Gly Gly Ile
```

530 535 540

Val Gly Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys Leu Ala Ile
545 550 555 560

Glu Met Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile His Pro His
565 570 575

Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly Thr Cys
580 585 590

Thr Asp Leu Pro Pro Gln Lys Lys Lys Gly Ser Phe Lys Leu Glu Lys
595 600 605

Glu Val Ala Glu Thr Gln His Gly Thr Val Leu Val Gln Val Lys Tyr
610 615 620

Gln Gly Thr Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Gln Asp Glu
625 630 635 640

Lys Gly Val Thr Gln Asn Arg Leu Ile Thr Ala Asn Pro Ile Val Thr
645 650 655

Asp Lys Glu Lys Pro Val Asn Ile Glu Thr Glu Pro Pro Phe Gly Glu
660 665 670

Ser Tyr Ile Val Val Gly Ala Gly Glu Lys Ala Leu Lys Gln Cys Trp
675 680 685

Phe Lys Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg
690 695 700

Gly Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly
705 710 715 720

Ser Ile Gly Gly

<210> 39
<211> 426
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(426)
<223> Nucleotidic sequence coding for the aminoacids 286 to 426 of the DEN-3 envelope protein

<400> 39
agactcaaga tggacaaatt gaaactcaag gggatgagct atgcaatgtg cttgaatacc 60
tttgtgttga agaaagaagt ctccgaaacg cagcatggga caatactcat taaggttgag 120
tacaaagggg aagatgcacc ctgcaagatt cctttctcca cggaggatgg acaagggaaa 180
gctcacaatg gcagactgat cacagccaat ccagtggtga ccaagaagga ggagcctgtc 240
aacattgagg ctgaacctcc ttttggggaa agtaatatag taattggaat tggagacaaa 300
gcccctgaaaa tcaactggta caggaaggga agctcgattg ggaagatgtt cgaggccact 360
gccagaggtg caaggcgcat ggccatcttg ggagacacag cctgggactt tggatcagtg 420
ggtggt 426

<210> 40
<211> 615
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(615)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pAZ1

<400> 40
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagattt ggatctagat 180
agactcaaga tggacaaatt gaaactcaag gggatgagct atgcaatgtg cttgaatacc 240
tttgtgttga agaaagaagt ctccgaaacg cagcatggga caatactcat taaggttgag 300
tacaaagggg aagatgcacc ctgcaagatt cctttctcca cggaggatgg acaagggaaa 360
gctcacaatg gcagactgat cacagccaat ccagtggtga ccaagaagga ggagcctgtc 420
aacattgagg ctgaacctcc ttttggggaa agtaatatag taattggaat tggagacaaa 480
gccctgaaaa tcaactggta caggaaggga agctcgattg ggaagatgtt cgaggccact 540
gccagaggtg caaggcgcat ggccatcttg ggagacacag cctgggactt tggttcagtg 600
ggtggttaag gatcc 615

<210> 41
<211> 194
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(194)
<223> Aminoacidic sequence of the PAZ1

<400> 41
His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
1 5 10 15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
20 25 30

Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
35 40 45

Thr Leu Asp Leu Asp Arg Leu Lys Met Asp Lys Leu Lys Leu Lys Gly
50 55 60

Met Ser Tyr Ala Met Cys Leu Asn Thr Phe Val Leu Lys Lys Glu Val
65 70 75 80

Ser Glu Thr His Gly Thr Ile Leu Ile Lys Val Glu Tyr Lys Gly Glu
85 90 95

Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr Glu Asp Gly Gln Gly Lys
100 105 110

Ala His Asn Gly Arg Leu Ile Thr Ala Asn Pro Val Val Thr Lys Lys
115 120 125

Glu Glu Pro Val Asn Ile Glu Ala Glu Pro Pro Phe Gly Glu Ser Asn

130 135 140

Ile Val Ile Gly Ile Gly Asp Lys Ala Leu Lys Ile Asn Trp Tyr Arg
145 150 155 160

Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ala Thr Ala Arg Gly Ala
165 170 175

Arg Arg Met Ala Ile Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val
180 185 190

Gly Gly

<210> 42
<211> 2253
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2253)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pAZ2

<400> 42
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagattt ggatctagat 180
agactcaaga tggacaaatt gaaactcaag gggatgagct atgcaatgtg cttgaatacc 240
tttgtgttga agaaagaagt ctccgaaacg cagcatggga caatactcat taaggttgag 300
tacaaagggg aagatgcacc ctgcaagatt cctttctcca cggaggatgg acaagggaaa 360
gctcacaatg gcagactgat cacagccaat ccagtggtga ccaagaagga ggagcctgtc 420
aacattgagg ctgaacctcc ttttggggaa agtaatatag taattggaat tggagacaaa 480
gccctgaaaa tcaactggta caggaaggga agctcgattg ggaagatgtt cgaggccact 540
gccagaggtg caaggcgcat ggccatcttg ggagacacag cctgggactt tggatctgtg 600
ggtggtgtga attcgatgaa ttcgatggac gtacctgctg aagttgcagg cgtagtcaaa 660
gaagttaaag ttaaagtcgg cgacaaaatc tctgaaggtg gtttgattgt cgtcgttgaa 720
gctgaaggca cggcagccgc tcctaaagcc gaagcggctg ccgccccggc gcaagaagcc 780
cctaaagctg ccgctcctgc tccgcaagcc gcgcaattcg gcggttctgc cgatgccgag 840
tacgacgtgg tcgtattggg tggcggtccc ggcggttact ccgctgcatt tgccgctgcc 900
gatgaaggct tgaaagtcgc catcgtcgaa cgttacaaaa ctttgggcgg cgtttgcctg 960
aacgtcggct gtatcccttc caaagccttg ttgcacaatg ccgccgttat cgacgaagtg 1020
cgccacttgg ctgccaacgg tatcaaatac cccgagccgg aactcgacat cgatatgctt 1080
cgcgcctaca aagacggcgt agtttcccgc ctcacgggcg gtttggcagg tatggcgaaa 1140
agccgtaaag tggacgttat ccaaggcgac gggcaattct tagatccgca ccacttggaa 1200
gtgtcgctga ctgccggcga cgcgtacgaa caggcagccc ctaccggcga gaaaaaaatc 1260
gttgccttca aaaactgtat cattgcagca ggcagccgcg taaccaaact gcctttcatt 1320
cctgaagatc cgcgcatcat cgattccagc ggcgcattgg ctctgaaaga agtaccgggc 1380
aaactgctga ttatcggcgg cggcattatc ggcctcgaga tgggtacggt ttacagcacg 1440
ctgggttcgc gtttggatgt ggttgaaatg atggacggcc tgatgcaagg cgcagaccgc 1500
gatttggtaa aagtatggca aaaacaaaac gaataccgtt ttgacaacat tatggtcaac 1560
accaaaaccg ttgcagttga gccgaaagaa gacggcgttt acgttacctt tgaaggcgcg 1620
aacgcgccta aagagccgca acgctacgat gccgtattgg ttgccgccgg ccgcgcgccc 1680
aacggcaaac tcatcagcgc ggaaaaagca ggcgttgccg taaccgatcg cggcttcatc 1740
