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(54) **Hybrid expression of neisserial proteins**  
Hybride Expression Neisserscher Proteine  
Expression hybride de protéines neisseriales

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**WO-A-92/16643 WO-A-99/36544**  
**US-A- 5 547 670**

- **GUILLEN G ET AL: "EXPRESSION IN**  
**ESCHERICHIA COLI AND IMMUNOLOGICAL**  
**CHARACTERIZATION OF A HYBRID CLASS 1-**  
**P64K PROTEIN FROM NEISSERIA**  
**MENINGITIDIS" BIOTECNOLOGIA APLICADA,**  
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**Description****TECHNICAL FIELD**

[0001] This invention is in the field of protein expression. In particular, it relates to the heterologous expression of proteins from *Neisseria* (e.g. *N.gonorrhoeae* or, preferably, *N.meningitidis*).

**BACKGROUND ART**

[0002] International patent applications WO99/24578, WO99/36544, WO99/57280 and WO00/22430 disclose proteins from *Neisseria meningitidis* and *Neisseria gonorrhoeae*. These proteins are typically described as being expressed in *E.coli* (i.e. heterologous expression) as either N-terminal GST-fusions or C-terminal His-tag fusions, although other expression systems, including expression in native *Neisseria*, are also disclosed.

[0003] Guillen et al (Biotechnologia Aplicada, 13(4), pg 1027-2852 (1996)) discloses a fusion of *Neisseria* P1.15 protein with *Neisseria* P64-k protein which is expressed in *E.coli*.

[0004] It is an object of the present invention to provide alternative and improved approaches for the heterologous expression of these proteins. These approaches will typically affect the level of expression, the ease of purification, the cellular localisation of expression, and/or the immunological properties of the expressed protein.

**DISCLOSURE OF THE INVENTION**

[0005] In accordance with the invention, two proteins of the invention are expressed as a single hybrid protein. It is preferred that no non-*Neisseria* fusion partner (e.g. GST or poly-His) is used.

[0006] This offers two advantages. Firstly, a protein that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem. Secondly, commercial manufacture is simplified - only one expression and purification need be employed in order to produce two separately-useful proteins.

[0007] Thus the invention provides a method for the simultaneous heterologous expression of two proteins of the invention, in which said two or more proteins of the invention are fused (i.e. they are translated as a single polypeptide chain).

[0008] The method will typically involve the steps of obtaining a first nucleic acid encoding a first protein of the invention; obtaining a second nucleic acid encoding a second protein of the invention; ligating the first and second nucleic acids. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

[0009] The hybrid protein can be represented by the formula  $\text{NH}_2\text{-A-B-COOH}$ . A comprises protein 287, and B comprises protein 961.

[0010] 287, is its poly-glycine deletions ( $\Delta\text{G}$ ) form  $\Delta\text{G-287}$ .

[0011] The hybrid protein of the invention is  $\Delta\text{G287-961}$ .

[0012] 287 is used at the N-terminus as a  $\Delta\text{G}$  form of 287 as the N-terminus of a hybrid with 961.

[0013] 287 is preferably from strain 2996 or from strain 394/98. Domain forms of 961 may be used.

[0014] Alignments of polymorphic forms of ORF46, 287, 919 and 953 are disclosed in WO00/66741. Any of these polymorphs can be used according to the present invention.

[0015] Preferably, the constituent proteins (A and B) in a hybrid protein according to the invention will be from the same strain.

[0016] The fused proteins in the hybrid are joined directly.

[0017] The fused proteins may lack native leader peptides or may include the leader peptide sequence of the N-terminal fusion partner

**Host**

[0018] It is preferred to utilise a heterologous host. The heterologous host may be prokaryotic or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*; *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (e.g. *M.tuberculosis*), yeast etc.

**Sequences**

[0019] The invention also provides a protein comprising the sequences of SEQ ID NO 8.

[0020] The degree of 'sequence identity' of the proteins is greater than 70%. This includes mutants and allelic variants [e.g. see WO00/66741]. Identity is preferably determined by the Smith-Waterman homology search algorithm as imple-

mented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1.

**[0021]** Preferred proteins of the invention are found in *N.meningitidis* serogroup B.

**[0022]** Preferred proteins for use according to the invention are those of serogroup B *N.meningitidis* strain 2996 or strain 394/98 (a New Zealand strain). Unless otherwise stated, proteins mentioned herein are from *N.meningitidis* strain 2996. It will be appreciated, however, that the invention is not in general limited by strain. References to a particular protein (e.g. '287', '919' etc.) may be taken to include that protein from any strain.

## BRIEF DESCRIPTION OF DRAWINGS

**[0023]** Figures 1 and 2 show hybrid proteins according to the invention.

## MODES FOR CARRYING OUT THE INVENTION

*Example 1 hybrids of ΔG287*

**[0024]** The deletion of the (Gly)<sub>6</sub> sequence in 287 was found to have a dramatic effect on protein expression. The protein lacking the N-terminal amino acids up to GGGGGG is called 'ΔG287'. In strain MC58, its basic sequence (leader peptide underlined) is:

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      SPDVKS ADTLSKPAAP VVSEKETEAK EDAPQAGSQG QGAPSAQGSQ DMAAVSEENT
GNGGAVTADN PKNEDEVAQN DMPQNAAGTD SSTPNHTPDP NMLAGNMENQ ATDAGESSQP
ANQPDMANAA DGMQGDPSA GGQNAGNTAA QGANQAGNNQ AAGSSDPIPA SNPAPANGGS
NFGRVDLANG VLIDGPSQNI TLTHCKGDSC SGNNFLDEEV QLKSEFEKLS DADKISNYKK
DGKNDKFVGL VADSVQMKGI NQYIIFYKPK PTSFARFRRS ARSRRLPÆE MPLIPVNQAD
TLIVDGEAVS LTGHSGNIFA PEGNYRYLTY GAEKLPGGSY ALRVQGEPAK GEMLAGAAVY
NGEVLHFHTE NGRPYPTRGR FAAKVDFGSK SVDGIIDSGD DLHMGTOQFK AAIDGNGFKG
TWTENGSGDV SGKFGYPAGE EVAGKYSYRP TDAEKGFGV FAGKKEQD*

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ΔG287, with or without His-tag ('ΔG287-His' and 'ΔG287K', respectively), are expressed at very good levels in comparison with the '287-His' or '287<sub>untagged</sub>'.

**[0025]** On the basis of gene variability data, variants of ΔG287-His were expressed in *E. coli* from a number of MenB strains, in particular from strains 2996, MC58, 1000, and BZ232. The results were also good - each of these gave high ELISA titres and also serum bactericidal titres of >8192. ΔG287K, expressed from pET-24b, gave excellent titres in ELISA and the serum bactericidal assay.

**[0026]** ΔG287 was fused directly in-frame upstream of 961 (sequence shown below)

ΔG287-961

ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCGGCTCCTGTTGTTGCTGAAAAAGAGACAGAG  
 GTAAAAGAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGGCAGCCAAGATATGGCGGCA  
 GTTTCGGCAGAAAATACAGGCAATGGCGGTGCGGCAACAACGGACAAAACCCAAAAATGAAGACGAGGGACCGCAAAAT  
 GATATGCCGCAAAATTCGCCGAATCCGCAATCAAACAGGGAACAACCAACCCGCCGATTCTTCAGATTCCGCCCC  
 GCGTCAAACCCCTGCACCTGCGAATGGCGGTAGCAATTTTGGAGGGTTGATTGCTAATGGCGTTTGGATTGATGGG  
 CCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGTAAATGGTGATAATTTATTGGATGAAGAAGCACCG  
 TCAAAATCAGAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAATTT  
 ACTAATTTGGTTGCGACAGCAGTTCAAGCTAATGGAACCTAACAAATATGTCATCATTTATAAAGACAAGTCCGCTTCA  
 TCTTCATCTGCGCGATTTCAGGCGTTCTGCACGGTCGAGGAGGTCGCTTCCGCGGAGATGCCGCTAATCCCCGTCAAT  
 CAGGCGGATACGCTGATTGTCGATGGGGAAGCGGTGACCGTGACGGGGCATTCGGGCAATATCTTCGCGCCCGAAGGG  
 AATTACCGGTATCTGACTTACGGGGCGGAAAATTTGCCCGCGGATCGTATGCCCTCCGTGTGCAAGGCGAAACCGGCA  
 AAAGGCGAAAATGCTTGTGCGCACGGCCGTGTACAACGGCGAAGTGCTGCATTTTCATACGGAAAACGGCCGTCCGTAC  
 CCGACTAGAGGCAGGTTTGCCTGCAAAAGTCGATTTCGGCAGCAAACTGTGTGGACGGCATTTATCGACAGCGGCGATGAT  
 TTGCATATGGGTACGCAAAAATTTCAAAGCCGCCATCGATGGAACCGGCTTTAAGGGGACTTGGACGGAATTTGGCGGC  
 GGGGATGTTTCCGGAAGGTTTACGGCCCGCGCGGAGAGTGGCGGGGAAAATACAGCTATCCGCCGACAGATGCG  
 GAAAAGGGCGGATTTCGGCGTGTTCGCCGCAAAAAGAGCAGGATGGATCCGGAGGAGGAGGAGCCACAAACGACGAC  
 GATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCACAAACATGGCCAAAGAAATCAACGGTTTCAAAGCTGGA  
 GAGACCATCTACGACATTTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGAC  
 TTTAAAGGTCTGGGTCTGAAAAAAGTCTGACTAACCCTGACCAAAACCGTCAATGAAAAACAACAAAACGTCGATGCC  
 AAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAAACAGTTAGCAGACACTGATGCCGCTTTAGCAGATAGT  
 GATGCCGCTCTGGATGCAACCACCAACGCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAAGAGACTAAG  
 ACAATATCGTAAAAATTTGATGAAAAATTTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTTCAACGAT  
 ATCGCCGATTCTATTTGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCAATGAAGCCAAACAGACGGCC  
 GAAGAAACCAACAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCTGCCGCTGGC  
 ACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAAC  
 AAAGATAATATTGCTAAAAAAGCAAAACAGTCCGACGTTACACCCAGAGAAGAGTCTGACAGCAAAATTTGTCAGAATT  
 GATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCAGGATACT  
 CGCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAAGAAACCCGCCAAGGCCCTTGCAAGCAACGCCGCGCTC  
 TCCGGTCTGTTCCAACTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCGGCGGCTACAAATCCGAATCGGCA  
 GTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGGCGTGGCAGTCGGCACTTCGTCGGGT  
 TCTTCGGCAGCTTACCATGTGCGGCTCAATTACGAGTGGTAACTCGAG

1 MASPDVKSAD TLSKPAAPVV AEKETEVKED APQAGSQGQ APSTQGSQDM  
 51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNQPADSS  
 101 DSAPASNPA ANGGSNFRV DLANGVLIDG PSQNLTLTHC KGDSNCGDNL  
 151 LDEEAPSKSE FENLNERI EKYKDKGSD KFTNLVATAV QANGTNKYVI  
 201 IYKDKSASS SARFRSARS RRSIPAEMPL IPVQADTLI VDGEAVSLTG  
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKSEM LAGTAVYNGE  
 301 VLHFHTENGR PYPTRGRFAA KVDFGSKSVD GIIDSGDDLH MGTQKFKAAI  
 351 DNGFGKWTW ENGSGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVVFAG  
 401 KKEQDGSGGG GATNDDDVKK AATVAIAAY NNGQEINGFK AGETIYDIDE  
 451 DGTITKKDAT AADVEADDFK GLGLKKVVTN LTKTVNENKQ NVDKVKAAE  
 501 SEIEKLITKL ADTDAALADT DAALDATINA LNKLGENTIT PAEBTKTNIV  
 551 KIDEKLEAVA DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE  
 601 TKQNVDAKVK AAETAAGKAE AAAGTANTAA DKAEVAKAV TDIKADIATN  
 651 KDNIAKKANS ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH  
 701 DTRLNGLDKT VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK  
 751 SESAVAIGTG FRFTENFAK AGVAVGTSSG SSAAYHVGVN YEW\*

