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(54) MENINGOCOCCAL ANTIGENS

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Related U.S. Application Data

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6,709,660, which is a continuation-in-part of application No. PCT/IB99/00103, filed on Jan. 14, 1999.

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Sep. 1, 1998	(GB)	9819015.0
Jan. 14, 1998	(GB)	9800760.2

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530/350; 530/388.4

(57)ABSTRACT

The invention provides proteins from Neisseria meningitidis (strains A & B), including amino acid sequences, the corresponding nucleotide sequences, expression data, and serological data. The proteins are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

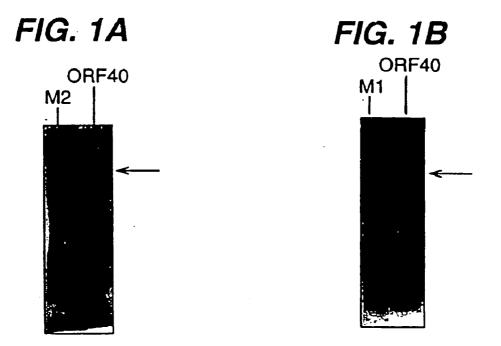
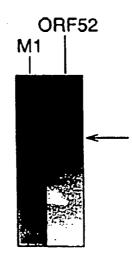
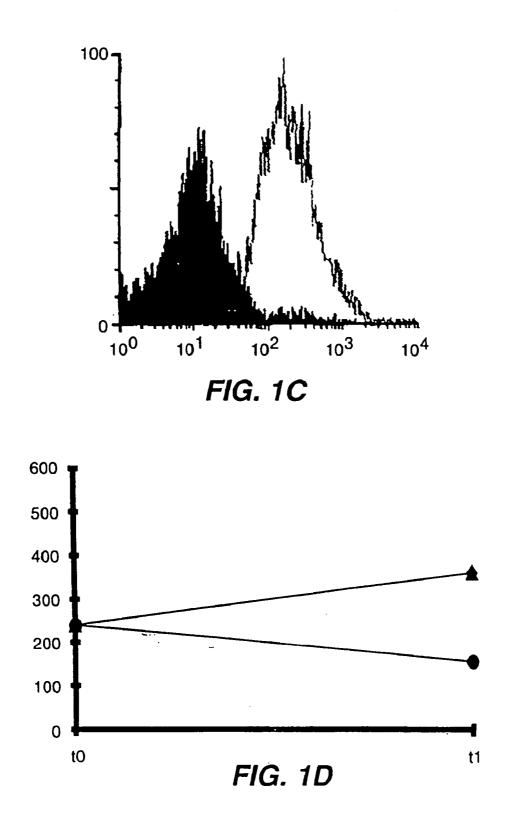


FIG. 4A





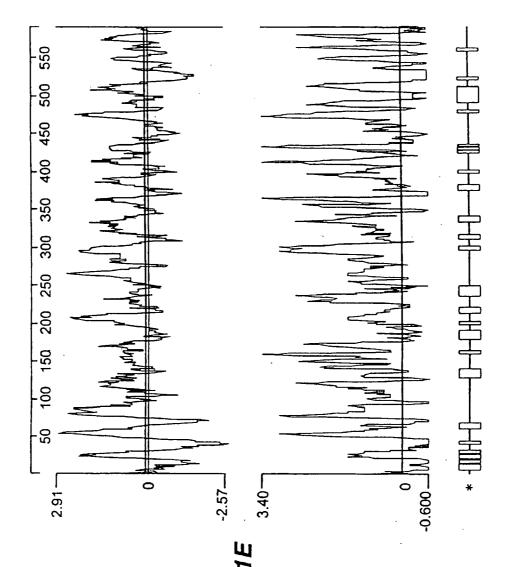


FIG. 1E

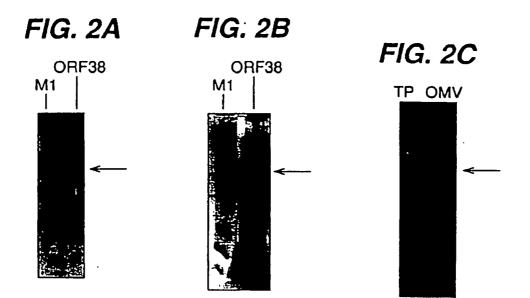
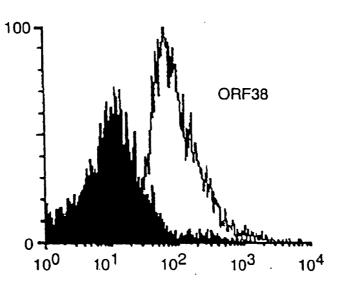


FIG. 2D



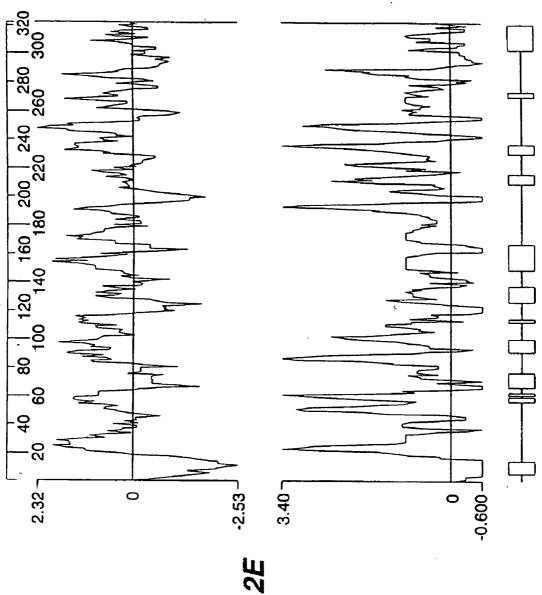


FIG. 2E

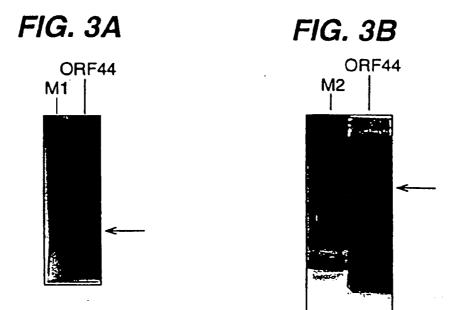
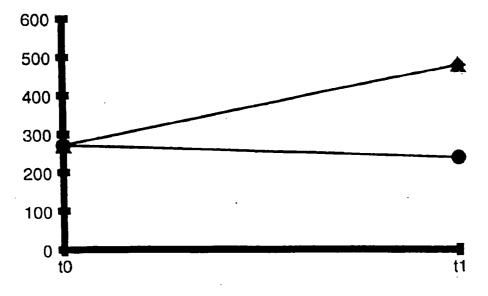