gaagtggaca aacaaatgcg taccaatgtg ccgcacatct acgccatcgg cgacatcgtc 1800
ggtcagccga tgttggcgca caaagccgtt cacgaaggcc acgttgccgc cgaaaactgc 1860
gccggccaca aagcctactt cgacgcacgc gtgattccgg gcgttgccta cacttccccc 1920
gaagtggcgt gggtgggcga aaccgaactg tccgccaaag cctccggccg caaaatcacc 1980
aaagccaact tcccgtgggc ggcttccggc cgtgcgattg ccaacggttg cgacaagccg 2040

tttaccaagc tgatttttga tgccgaaacc ggccgcatca tcggcggcgg cattgtcggt 2100
ccgaacggtg gcgatatgat cggcgaagtc tgccttgcca tcgaaatggg ctgcgacgcg 2160
gcagacatcg gcaaaaccat ccacccgcac ccgggcgaat ccatcggtat ggcggcggaa 2220
gtggcattgg gtacttgtac cgacaaaaaa aaa 2253

<210> 43
<211> 747
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(747)
<223> Aminoacidic sequence of the PAZ2

<400> 43

His	His	His	His	His	His	Met	Val	Asp	Lys	Arg	Met	Ala	Leu	Val	Glu
1				5				10					15		
Leu	Lys	Val	Pro	Asp	Ile	Gly	Gly	His	Glu	Asn	Val	Asp	Ile	Ile	Ala
			20					25					30		
Val	Glu	Val	Asn	Val	Gly	Asp	Thr	Ile	Ala	Val	Asp	Asp	Thr	Leu	Ile
		35					40					45			
Thr	Leu	Asp	Leu	Asp	Arg	Leu	Lys	Met	Asp	Lys	Leu	Lys	Leu	Lys	Gly
	50					55					60				
Met	Ser	Tyr	Ala	Met	Cys	Leu	Asn	Thr	Phe	Val	Leu	Lys	Lys	Glu	Val
65					70					75					80
Ser	Glu	Thr	His	Gly	Thr	Ile	Leu	Ile	Lys	Val	Glu	Tyr	Lys	Gly	Glu
				85					90					95	
Asp	Ala	Pro	Cys	Lys	Ile	Pro	Phe	Ser	Thr	Glu	Asp	Gly	Gln	Gly	Lys
			100					105					110		
Ala	His	Asn	Gly	Arg	Leu	Ile	Thr	Ala	Asn	Pro	Val	Val	Thr	Lys	Lys
		115					120					125			
Glu	Glu	Pro	Val	Asn	Ile	Glu	Ala	Glu	Pro	Pro	Phe	Gly	Glu	Ser	Asn
	130					135					140				
Ile	Val	Ile	Gly	Ile	Gly	Asp	Lys	Ala	Leu	Lys	Ile	Asn	Trp	Tyr	Arg
145					150					155					160
Lys	Gly	Ser	Ser	Ile	Gly	Lys	Met	Phe	Glu	Ala	Thr	Ala	Arg	Gly	Ala
				165					170					175	
Arg	Arg	Met	Ala	Ile	Leu	Gly	Asp	Thr	Ala	Trp	Asp	Phe	Gly	Ser	Val
			180					185					190		
Gly	Gly	Val	Asn	Ser	Met	Asn	Ser	Met	Asp	Val	Pro	Ala	Glu	Val	Ala
		195					200					205			
Gly	Val	Val	Lys	Glu	Val	Lys	Val	Lys	Val	Gly	Asp	Lys	Ile	Ser	Glu
	210					215					220				
Gly	Gly	Leu	Ile	Val	Val	Val	Glu	Ala	Glu	Gly	Thr	Ala	Ala	Ala	Pro
225					230					235					240

Lys Ala Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala
245 250 255

Ala Pro Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu
260 265 270

Tyr Asp Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala
275 280 285

Phe Ala Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr
290 295 300

Lys Thr Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys
305 310 315 320

Ala Leu Leu His Asn Ala Ala Val Ile Asp Glu Val Arg His Leu Ala
325 330 335

Ala Asn Gly Ile Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met Leu
340 345 350

Arg Ala Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala
355 360 365

Gly Met Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln
370 375 380

Phe Leu Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala
385 390 395 400

Tyr Glu Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys
405 410 415

Asn Cys Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile
420 425 430

Pro Glu Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys
435 440 445

Glu Val Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu
450 455 460

Glu Met Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val
465 470 475 480

Glu Met Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys
485 490 495

Val Trp Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn
500 505 510

Thr Lys Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr
515 520 525

Phe Glu Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val
530 535 540

Leu Val Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu
545 550 555 560

Lys Ala Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys
565 570 575

Gln Met Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val
580 585 590

Gly Gln Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala
595 600 605

Ala Glu Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile
610 615 620

Pro Gly Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr
625 630 635 640

Glu Leu Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe
645 650 655

Pro Trp Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro
660 665 670

Phe Thr Lys Leu Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly Gly
675 680 685

Gly Ile Val Gly Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys Leu
690 695 700

Ala Ile Glu Met Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile His
705 710 715 720

Pro His Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly
725 730 735

Thr Cys Thr Asp Leu Pro Pro Gln Lys Lys Lys
740 745

<210> 44
<211> 2256
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2256)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pAZ3

<400> 44
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg acacgaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagaaat ggacgtacct 180
gctgaagttg caggcgtagt caaagaagtt aaagttaaag tcggcgacaa aatctctgaa 240
ggtggtttga ttgtcgtcgt tgaagctgaa ggcacggcag ccgctcctaa agccgaagcg 300
gctgccgccc cggcgcaaga agcccctaaa gctgccgctc ctgctccgca agccgcgcaa 360
ttcggcggtt ctgccgatgc cgagtacgac gtggtcgtat tgggtggcgg tcccggcggt 420
tactccgctg catttgccgc tgccgatgaa ggcttgaaag tcgccatcgt cgaacgttac 480
aaaactttgg gcggcgtttg cctgaacgtc ggctgtatcc cttccaaagc cttgttgcac 540
aatgccgccg ttatcgacga agtgcgccac ttggctgcca acggtatcaa ataccccgag 600
ccggaactcg acatcgatat gcttcgcgcc tacaaagacg gcgtagtttc ccgcctcacg 660
ggcggtttgg caggtatggc gaaaagccgt aaagtggacg ttatccaagg cgacgggcaa 720