	ELISA	Bactericidal
ΔG287-961-His	108627	65536

[0027] The same hybrid proteins were made using New Zealand strain 394/98 rather than 2996:

AG287NZ-961

ATGGCTAGCCCCGATGTCAAGTCGGCGGACACGCTGTCAAAACCTGCCGCCCTGTTGTTTCTGAAAAAGAGACAGAG  
 GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCGCACAAGGCGGTCAAGATATGCGGCGG  
 GTTTCGGAAGAAAATACAGGCAATGGCGGTGCGGCAGCAACGGACAAACCCAAAAATGAAGACGAGGGGGCGCAAAAT  
 5 GATATGCCGCCAAAATGCCGCCGATACAGATAGTTTGACACCGAATCACACCCCGGCTTCGAATATGCCGCCGCGAAAT  
 ATGGAACCAAGCACCGGATGCCGGGGAATCGGAGCAGCCGGCAACCAACCGGATATGGCAAATACGGCGGACGGA  
 ATGCAGGGTGACGATCCGTCGGCAGGCGGGGAAAATGCCGGCAATACGGCTGECGAAGGTACAAATCAAGCCGAAAAC  
 AATCAAACCGCCGGTTCTCAAAATCCTGCCTCTTCAACCAATCCTAGCGCCACGAATAGCGGTGGTGATTTTGGAAAGG  
 ACGAACGTGGGCAATTCTGTTGTGATTGACGGGCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGT  
 10 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGCTAAATCAGAATTTGAAAAATTAAAGTATGCAGACAAAATAAGT  
 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTTGTCGGTTTGGTTGCCGATAGTGTGCAGATGAAG  
 GGAATCAATCAATATATTATCTTTTATAAACCTAAACCCACTTCATTTGCGCGATTAGGCGTTCTGCACGGTTCGAGG  
 CGGTGCTTCCCGGCCGAGATGCCGCTGATTCCCGTCAATCAGGCGGATACGCTGATTGTGATGGGGAAGCGGTACAGC  
 CTGACGGGGCATTCCGGCAATATCTTCCGCGCCGAAGGGAATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCCC  
 GCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAGGCGGAAATGCTCGCGGGCACGGCAGTGTACAACGGC  
 15 GAAGTGTGCTATTTTCATACGGAACCGCCGTCCTCCCGTCCAGAGGCAGGTTTGCCGCAAAAGTCGATTTCCGGC  
 AGCAAATCTGTGGACGGCATTATCGACAGCGGCGATGGTTTGCATATGGGTACGCAAAATTCAAAGCCGCCATCGAT  
 GGAACGGCTTTAAGGGGACTTGGACGGAAAAATGGCGGCGGGGATGTTTCCGGAAGTTTACGGCCCGCGCGGAG  
 GAAGTGGCGGGAAAAATACAGCTATCGCCCAACAGATGCGGAAAAGGCGGATTTCGGCGTGTTCGCGGCAAAAAAGAG  
 CAGGATGGATCCGGAGGAGGAGGAGCCACAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCC  
 TACAACATGGCCAAGAAATCAACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACC  
 20 AAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTG  
 ACCAAACCGTCAATGAAAACAAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGAAATAGAAAAGTTAACA  
 ACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATCTGATGCCGCTCTGGATGCAACCACCAACGCTTGAATAAA  
 TTGGGAGAAAAATATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTG  
 GCTGATACCGTCGACAAGCATGCCGAAGCATTCACGATATCGCCGATTTCATTGGATGAAACCAACACTAAGGCAGAC  
 GAAGCCGTCAAAACCGCCAAATGAAGCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCT  
 25 GCAGAACTGCAGCAGGCAAGCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCTGCT  
 GCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAAACAGTCCCGACGTG  
 TACACCAGAGAAGAGTCTGACAGCAAAATTTGTGAGAATTGATGGTCTGAACGCTACTACCGAAAAATGGACACACGC  
 TTGGCTTCTGCTGAAAAATCCATTGCCGATCAGGATCTCGCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGC  
 AAAGAAACCCGCCAAGGCCTTGCAGAACAGCCGCGCTCTCCGCTCTGTTCCAACCTTACAACGTGGTTCGGTTCAAT  
 30 GTAACGCTGCAGTCCGGCGCTACAAATCCGAATCGGCAGTCCGCATCGGTACCGGCTTCCGCTTTACCGAAAACTTT  
 GCCGCCAAAGCAGGCGTGGCAGTCCGCACCTTCGTCGGGTTCTTCCGCGAGCTACCATGTCCGGCTCAATTACGAGTGG  
 TAAAAGCTT

1 MASPDVKSAD TLSKPAAPVV SEKETEAKED APQAGSQGQG APSAQGGQDM  
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM  
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG  
 151 TNQAEENQTA GSQNPASSTN PSATNSGGDF GRTNVGNSV IDGPSQNIITL  
 201 THCKGDSQSG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKDV  
 251 GLVADSVQMK GINQYIIFYK PKPTSFARFR RSARSRRSLP AEMPLIPVNF  
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGEF  
 40 351 SKGEMLAGTA VYNGEVLHFP TENGRPSPSR GRFAAKVDFG SKSVDGIIDS  
 401 GDGLHMGTOX FKAIDGNPF KGTWTENGGG DVSGKFYGPA GBEVAGKYSY  
 451 RPTDAEKGGF GVFAKKEQD GSGGGGATND DDVKKAATVA IAAAYNNQGE  
 501 INGFKAGETI YDIDEDGIT KKDATAADVE ADDFKGLGLK KVVTNLTKTIV  
 551 NENKQNVDAK VKAESBIEK LTTKLADTDA ALADTDAALD ATTNALNKLK  
 601 ENITFAEET KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD  
 45 651 EAVKTANEAK QTARETRQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA  
 701 VAAKVTDIKA DIATNKDNLK KANSADVYT REESDSKFVR IDGLNATTEK  
 751 LDTRLASAEL SIADHDTRLN GLDKTVSDLR KETROGLAEQ AALSGLFQPY  
 801 NVGRPNVTAA VGGYKSESAV AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY  
 851 HVGVNYEW\*

**Example 2 - hybrids of 287**

**[0028]** Expression of 287 as full-length with a C-terminal His-tag, or without its leader peptide but with a C-terminal His-tag, gives fairly low expression levels. Better expression is achieved using a N-terminal GST-fusion.

**[0029]** When fused to protein 961 [NH<sub>2</sub>-AG287-961-COOH - sequence shown above], the resulting protein is insoluble and must be denatured and renatured for purification. Following denaturation, around 50% of the protein was found to remain insoluble. The soluble and insoluble proteins were compared, and much better bactericidal titres were obtained

with the soluble protein (FCA as adjuvant):

	2996	BZ232	MC58	NGH38	F6124	BZ133
<b>Soluble</b>	65536	128	4096	>2048	>2048	4096
<b>Insoluble</b>	8192	<4	<4	16	n.d.	n.d.

**[0030]** Titres with the insoluble form were, however, improved by using alum adjuvant instead:

<b>Insoluble</b>	32768	128	4096	>2048	>2048	2048
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**[0031]** 961c was also used in hybrid proteins (see above). As 961 and its domain variants direct efficient expression, they are ideally suited as the N-terminal portion of a hybrid protein.

## EXPERIMENTAL DETAILS

### *Cloning strategy and oligonucleotide design*

**[0032]** Genes coding for antigens of interest were amplified by PCR, using oligonucleotides designed on the basis of the genomic sequence of *N. meningitidis* B MC58. Genomic DNA from strain 2996 was always used as a template in PCR reactions, unless otherwise specified, and the amplified fragments were cloned in the expression vector pET21b+ (Novagen) to express the protein as C-terminal His-tagged product, or in pET-24b+ (Novagen) to express the protein in 'untagged' form (e.g. ΔG287K).

**[0033]** Where a protein was expressed without a fusion partner and with its own leader peptide (if present), amplification of the open reading frame (ATG to STOP codons) was performed.

**[0034]** Where a protein was expressed in 'untagged' form, the leader peptide was omitted by designing the 5'-end amplification primer downstream from the predicted leader sequence.

**[0035]** The melting temperature of the primers used in PCR depended on the number and type of hybridising nucleotides in the whole primer, and was determined using the formulae:

$$T_{m1} = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_{m2} = 64.9 + 0.41 (\% GC) - 600/N \quad (\text{whole primer})$$

**[0036]** The melting temperatures of the selected oligonucleotides were usually 65–70°C for the whole oligo and 50–60°C for the hybridising region alone.

**[0037]** Oligonucleotides were synthesised using a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2.0ml NH<sub>4</sub>OH, and deprotected by 5 hours incubation at 56°C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were centrifuged and the pellets resuspended in water.

		Sequences	Restriction site
fu (961)-741(MC58)-His	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCTG	BamHI
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAGGC	XhoI
fu (961)-983-His	Fwd	CGCGGATCC - GGCGGAGGCGGCACCTT	BamHI
	Rev	CCCGCTCGAG-GAACC GG TAGCCTACG	XhoI
fu (961)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGT-TCAGATTTGGCAAACGATTTC	BamHI
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu (961c-L)-741(MC58)	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCTG	BamHI
	Rev	CCCGCTCGAG-TTATTGCTTGGCGGCAAG	XhoI
fu (961c-L)-983	Fwd	CGCGGATCC - GGCGGAGGCGGCACCTT	BamHI
	Rev	CCCGCTCGAG-TCAGAACCGGTAGCCTAC	XhoI
fu (961c-L)-Orf46.1	Fwd	CGCGGATCCGGTGGTGGT-TCAGATTTGGCAAACGATTTC	BamHI
	Rev	CCCGCTCGAG-TTACGTATCATATTTACGTGC	XhoI
fu-(ΔG287)-919-His	Fwd	CGCGGATCCGGTGGTGGT-CAAAGCAAGAGCATCCAAACC	BamHI
	Rev	CCCAAGCTT-TTCGGGCGGTATTCGGGCTTC	HindIII
fu-(ΔG287)-953-His	Fwd	CGCGGATCCGGTGGTGGT-GCCACCTACAAAGTGGAC	BamHI
	Rev	GCCCAAGCTT-TTGTTTGGCTGCCTCGAT	HindIII
fu-(ΔG287)-961-His	Fwd	CGCGGATCCGGTGGTGGT-ACAAGCGACGACG	BamHI
	Rev	GCCCAAGCTT-CCACTCGTAATTGACGCC	HindIII
fu-(ΔG287)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGT-TCAGATTTGGCAAACGATTTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu-(ΔG287-919)-Orf46.1-His	Fwd	CCCAAGCTTGGTGGTGGT-TCAGATTTGGCAAACGATTTC	HindIII
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu-(ΔG287-Orf46.1)-919-His	Fwd	CCCAAGCCTGGTGGTGGT-CAAAGCAAGAGCATCCAAACC	HindIII
	Rev	CCCGCTCGAG-CGGGCGGTATTCGGGCTT	XhoI