FIG. 3C



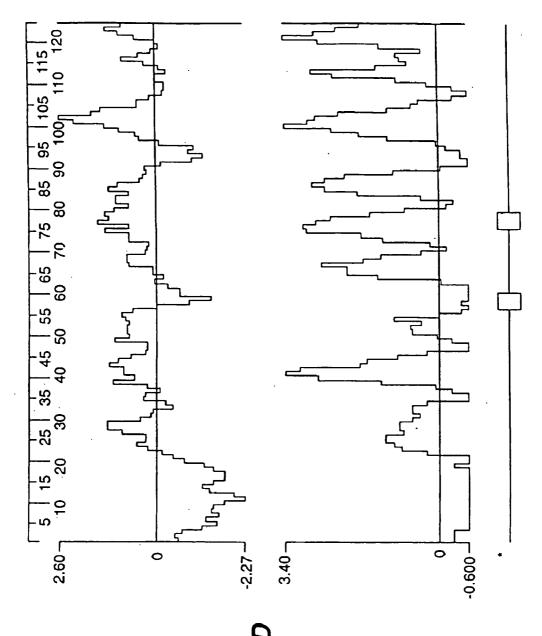
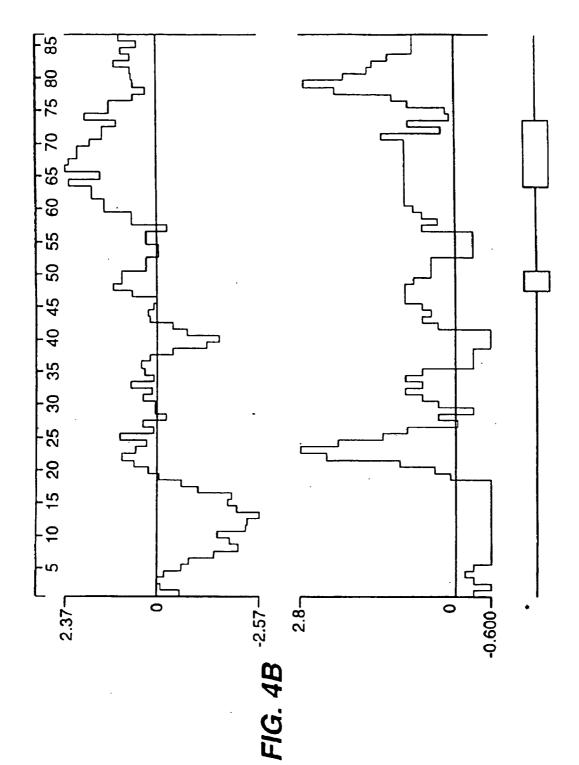
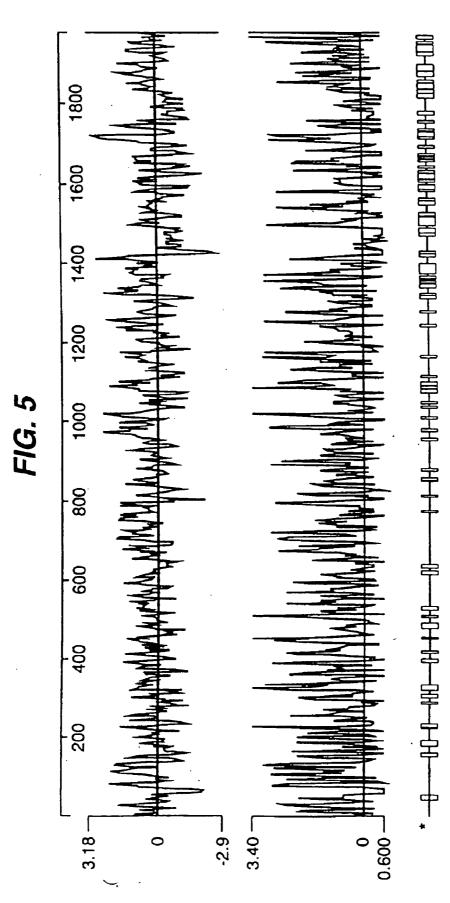