```
ttcttagatc cgcaccactt ggaagtgtcg ctgactgccg gcgacgcgta cgaacaggca 780
gcccctaccg gcgagaaaaa aatcgttgcc ttcaaaaact gtatcattgc agcaggcagc 840
cgcgtaacca aactgccttt cattcctgaa gatccgcgca tcatcgattc cagcggcgca 900
ttggctctga aagaagtacc gggcaaactg ctgattatcg gcggcggcat tatcggcctc 960
gagatgggta cggtttacag cacgctgggt tcgcgtttgg atgtggttga aatgatggac 1020
ggcctgatgc aaggcgcaga ccgcgatttg gtaaaagtat ggcaaaaaca aaacgaatac 1080
cgttttgaca acattatggt caacaccaaa accgttgcag ttgagccgaa agaagacggc 1140
gtttacgtta cctttgaagg cgcgaacgcg cctaaagagc cgcaacgcta cgatgccgta 1200
ttggttgccg ccggccgcgc gcccaacggc aaactcatca gcgcggaaaa agcaggcgtt 1260
gccgtaaccg atcgcggctt catcgaagtg gacaaacaaa tgcgtaccaa tgtgccgcac 1320
atctacgcca tcggcgacat cgtcggtcag ccgatgttgg cgcacaaagc cgttcacgaa 1380
ggccacgttg ccgccgaaaa ctgcgccggc cacaaagcct acttcgacgc acgcgtgatt 1440
ccgggcgttg cctacacttc ccccgaagtg gcgtgggtgg gcgaaaccga actgtccgcc 1500
aaagcctccg gccgcaaaat caccaaagcc aacttcccgt gggcggcttc cggccgtgcg 1560
attgccaacg gttgcgacaa gccgtttacc aagctgattt ttgatgccga aaccggccgc 1620
atcatcggcg gcggcattgt cggtccgaac ggtggcgata tgatcggcga agtctgcctt 1680
gccatcgaaa tgggctgcga cgcggcagac atcggcaaaa ccatccaccc gcacccgacc 1740
ttgggcgaat ccatcggtat ggcggcggaa gtggcattgg gtacttgtac cgacctgcct 1800
ccgcaaaaga aaaaaggatc cagactcaag atggacaaat tgaaactcaa ggggatgagc 1860
tatgcaatgt gcttgaatac ctttgtgttg aagaaagaag tctccgaaac gcagcatggg 1920
acaatactca ttaaggttga gtacaaaggg gaagatgcac cctgcaagat tcctttctcc 1980
acggaggatg gacaagggaa agctcacaat ggcagactga tcacagccaa tccagtggtg 2040
accaagaagg aggagcctgt caacattgag gctgaacctc cttttgggga aagtaatata 2100
gtaattggaa ttggagacaa agccctgaaa atcaactggt acaggaaggg aagctcgatt 2160
gggaagatgt tcgaggccac tgccagaggt gcaaggcgca tggccatctt gggagacaca 2220
gcctgggact ttggttcagt gggtggttaa ggatcc                          2256
```

<210> 45
<211> 744
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(744)
<223> Aminoacidic sequence of the PAZ3

<400> 45

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His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
  1               5                  10                  15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
             20                  25                  30

Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
         35                  40                  45

Thr Leu Asp Met Asn Ser Met Asp Val Pro Ala Glu Val Ala Gly Val
     50                  55                  60

Val Lys Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser Glu Gly Gly
 65                  70                  75                  80

Leu Ile Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala Pro Lys Ala
                 85                  90                  95

Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala Ala Pro
            100                 105                 110

Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu Tyr Asp
```

```
          115                 120                 125

Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala Phe Ala
    130                 135                 140

Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr Lys Thr
145                 150                 155                 160

Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys Ala Leu
                165                 170                 175

Leu His Asn Ala Ala Val Ile Asp Glu Val Arg His Leu Ala Ala Asn
            180                 185                 190

Gly Ile Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met Leu Arg Ala
        195                 200                 205

Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala Gly Met
    210                 215                 220

Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln Phe Leu
225                 230                 235                 240

Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala Tyr Glu
                245                 250                 255

Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys Asn Cys
            260                 265                 270

Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile Pro Glu
        275                 280                 285

Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys Glu Val
    290                 295                 300

Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu Glu Met
305                 310                 315                 320

Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val Glu Met
                325                 330                 335

Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys Val Trp
            340                 345                 350

Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn Thr Lys
        355                 360                 365

Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr Phe Glu
    370                 375                 380

Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val Leu Val
385                 390                 395                 400

Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu Lys Ala
                405                 410                 415

Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys Gln Met
            420                 425                 430

Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val Gly Gln
        435                 440                 445
```

Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala Ala Glu
450 455 460

Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile Pro Gly
465 470 475 480

Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr Glu Leu
485 490 495

Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe Pro Trp
500 505 510

Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro Phe Thr
515 520 525

Lys Leu Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly Gly Gly Ile
530 535 540

Val Gly Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys Leu Ala Ile
545 550 555 560

Glu Met Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile His Pro His
565 570 575

Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly Thr Cys
580 585 590

Thr Asp Leu Pro Pro Gln Lys Lys Lys Gly Ser Arg Leu Lys Met Asp
595 600 605

Lys Leu Lys Leu Lys Gly Met Ser Tyr Ala Met Cys Leu Asn Thr Phe
610 615 620

Val Leu Lys Lys Glu Val Ser Glu Thr His Gly Thr Ile Leu Ile Lys
625 630 635 640

Val Glu Tyr Lys Gly Glu Asp Ala Pro Cys Lys Ile Pro Phe Ser Thr
645 650 655

Glu Asp Gly Gln Gly Lys Ala His Asn Gly Arg Leu Ile Thr Ala Asn
660 665 670

Pro Val Val Thr Lys Lys Glu Glu Pro Val Asn Ile Glu Ala Glu Pro
675 680 685

Pro Phe Gly Glu Ser Asn Ile Val Ile Gly Ile Gly Asp Lys Ala Leu
690 695 700

Lys Ile Asn Trp Tyr Arg Lys Gly Ser Ser Ile Gly Lys Met Phe Glu
705 710 715 720

Ala Thr Ala Arg Gly Ala Arg Arg Met Ala Ile Leu Gly Asp Thr Ala
725 730 735

Trp Asp Phe Gly Ser Val Gly Gly
740

<210> 46
<211> 426

<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(426)
<223> Nucleotidic sequence coding for the aminoacids 286 to 426 of the DEN-4 envelope protein

<400> 46
aaagtccgta tggagaaatt gagaatcaag ggaatgtcat acacgatgtg ttcaggaaag 60
ttttcaattg acaaagagat ggcagaaaca cagcatggga caacagtggt gaaagtcaag 120
tatgaaggtg ctggagctcc gtgtaaagtc cccatagaga taagagatgt aaacaaggaa 180
aaagtggttg ggcgtatcat ctcatccacc cctttggctg agaataccaa cagtgtaacc 240
aacatagaat tagaaccccc ctttggggac agctacatag tgataggtgt tggaaacagc 300
gcattaacac tccattggtt caggaaaggg agttccattg gcaagatgtt tgagtccaca 360
tacagaggtg caaaacgaat ggccattcta ggtgaaacag cttgggattt tggttccgtt 420
ggtgga 426

<210> 47
<211> 615
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(615)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pID1

<400> 47
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagattt ggatctagac 180
aaagtgcgta tggagaaatt gagaatcaag ggaatgtcat acacgatgtg ttcaggaaag 240
ttttcaattg acaaagagat ggcagaaaca cagcatggga caacagtggt gaaagtcaag 300
tatgaaggtg ctggagctcc gtgtaaagtc cccatagaga taagagatgt aaacaaggaa 360
aaagtggttg ggcgtatcat ctcatccacc cctttggctg agaataccaa cagtgtaacc 420
aacatagaat tagaaccccc ctttggggac agctacatag tgataggtgt tggaaacagc 480
gcattaacac tccattggtt caggaaaggg agttccattg gcaagatgtt tgagtccaca 540
tacagaggtg caaaacgaat ggccattcta ggtgaaacag cttgggattt tggttccgtt 600
ggtggataag gatcc 615

<210> 48
<211> 192
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(192)
<223> Aminoacidic sequence of the PID1

<400> 48
His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
1 5 10 15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
20 25 30

```
Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
         35                  40                  45

Thr Leu Asp Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly Met Ser
     50                  55                  60

Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met Ala Glu
 65                  70                  75                  80

Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly Ala Gly
                 85                  90                  95

Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys Glu Lys
            100                 105                 110

Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn Thr Asn
        115                 120                 125

Ser Val Thr Asn Ile Glu Leu Glu Arg Pro Leu Asp Ser Tyr Ile Val
    130                 135                 140

Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe Arg Lys Gly
145                 150                 155                 160

Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly Ala Lys Arg
                165                 170                 175

Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser Val Gly Gly
            180                 185                 190
```

<210> 49
<211> 2241
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2241)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pID2

<400> 49
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aaagtgcccg acattggcgg aacagaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagacaa agtccgtatg 180
gagaaattga gaatcaaggg aatgtcatac acgatgtgtt caggaaagtt ttcaattgac 240
aaagagatgg cagaaacaca gcatgggaca acagtggtga aagtcaagta tgaaggtgct 300
ggagctccgt gtaaagtccc catagagata agagatgtaa acaaggaaaa agtggttggg 360
cgtatcatct catccacccc tttggctgag aataccaaca gtgtaaccaa catagaatta 420
gaaccccccct ttggggacag ctacatagtg ataggtgttg gaaacagcgc attaacactc 480
cattggttca ggaaagggag ttccattggc aagatgtttg agtccacata cagaggtgca 540
aaacgaatgg ccattctagg tgaaacagct tgggattttg gtagcgttgg tggactgaat 600
tcgatgaatt cgatggacgt acctgctgaa gttgcaggcg tagtcaaaga agttaaagtt 660
aaagtcggcg acaaaatctc tgaaggtggt ttgattgtcg tcgttgaagc tgaaggcacg 720
gcagccgctc ctaaagccga agcggctgcc gccccggcgc aagaagcccc taaagctgcc 780
gctcctgctc cgcaagccgc gcaattcggc ggttctgccg atgccgagta cgacgtggtc 840