(continued)

		Sequences	Restriction site
fu ΔG287(394.98)-...	Fwd	CGCGGATCCGCTAGC-CCCGATGTTAAATCGGC	NheI
	Rev	CGGGGATCC-ATCCTGCTCTTTTTTGCCGG	BamHI
fu Orf1-(Orf46.1)-His	Fwd	CGCGGATCCGCTAGC-GGACACACTTATTTGCGCATC	NheI
	Rev	CGCGGATCC-CCAGCGGTAGCGTTAATTTGAT	
fu (Orf1)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGT-TCAGATTTGGCAACGATTTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACAGTGC	HindIII
fu (919)-Orf46.1-His	Fwd1	GCGGCGTCGACGGTGGCGGAGGCACTGGATCCTCAG	Sall
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Fu (orf46)-287-His	Rev	CCCGCTCGAG-CGTATCATATTTACAGTGC	XhoI
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	Fwd	CCCAAGCTTGGTGGTGGTGGTCAAGCAAGAGCAT CCAAACC	HindIII
(ΔG741)-961c-His	Rev	CCCGCTCGAGCGGCGGTATTCGGGCTT	XhoI
	Fwd1	GGAGGCACTGGATCCGAGCCACAAACGACGACGA	XhoI
(ΔG741)-961-His	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(ΔG741)-961-His	Fwd1	GGAGGCACTGGATCCGAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
(ΔG741)-961-His	Rev	CCCGCTCGAG-CCACTCGTAAATTGACGCC	XhoI
	Fwd	CCCGCTCGAG-CCACTCGTAAATTGACGCC	



(continued)

		Sequences	Restriction site
(ΔG741)-983-His	Fwd	<u>GGGCGCTCGAG</u> - GGATCCGCGGAGCGGCACTTCTGCG	XhoI
	Rev	CCCGCTCGAG-GAACC GG TAGCCTACG	XhoI
(ΔG741 )-orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTGGCAAACGATTG	Sall
	Fwd2	GCGGCCGTCGACGGTGGCGGAGGCACTGGATCCTCAGA	
(ΔG983)-741(MC58) -His	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
	Fwd	GCGGCCGTCGAG-GGATCCGAGGGGGTGGTGTGCGCC	XhoI
(ΔG983)-961c-His	Rev	CCCGCTCGAG-ITGCTTGGCGGCAAG	XhoI
	Fwd1	GGAGGCACTGGATCCGCGAGCCACAAACGACGACGA	XhoI
(ΔG983)-961-His	Fwd2	GCGGCCGTCGAG-GGTGGCGGAGGCACTGGATCCGCGAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(ΔG983)-961-His	Fwd1	GGAGGCACTGGATCCGCGAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCGTCGAG-GGTGGCGGAGGCACTGGATCCGCGAG	
(ΔG983)- Orf46.1-His	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
	Fwd1	GGAGGCACTGGATCCTCAGATTGGCAAACGATTG	Sall
(ΔG983)- Orf46.1-His	Fwd2	GCGGCCGTCGACGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
<p>*This primer was used as a Reverse primer for all the C terminal fusions of 287 to the His-tag.  § Forward primers used in combination with the 287-His Reverse primer.  NB-All PCR reactions use strain 2996 unless otherwise specified (e.g. strain MC58)</p>			

**[0038]** In all constructs starting with an ATG not followed by a unique *NheI* site, the ATG codon is part of the *NdeI* site used for cloning. The constructs made using *NheI* as a cloning site at the 5' end (e.g. all those containing 287 at the N-terminus) have two additional codons (GCT AGC) fused to the coding sequence of the antigen.

## 5 **Preparation of chromosomal DNA templates**

**[0039]** *N.meningitidis* strains 2996, MC58, 394.98, 1000 and BZ232 (and others) were grown to exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in 5ml buffer (20% w/v sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10ml of lysis solution (50mM NaCl, 1% Na-Sarkosyl, 50µg/ml Proteinase K), and the suspension incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one CHCl<sub>3</sub>/isoamylalcohol (24: 1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes of ethanol, and collected by centrifugation. The pellet was washed once with 70%(v/v) ethanol and redissolved in 4.0ml TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The DNA concentration was measured by reading OD<sub>260</sub>.

## 15 **PCR Amplification**

**[0040]** The standard PCR protocol was as follows: 200ng of genomic DNA from 2996, MC581000, or BZ232 strains or 10ng of plasmid DNA preparation of recombinant clones were used as template in the presence of 40µM of each oligonucleotide primer, 400-800 µM dNTPs solution, 1x PCR buffer (including 1.5mM MgCl<sub>2</sub>), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, Boehringer Mannheim Expand™ Long Template).

**[0041]** After a preliminary 3 minute incubation of the whole mix at 95°C, each sample underwent a two-step amplification: the first 5 cycles were performed using the hybridisation temperature that excluded the restriction enzymes tail of the primer (T<sub>m1</sub>). This was followed by 30 cycles according to the hybridisation temperature calculated for the whole length oligos (T<sub>m2</sub>). Elongation times, performed at 68°C for 72°C, varied according to the length of the Orf to be amplified. In the case of Orf1 the elongation time, starting from 3 minutes, was increased by 15 seconds each cycle. The cycles were completed with a 10 minute extension step at 72°C.

**[0042]** The amplified DNA was either loaded directly on a 1% agarose gel. The DNA fragment corresponding to the band of correct size was purified from the gel using the Qiagen Gel Extraction Kit, following the manufacturer's protocol.

## 30 **Digestion of PCR fragments and of the cloning vectors**

**[0043]** The purified DNA corresponding to the amplified fragment was digested with the appropriate restriction enzymes for cloning into pET-21b+, pET22b+ or pET-24b+. Digested fragments were purified using the QIAquick PCR purification kit (following the manufacturer's instructions) and eluted with either H<sub>2</sub>O or 10mM Tris, pH 8.5. Plasmid vectors were digested with the appropriate restriction enzymes, loaded onto a 1.0% agarose gel and the band corresponding to the digested vector purified using the Qiagen QIAquick Gel Extraction Kit.

## 40 **Cloning**

**[0044]** The fragments corresponding to each gene, previously digested and purified, were ligated into pET21b+, pET22b+ or pET-24b+. A molar ratio of 3:1 fragment/vector was used with T4 DNA ligase in the ligation buffer supplied by the manufacturer.

**[0045]** Recombinant plasmid was transformed into competent *E.coli* DH5 or HB101 by incubating the ligase reaction solution and bacteria for 40 minutes on ice, then at 37°C for 3 minutes. This was followed by the addition of 800µl LB broth and incubation at 37°C for 20 minutes. The cells were centrifuged at maximum speed in an Eppendorf microfuge, resuspended in approximately 200µl of the supernatant and plated onto LB ampicillin (100mg/ml) agar.

**[0046]** Screening for recombinant clones was performed by growing randomly selected colonies overnight at 37°C in 4.0ml of LB broth + 100µg/ml ampicillin. Cells were pelleted and plasmid DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions. Approximately 1µg of each individual miniprep was digested with the appropriate restriction enzymes and the digest loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1kb DNA Ladder, GIBCO). Positive clones were selected on the basis of the size of insert.

## 55 **Expression**

**[0047]** After cloning each gene into the expression vector, recombinant plasmids, were transformed into *E.coli* strains suitable for expression of the recombinant protein. 1µl of each construct was used to transform *E.coli* BL21-DE3 as

described above. Single recombinant-colonies were inoculated into 2ml LB+Amp (100µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100µg/ml) in 100ml flasks, to give an OD<sub>600</sub> between 0.1 and 0.2. The flasks were incubated at 30°C or at 37°C in a gyratory water bath shaker until OD<sub>600</sub> indicated exponential growth suitable for induction of expression (0.4-0.8 OD). Protein expression was induced by addition of 1.0mM IPTG. After 3 hours incubation at 30°C or 37°C the OD<sub>600</sub> was measured and expression examined. 1.0ml of each sample was centrifuged in a microfuge, the pellet resuspended in PBS and analysed by SDS-PAGE and Coomassie Blue staining.

#### **Purification of His-tagged proteins**

**[0048]** Various forms of 287 were cloned from strains 2996 and MC58. They were constructed with a C-terminus His-tagged fusion and included a mature form (aa 18-427), constructs with deletion (Δ1, Δ2, Δ3 and Δ4) and clones composed of either B or C domains. For each clone purified as a His-fusion, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 µg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 µg/ml) liquid medium and grown overnight at 37°C with shaking. The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 µg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD<sub>550</sub> reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000g for 15 min at 4°C. The bacterial pellet was resuspended in 7.5 ml of either (i) cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0) for soluble proteins or (ii) buffer B (10mM Tris-HCl, 100 mM phosphate buffer, pH 8.8 and, optionally, 8M urea) for insoluble proteins. Proteins purified in a soluble form included 287-His, Δ1, Δ2, Δ3 and Δ4287-His, Δ4287MC58-His, 287c-His and 287cMC58-His. Protein 287bMC58-His was insoluble and purified accordingly. Cells were disrupted by sonication on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13000xg for 30 min at 4°C. For insoluble proteins, pellets were resuspended in 2.0 ml buffer C (6 M guanidine hydrochloride; 100 mM phosphate buffer, 10 mM Tris- HCl, pH 7.5 and treated with 10 passes of a Dounce homogenizer. The homogenate was centrifuged at 13000g for 30 min and the supernatant retained. Supernatants for both soluble and insoluble preparations were mixed with 150µl Ni<sup>2+</sup>-resin (previously equilibrated with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700g for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml buffer A or B for 10 min, resuspended in 1.0 ml buffer A or B and loaded onto a disposable column. The resin continued to be washed with either (i) buffer A at 4°C or (ii) buffer B at room temperature, until the OD<sub>280</sub> of the flow-through reached 0.02-0.01. The resin was further washed with either (i) cold buffer C (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8.0) or (ii) buffer D (10mM Tris-HCl, 100mM phosphate buffer, pH 6.3 and, optionally, 8M urea) until OD<sub>280</sub> of the flow-through reached 0.02-0.01. The His-fusion protein was eluted by addition of 700µl of either (i) cold elution buffer A (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) or (ii) elution buffer B (10 mM Tris-HCl, 100 mM phosphate buffer, pH 4.5 and, optionally, 8M urea) and fractions collected until the OD<sub>280</sub> indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analysed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

#### **Renaturation of denatured His-fusion proteins.**

**[0049]** Denaturation was required to solubilize 287bMC8, so a renaturation step was employed prior to immunisation. Glycerol was added to the denatured fractions obtained above to give a final concentration of 10% v/v. The proteins were diluted to 200 µg/ml using dialysis buffer I (10% v/v glycerol, 0.5M arginine, 50 mM phosphate buffer, 5.0 mM reduced glutathione, 0.5 mM oxidised glutathione, 2.0M urea, pH 8.8) and dialysed against the same buffer for 12-14 hours at 4°C. Further dialysis was performed with buffer II (10% v/v glycerol, 0.5M arginine, 50mM phosphate buffer, 5.0mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was estimated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times \text{OD}_{280}) - (0.76 \times \text{OD}_{260})$$

#### **Immunization**

**[0050]** Balb/C mice were immunized with antigens on days 0, 21 and 35 and sera analyzed at day 49.

**Sera analysis - ELISA**

**[0051]** The acapsulated MenB M7 and the capsulated strains were plated on chocolate agar plates and incubated overnight at 37°C with 5% CO<sub>2</sub>. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.4-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and bacteria were washed twice with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 1 hour at 37°C and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200µl of saturation buffer (2.7% polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 100µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100µl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10µl of H<sub>2</sub>O<sub>2</sub>) were added to each well and the plates were left at room temperature for 20 minutes. 100µl 12.5% H<sub>2</sub>SO<sub>4</sub> was added to each well and OD<sub>490</sub> was followed. The ELISA titers were calculated arbitrarily as the dilution of sera which gave an OD<sub>490</sub> value of 0.4 above the level of preimmune sera. The ELISA was considered positive when the dilution of sera with OD<sub>490</sub> of 0.4 was higher than 1:400.