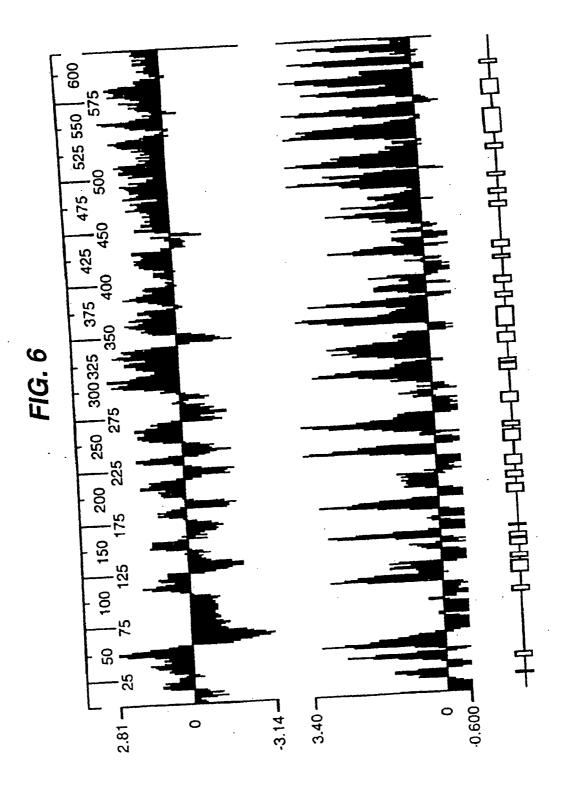
FIG. 3D

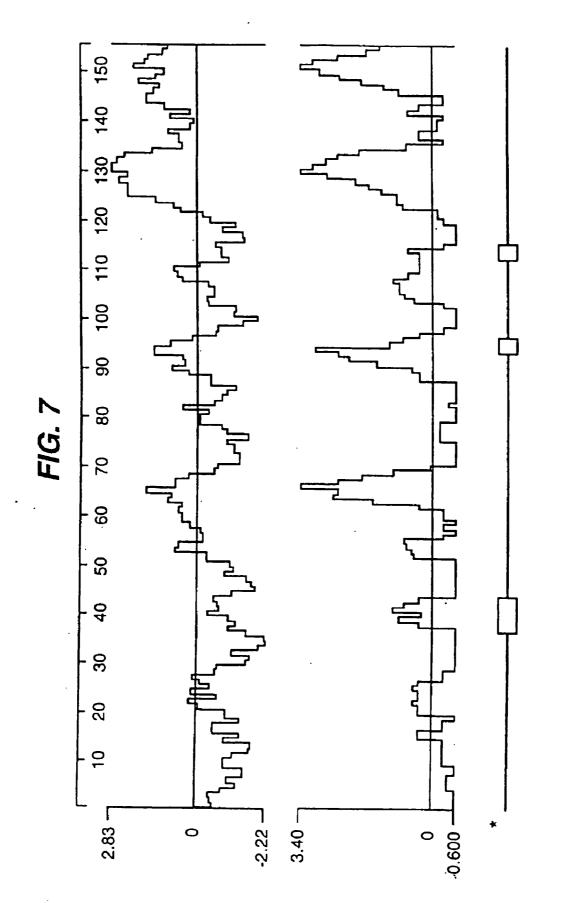






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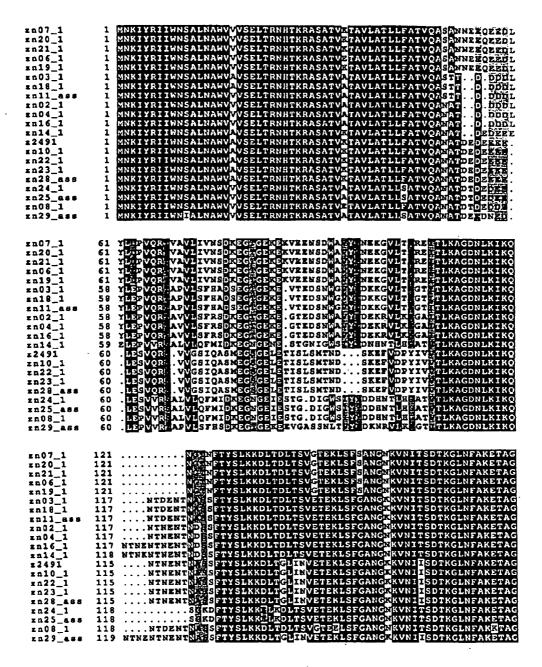