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gtattgggtg gcggtcccgg cggttactcc gctgcatttg ccgctgccga tgaaggcttg 900
aaagtcgcca tcgtcgaacg ttacaaaact ttgggcggcg tttgcctgaa cgtcggctgt 960
atcccttcca aagccttgtt gcacaatgcc gccgttatcg acgaagtgcg ccacttggct 1020
gccaacggta tcaaataccc cgagccggaa ctcgacatcg atatgcttcg cgcctacaaa 1080
gacggcgtag tttcccgcct cacgggcggt ttggcaggta tggcgaaaag ccgtaaagtg 1140
gacgttatcc aaggcgacgg gcaattctta gatccgcacc acttggaagt gtcgctgact 1200
gccggcgacg cgtacgaaca ggcagcccct accggcgaga aaaaaatcgt tgccttcaaa 1260
aactgtatca ttgcagcagg cagccgcgta accaaactgc ctttcattcc tgaagatccg 1320
cgcatcatcg attccagcgg cgcattggct ctgaaagaag taccgggcaa actgctgatt 1380
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gtatggcaaa aacaaaacga ataccgtttt gacaacatta tggtcaacac caaaaccgtt 1560
gcagttgagc cgaaagaaga cggcgtttac gttacctttg aaggcgcgaa cgcgcctaaa 1620
gagccgcaac gctacgatgc cgtattggtt gccgccggcc gcgcgcccaa cggcaaactc 1680
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caaatgcgta ccaatgtgcc gcacatctac gccatcggcg acatcgtcgg tcagccgatg 1800
ttggcgcaca aagccgttca cgaaggccac gttgccgccg aaaactgcgc cggccacaaa 1860
gcctacttcg acgcacgcgt gattccgggc gttgcctaca cttcccccga agtggcgtgg 1920
gtgggcgaaa ccgaactgtc cgccaaagcc tccggccgca aaatcaccaa agccaacttc 1980
ccgtgggcgg cttccggccg tgcgattgcc aacggttgcg acaagccgtt taccaagctg 2040
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gatatgatcg gcgaagtctg ccttgccatc gaaatgggct gcgacgcggc agacatcggc 2160
aaaaccatcc acccgcaccc gggcgaatcc atcggtatgg cggcggaagt ggcattgggt 2220
acttgtaccg acaaaaaaaa a                                          2241
```

<210> 50
<211> 747
<212> PRT
<213> Escherichia coli

<220>
<221> CHAIN
<222> (1)..(747)
<223> Aminoacidic sequence of the PID2

<400> 50

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His His His His His His Met Val Asp Lys Arg Met Ala Leu Val Glu
  1               5                  10                  15

Leu Lys Val Pro Asp Ile Gly Gly His Glu Asn Val Asp Ile Ile Ala
             20                  25                  30

Val Glu Val Asn Val Gly Asp Thr Ile Ala Val Asp Asp Thr Leu Ile
         35                  40                  45

Thr Leu Asp Leu Asp Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly
     50                  55                  60

Met Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met
 65                  70                  75                  80

Ala Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly
                 85                  90                  95

Ala Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys
            100                 105                 110

Glu Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn
        115                 120                 125
```

```
Thr Asn Ser Val Thr Asn Ile Glu Leu Glu Arg Pro Leu Asp Ser Tyr
    130                 135                 140

Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe Arg
145                 150                 155                 160

Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly Ala
                165                 170                 175

Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser Val
            180                 185                 190

Gly Gly Leu Asn Ser Met Asn Ser Met Asp Val Pro Ala Glu Val Ala
        195                 200                 205

Gly Val Val Lys Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser Glu
    210                 215                 220

Gly Gly Leu Ile Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala Pro
225                 230                 235                 240

Lys Ala Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala
                245                 250                 255

Ala Pro Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu
            260                 265                 270

Tyr Asp Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala
        275                 280                 285

Phe Ala Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr
    290                 295                 300

Lys Thr Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys
305                 310                 315                 320

Ala Leu Leu His Asn Ala Ala Val Ile Asp Glu Val Arg His Leu Ala
                325                 330                 335

Ala Asn Gly Ile Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met Leu
            340                 345                 350

Arg Ala Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala
        355                 360                 365

Gly Met Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln
    370                 375                 380

Phe Leu Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala
385                 390                 395                 400

Tyr Glu Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys
                405                 410                 415

Asn Cys Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile
            420                 425                 430

Pro Glu Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys
        435                 440                 445

Glu Val Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu
```

```
     450                 455                 460

Glu Met Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val
465                 470                 475                 480

Glu Met Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys
                485                 490                 495

Val Trp Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn
            500                 505                 510

Thr Lys Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr
        515                 520                 525

Phe Glu Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val
    530                 535                 540

Leu Val Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu
545                 550                 555                 560

Lys Ala Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys
                565                 570                 575

Gln Met Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val
            580                 585                 590

Gly Gln Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala
        595                 600                 605

Ala Glu Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile
    610                 615                 620

Pro Gly Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr
625                 630                 635                 640

Glu Leu Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe
                645                 650                 655

Pro Trp Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro
            660                 665                 670

Phe Thr Lys Leu Ile Phe Asp Ala Glu Thr Gly Arg Ile Ile Gly Gly
        675                 680                 685

Gly Ile Val Gly Pro Asn Gly Gly Asp Met Ile Gly Glu Val Cys Leu
    690                 695                 700

Ala Ile Glu Met Gly Cys Asp Ala Ala Asp Ile Gly Lys Thr Ile His
705                 710                 715                 720

Pro His Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly
                725                 730                 735

Thr Cys Thr Asp Leu Pro Pro Gln Lys Lys Lys
            740                 745
```

<210> 51
<211> 2256
<212> DNA
<213> Escherichia coli

<220>
<221> gene
<222> (1)..(2256)
<223> Nucleotidic sequence coding for the quimeric protein in the plasmid pID3