**Sera analysis - FACS Scan bacteria binding assay**

**[0052]** The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. with 5% CO<sub>2</sub>. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA in PBS, 0.4% NaN<sub>3</sub>) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD<sub>620</sub> of 0.05. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:100, 1:200, 1:400) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerythrin conjugated F(ab)<sub>2</sub> goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan (Laser Power 15mW) setting were: FL2 on; FSC-H threshold:92; FSC PMT Voltage: E 01; SSC PMT: 474; Amp. Gains 6.1; FL-2 PMT: 586; compensation values: 0.

**Sera analysis - bactericidal assay**

**[0053]** *N. meningitidis* strain 2996 was grown overnight at 37°C on chocolate agar plates (starting from a frozen stock) with 5% CO<sub>2</sub>. Colonies were collected and used to inoculate 7ml Mueller-Hinton broth, containing 0.25% glucose to reach an OD<sub>620</sub> of 0.05-0.08. The culture was incubated for approximately 1.5 hours at 37 degrees with shaking until the OD<sub>620</sub> reached the value of 0.23-0.24. Bacteria were diluted in 50mM Phosphate buffer pH 7.2 containing 10mM MgCl<sub>2</sub>, 10mM CaCl<sub>2</sub> and 0.5% (w/v) BSA (assay buffer) at the working dilution of 10<sup>5</sup> CFU/ml. The total volume of the final reaction mixture was 50 µl with 25 µl of serial two fold dilution of test serum, 12.5 µl of bacteria at the working dilution, 12.5 µl of baby rabbit complement (final concentration 25%).

**[0054]** Controls included bacteria incubated with complement serum, immune sera incubated with bacteria and with complement inactivated by heating at 56°C for 30'. Immediately after the addition of the baby rabbit complement, 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 0). The 96-wells plate was incubated for 1 hour at 37°C with rotation. 7µl of each sample were plated on Mueller-Hinton agar plates as spots, whereas 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 1). Agar plates were incubated for 18 hours at 37 degrees and the colonies corresponding to time 0 and time 1 were counted.

**Sera analysis - western blots**

**[0055]** Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded onto a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The transfer

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was performed for 2 hours at 150mA at 4°C, using transfer buffer (0.3% Tris base, 1.44% glycine, 20% (v/v) ethanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labelled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

**[0056]** The OMVs were prepared as follows: *N. meningitidis* strain 2996 was grown overnight at 37 degrees with 5% CO<sub>2</sub> on 5 GC plates, harvested with a loop and resuspended in 10 ml of 20mM Tris-HCl pH 7.5, 2 mM EDTA. Heat inactivation was performed at 56°C for 45 minutes and the bacteria disrupted by sonication for 5 minutes on ice (50% duty cycle, 50% output, Branson sonifier 3 mm microtip). Unbroken cells were removed by centrifugation at 5000g for 10 minutes, the supernatant containing the total cell envelope fraction recovered and further centrifuged overnight at 50000g at the temperature of 4°C. The pellet containing the membranes was resuspended in 2% sarkosyl, 20mM Tris-HCl pH 7.5, 2 mM EDTA and incubated at room temperature for 20 minutes to solubilise the inner membranes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, the supernatant was further centrifuges at 50000g for 3 hours. The pellet, containing the outer membranes was washed in PBS and resuspended in the same buffer. Protein concentration was measured by the D.C. Bio-Rad Protein assay (Modified Lowry method), using BSA as a standard.

**[0057]** Total cell extracts were prepared as follows: *N. meningitidis* strain 2996 was grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes.

### SEQUENCE LISTING

#### **[0058]**

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Phe Ser Thr Thr Ile Asp Arg Thr Lys Trp Gly Val Asp Tyr Leu Val  
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Ala Lys Gln

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<400> 8

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5	His	Thr	Glu	Asn	Gly	Arg	Pro	Tyr	Pro	Thr	Arg	Gly	Arg	Phe	Ala	Ala	
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					645					650					655	
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25	Val	Gly	Arg	Phe	Asn	Val	Thr	Ala	Ala	Val	Gly	Gly	Tyr	Lys	Ser	Glu
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	Ser	Ala	Val	Ala	Ile	Gly	Thr	Gly	Phe	Arg	Phe	Thr	Glu	Asn	Phe	Ala
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<400> 10



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	Ala	Thr	Asp	Lys	Pro	Lys	Asn	Glu	Asp	Glu	Gly	Ala	Gln	Asn	Asp	Met
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	Pro	Gln	Asn	Ala	Ala	Asp	Thr	Asp	Ser	Leu	Thr	Pro	Asn	His	Thr	Pro
					85					90					95	
	Ala	Ser	Asn	Met	Pro	Ala	Gly	Asn	Met	Glu	Asn	Gln	Ala	Pro	Asp	Ala
20				100					105					110		
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10	Gly Ser Gln Asn Pro Ala Ser Ser Thr Asn Pro Ser Ala Thr Asn Ser			165		170											175
	Gly Gly Asp Phe Gly Arg Thr Asn Val Gly Asn Ser Val Val Ile Asp			180		185											190
15	Gly Pro Ser Gln Asn Ile Thr Leu Thr His Cys Lys Gly Asp Ser Cys			195		200											205
	Ser Gly Asn Asn Phe Leu Asp Glu Glu Val Gln Leu Lys Ser Glu Phe				210	215											220
20	Glu Lys Leu Ser Asp Ala Asp Lys Ile Ser Asn Tyr Lys Lys Asp Gly				225	230											240
	Lys Asn Asp Gly Lys Asn Asp Lys Phe Val Gly Leu Val Ala Asp Ser				245												255
25	Val Gln Met Lys Gly Ile Asn Gln Tyr Ile Ile Phe Tyr Lys Pro Lys				260												270
	Pro Thr Ser Phe Ala Arg Phe Arg Arg Ser Ala Arg Ser Arg Arg Ser				275												285
30	Leu Pro Ala Glu Met Pro Leu Ile Pro Val Asn Gln Ala Asp Thr Leu				290	295											300
	Ile Val Asp Gly Glu Ala Val Ser Leu Thr Gly His Ser Gly Asn Ile				305	310											320
35	Phe Ala Pro Glu Gly Asn Tyr Arg Tyr Leu Thr Tyr Gly Ala Glu Lys				325												335
	Leu Pro Gly Gly Ser Tyr Ala Leu Arg Val Gln Gly Glu Pro Ser Lys				340												350
40	Gly Glu Met Leu Ala Gly Thr Ala Val Tyr Asn Gly Glu Val Leu His				355												365
	Phe His Thr Glu Asn Gly Arg Pro Ser Pro Ser Arg Gly Arg Phe Ala				370												380
45	Ala Lys Val Asp Phe Gly Ser Lys Ser Val Asp Gly Ile Ile Asp Ser				385	390											400
	Gly Asp Gly Leu His Met Gly Thr Gln Lys Phe Lys Ala Ala Ile Asp				405												415
50	Gly Asn Gly Phe Lys Gly Thr Trp Thr Glu Asn Gly Gly Gly Asp Val				420												430
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	Gly	Tyr	Val	Trp	Gln	Leu	Leu	Pro	Asn	Gly	Met	Lys	Pro	Glu	Tyr	Arg
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<400> 11

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<220>  
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<400> 12

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25	Asp	Gly	Met	Gln	Gly	Asp	Asp	Pro	Ser	Ala	Gly	Gly	Glu	Asn	Ala	Gly	
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35	Gly	Pro	Ser	Gln	Asn	Ile	Thr	Leu	Thr	His	Cys	Lys	Gly	Asp	Ser	Cys	
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	225				230					235				240
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	Ser	Tyr	Arg	Pro	Thr	Asp	Ala	Glu	Lys	Gly	Gly	Phe	Gly	Val
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	Gly	Lys	Lys	Glu	Gln	Asp	Gly	Ser	Gly	Gly	Gly	Gly	Ala	Thr
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														Lys
	Val	Asp	Glu	Tyr	His	Ala	Asn	Ala	Arg	Phe	Ala	Ile	Asp	His
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	Thr	Ser	Thr	Asn	Val	Gly	Gly	Phe	Tyr	Gly	Leu	Thr	Gly	Ser
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	Phe	Asp	Gln	Ala	Lys	Arg	Asp	Gly	Lys	Ile	Asp	Ile	Thr	Ile
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	Ala	Asn	Leu	Gln	Ser	Gly	Ser	Gln	His	Phe	Thr	Asp	His	Leu
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	545					550					555			Val
														Ser
														560

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	Thr	Lys	Phe	Asn	Phe	Asn	Gly	Lys	Lys	Leu	Val	Ser	Val	Asp	Gly	Asn
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5	Leu	Thr	Met	His	Gly	Lys	Thr	Ala	Pro	Val	Lys	Leu	Lys	Ala	Glu	Lys
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	Phe	Asn	Cys	Tyr	Gln	Ser	Pro	Met	Ala	Lys	Thr	Glu	Val	Cys	Gly	Gly
			595					600					605			
10	Asp	Phe	Ser	Thr	Thr	Ile	Asp	Arg	Thr	Lys	Trp	Gly	Val	Asp	Tyr	Leu
	610						615					620				
	Val	Asn	Val	Gly	Met	Thr	Lys	Ser	Val	Arg	Ile	Asp	Ile	Gln	Ile	Glu
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<220>