FIG. 8Å

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±n07_1	171 TNGDTTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 171 TNGDTTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 171 TNGDTTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 171 TNGDTTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 173 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 173 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 173 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 174 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 175 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 176 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP 177 TNGDTVHLNGIGSTLTDTLLN GA TNVTND VIDDIKKRAASUKDVLNAGWNIKGVKP
zn20_1	171 INGDTTVHLNGIGSTLTDTLLNEGATINVTNDEVTDDEKKRAASOKDVLNAGWNIKGVKP
zn21_1	171 TNGDTTVHLNGIGSTLTDTLLNGGATTNVTNDEVTDDEXERAASGKDVLNAGWNIKGVKP
rn06_1	171 INGDTTVHLNGIGSTLTDTLLN <mark>BG</mark> ANTNVINDAVIDDEKKRAASSKDVLNAGWNIKGVKP
xn19_1	171 INGDTIVHLNGIGSTLTDTLLNGA THVIND VTDDEKERAAS KOVLNAGWNIKGVKP
zn03_1	173 INGDITVHLNGIGSTLIDTLLN GA TNVINDEVIDDEKKRAAS KOVLNAGWNIKGVKP
xn18_1	173 INGDTTVILNGIGSTLTDTLLBGA TNVTND VTDDEKKRAASVKDVLNAGHNIKGVKP
rn11_ass	173 TNGDTTVHLNGIGSTLTDTLLH CAL TNVTND VTDDEXKRAASIKDVLNAGWNIKGVKP
1102_1	173 TNGDPTVHLNGIGSTLTDTLLN GA INVTHDEVTDDEKKRAASUKDVLNAGWNIKGVKP
zn04_1	173 TNGDPTVHLNGIGSTLTDTLLNGGA INVTHDIVTDDBKKRAASWKDVLNAGWNIKGVKP 177 TNGDPIVHLNGIGSTLTDTLLNGGA INVTHDIVIDDEKKRAASWKDVLNAGWNIKGVKP
<pre>rn16_1 rn14_1</pre>	178 INGDETVHLNGIGSTLTDTLLNGGA INVINDERKRAASEKDVLNAGWNIKGVKP
z2491	178 TNGDTTVHLNGIGSTLTDTLLAF GAL INVIKDS VIDDEKERAASIKDVLNAGWNIKGVKP 171 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 171 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 171 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 171 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 171 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 168 TNGDTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 168 TNGDTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 174 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 175 TNGDTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 174 TNGDTTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 175 TNGDTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 176 TNGDTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 177 TNGDTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT 179 TNGDFTVHLNGIGSTLTDTLAG SAL HVDAGH STHYRAASIKDVLNAGWNIKGVKT
£n10_1	171 TNGDTTVHLNGIGSTLTDTLAG SAL EVDAGN ST BYTRAASEKDVLNAGWNIKGVKT
zn22_1	171 INGDITVHLNGIGSTLTDTLAG SAMEYDAGN ST BYTRAASYKDVLNAGWNIKGVKT
1n23_1	171 INGDTTVHLNGIGSTLTDTLAG SA BVDAGN ST. BYTRAASTKDVLNAGWNIKGVKT
1028_448	171 INGDTTVHLNGIGSTLTDULLNGA THVTND VTDDELSRAASVKDVLNAGWNIKGVKP
sn24_1	168 INGD TVHLNGIGSTLIDTLAG SA HVDAGN ST HYTRAAS KDVLNAGWNIKGVKT
zn25_488	168 TNGDPTVHLNGIGSTLTDTLAG SA HVDAGN ST HYTRAAS KDVLNAGWNIKGVKT
zn08_1	174 INGDITVHLNGIGSTLIDTLAGUSAUUVDAGN ST HYTRAASEKDVLNAGWNIKGVKT
zn29_48#	179 TNGDPTVHLNGIGSTLTDTLAG SALEVDAGN ST HYTRAAS KDVLNAGWNIKGVKT
	231 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 231 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 231 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 231 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 233 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 234 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 235 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 236 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 237 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL 238 GTA. SINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn07_1	231 GTA. SUNVDEVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
1 120_1	231 GTA. SUNVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn21_1 zn06_1	231 GTA. SONVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKI 231 GTA. SONVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKI
zn19_1	231 GTA. SONVDEVRTYDIVEFLSADIKTTIVNVESKDNGKGTEVKIGAKTSVIKEKDGKL
zn03_1	233 GHTA. SUNVDEVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn18_1	233 GTT S NVDFVRTYDTVEFLSADTKTTTVNVESKDNCK TEVKIGAKTSVIKEKDGKL
zn11_ass	233 GTTA. SANVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn02_1	233 GTTASNVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn04_1	233 GGTASENVDEVRTYDTVEFLSADTKTTTVNVESKDNCKSTEVKIGAKTSVIKEKDGKL
zn16_1	237 GHTASHNVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn14_1	 237 GTTAS. NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 238 GTTAS. NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 229 GTTGOS, NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 229 GTTGOS, NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 229 GTTGOS, NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 231 GTTAS. NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 232 GTTGOS, NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 246 GTTGOS, NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 246 GTTGOS, NVDFVRTYDTVEFLSADTKTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL 246 GTTGOS, NVDFVRTYDTVEFLSADTKTTVNVESKDNGK, TEVKIGAKTSVIKEKDGKL
z2491	229 GSTIGOSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKETEVKIGAKTSVIKEKDGKL
· zn10_1	229 GTTGOSTNVDFVRTYDTVEFLSADTKTTTVVVESKDNGK" TEVKIGAKTSVIKEKDGKL
zn22_1	229 GETTGOSINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK®TEVKIGAKTSVIKEKDGKL 229 GETTGOSINVDFVRTYDTVEFLSADTKTTTVNVESKDNGK®TEVKIGAKTSVIKEKDGKL
1223_1 1228_85	231 GETA. SNVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
sn24_1	226 GSTTGOSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKUTEVKIGAKTSVIKEKDGKL
1125_455	226 GHTTGCSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
zn08_1	226 GETTGCSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKETEVKIGAKTSVIKEKDGKL 232 GETTGCSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKETEVKIGAKTSVIKEKDGKL
1129_458	237 GTTGCS NVDFVRTYDTVEFLSADTKTTTVNVESKDNGK TEVKIGAKTSVIKEKDGKL
_	
zn07_1	289 VTGKOKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTMV
zn20_1	289 VTGKOKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV 289 VTGKOKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn21_1 zn06_1	289 VTGKOKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV 289 VTGKOKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
2n19_1	289 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
xn03_1	291 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGT
	291 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGOTGOADKFETVTSGT
zn11_ass	291 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGOTGOADKFETVTSGTAV
fn02_1	291 VTGKGKDENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn04_1	291 VTGKGKDENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTAV
zn16_1	295 VTGKGKDENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGT
· sn14_1	296 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
z2491	289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn10_1	289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTAV 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADXFETVTSGTAV
zn22_1 zn23_1	289 VTGKGKGENGSSTDEGEGEVTAKEVIDAVNAKAGHKMATTTANGQTGQADKFETVISGTAV 289 VTGKGKGENGSSTDEGEGEVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTAV
1125_1 1128_455	289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGOTGQADKFETVTSGTNV
zn24_1	286 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn25_ass	286 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGOTGOADKFETVTSGT
zn08_1	292 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn29_ass	297 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGOTGOADKFETVTSGT
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FIG. 8B