<400> 51
atgggccacc accaccacca ccacgccatg gtagataaaa gaatggcttt agttgaattg 60
aaagtgcccg acattggcgg acacgaaaat gtagatatta tcgcggttga agtaaacgtg 120
ggcgacacta ttgctgtgga cgataccctg attactttgg atctagaaat ggacgtacct 180
gctgaagttg caggcgtagt caaagaagtt aaagttaaag tcggcgacaa aatctctgaa 240
ggtggtttga ttgtcgtcgt tgaagctgaa ggcacggcag ccgctcctaa agccgaagcg 300
gctgccgccc cggcgcaaga agcccctaaa gctgccgctc ctgctccgca agccgcgcaa 360
ttcggcggtt ctgccgatgc cgagtacgac gtggtcgtat tgggtggcgg tcccggcggt 420
tactccgctg catttgccgc tgccgatgaa ggcttgaaag tcgccatcgt cgaacgttac 480
aaaactttgg gcggcgtttg cctgaacgtc ggctgtatcc cttccaaagc cttgttgcac 540
aatgccgccg ttatcgacga agtgcgccac ttggctgcca acggtatcaa ataccccgag 600
ccggaactcg acatcgatat gcttcgcgcc tacaaagacg gcgtagtttc ccgcctcacg 660
ggcggtttgg caggtatggc gaaaagccgt aaagtggacg ttatccaagg cgacgggcaa 720
ttcttagatc cgcaccactt ggaagtgtcg ctgactgccg gcgacgcgta cgaacaggca 780
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cgcgtaacca aactgccttt cattcctgaa gatccgcgca tcatcgattc cagcggcgca 900
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gagatgggta cggtttacag cacgctgggt tcgcgtttgg atgtggttga aatgatggac 1020
ggcctgatgc aaggcgcaga ccgcgatttg gtaaaagtat ggcaaaaaca aaacgaatac 1080
cgttttgaca acattatggt caacaccaaa accgttgcag ttgagccgaa agaagacggc 1140
gtttacgtta cctttgaagg cgcgaacgcg cctaaagagc cgcaacgcta cgatgccgta 1200
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gccgtaaccg atcgcggctt catcgaagtg gacaaacaaa tgcgtaccaa tgtgccgcac 1320
atctacgcca tcggcgacat cgtcggtcag ccgatgttgg cgcacaaagc cgttcacgaa 1380
ggccacgttg ccgccgaaaa ctgcgccggc cacaaagcct acttcgacgc acgcgtgatt 1440
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aaagcctccg gccgcaaaat caccaaagcc aacttcccgt gggcggcttc cggccgtgcg 1560
attgccaacg gttgcgacaa gccgtttacc aagctgattt ttgatgccga aaccggccgc 1620
atcatcggcg gcggcattgt cggtccgaac ggtggcgata tgatcggcga agtctgcctt 1680
gccatcgaaa tgggctgcga cgcggcagac atcggcaaaa ccatccaccc gcacccgacc 1740
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ccgcaaaaga aaaaaggatc caaagtgcgt atggagaaat tgagaatcaa gggaatgtca 1860
tacacgatgt gttcaggaaa gttttcaatt gacaaagaga tggcagaaac acagcatggg 1920
acaacagtgg tgaaagtcaa gtatgaaggt gctggagctc cgtgtaaagt ccccatagag 1980
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        35                  40                  45

Thr Leu Asp Met Asn Ser Met Asp Val Pro Ala Glu Val Ala Gly Val
    50                  55                  60

Val Lys Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser Glu Gly Gly
65                  70                  75                  80

Leu Ile Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala Pro Lys Ala
                85                  90                  95

Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala Ala Pro
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Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu Tyr Asp
        115                 120                 125

Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala Phe Ala
    130                 135                 140

Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr Lys Thr
145                 150                 155                 160

Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys Ala Leu
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Leu His Asn Ala Ala Val Ile Asp Glu Val Arg His Leu Ala Ala Asn
            180                 185                 190

Gly Ile Lys Tyr Pro Glu Pro Glu Leu Asp Ile Asp Met Leu Arg Ala
        195                 200                 205

Tyr Lys Asp Gly Val Val Ser Arg Leu Thr Gly Gly Leu Ala Gly Met
    210                 215                 220

Ala Lys Ser Arg Lys Val Asp Val Ile Gln Gly Asp Gly Gln Phe Leu
225                 230                 235                 240

Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala Tyr Glu
                245                 250                 255

Gln Ala Ala Pro Thr Gly Glu Lys Lys Ile Val Ala Phe Lys Asn Cys
            260                 265                 270

Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile Pro Glu
        275                 280                 285

Asp Pro Arg Ile Ile Asp Ser Ser Gly Ala Leu Ala Leu Lys Glu Val
    290                 295                 300

Pro Gly Lys Leu Leu Ile Ile Gly Gly Gly Ile Ile Gly Leu Glu Met
305                 310                 315                 320

Gly Thr Val Tyr Ser Thr Leu Gly Ser Arg Leu Asp Val Val Glu Met
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Met Asp Gly Leu Met Gln Gly Ala Asp Arg Asp Leu Val Lys Val Trp
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Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn Thr Lys
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Thr Val Ala Val Glu Pro Lys Glu Asp Gly Val Tyr Val Thr Phe Glu
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Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val Leu Val
385                 390                 395                 400

Ala Ala Gly Arg Ala Pro Asn Gly Lys Leu Ile Ser Ala Glu Lys Ala
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Arg Thr Asn Val Pro His Ile Tyr Ala Ile Gly Asp Ile Val Gly Gln
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Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala Ala Glu
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Asn Cys Ala Gly His Lys Ala Tyr Phe Asp Ala Arg Val Ile Pro Gly
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Val Ala Tyr Thr Ser Pro Glu Val Ala Trp Val Gly Glu Thr Glu Leu
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Ser Ala Lys Ala Ser Gly Arg Lys Ile Thr Lys Ala Asn Phe Pro Trp
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Ala Ala Ser Gly Arg Ala Ile Ala Asn Gly Cys Asp Lys Pro Phe Thr
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                565                 570                 575

Pro Gly Glu Ser Ile Gly Met Ala Ala Glu Val Ala Leu Gly Thr Cys
            580                 585                 590

Thr Asp Leu Pro Pro Gln Lys Lys Lys Gly Ser Lys Val Arg Met Glu
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Lys Leu Arg Ile Lys Gly Met Ser Tyr Thr Met Cys Ser Gly Lys Phe
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Ser Ile Asp Lys Glu Met Ala Glu Thr Gln His Gly Thr Thr Val Val
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Lys Val Lys Tyr Glu Gly Ala Gly Ala Pro Cys Lys Val Pro Ile Glu
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Ile Arg Asp Val Asn Lys Glu Lys Val Val Gly Arg Ile Ile Ser Ser
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660 665 670