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			35					40					45				
10	Asp	Met	Ala	Ala	Val	Ser	Glu	Glu	Asn	Thr	Gly	Asn	Gly	Gly	Ala	Ala	
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	Ala	Thr	Asp	Lys	Pro	Lys	Asn	Glu	Asp	Glu	Gly	Ala	Gln	Asn	Asp	Met	
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					85					90					95		
	Ala	Ser	Asn	Met	Pro	Ala	Gly	Asn	Met	Glu	Asn	Gln	Ala	Pro	Asp	Ala	
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	Gly	Pro	Ser	Gln	Asn	Ile	Thr	Leu	Thr	His	Cys	Lys	Gly	Asp	Ser	Cys	
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	210		215		220
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10	Val Gln Met Lys Gly Ile Asn Gln Tyr Ile Ile Phe Tyr Lys Pro Lys	260	265	270	
	Pro Thr Ser Phe Ala Arg Phe Arg Arg Ser Ala Arg Ser Arg Arg Ser	275	280	285	
15	Leu Pro Ala Glu Met Pro Leu Ile Pro Val Asn Gln Ala Asp Thr Leu	290	295	300	
	Ile Val Asp Gly Glu Ala Val Ser Leu Thr Gly His Ser Gly Asn Ile	305	310	315	320
20	Phe Ala Pro Glu Gly Asn Tyr Arg Tyr Leu Thr Tyr Gly Ala Glu Lys	325	330	335	
	Leu Pro Gly Gly Ser Tyr Ala Leu Arg Val Gln Gly Glu Pro Ser Lys	340	345	350	
25	Gly Glu Met Leu Ala Gly Thr Ala Val Tyr Asn Gly Glu Val Leu His	355	360	365	
	Phe His Thr Glu Asn Gly Arg Pro Ser Pro Ser Arg Gly Arg Phe Ala	370	375	380	
30	Ala Lys Val Asp Phe Gly Ser Lys Ser Val Asp Gly Ile Ile Asp Ser	385	390	395	400
	Gly Asp Gly Leu His Met Gly Thr Gln Lys Phe Lys Ala Ala Ile Asp	405	410	415	
35	Gly Asn Gly Phe Lys Gly Thr Trp Thr Glu Asn Gly Gly Gly Asp Val	420	425	430	
	Ser Gly Lys Phe Tyr Gly Pro Ala Gly Glu Glu Val Ala Gly Lys Tyr	435	440	445	
40	Ser Tyr Arg Pro Thr Asp Ala Glu Lys Gly Gly Phe Gly Val Phe Ala	450	455	460	
	Gly Lys Lys Glu Gln Asp Gly Ser Gly Gly Gly Gly Ala Thr Asn Asp	465	470	475	480
45	Asp Asp Val Lys Lys Ala Ala Thr Val Ala Ile Ala Ala Ala Tyr Asn	485	490	495	
	Asn Gly Gln Glu Ile Asn Gly Phe Lys Ala Gly Glu Thr Ile Tyr Asp	500	505	510	
50	Ile Asp Glu Asp Gly Thr Ile Thr Lys Lys Asp Ala Thr Ala Ala Asp	515	520	525	
55	Val Glu Ala Asp Asp Phe Lys Gly Leu Gly Leu Lys Lys Val Val Thr	530	535	540	

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5	Val	Lys	Ala	Ala	Glu	Ser	Glu	Ile	Glu	Lys	Leu	Thr	Thr	Lys	Leu	Ala	
					565					570					575		
	Asp	Thr	Asp	Ala	Ala	Leu	Ala	Asp	Thr	Asp	Ala	Ala	Leu	Asp	Ala	Thr	
				580					585					590			
10	Thr	Asn	Ala	Leu	Asn	Lys	Leu	Gly	Glu	Asn	Ile	Thr	Thr	Phe	Ala	Glu	
			595					600					605				
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		610					615					620					
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	625					630					635					640	
	Ser	Leu	Asp	Glu	Thr	Asn	Thr	Lys	Ala	Asp	Glu	Ala	Val	Lys	Thr	Ala	
20					645					650					655		
	Asn	Glu	Ala	Lys	Gln	Thr	Ala	Glu	Glu	Thr	Lys	Gln	Asn	Val	Asp	Ala	
				660					665					670			
25	Lys	Val	Lys	Ala	Ala	Glu	Thr	Ala	Ala	Gly	Lys	Ala	Glu	Ala	Ala	Ala	
			675					680					685				
	Gly	Thr	Ala	Asn	Thr	Ala	Ala	Asp	Lys	Ala	Glu	Ala	Val	Ala	Ala	Lys	
		690					695					700					
30	Val	Thr	Asp	Ile	Lys	Ala	Asp	Ile	Ala	Thr	Asn	Lys	Asp	Asn	Ile	Ala	
	705					710					715					720	
	Lys	Lys	Ala	Asn	Ser	Ala	Asp	Val	Tyr	Thr	Arg	Glu	Glu	Ser	Asp	Ser	
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35	Lys	Phe	Val	Arg	Ile	Asp	Gly	Leu	Asn	Ala	Thr	Thr	Glu	Lys	Leu	Asp	
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	Thr	Arg	Leu	Ala	Ser	Ala	Glu	Lys	Ser	Ile	Ala	Asp	His	Asp	Thr	Arg	
			755					760					765				
40	Leu	Asn	Gly	Leu	Asp	Lys	Thr	Val	Ser	Asp	Leu	Arg	Lys	Glu	Thr	Arg	
		770					775					780					
	Gln	Gly	Leu	Ala	Glu	Gln	Ala	Ala	Leu	Ser	Gly	Leu	Phe	Gln	Pro	Tyr	
	785					790					795					800	
45	Asn	Val	Gly	Arg	Phe	Asn	Val	Thr	Ala	Ala	Val	Gly	Gly	Tyr	Lys	Ser	
				805						810					815		
	Glu	Ser	Ala	Val	Ala	Ile	Gly	Thr	Gly	Phe	Arg	Phe	Thr	Glu	Asn	Phe	
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	Ala	Ala	Lys	Ala	Gly	Val	Ala	Val	Gly	Thr	Ser	Ser	Gly	Ser	Ser	Ala	
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5	Met	Ala	Leu	Ala	Val	Ala	Thr	Thr	Leu	Ser	Ala	Cys	Leu	Gly	Gly	Gly	
				20					25					30			
	Gly	Gly	Gly	Thr	Ser	Ala	Pro	Asp	Phe	Asn	Ala	Gly	Gly	Thr	Gly	Ile	
			35					40					45				
10	Gly	Ser	Asn	Ser	Arg	Ala	Thr	Thr	Ala	Lys	Ser	Ala	Ala	Val	Ser	Tyr	
		50					55					60					
	Ala	Gly	Ile	Lys	Asn	Glu	Met	Cys	Lys	Asp	Arg	Ser	Met	Leu	Cys	Ala	
	65					70					75					80	
15	Gly	Arg	Asp	Asp	Val	Ala	Val	Thr	Asp	Arg	Asp	Ala	Lys	Ile	Asn	Ala	
					85					90					95		
	Pro	Pro	Pro	Asn	Leu	His	Thr	Gly	Asp	Phe	Pro	Asn	Pro	Asn	Asp	Ala	
				100					105					110			
20	Tyr	Lys	Asn	Leu	Ile	Asn	Leu	Lys	Pro	Ala	Ile	Glu	Ala	Gly	Tyr	Thr	
			115					120					125				
	Gly	Arg	Gly	Val	Glu	Val	Gly	Ile	Val	Asp	Thr	Gly	Glu	Ser	Val	Gly	
25		130					135					140					
	Ser	Ile	Ser	Phe	Pro	Glu	Leu	Tyr	Gly	Arg	Lys	Glu	His	Gly	Tyr	Asn	
	145					150					155					160	
30	Glu	Asn	Tyr	Lys	Asn	Tyr	Thr	Ala	Tyr	Met	Arg	Lys	Glu	Ala	Pro	Glu	
					165					170					175		
	Asp	Gly	Gly	Gly	Lys	Asp	Ile	Glu	Ala	Ser	Phe	Asp	Asp	Glu	Ala	Val	
				180					185					190			
35	Ile	Glu	Thr	Glu	Ala	Lys	Pro	Thr	Asp	Ile	Arg	His	Val	Lys	Glu	Ile	
			195					200					205				
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	225				230						235					240	
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	275		280		285
5	Ala Asn Ser Glu Glu Gln Tyr Arg Gln Ala Leu Leu Asp Tyr Ser Gly 290 295 300				
	Gly Asp Lys Thr Asp Glu Gly Ile Arg Leu Met Gln Gln Ser Asp Tyr 305 310 315 320				
10	Gly Asn Leu Ser Tyr His Ile Arg Asn Lys Asn Met Leu Phe Ile Phe 325 330 335				
	Ser Thr Gly Asn Asp Ala Gln Ala Gln Pro Asn Thr Tyr Ala Leu Leu 340 345 350				
15	Pro Phe Tyr Glu Lys Asp Ala Gln Lys Gly Ile Ile Thr Val Ala Gly 355 360 365				
	Val Asp Arg Ser Gly Glu Lys Phe Lys Arg Glu Met Tyr Gly Glu Pro 370 375 380				
20	Gly Thr Glu Pro Leu Glu Tyr Gly Ser Asn His Cys Gly Ile Thr Ala 385 390 395 400				
	Met Trp Cys Leu Ser Ala Pro Tyr Glu Ala Ser Val Arg Phe Thr Arg 405 410 415				
25	Thr Asn Pro Ile Gln Ile Ala Gly Thr Ser Phe Ser Ala Pro Ile Val 420 425 430				
	Thr Gly Thr Ala Ala Leu Leu Leu Gln Lys Tyr Pro Trp Met Ser Asn 435 440 445				
30	Asp Asn Leu Arg Thr Thr Leu Leu Thr Thr Ala Gln Asp Ile Gly Ala 450 455 460				
	Val Gly Val Asp Ser Lys Phe Gly Trp Gly Leu Leu Asp Ala Gly Lys 465 470 475 480				
35	Ala Met Asn Gly Pro Ala Ser Phe Pro Phe Gly Asp Phe Thr Ala Asp 485 490 495				
	Thr Lys Gly Thr Ser Asp Ile Ala Tyr Ser Phe Arg Asn Asp Ile Ser 500 505 510				
40	Gly Thr Gly Gly Leu Ile Lys Lys Gly Gly Ser Gln Leu Gln Leu His 515 520 525				
	Gly Asn Asn Thr Tyr Thr Gly Lys Thr Ile Ile Glu Gly Gly Ser Leu 530 535 540				
45	Val Leu Tyr Gly Asn Asn Lys Ser Asp Met Arg Val Glu Thr Lys Gly 545 550 555 560				
	Ala Leu Ile Tyr Asn Gly Ala Ala Ser Gly Gly Ser Leu Asn Ser Asp 565 570 575				
50	Gly Ile Val Tyr Leu Ala Asp Thr Asp Gln Ser Gly Ala Asn Glu Thr 580 585 590				
55	Val His Ile Lys Gly Ser Leu Gln Leu Asp Gly Lys Gly Thr Leu Tyr 595 600 605				