xn07_1	349	TFASG GTTATVSKDDQGNITV YDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn20_1	349	TFASG <mark>R</mark> GTTATVSKDDQGNITVN <mark>Y</mark> DVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASG <mark>R</mark> GTTATVSKDDQGNITVN <mark>Y</mark> DVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn21_1	349	TFASG GTTATVSKDDQGNITV YDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
za06_1	349	TFASG GTTATVSKDDQGNITV YDVNVGDALNVNOLONSGWNLDSKAVAGSSGKVISGN
zn19_1	349	TFASGINGTTATVSKDDOGNITVNYDVNVGDALNVNOLONSGWNLDSKAVAGSSGKVISGN TFASGINGTTATVSKDDOGNITVNYDVNVGDALNVNELONSGWELDSKAVAGSSGKVISGN TFASGINGTTATVSKDDOGNITVNYDVNVGDALNVNQLONSGWNLDSKAVAGSSGKVISGN
1 203	351	TFASG GTTATVSKDDOGNITV YDVNVGDALNVNOLONSGWNLDSKAVAGSSGKVISGN
zn18_1	351	TFASGEGTTATV5KDDOGN1TVEIYDVNVGDALNVNOLONSGWNIDSKAVAGSSGKUTSGN
snll_ass	351	TFASGNGTTATVSKDDOGNITVNYDVNVGDALNVNOLONSGWNLDSKAVAGSSCKVISGN TFASGNGTTATVSKDDOGNITVNYDVNVGDALNVNQLONSGWNLDSKAVAGSSCKVISGN
zn02_1	351	TEASGUGTTATVSKDDOGNITVNYDVNVGDALNVNOLONSGWNLDSKAVAGSSGKVLSGN
xa04_1	351	TFASGAGTTATVSKDDQGNITVATDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQGNITVATDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQGNITVATDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQGNITVATDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQGNITVATDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
£n16_1	355	
sn14_1	356	
x2491	349	
	349	
zn10_1 zn22_1	349	
	349	IFASG GITAT VSRDDQGITT VTDVNVGDALNVNQLQNSGWNLDSKNVACSSGRVISGN
zn23_1	349	TFASGAGTTATVSKDDQCNITVAYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQCNITVAYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQGNITVAYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN TFASGAGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn28_ass		IF ASGAGT I A TV SKUDUGN I I VITUVNVGDALNVNOLONSGWNLUSKAVAGSSGRV I SGN
2n24_1	346	TFASGNGTTATVSKDDOGN1TVN1DVNVGDALNVNOLONSGWNLDSKAVAGSSGRVISGN
zn25_e##	346	TFASGNGTTATVSKDDOGNITVNYDVNVGDALNVNOLONSGWNLDSKAVAGSSGRVISGN
zn08_1	352	IFASGEGTTATVSKDDQGNITVEYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISCN
zn29_ass	357	TFASG <mark>N</mark> GTTATVSKDDQGNITV <mark>X</mark> YDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISCN
za07_1	409	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVD <mark>G</mark> , A VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDG VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVD <mark>G</mark> , A
zn20_1	409	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVD <mark>G</mark> , a
zn21_1	409	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVD <mark>G</mark> . A
zn06_1	409	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVD <mark>G</mark> F.A
zn19_1	409	VSPSKGKMDETVNINACNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVD <mark>G</mark> .A
zn03_1	411	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGAÐAPTLSVDDÖGA
zn18_1	411	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDD <mark>.</mark> Ga
zn11_ass	411	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGAGADAPTLSVDD.GA
±n02_1	411	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMPOFSSVSLGAGADAPTLSVDDÓ.GA
zn04_1	411	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSM <mark>A</mark> POFSSVSLGAGADAPTLSVDD ¹ GA
xn16_1	415	VSPSKCKMDETVNINAGNNIEITRNCKNIDIATSMTPOFSSVSLGAGADAPTLSVDD GA
2014_1	416	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGAGADAPTLSVDDØGA
12491	409	VSPSKGKMDETVNINAGNNIEI RNGKNIDIATSM POFSSVSLGAGADAPTLSVDD GA
za10_1	409	VSPSKGKMDETVNINAGNNIEI.RNGKNIDIATSM POFSSVSLGAGADAPTLSVDD [#] GA
xn22_1	409	VSPSKGKMDETVNINAGNNIEI RNGKNIDIATSM POFSSVSLGAGADAPTLSVDD CA
zn23_1	409	VSPSKGKMDETVNINAGNNIEI_RNGKNIDIATSM <mark>A</mark> POFSSVSLGAGADAPTLSVDD ^N GA
1028_ass	409	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGAGADAPTLSVDDIGA
zn24_1	406	VSPSKCKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGACADAPTLSVDDÖGA
zn25_ass	406	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGAGADAPTLSVDDEGA
xn08_1	412	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGAGADAPTLSVDDHGA
xn29_888	417	VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPOFSSVSLGAGADAPTLSVDDMGA
aus/_600	•••	
£n07_1	468	LNVGSKUDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn20_1	468	LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn21_1	468	LNVGSKEDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn06_1	468	LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
1000_1 119_1	468	LNVGSKADARFVRITNVAPGVÆGDVINVAGLKGVAGNINNRIDNVDGNARAGIAGAIAT
	471	LNVGSKDINKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNEIDNVDGNARAGIAQAIAT
zn03_1	471	LNVGSKDUNKPVRITNVAPGVKEGDVINVAQLKGVAQNLNMIDNVDGNARAGIAQAIAT
zn18_1	471	LNVGSKDANKPVRITNVAPGVKEGDVINVAQLKGVAQNLNNHIDNVDGNARAGIAQAIAT LNVGSKDANKPVRITNVAPGVKEGDVINVAQLKGVAQNLNNHIDNVDGNARAGIAQAIAT
xn11_ass	471	LNVGSKD-NKPVRITNVAPGVREGDVINVAQLKGVAQNLNNBIDNVDGNARAGIAQAIAT LNVGSKD <mark>-</mark> NKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNBIDNVDGNARAGIAQAIAT
En02_1	471	LNVGSKDMNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn04_1 zn16_1	475	
zn14_1	476	
z2491	469	LNVGSKDUNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn10_1	469	
zn22_1	-469	
zn23_1	469	LNVGSKDNNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn28_868	469	
zn24_1	466	
zn25_ass	466	LNVGSKD <mark>A</mark> NKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn08_1	472	
zn29_ess	477	LNVGSKD <mark>ANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT</mark>