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Arg Pro Leu Asp Ser Tyr Ile Val Ile Gly Val Gly Asn Ser Ala Leu
690 695 700

Thr Leu His Trp Phe Arg Lys Gly Ser Ser Ile Gly Lys Met Phe Glu
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Ser Thr Tyr Arg Gly Ala Lys Arg Met Ala Ile Leu Gly Glu Thr Ala
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Thr Leu Asp Leu Asp Lys Val Arg Met Glu Lys Leu Arg Ile Lys Gly
     50                  55                  60

Met Ser Tyr Thr Met Cys Ser Gly Lys Phe Ser Ile Asp Lys Glu Met
 65                  70                  75                  80

Ala Glu Thr Gln His Gly Thr Thr Val Val Lys Val Lys Tyr Glu Gly
                 85                  90                  95

Ala Gly Ala Pro Cys Lys Val Pro Ile Glu Ile Arg Asp Val Asn Lys
            100                 105                 110

Glu Lys Val Val Gly Arg Ile Ile Ser Ser Thr Pro Leu Ala Glu Asn
        115                 120                 125

Thr Asn Ser Val Thr Asn Ile Glu Leu Glu Arg Pro Leu Asp Ser Tyr
    130                 135                 140

Ile Val Ile Gly Val Gly Asn Ser Ala Leu Thr Leu His Trp Phe Arg
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Lys Gly Ser Ser Ile Gly Lys Met Phe Glu Ser Thr Tyr Arg Gly Ala
                165                 170                 175

Lys Arg Met Ala Ile Leu Gly Glu Thr Ala Trp Asp Phe Gly Ser Val
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195 200 205

Gly Val Val Lys Glu Val Lys Val Lys Val Gly Asp Lys Ile Ser Glu
210 215 220

Gly Gly Leu Ile Val Val Val Glu Ala Glu Gly Thr Ala Ala Ala Pro
225 230 235 240

Lys Ala Glu Ala Ala Ala Ala Pro Ala Gln Glu Ala Pro Lys Ala Ala
245 250 255

Ala Pro Ala Pro Gln Ala Ala Gln Phe Gly Gly Ser Ala Asp Ala Glu
260 265 270

Tyr Asp Val Val Val Leu Gly Gly Gly Pro Gly Gly Tyr Ser Ala Ala
275 280 285

Phe Ala Ala Ala Asp Glu Gly Leu Lys Val Ala Ile Val Glu Arg Tyr
290 295 300

Lys Thr Leu Gly Gly Val Cys Leu Asn Val Gly Cys Ile Pro Ser Lys
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Phe Leu Asp Pro His His Leu Glu Val Ser Leu Thr Ala Gly Asp Ala
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Asn Cys Ile Ile Ala Ala Gly Ser Arg Val Thr Lys Leu Pro Phe Ile
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Val Trp Gln Lys Gln Asn Glu Tyr Arg Phe Asp Asn Ile Met Val Asn
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Phe Glu Gly Ala Asn Ala Pro Lys Glu Pro Gln Arg Tyr Asp Ala Val
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545                 550                 555                 560

Lys Ala Gly Val Ala Val Thr Asp Arg Gly Phe Ile Glu Val Asp Lys
                565                 570                 575

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Gly Gln Pro Met Leu Ala His Lys Ala Val His Glu Gly His Val Ala
        595                 600                 605

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    610                 615                 620

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625                 630                 635                 640

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            740                 745                 750

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        755                 760                 765

Lys Phe Lys Ile Val Lys Glu Ile Ala Glu Thr Gln His Gly Thr Ile
    770                 775                 780

Val Ile Arg Val Gln Tyr Glu Gly Asp Gly Ser Pro Cys Lys Ile Pro
785                 790                 795                 800

Phe Glu Ile Met Asp Leu Glu Lys Arg His Val Leu Gly Arg Leu Ile
                805                 810                 815

Thr Val Asn Pro Ile Val Thr Glu Lys Asp Ser Pro Val Asn Ile Glu
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Ala Glu Pro Pro Phe Gly Asp Ser Tyr Ile Ile Ile Gly Val Glu Pro
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Gly Gln Leu Lys Leu Asn Trp Phe Lys Lys Gly Ser Ser Ile Gly Gln
850 855 860

Met Phe Glu Thr Thr Met Arg Gly Ala Lys Arg Met Ala Ile Leu Gly
865 870 875 880

Asp Thr Ala Trp Asp Phe Gly Ser Leu Gly Gly
885 890

CLAIMS

1. Chimeric nucleotide chains characterized by being the specific combination of sequences corresponding to fragments of the E protein from Flavivirus, with sequences corresponding to the codifying gene for the mutated MDH protein, identified in the sequencing list as Sequence No.26, Sequence No.29 or with the fragment of these identified as Sequence No.23, which codify for chimeric proteins capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient, against the Flavivirus, when these proteins are present in a pharmaceutical preparation.
2. Chimeric nucleotide chains according to claim 1, characterized by being the specific combination of one or several sequences that codify for the amino acids 286 to 426 of the E protein from Dengue viruses with the nucleotide sequence of the MDH protein, where these sequences are inserted and/or fused to the sequences identified in the sequencing list as Sequence No.26, Sequence No.29 or Sequence No.23, which codify for chimeric proteins capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient, serotype-specific against each serotype of Dengue virus.
3. Chimeric nucleotide chains according to claims 1 and 2, characterized by being formed by the codifying sequences for the amino acids 286 to 426 of the E protein from each serotype 1, 2, 3 and 4 of Dengue virus, fused to the 3' end region that codifies for the first 45 amino acids of the MDH protein, identified as Sequence No.23.
4. Chimeric nucleotide chains according to claims 1 and 2, characterized by being formed by the codifying sequences for the amino acids 286 to 426 of the E protein

from each serotype 1, 2, 3 and 4 of Dengue virus, fused to the 3' end region that codifies for the amino acid 45 of the MDH protein, identified as Sequence No.26.