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	Thr	Arg	Leu	Gly	Lys	Leu	Leu	Lys	Val	Asp	Gly	Thr	Ala	Ile	Ile	Gly
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5	Gly	Lys	Leu	Tyr	Met	Ser	Ala	Arg	Gly	Lys	Gly	Ala	Gly	Tyr	Leu	Asn
	625					630					635					640
	Ser	Thr	Gly	Arg	Arg	Val	Pro	Phe	Leu	Ser	Ala	Ala	Lys	Ile	Gly	Gln
					645					650					655	
10	Asp	Tyr	Ser	Phe	Phe	Thr	Asn	Ile	Glu	Thr	Asp	Gly	Gly	Leu	Leu	Ala
				660					665					670		
	Ser	Leu	Asp	Ser	Val	Glu	Lys	Thr	Ala	Gly	Ser	Glu	Gly	Asp	Thr	Leu
			675					680					685			
15	Ser	Tyr	Tyr	Val	Arg	Arg	Gly	Asn	Ala	Ala	Arg	Thr	Ala	Ser	Ala	Ala
		690					695					700				
	Ala	His	Ser	Ala	Pro	Ala	Gly	Leu	Lys	His	Ala	Val	Glu	Gln	Gly	Gly
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	Ser	Asn	Leu	Glu	Asn	Leu	Met	Val	Glu	Leu	Asp	Ala	Ser	Glu	Ser	Ser
					725					730					735	
	Ala	Thr	Pro	Glu	Thr	Val	Glu	Thr	Ala	Ala	Ala	Asp	Arg	Thr	Asp	Met
25				740					745						750	
	Pro	Gly	Ile	Arg	Pro	Tyr	Gly	Ala	Thr	Phe	Arg	Ala	Ala	Ala	Ala	Val
			755					760					765			
30	Gln	His	Ala	Asn	Ala	Ala	Asp	Gly	Val	Arg	Ile	Phe	Asn	Ser	Leu	Ala
		770					775					780				
	Ala	Thr	Val	Tyr	Ala	Asp	Ser	Thr	Ala	Ala	His	Ala	Asp	Met	Gln	Gly
		785				790					795					800
35	Arg	Arg	Leu	Lys	Ala	Val	Ser	Asp	Gly	Leu	Asp	His	Asn	Gly	Thr	Gly
					805					810					815	
	Leu	Arg	Val	Ile	Ala	Gln	Thr	Gln	Gln	Asp	Gly	Gly	Thr	Trp	Glu	Gln
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	Ala	Ala	Lys	Thr	Gly	Glu	Asn	Thr	Thr	Ala	Ala	Ala	Thr	Leu	Gly	Met
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	Leu	Lys	Gly	Leu	Phe	Ser	Tyr	Gly	Arg	Tyr	Lys	Asn	Ser	Ile	Ser	Arg
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55	Ser	Thr	Gly	Ala	Asp	Glu	His	Ala	Glu	Gly	Ser	Val	Asn	Gly	Thr	Leu
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	Met	Gln	Leu	Gly	Ala	Leu	Gly	Gly	Val	Asn	Val	Pro	Phe	Ala	Ala	Thr	
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5	Gly	Asp	Leu	Thr	Val	Glu	Gly	Gly	Leu	Arg	Tyr	Asp	Leu	Leu	Lys	Gln	
	945					950					955					960	
	Asp	Ala	Phe	Ala	Glu	Lys	Gly	Ser	Ala	Leu	Gly	Trp	Ser	Gly	Asn	Ser	
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10	Leu	Thr	Glu	Gly	Thr	Leu	Val	Gly	Leu	Ala	Gly	Leu	Lys	Leu	Ser	Gln	
				980					985					990			
	Pro	Leu	Ser	Asp	Lys	Ala	Val	Leu	Phe	Ala	Thr	Ala	Gly	Val	Glu	Arg	
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	Lys	Asn	Glu	Met	Cys	Lys	Asp	Arg	Ser	Met	Leu	Cys	Ala	Gly	Arg	Asp
			35					40					45			
10	Asp	Val	Ala	Val	Thr	Asp	Arg	Asp	Ala	Lys	Ile	Asn	Ala	Pro	Pro	Pro
		50					55					60				
	Asn	Leu	His	Thr	Gly	Asp	Phe	Pro	Asn	Pro	Asn	Asp	Ala	Tyr	Lys	Asn
15	65					70					75					80
	Leu	Ile	Asn	Leu	Lys	Pro	Ala	Ile	Glu	Ala	Gly	Tyr	Thr	Gly	Arg	Gly
					85					90					95	
	Val	Glu	Val	Gly	Ile	Val	Asp	Thr	Gly	Glu	Ser	Val	Gly	Ser	Ile	Ser
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	Phe	Pro	Glu	Leu	Tyr	Gly	Arg	Lys	Glu	His	Gly	Tyr	Asn	Glu	Asn	Tyr

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5	Lys	Asn	Tyr	Thr	Ala	Tyr	Met	Arg	Lys	Glu	Ala	Pro	Glu	Asp	Gly	Gly	
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	Gly	Lys	Asp	Ile	Glu	Ala	Ser	Phe	Asp	Asp	Glu	Ala	Val	Ile	Glu	Thr	
	145					150					155					160	
	Glu	Ala	Lys	Pro	Thr	Asp	Ile	Arg	His	Val	Lys	Glu	Ile	Gly	His	Ile	
10					165					170					175		
	Asp	Leu	Val	Ser	His	Ile	Ile	Gly	Gly	Arg	Ser	Val	Asp	Gly	Arg	Pro	
				180					185					190			
	Ala	Gly	Gly	Ile	Ala	Pro	Asp	Ala	Thr	Leu	His	Ile	Met	Asn	Thr	Asn	
15			195					200					205				
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	Thr	Thr	Ser	Arg	Ala	Gly	Thr	Ala	Asp	Leu	Phe	Gln	Ile	Ala	Asn	Ser	
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	Thr	Asp	Glu	Gly	Ile	Arg	Leu	Met	Gln	Gln	Ser	Asp	Tyr	Gly	Asn	Leu	
			275					280					285				
	Ser	Tyr	His	Ile	Arg	Asn	Lys	Asn	Met	Leu	Phe	Ile	Phe	Ser	Thr	Gly	
30		290					295					300					
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	Glu	Lys	Asp	Ala	Gln	Lys	Gly	Ile	Ile	Thr	Val	Ala	Gly	Val	Asp	Arg	
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40	Pro	Leu	Glu	Tyr	Gly	Ser	Asn	His	Cys	Gly	Ile	Thr	Ala	Met	Trp	Cys	
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	Asp	Ser	Lys	Phe	Gly	Trp	Gly	Leu	Leu	Asp	Ala	Gly	Lys	Ala	Met	Asn	
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5	Thr	Ser	Asp	Ile	Ala	Tyr	Ser	Phe	Arg	Asn	Asp	Ile	Ser	Gly	Thr	Gly	
	465					470					475					480	
	Gly	Leu	Ile	Lys	Lys	Gly	Gly	Ser	Gln	Leu	Gln	Leu	His	Gly	Asn	Asn	
				485						490					495		
10	Thr	Tyr	Thr	Gly	Lys	Thr	Ile	Ile	Glu	Gly	Gly	Ser	Leu	Val	Leu	Tyr	
				500					505					510			
	Gly	Asn	Asn	Lys	Ser	Asp	Met	Arg	Val	Glu	Thr	Lys	Gly	Ala	Leu	Ile	
			515					520					525				
15	Tyr	Asn	Gly	Ala	Ala	Ser	Gly	Gly	Ser	Leu	Asn	Ser	Asp	Gly	Ile	Val	
	530						535					540					
	Tyr	Leu	Ala	Asp	Thr	Asp	Gln	Ser	Gly	Ala	Asn	Glu	Thr	Val	His	Ile	
20	545					550					555					560	
	Lys	Gly	Ser	Leu	Gln	Leu	Asp	Gly	Lys	Gly	Thr	Leu	Tyr	Thr	Arg	Leu	
				565						570					575		
	Gly	Lys	Leu	Leu	Lys	Val	Asp	Gly	Thr	Ala	Ile	Ile	Gly	Gly	Lys	Leu	
25				580					585					590			
	Tyr	Met	Ser	Ala	Arg	Gly	Lys	Gly	Ala	Gly	Tyr	Leu	Asn	Ser	Thr	Gly	
		595						600					605				
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	Phe	Phe	Thr	Asn	Ile	Glu	Thr	Asp	Gly	Gly	Leu	Leu	Ala	Ser	Leu	Asp	
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			675					680					685				
	Glu	Asn	Leu	Met	Val	Glu	Leu	Asp	Ala	Ser	Glu	Ser	Ser	Ala	Thr	Pro	
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	Glu	Thr	Val	Glu	Thr	Ala	Ala	Ala	Asp	Arg	Thr	Asp	Met	Pro	Gly	Ile	
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5	Ile	Ala	Gln	Thr	Gln	Gln	Asp	Gly	Gly	Thr	Trp	Glu	Gln	Gly	Gly	Val	
	785					790					795					800	
	Glu	Gly	Lys	Met	Arg	Gly	Ser	Thr	Gln	Thr	Val	Gly	Ile	Ala	Ala	Lys	
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	Thr	Gly	Glu	Asn	Thr	Thr	Ala	Ala	Ala	Thr	Leu	Gly	Met	Gly	Arg	Ser	
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	Thr	Trp	Ser	Glu	Asn	Ser	Ala	Asn	Ala	Lys	Thr	Asp	Ser	Ile	Ser	Leu	
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20	Leu	Phe	Ser	Tyr	Gly	Arg	Tyr	Lys	Asn	Ser	Ile	Ser	Arg	Ser	Thr	Gly	
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					885					890					895		
25	Gly	Ala	Leu	Gly	Gly	Val	Asn	Val	Pro	Phe	Ala	Ala	Thr	Gly	Asp	Leu	
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15	Phe Ala Glu Lys Gly Ser Ala Leu Gly Trp Ser Gly Asn Ser Leu Thr 930		935		940	
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20	Ser Asp Lys Ala Val Leu Phe Ala Thr Ala Gly Val Glu Arg Asp Leu 965		970		975	
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 <213> Artificial Sequence

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15																
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55																



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	Asp	Asp	Val	Ala	Val	Thr	Asp	Arg	Asp	Ala	Lys	Ile	Asn	Ala	Pro	Pro	
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5	Pro	Asn	Leu	His	Thr	Gly	Asp	Phe	Pro	Asn	Pro	Asn	Asp	Ala	Tyr	Lys	
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15	Tyr	Lys	Asn	Tyr	Thr	Ala	Tyr	Met	Arg	Lys	Glu	Ala	Pro	Glu	Asp	Gly	
	130						135					140					
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					165					170					175		
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25				180					185					190			
	Pro	Ala	Gly	Gly	Ile	Ala	Pro	Asp	Ala	Thr	Leu	His	Ile	Met	Asn	Thr	
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	Trp	Val	Lys	Leu	Gly	Glu	Arg	Gly	Val	Arg	Ile	Val	Asn	Asn	Ser	Phe	
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	Gly	Thr	Thr	Ser	Arg	Ala	Gly	Thr	Ala	Asp	Leu	Phe	Gln	Ile	Ala	Asn	
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5	Pro	Ile	Gln	Ile	Ala	Gly	Thr	Ser	Phe	Ser	Ala	Pro	Ile	Val	Thr	Gly
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	Thr	Ala	Ala	Leu	Leu	Leu	Gln	Lys	Tyr	Pro	Trp	Met	Ser	Asn	Asp	Asn
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15	Asn	Gly	Pro	Ala	Ser	Phe	Pro	Phe	Gly	Asp	Phe	Thr	Ala	Asp	Thr	Lys
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	465					470					475					480
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	Asn	Thr	Tyr	Thr	Gly	Lys	Thr	Ile	Ile	Glu	Gly	Gly	Ser	Leu	Val	Leu
				500					505					510		
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	625					630					635					640
	Asp	Ser	Val	Glu	Lys	Thr	Ala	Gly	Ser	Glu	Gly	Asp	Thr	Leu	Ser	Tyr
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50	Tyr	Val	Arg	Arg	Gly	Asn	Ala	Ala	Arg	Thr	Ala	Ser	Ala	Ala	Ala	His
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	705					710					715					720	
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10	Ala	Asn	Ala	Ala	Asp	Gly	Val	Arg	Ile	Phe	Asn	Ser	Leu	Ala	Ala	Thr	
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	Val	Tyr	Ala	Asp	Ser	Thr	Ala	Ala	His	Ala	Asp	Met	Gln	Gly	Arg	Arg	
			755					760					765				
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45	Glu	Gly	Thr	Leu	Val	Gly	Leu	Ala	Gly	Leu	Lys	Leu	Ser	Gln	Pro	Leu	
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	Glu Thr Ile Tyr Asp Ile Asp Glu Asp Gly Thr Ile Thr Lys Lys Asp	
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	Ala Val Lys Thr Ala Asn Glu Ala Lys Gln Thr Ala Glu Glu Thr Lys	
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	210						215					220					
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<213> Artificial Sequence