FIG. 8C

zn07_1	528	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn20_1	528	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn21_1	528	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
xn06_1	528	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn19_1	528	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn03_1	531	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn18_1	531	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
znll_ass	531	ASLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn02_1	531	AGLVQAYLPGKSMMAIGGDTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn04_1	531	AGLVQAYLPGKSMMAIGG <mark>D</mark> TYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn16_1	535	AGLAQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISD <mark>.</mark> GNW <u>H</u> ikgtasgnsrghfgasasv
zo14_1	536	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
z2491	529	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
za10_1	529	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn22_1	529	AGLVQAYLPGK5MMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
xn23_1	529	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn28_ass	529	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn24_1	526	AGL <mark>R</mark> QAYLPGKSMMAIGGGTYRGEAGYAIGYSSISD <mark>E</mark> GNWÄIKGTASGNSRGHFG <mark>E</mark> SASV
zn25_ass	526	AGU _ QAYLPGKSMMAIGGGTYRGEAGYAIGYSSISD <mark>⊡</mark> GNW <u>Q</u> IKGTASCNSRGHFG <mark>⊡</mark> SASV
xn08_1	532	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASV
zn29_ass	537	AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTÁSGNSRGHFGASASV
zn07_1	588	GYQW
zn20_1	588	GYQW®
zn21_1	588	GYQW*
zn06_1	598	GYQW*
zn19_1	588	GYQW*
zn03_1	591	
gn18_1	591	GYQW*
sn11_ass	591	GYQW ⁴
02 1	5 6 1	GYOR T

 xn11_xss
 591
 GYQW

 xn02_1
 591
 GYQW

 xn04_1
 591
 GYQW

 xn16_1
 595
 GYQW

 xn14_1
 596
 GYQW

 xn10_1
 589
 GYQW

 xn22_1
 589
 GYQW

 xn23_1
 589
 GYQW

 xn28_ass
 589
 GYQW

 xn25_ass
 586
 GYQW

 xn29_ass
 597
 GYQW

FIG. 8D

MENINGOCOCCAL ANTIGENS

[0001] This application is a continuation-in-part of international patent application PCT/IB99/00103, filed Jan. 14, 1999, from which priority is claimed under 35 U.S.C. § 119.

[0002] This invention relates to antigens from the bacterium *Neisseria meningitidis*.

BACKGROUND

[0003] Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonises the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N. gonorrhoeae*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

[0004] *N. meningitidis* causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks (see Lieberman el al. (1996) Safety and Immunogenicity of a Serogroups A/C Neisseria meningitidis Oligosaccharide-Protein Conjugate Vaccine in Young Children. JAMA 275(19):1499-1503; Schuchat et al (1997) Bacterial Meningitis in the United States in 1995. N Engl J Med 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against Haemophilus influenzae, N. meningitidis is the major cause of bacterial meningitis at all ages in the United States (Schuchat et al (1997) supra).

[0005] Based on the organism's capsular polysaccharide, 12 serogroups of N. meningitidis have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants [eg. Morbidity and Mortality weekly report, Vol. 46, No. RR-5 (1997)]. This is because polysaccharides are T-cell independent antigens that induce a weak immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against H. influenzae, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger W D "New and Improved Vaccines Against Meningococcal Disease" in: New Generation Vaccines, supra, pp. 469-488; Lieberman et al (1996) supra; Costantino et al (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A and C. Vaccine 10:691-698).

[0006] Meningococcus B remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of α (2-8)-linked N-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the N-acetyl groups with N-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular?*Clin Microbiol Rev* 7(4):559-575).

[0007] Alternative approaches to menB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (eg. Poolman J T (1992) Development of a meningococcal vaccine. Infect. Agents Dis. 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (eg. Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. Vaccine 14(1):49-53).

[0008] A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (eg. EP-A-0467714, WO96/29412), but this is by no means complete. The provision of further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*.

[0009] The Invention

[0010] The invention provides proteins comprising the *N*. *meningitidis* amino acid sequences disclosed in the examples.

[0011] It also provides proteins comprising sequences homologous (ie. having sequence identity) to the *N. meningitidis* amino acid sequences disclosed in the examples. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more). These homologous proteins include mutants and allelic variants of the sequences disclosed in the examples. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between the proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters gap open penalty=12 and gap extension penalty=1.

[0012] The invention further provides proteins comprising fragments of the *N. meningitidis* amino acid sequences disclosed in the examples. The fragments should comprise at least n consecutive amino acids from the sequences and, depending on the particular sequence, n is 7 or more (eg. 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

[0013] The proteins of the invention can, of course, be prepared by various means (eg. recombinant expression, purification from cell culture, chemical synthesis etc.) and in various forms (eg. native, fusions etc.). They are preferably prepared in substantially pure form (ie. substantially free from other *N. meningitidis* or host cell proteins).

[0014] According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means.

[0015] According to a further aspect, the invention provides nucleic acid comprising the *N. meningitidis* nucleotide sequences disclosed in the examples. In addition, the invention provides nucleic acid comprising sequences homologous (ie. having sequence identity) to the *N. meningitidis* nucleotide sequences disclosed in the examples.

[0016] Furthermore, the invention provides nucleic acid which can hybridise to the *N. meningitidis* nucleic acid disclosed in the examples, preferably under "high stringency" conditions (eg. 65° C. in a 0.1×SSC, 0.5% SDS solution).

[0017] Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least n consecutive nucleotides from the *N. meningitidis* sequences and, depending on the particular sequence, n is 10 or more (eg 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

[0018] According to a further aspect, the invention provides nucleic acid encoding the proteins and protein fragments of the invention.

[0019] It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (eg. for antisense or probing purposes).

[0020] Nucleic acid according to the invention can, of course, be prepared in many ways (eg. by chemical synthesis, from genomic or cDNA libraries, from the organism itself etc.) and can take various forms (eg. single stranded, double stranded, vectors, probes etc.).

[0021] In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) etc.

[0022] According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (eg. expression vectors) and host cells transformed with such vectors.

[0023] According to a further aspect, the invention provides compositions comprising protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions.

[0024] The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (eg. as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria Said Neisserial bacteria may be any species or strain (such as *N. gonorrhoeae*) but are preferably *N. meningitidis*, especially strain A, strain B or strain C.

[0025] The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

[0026] According to further aspects, the invention provides various processes.