5. Chimeric nucleotide chains according to claims 1 and 2, characterized by being formed by the codifying sequences for the amino acids 286 to 426 of the E protein from each serotype 1, 2, 3and4 of Dengue virus, fused to the 3' end region that codifies for the MDH protein, identified as Sequence No.29.

6. Chimeric nucleotide chains according to claims 1 and 2, characterized by being the combination of the nucleotide sequences that codify for the amino acids 286 to 426 of the E protein from two different serotypes of Dengue virus, with the sequence of the MDH, where one of the sequences corresponding to the E protein is inserted in the region that codifies for the amino acid 45 of the MDH protein and the other is fused to the 3' region of the same MDH sequence, identified as sequence No.29.

7. Recombinant chimeric proteins characterized by being the expression products of the nucleotide chains from claims 1, 2, 3, 4, 5 and 6, and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient, serotype-specific against each serotype of Dengue virus, when these proteins are present in a pharmaceutical preparation.

8. Chimeric recombinant proteins characterized by being the expression products of the nucleotide chains from claims 1 and 6, and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient, specific against the two homologous serotypes of Dengue virus.

9. Chimeric nucleotide chains, identified essentially by the Sequence No.24, according to claims 1, 2 and 3 characterized by being the combination of the sequences identified in the sequencing list as Sequence No.22 and Sequence No.23, which codifies for the chimeric protein PLL1.

10. Chimeric protein PLL1, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.25, be the expression product of the Sequence No.24 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-2 virus, when PLL1 is present in a pharmaceutical preparation.

11. Chimeric nucleotide chains according to claims 1, 2 and 4 identified essentially as Sequence No.27, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.22 and Sequence No.26, which codifies for the chimeric protein PLL2.

12. Chimeric protein PLL2, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.28, be the expression product of the Sequence No. 27 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-2 virus, when PLL2 is present in a pharmaceutical preparation.

13. Chimeric nucleotide chains according to claims 1, 2 and 5 identified essentially as Sequence No.30, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.22 and Sequence No.29, which codifies for the chimeric protein PLL3.

14. Chimeric protein PLL3, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.31, be the expression product of the Sequence No.30 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-2 virus, when PLL3 is present in a pharmaceutical preparation.

15. Chimeric nucleotide chains according to claims 1, 2 and 3 identified essentially as Sequence No.33, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.23 and Sequence No.32, which codifies for the chimeric protein PLH1.

16. Chimeric protein PLH1, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.34, be the expression product of the Sequence No.33 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-1 virus, when PLH1 is present in a pharmaceutical preparation.

17. Chimeric nucleotide chains according to claims 1, 2 and 4 identified essentially as Sequence No.35, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.26 and Sequence No.32, which codifies for the chimeric protein PLH2.

18. Chimeric protein PLH2, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.36, be the expression product of the Sequence No.35 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-1 virus, when PLH2 is present in a pharmaceutical preparation.

19. Chimeric nucleotide chains according to claims 1, 2 and 5 identified essentially as Sequence No.37, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.29 and Sequence No.32, which codifies for the chimeric protein PLH3.

20. Chimeric protein PLH3, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.38, be the expression product of the Sequence No.37 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-1 virus, when PLH3 is present in a pharmaceutical preparation.

21. Chimeric nucleotide chains according to claims 1, 2 and 3 identified essentially as Sequence No.40, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.23 and Sequence No.39, which codifies for the chimeric protein PAZ1.

22. Chimeric protein PAZ1, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.41, be the expression product of the Sequence No.40 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-3 virus, when PAZ1 is present in a pharmaceutical preparation.

23. Chimeric nucleotide chains according to claims 1, 2 and 4 identified essentially as Sequence No.42, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.26 and Sequence No.39, which codifies for the chimeric protein PAZ2.

24. Chimeric protein PAZ2, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.43, be the expression product of the Sequence No.42 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-3 virus, when PAZ2 is present in a pharmaceutical preparation.

25. Chimeric nucleotide chains according to claims 1, 2 and 5 identified essentially as Sequence No.44, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.29 and Sequence No.39, which codifies for the chimeric protein PAZ3.

26. Chimeric protein PAZ3, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.45, be the expression product of the Sequence No.44 and being capable of

inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-3 virus when, PAZ3 is present in a pharmaceutical preparation.

27. Chimeric nucleotide chains according to claims 1, 2 and 3 identified essentially as Sequence No.47, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.23 and Sequence No.46, which codifies for the chimeric protein PID1.

28. Chimeric protein PID1, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.48, be the expression product of the Sequence No.47 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-4 virus, when PID1 is present in a pharmaceutical preparation.

29. Chimeric nucleotide chains according to claims 1, 2 and 4 identified essentially as Sequence No.49, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.26 and Sequence No.46, which codifies for the chimeric protein PID2.

30. Chimeric protein PID2, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.50 be the expression product of the Sequence No.49 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-4 virus, when PID2 is present in a pharmaceutical preparation.

31. Chimeric nucleotide chains according to claims 1, 2 and 5 identified essentially as Sequence No.51, characterised by being the combination of the sequences identified in the sequencing list as Sequence No.29 and Sequence No.46, which codifies for the chimeric protein PID3.

32. Chimeric protein PID3, according to claims 1,2 and 7 characterized by having essentially the amino acid sequence identified in the sequencing list as Sequence No.52 be the expression product of the Sequence No.51 and being capable of inducing a humoral immune response of neutralizing and protective antibodies in the recipient against the Dengue-4 virus, when PID3 is present in a pharmaceutical preparation.

33. A pharmaceutical preparation capable of inducing in the recipient a protective immune response, specific against the Dengue virus, according to claim 1, characterized by having two or more of the chimeric proteins described in claims

1, 2, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32, and a pharmacological vehicle.

34. A pharmaceutical preparation according to claim 33, characterized by being a preventive or therapeutic agent against the Dengue viruses, for oral, intramuscular, subcutaneous, mucosal or intravenous use.

35. A diagnostic mean characterized by having one or several of the proteins described in claims 1, 2, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32, useful for the diagnosis and serotyping of the Dengue virus.