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<223> deltaG741

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<400> 26

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25	Ser	Ala	Leu	Thr	Ala	Phe	Gln	Thr	Glu	Gln	Ile	Gln	Asp	Ser	Glu	His	100	105	110	
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30	Gly	Glu	His	Thr	Ser	Phe	Asp	Lys	Leu	Pro	Glu	Gly	Gly	Arg	Ala	Thr	130	135	140	
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[illegible]



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35 <212> PRT

<213> Artificial Sequence

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<400> 46

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5	Ala	Ala	Ala	Tyr	Asn	Asn	Gly	Gln	Glu	Ile	Asn	Gly	Phe	Lys	Ala	Gly
				20					25					30		
	Glu	Thr	Ile	Tyr	Asp	Ile	Asp	Glu	Asp	Gly	Thr	Ile	Thr	Lys	Lys	Asp
			35					40					45			
10	Ala	Thr	Ala	Ala	Asp	Val	Glu	Ala	Asp	Asp	Phe	Lys	Gly	Leu	Gly	Leu
		50					55					60				
	Lys	Lys	Val	Val	Thr	Asn	Leu	Thr	Lys	Thr	Val	Asn	Glu	Asn	Lys	Gln
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	Asn	Val	Asp	Ala	Lys	Val	Lys	Ala	Ala	Glu	Ser	Glu	Ile	Glu	Lys	Leu
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	Thr	Thr	Lys	Leu	Ala	Asp	Thr	Asp	Ala	Ala	Leu	Ala	Asp	Thr	Asp	Ala
20				100					105					110		

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	Ala	Leu	Asp	Ala	Thr	Thr	Asn	Ala	Leu	Asn	Lys	Leu	Gly	Glu	Asn	Ile	
			115					120					125				
5	Thr	Thr	Phe	Ala	Glu	Glu	Thr	Lys	Thr	Asn	Ile	Val	Lys	Ile	Asp	Glu	
		130					135					140					
	Lys	Leu	Glu	Ala	Val	Ala	Asp	Thr	Val	Asp	Lys	His	Ala	Glu	Ala	Phe	
	145					150					155					160	
10	Asn	Asp	Ile	Ala	Asp	Ser	Leu	Asp	Glu	Thr	Asn	Thr	Lys	Ala	Asp	Glu	
				165						170					175		
	Ala	Val	Lys	Thr	Ala	Asn	Glu	Ala	Lys	Gln	Thr	Ala	Glu	Glu	Thr	Lys	
				180					185					190			
15	Gln	Asn	Val	Asp	Ala	Lys	Val	Lys	Ala	Ala	Glu	Thr	Ala	Ala	Gly	Lys	
			195					200						205			
	Ala	Glu	Ala	Ala	Ala	Gly	Thr	Ala	Asn	Thr	Ala	Ala	Asp	Lys	Ala	Glu	
	210						215					220					
20	Ala	Val	Ala	Ala	Lys	Val	Thr	Asp	Ile	Lys	Ala	Asp	Ile	Ala	Thr	Asn	
	225					230					235					240	
	Lys	Asp	Asn	Ile	Ala	Lys	Lys	Ala	Asn	Ser	Ala	Asp	Val	Tyr	Thr	Arg	
				245					250						255		
25	Glu	Glu	Ser	Asp	Ser	Lys	Phe	Val	Arg	Ile	Asp	Gly	Leu	Asn	Ala	Thr	
				260					265					270			
	Thr	Glu	Lys	Leu	Asp	Thr	Arg	Leu	Ala	Ser	Ala	Glu	Lys	Ser	Ile	Ala	
			275					280					285				
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	290						295					300					
	Arg	Lys	Glu	Thr	Arg	Gln	Gly	Leu	Ala	Glu	Gln	Ala	Ala	Leu	Ser	Gly	
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35	Leu	Phe	Gln	Pro	Tyr	Asn	Val	Gly	Arg	Phe	Asn	Val	Thr	Ala	Ala	Val	
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40	Phe	Thr	Glu	Asn	Phe	Ala	Ala	Lys	Ala	Gly	Val	Ala	Val	Gly	Thr	Ser	
			355					360					365				
45	Ser	Gly	Ser	Ser	Ala	Ala	Tyr	His	Val	Gly	Val	Asn	Tyr	Glu	Trp	Gly	
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55	Cys	Ala	Gly	Arg	Asp	Asp	Val	Ala	Val	Thr	Asp	Arg	Asp	Ala	Lys	Ile	

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5	Asn Ala Pro Pro Pro Asn Leu His Thr Gly Asp Phe Pro Asn Pro Asn 450 455 460			
	Asp Ala Tyr Lys Asn Leu Ile Asn Leu Lys Pro Ala Ile Glu Ala Gly 465 470 475 480			
10	Tyr Thr Gly Arg Gly Val Glu Val Gly Ile Val Asp Thr Gly Glu Ser 485 490 495			
	Val Gly Ser Ile Ser Phe Pro Glu Leu Tyr Gly Arg Lys Glu His Gly 500 505 510			
15	Tyr Asn Glu Asn Tyr Lys Asn Tyr Thr Ala Tyr Met Arg Lys Glu Ala 515 520 525			
	Pro Glu Asp Gly Gly Gly Lys Asp Ile Glu Ala Ser Phe Asp Asp Glu 530 535 540			
20	Ala Val Ile Glu Thr Glu Ala Lys Pro Thr Asp Ile Arg His Val Lys 545 550 555 560			
	Glu Ile Gly His Ile Asp Leu Val Ser His Ile Ile Gly Gly Arg Ser 565 570 575			
25	Val Asp Gly Arg Pro Ala Gly Gly Ile Ala Pro Asp Ala Thr Leu His 580 585 590			
	Ile Met Asn Thr Asn Asp Glu Thr Lys Asn Glu Met Met Val Ala Ala 595 600 605			
30	Ile Arg Asn Ala Trp Val Lys Leu Gly Glu Arg Gly Val Arg Ile Val 610 615 620			
	Asn Asn Ser Phe Gly Thr Thr Ser Arg Ala Gly Thr Ala Asp Leu Phe 625 630 635 640			
35	Gln Ile Ala Asn Ser Glu Glu Gln Tyr Arg Gln Ala Leu Leu Asp Tyr 645 650 655			
	Ser Gly Gly Asp Lys Thr Asp Glu Gly Ile Arg Leu Met Gln Gln Ser 660 665 670			
40	Asp Tyr Gly Asn Leu Ser Tyr His Ile Arg Asn Lys Asn Met Leu Phe 675 680 685			
	Ile Phe Ser Thr Gly Asn Asp Ala Gln Ala Gln Pro Asn Thr Tyr Ala 690 695 700			
45	Leu Leu Pro Phe Tyr Glu Lys Asp Ala Gln Lys Gly Ile Ile Thr Val 705 710 715 720			
	Ala Gly Val Asp Arg Ser Gly Glu Lys Phe Lys Arg Glu Met Tyr Gly 725 730 735			
50	Glu Pro Gly Thr Glu Pro Leu Glu Tyr Gly Ser Asn His Cys Gly Ile 740 745 750			
55	Thr Ala Met Trp Cys Leu Ser Ala Pro Tyr Glu Ala Ser Val Arg Phe 755 760 765			

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	Thr	Arg	Thr	Asn	Pro	Ile	Gln	Ile	Ala	Gly	Thr	Ser	Phe	Ser	Ala	Pro	
	770						775					780					
5	Ile	Val	Thr	Gly	Thr	Ala	Ala	Leu	Leu	Leu	Gln	Lys	Tyr	Pro	Trp	Met	
	785					790					795					800	
	Ser	Asn	Asp	Asn	Leu	Arg	Thr	Thr	Leu	Leu	Thr	Thr	Ala	Gln	Asp	Ile	
					805					810					815		
10	Gly	Ala	Val	Gly	Val	Asp	Ser	Lys	Phe	Gly	Trp	Gly	Leu	Leu	Asp	Ala	
				820					825					830			
	Gly	Lys	Ala	Met	Asn	Gly	Pro	Ala	Ser	Phe	Pro	Phe	Gly	Asp	Phe	Thr	
			835					840					845				
15	Ala	Asp	Thr	Lys	Gly	Thr	Ser	Asp	Ile	Ala	Tyr	Ser	Phe	Arg	Asn	Asp	
	850						855					860					
	Ile	Ser	Gly	Thr	Gly	Gly	Leu	Ile	Lys	Lys	Gly	Gly	Ser	Gln	Leu	Gln	
20	865					870					875					880	
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				885						890					895		
	Ser	Leu	Val	Leu	Tyr	Gly	Asn	Asn	Lys	Ser	Asp	Met	Arg	Val	Glu	Thr	
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	Glu	Thr	Val	His	Ile	Lys	Gly	Ser	Leu	Gln	Leu	Asp	Gly	Lys	Gly	Thr	
	945					950					955					960	
35	Leu	Tyr	Thr	Arg	Leu	Gly	Lys	Leu	Leu	Lys	Val	Asp	Gly	Thr	Ala	Ile	
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	Ile	Gly	Gly	Lys	Leu	Tyr	Met	Ser	Ala	Arg	Gly	Lys	Gly	Ala	Gly	Tyr	
				980					985					990			
40	Leu	Asn	Ser	Thr	Gly	Arg	Arg	Val	Pro	Phe	Leu	Ser	Ala	Ala	Lys	Ile	
			995					1000						1005			
	Gly	Gln	Asp	Tyr	Ser	Phe	Phe	Thr	Asn	Ile	Glu	Thr	Asp	Gly	Gly	Leu	
		1010					1015					1020					
45	Leu	Ala	Ser	Leu	Asp	Ser	Val	Glu	Lys	Thr	Ala	Gly	Ser	Glu	Gly	Asp	
	1025					1030					1035					1040	
	Thr	Leu	Ser	Tyr	Tyr	Val	Arg	Arg	Gly	Asn	Ala	Ala	Arg	Thr	Ala	Ser	
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	Ala	Ala	Ala	His	Ser	Ala	Pro	Ala	Gly	Leu	Lys	His	Ala	Val	Glu	Gln	
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	Gly	Gly	Ser	Asn	Leu	Glu	Asn	Leu	Met	Val	Glu	Leu	Asp	Ala	Ser	Glu	
55			1075					1080					1085				