[0027] A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

[0028] A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

[0029] A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

[0030] A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

[0031] Unlike the sequences disclosed in PCT/IB98/ 01665, the sequences disclosed in the present application are believed not to have any significant homologs in *N. gonorrhoeae*. Accordingly, the sequences of the present invention also find use in the preparation of reagents for distinguishing between *N. meningitidis* and *N. gonorrhoeae*.

[0032] A summary of standard techniques and procedures which may be employed in order to perform the invention (eg. to utilise the disclosed sequences for vaccination or diagnostic purposes) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

[0033] General

[0034] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature eg. Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D. N Glover ed. 1985); *Oligonucleotide Synthesis* (M. J. Gait ed, 1984); *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription and Translation* (B. D. Hames &

S. J. Higgins eds. 1984); Animal Cell Culture (R. I. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984); the Methods in Enzymology series (Academic Press, Inc.), especially volumes 154 & 155; Gene Transfer Vectors for Mammalian Cells (J. H. Miller and M. P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), Immunochemical Methods in Cell and Molecular Biology (Academic Press, London); Scopes, (1987) Protein Purification: Principles and Practice, Second Edition (Springer-Verlag, N.Y.), and Handbook of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell eds 1986).

[0035] Standard abbreviations for nucleotides and amino acids are used in this specification.

[0036] All publications, patents, and patent applications cited herein are incorporated in full by reference. In particular, the contents of UK patent applications 9800760.2, 9819015.0 and 9822143.5 are incorporated herein.

[0037] Definitions

[0038] A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

[0039] The term "comprising" means "including" as well as "consisting" eg. a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

[0040] A "conserved"*Neisseria* amino acid fragment or protein is one that is present in a particular Neisserial protein in at least x % of *Neisseria*. The value of x may be 50% or more, e.g., 66%, 75%, 80%, 90%, 95% or even 100% (i.e. the amino acid is found in the protein in question in all *Neisseria*). In order to determine whether an animo acid is "conserved" in a particular Neisserial protein, it is necessary to compare that amino acid residue in the sequences of the protein in question from a plurality of different *Neisseria* (a reference population). The reference population may include a number of different *Neisseria* species or may include a single species. The reference population may include a number of different serogroups of a particular species or a single serogroup. A preferred reference population consists of the 5 most common *Neisseria*.

[0041] The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature.

[0042] An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own

control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

[0043] A "mutant" sequence is defined as DNA, RNA or amino acid sequence differing from but having sequence identity with the native or disclosed sequence. Depending on the particular sequence, the degree of sequence identity between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more, calculated using the Smith-Waterman algorithm as described above). As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions (eg. see U.S. Pat. No. 5,753,235).

[0044] Expression Systems

[0045] The Neisserial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

[0046] i. Mammalian Systems

[0047] Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (eg. structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In Molecular Cloning: A Laboratory Manual, 2nd ed.]

[0048] Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallotheionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormoneresponsive cells. [0049] The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) Science 236:1237; Alberts et al. (1989) Molecular Biology of the Cell, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer [Dijkema et al (1985) EMBO J. 4:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al. (1982b) Proc. Natl. Acad. Sci. 79:6777] and from human cytomegalovirus [Boshart et al. (1985) Cell 41:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) Trends Genet. 2:215; Maniatis et al. (1987) Science 236:1237].

[0050] A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by in vitro incubation with cyanogen bromide.

[0051] Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either in vivo or in vitro. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus triparite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

[0052] Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) Cell 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In Transcription and splicing (ed. B. D. Hames and D. M. Glover); Proudfoot (1989) Trends Biochem. Sci. 14:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminater/ polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In Molecular Cloning: A Laboratory Manual].

[0053] Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription

termination sequence are put together into expression constructs. Enhancers; introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (eg. plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 [Gluzman (1981) Cell 23:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) Mol. Cell. Biol. 9:946] and pHEBO [Shimizu et al. (1986) Mol. Cell. Biol. 6:1074].

[0054] The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0055] Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (eg. Hep G2), and a number of other cell lines.

[0056] ii. Baculovirus Systems

[0057] The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

[0058] After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant-virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, inter alia, Invitrogen, San Diego Calif. ("MaxBac" kit). These techniques are generally

known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No.* 1555 (1987) (hereinafter "Summers and Smith").

[0059] Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (eg. plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

[0060] Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) 17:31.

[0061] The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, 42:177) and a prokaryotic ampicillin-resistance (amp) gene and origin of replication for selection and propagation in *E. coli*.

[0062] Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3) transcription of a coding sequence (eg. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

[0063] Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* 69:765.

[0064] DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, 73:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the

signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human α -interferon, Maeda et al., (1985), *Nature* 315:592; human gastrin-releasing peptide, Lebacq-Verheyden et al., (1988), *Molec. Cell. Biol.* 8:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, 82:8404; mouse IL-3, (Miyajima et al., (1987) *Gene* 58:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, 7:99, can also be used to provide for secretion in insects.

[0065] A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by in vitro incubation with cyanogen bromide.

[0066] Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

[0067] After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus—usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5 kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art (See Summers and Smith supra; Ju et al. (1987); Smith et al., Mol. Cell. Biol. (1983) 3:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), Bioessays 4:91 The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrinspecific sequences and is positioned downstream of the polyhedrin promoter.

[0068] The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μ m in

size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, supra; Miller et al. (1989).

[0069] Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, inter alia: *Aedes aegypti, Autographa californica, Bombyx mori, Drosophila melanogaster, Spodoptera frugiperda,* and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol.* 56:153; Wright (1986) *Nature* 321:718; Smith et al., (1983) *Mol. Cell. Biol.* 3:2156; and see generally, Fraser, et al. (1989) *In Vitro Cell. Dev. Biol.* 25:225).

[0070] Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. See, eg. Summers and Smith supra.

[0071] The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, eg. HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, eg. proteins, lipids and polysaccharides.