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	Ser	Ser	Ala	Thr	Pro	Glu	Thr	Val	Glu	Thr	Ala	Ala	Ala	Asp	Arg	Thr	
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5	Asp	Met	Pro	Gly	Ile	Arg	Pro	Tyr	Gly	Ala	Thr	Phe	Arg	Ala	Ala	Ala	
	1105					1110					1115					1120	
	Ala	Val	Gln	His	Ala	Asn	Ala	Ala	Asp	Gly	Val	Arg	Ile	Phe	Asn	Ser	
				1125						1130					1135		
10	Leu	Ala	Ala	Thr	Val	Tyr	Ala	Asp	Ser	Thr	Ala	Ala	His	Ala	Asp	Met	
				1140					1145					1150			
	Gln	Gly	Arg	Arg	Leu	Lys	Ala	Val	Ser	Asp	Gly	Leu	Asp	His	Asn	Gly	
			1155					1160					1165				
15	Thr	Gly	Leu	Arg	Val	Ile	Ala	Gln	Thr	Gln	Gln	Asp	Gly	Gly	Thr	Trp	
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	Gly	Met	Gly	Arg	Ser	Thr	Trp	Ser	Glu	Asn	Ser	Ala	Asn	Ala	Lys	Thr	
				1220					1225					1230			
25	Asp	Ser	Ile	Ser	Leu	Phe	Ala	Gly	Ile	Arg	His	Asp	Ala	Gly	Asp	Ile	
			1235					1240					1245				
	Gly	Tyr	Leu	Lys	Gly	Leu	Phe	Ser	Tyr	Gly	Arg	Tyr	Lys	Asn	Ser	Ile	
30		1250				1255						1260					
	Ser	Arg	Ser	Thr	Gly	Ala	Asp	Glu	His	Ala	Glu	Gly	Ser	Val	Asn	Gly	
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35				1285					1290						1295		
	Ala	Thr	Gly	Asp	Leu	Thr	Val	Glu	Gly	Gly	Leu	Arg	Tyr	Asp	Leu	Leu	
				1300					1305					1310			
	Lys	Gln	Asp	Ala	Phe	Ala	Glu	Lys	Gly	Ser	Ala	Leu	Gly	Trp	Ser	Gly	
40		1315					1320						1325				
	Asn	Ser	Leu	Thr	Glu	Gly	Thr	Leu	Val	Gly	Leu	Ala	Gly	Leu	Lys	Leu	
		1330					1335					1340					
45	Ser	Gln	Pro	Leu	Ser	Asp	Lys	Ala	Val	Leu	Phe	Ala	Thr	Ala	Gly	Val	
	1345				1350						1355					1360	
	Glu	Arg	Asp	Leu	Asn	Gly	Arg	Asp	Tyr	Thr	Val	Thr	Gly	Gly	Phe	Thr	
				1365					1370						1375		
50	Gly	Ala	Thr	Ala	Ala	Thr	Gly	Lys	Thr	Gly	Ala	Arg	Asn	Met	Pro	His	
			1380					1385					1390				
	Thr	Arg	Leu	Val	Ala	Gly	Leu	Gly	Ala	Asp	Val	Glu	Phe	Gly	Asn	Gly	
		1395					1400						1405				
55	Trp	Asn	Gly	Leu	Ala	Arg	Tyr	Ser	Tyr	Ala	Gly	Ser	Lys	Gln	Tyr	Gly	

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	gacggcacia ttaccaaaaa agacgcaact gcagccgatg ttgaagccga cgactttaaa 180			
	ggctctgggtc tgaaaaaagt cgtgactaac ctgaccaaaa ccgtcaatga aaacaaacaa 240			
25	aacgtcgatg ccaaagtaaa agctgcagaa tctgaaatag aaaagttaac aaccaagtta 300			
	gcagacactg atgccgcttt agcagatact gatgccgctc tggatgcaac caccaacgcc 360			
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	aaaattgatg aaaaattaga agccgtggct gataccgtcg acaagcatgc cgaagcattc 480			
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	aaagataata ttgctaaaaa agcaaacagt gccgacgtgt acaccagaga agagtctgac 780			
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40	attgtccgct tttccgatca cgggcacgaa gtccattccc ctttcgacaa ccatgcctca 1260			
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	cccgtcccca aaggcgcgag ggatatatac agctacgaca taaaaggcgt tgcccaaaat 1440			
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45	aatgccggta gtatgctgac gcaaggagta ggcgacggat tcaaaccgcg cacccgatac 1560			
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55	ccgaagacag gcgtaccgtt tgaagggtaaa gggtttccga attttgagaa gcacgtgaaa 2220			
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<220>  
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<400> 48

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10	Ala	Thr	Ala	Ala	Asp	Val	Glu	Ala	Asp	Asp	Phe	Lys	Gly	Leu	Gly	Leu	
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	Thr	Thr	Lys	Leu	Ala	Asp	Thr	Asp	Ala	Ala	Leu	Ala	Asp	Thr	Asp	Ala	
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	Ala	Leu	Asp	Ala	Thr	Thr	Asn	Ala	Leu	Asn	Lys	Leu	Gly	Glu	Asn	Ile	
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	Lys	Leu	Glu	Ala	Val	Ala	Asp	Thr	Val	Asp	Lys	His	Ala	Glu	Ala	Phe	
	145					150				155						160	
30	Asn	Asp	Ile	Ala	Asp	Ser	Leu	Asp	Glu	Thr	Asn	Thr	Lys	Ala	Asp	Glu	
					165					170					175		
	Ala	Val	Lys	Thr	Ala	Asn	Glu	Ala	Lys	Gln	Thr	Ala	Glu	Glu	Thr	Lys	
35				180					185					190			
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			195					200					205				
	Ala	Glu	Ala	Ala	Ala	Gly	Thr	Ala	Asn	Thr	Ala	Ala	Asp	Lys	Ala	Glu	
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	225					230					235					240	
45	Lys	Asp	Asn	Ile	Ala	Lys	Lys	Ala	Asn	Ser	Ala	Asp	Val	Tyr	Thr	Arg	
					245					250					255		
	Glu	Glu	Ser	Asp	Ser	Lys	Phe	Val	Arg	Ile	Asp	Gly	Leu	Asn	Ala	Thr	
				260					265					270			
50	Thr	Glu	Lys	Leu	Asp	Thr	Arg	Leu	Ala	Ser	Ala	Glu	Lys	Ser	Ile	Ala	
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	Asp	His	Asp	Thr	Arg	Leu	Asn	Gly	Leu	Asp	Lys	Thr	Val	Ser	Asp	Leu	
55		290					295					300					

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	Glu	Arg	Ser	Gly	His	Ile	Gly	Leu	Gly	Lys	Ile	Gln	Ser	His	Gln	Leu	
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20	Asn	His	Ala	Ser	His	Ser	Asp	Ser	Asp	Glu	Ala	Gly	Ser	Pro	Val	Asp	
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	Gly	Phe	Ser	Leu	Tyr	Arg	Ile	His	Trp	Asp	Gly	Tyr	Glu	His	His	Pro	
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25	Ala	Asp	Gly	Tyr	Asp	Gly	Pro	Gln	Gly	Gly	Gly	Tyr	Pro	Ala	Pro	Lys	
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	Gly	Ala	Arg	Asp	Ile	Tyr	Ser	Tyr	Asp	Ile	Lys	Gly	Val	Ala	Gln	Asn	
	465				470						475					480	
30	Ile	Arg	Leu	Asn	Leu	Thr	Asp	Asn	Arg	Ser	Thr	Gly	Gln	Arg	Leu	Ala	
				485						490					495		
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35	Gly	Phe	Lys	Arg	Ala	Thr	Arg	Tyr	Ser	Pro	Glu	Leu	Asp	Arg	Ser	Gly	
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 5 Ser Glu Asn Ser Ala Asn Ala Lys Thr Asp Ser Ile Ser Leu Phe Ala  
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 10 Ser Tyr Gly Arg Tyr Lys Asn Ser Ile Ser Arg Ser Thr Gly Ala Asp  
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 1235 1240 1245  
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 25 Leu Val Gly Leu Ala Gly Leu Lys Leu Ser Gln Pro Leu Ser Asp Lys  
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 35 Gly Ala Asp Val Glu Phe Gly Asn Gly Trp Asn Gly Leu Ala Arg Tyr  
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	Thr	Ile	Thr	Lys	Lys	Asp	Ala	Thr	Ala	Ala	Asp	Val	Glu	Ala	Asp	Asp
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25																
30																
35																
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45																
50																
55																



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55	Lys	Gly	Asn	Ile	Gly	Tyr	Ile	Val	Arg	Phe	Ser	Asp	His	Gly	His	Glu	
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# EP 1 947 187 B9

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20																
25																
30																
35																
40																
45																
50																
55																

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	Ala	Asp	Ile	Ala	Thr	Asn	Lys	Asp	Asn	Ile	Ala	Lys	Lys	Ala	Asn	Ser	
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 <223> 961cL-983

<400> 57

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<400> 58

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25	Leu	Ala	Asp	Thr	Asp	Ala	Ala	Leu	Asp	Ala	Thr	Thr	Asn	Ala	Leu	Asn	
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	Ala	Asp	Val	Tyr	Thr	Arg	Glu	Glu	Ser	Asp	Ser	Lys	Phe	Val	Arg	Ile	
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## Claims

35

1. A hybrid protein of formula  $\text{NH}_2\text{-A-B-COOH}$ , wherein A comprises the Neisserial protein  $\Delta\text{G287}$  and B comprises the Neisserial protein 961, and wherein the amino acid sequence of the hybrid protein is as disclosed in SEQ ID NO: 8 or is a sequence having greater than 70% sequence identity thereto.
2. The protein of claim 1, wherein  $\Delta\text{G287}$  is from strain 2996 or 394/98.
3. The protein of claim 1, wherein 961 is from strain 2996 or 394/98.
4. The protein of claim 1, wherein A and B are from the same strain.
5. The hybrid protein of claim 1, comprising the amino acid sequence recited in SEQ ID NO: 8.

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## Patentansprüche

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1. Ein Hybridprotein mit der Formel  $\text{NH}_2\text{-A-B-COOH}$ , wobei A das Neisseriale Protein  $\Delta\text{G287}$  und B das Neisseriale Protein 961 umfasst, und wobei die Aminosäuresequenz des Hybridproteins wie in SEQ ID Nr. 8 offenbart oder eine Sequenz, welche eine mehr als 70% Sequenzidentität dazu hat, ist.
2. Das Protein gemäß Anspruch 1, wobei  $\Delta\text{G287}$  vom Stamm 2996 oder 394/98 ist.
3. Das Protein gemäß Anspruch 1, wobei 961 vom Stamm 2996 oder 394/98 ist.

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4. Das Protein gemäß Anspruch 1, wobei A und B vom selben Stamm sind.
5. Das Hybridprotein gemäß Anspruch 1, umfassend die in SEQ ID Nr. 8 angegebene Aminosäuresequenz.

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#### Revendications

1. Protéine hybride de formule  $\text{NH}_2\text{-A-B-COOH}$ , dans laquelle A comprend la protéine  $\Delta\text{G287}$  de Neisseria et B comprend la protéine 961 de Neisseria, et dans laquelle la séquence d'acides aminés de la protéine hybride est telle que décrite par SEQ ID NO : 8 ou est une séquence présentant une identité de séquence supérieure à 70 % avec celle-ci.
2. Protéine selon la revendication 1, dans laquelle  $\Delta\text{G287}$  provient de la souche 2996 ou 394/98.
3. Protéine selon la revendication 1, dans laquelle 961 provient de la souche 2996 ou 394/98.
4. Protéine selon la revendication 1, dans laquelle A et B proviennent de la même souche.
5. Protéine hybride selon la revendication 1, comprenant la séquence d'acides aminés représentée par SEQ ID NO : 8.

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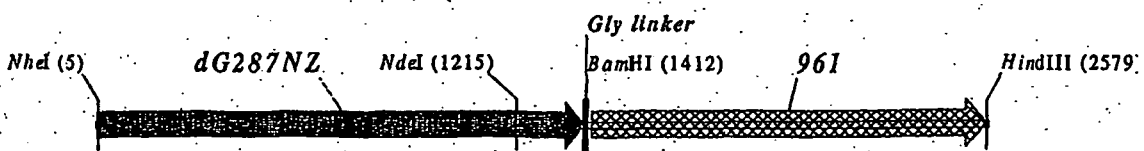
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**FIGURE 1 —  $\Delta G287-961$**



**FIGURE 2 —  $\Delta G287NZ-961$**



## REFERENCES CITED IN THE DESCRIPTION

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