[0072] In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

[0073] iii. Plant Systems

[0074] There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: U.S. Pat. No. 5,693,506; U.S. Pat. No. 5,659,122; and U.S. Pat. No. 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found

in addition to the references described above in Vaulcombe et al., Mol. Gen. Genet. 209:33-40 (1987); Chandler et al., Plant Molecular Biology 3:407-418 (1984); Rogers, J. Biol. Chem. 260:3731-3738 (1985); Rothstein et al., Gene 55:353-356 (1987); Whittier et al., Nucleic Acids Research 15:2515-2535 (1987); Wirsel et al., Molecular Microbiology 3:3-14 (1989); Yu et al., Gene 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R. L. Jones and J. MacMillin, Gibberellins: in: Advanced Plant Physiology, Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, Plant Cell, 2:1027-1038 (1990); Maas et al., EMBO J. 9:3447-3452 (1990); Benkel and Hickey, Proc. Natl. Acad. Sci 84:1337-1339 (1987)

[0075] Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker, and, for Agrobacterium transformations, T DNA sequences for Agrobacterium-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, Plant Mol. Biol. Reptr, 11(2):165-185.

[0076] Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

[0077] The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

[0078] A heterologous coding sequence may be for any protein relating to the present invention. The sequence

encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

[0079] Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

[0080] The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, Mol. Gen. Genet, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., Nature, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., Nature, 327, 70-73, 1987 and Knudsen and Muller, 1991, Planta, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., Proc. Natl. Acad. Sci. USA, 79, 1859-1863, 1982.

[0081] The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

[0082] All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersion, Nicotiana, Solanum, Petunia, Digitalis,* Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Hererocallis, Nemesia, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Lolium, Zea, Triticum, Sorghum, and Datura.

[0083] Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

[0084] In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryo-less-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

[0085] iv. Bacterial Systems

[0086] Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (eg. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (E. coli) [Raibaud et al. (1984) Annu. Rev. Genet. 18:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

[0087] Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples

include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (lac) [Chang et al. (1977) *Nature* 198:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (trp) [Goeddel et al. (1980) *Nuc. Acids Res.* 8:4057; Yelverton et al. (1981) *Nucl. Acids Res.* 9:731; U.S. Pat. No. 4,738,921; EP-A-0036776 and EP-A-0121775]. The g-laotamase (bla) promoter system [Weissmann (1981) "The cloning of interferon and other mistates." In *Interferon* 3 (ed I. Gresser)], bacteriophage lambda PL [Shimatake et al. (1981) *Nature* 292:128] and T5 [U.S. Pat. No. 4,689,406] promoter systems also provide useful promoter sequences.

[0088] In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [U.S. Pat. No. 4,551, 433]. For example, the tac promoter is a hybrid trp-lac promoter comprised of both trp promoter and lac operon sequences that is regulated by the lac repressor [Amann et al. (1983) Gene 25:167; de Boer et al. (1983) Proc. Natl. Acad. Sci. 80:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier et al. (1986) J. Mol. Biol. 189:113; Tabor et al. (1985) Proc Natl. Acad. Sci. 82:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an E. coli operator region (EPO-A-0 267 851).

[0089] In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In E. coli, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine et al. (1975) Nature 254:34). The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of E. coli 16S rRNA [Steitz et al. (1979) "Genetic signals and nucleotide sequences in messenger RNA." In Biological Regulation and Development: Gene Expression (ed. R. F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook et al. (1989) "Expression of cloned genes in Escherichia coli." In Molecular Cloning: A Laboratory Manual].

[0090] A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by in vitro incubation with cyanogen bromide or by either in vivo on in vitro incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

[0091] Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai et al. (1984) Nature 309:810]. Fusion proteins can also be made with sequences from the lacZ [Jia et al. (1987) Gene 60:197], trpE (Allen et al. (1987) J. Biotechnol. 5:93; Makoff et al. (1989) J. Gen. Microbiol. 135:11], and Chey [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (eg. ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated [Miller et al. (1989) Bio/Technology 7:698].

[0092] Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [U.S. Pat. No. 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either in vivo or in vitro encoded between the signal peptide fragment and the foreign gene.

[0093] DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (ompA) [Masui et al. (1983), in: *Experimental Manipulation of Gene Expression*; Ghrayeb et al. (1984) *EMBO J.* 3:2437] and the *E. coli* alkaline phosphatase signal sequence (phoA) [Oka et al. (1985) *Proc. Natl. Acad. Sci.* 82:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various *Bacillus* strains can be used to secrete heterologous proteins from *B. subtilis* [Palva et al. (1982) *Proc. Natl. Acad. Sci.* USA 79:5582; EP-A-0 244 042].

[0094] Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the trip gene in *E. coli* as well as other biosynthetic genes.

[0095] Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (eg. plasmids) capable of stable maintenance in a host, such as bacteria The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

[0096] Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EP-A-0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

[0097] Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies et al. (1978) *Annu. Rev. Microbiol.* 32:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

[0098] Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

[0099] Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria For example, expression vectors have been developed for, inter alia, the following bacteria: *Bacillus subtilis* [Palva et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], *Escherichia coli* [Shimatake et al. (1981) *Nature* 292:128; Amann et al. (1985) *Gene* 40:183; Studier et al. (1986) *J. Mol. Biol.* 189:113; EP-A-0 036 776, EP-A-0 136 829 and EP-A-0 136 907], *Streptococcus cremoris* [Powell et al. (1988) *Appl. Environ. Microbiol.* 54:655], *Streptomyces lividans* [U.S. Pat. No. 4,745,056].

[0100] Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial

species to be transformed. See eg. [Masson et al. (1989) FEMS Microbiol. Lett. 60:273; Palva et al. (1982) Proc. Natl. Acad. Sci USA 79:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, Bacillus], [Miller et al. (1988) Proc. Natl. Acad. Sci. 85:856; Wang et al. (1990) J. Bacteriol. 172:949, Campylobacter], [Cohen et al. (1973) Proc. Natl. Acad. Sci. 69:2110; Dower et al. (1988) Nucleic Acids Res. 16:6127; Kushner (1978) "An improved method for transformation of Escherichia coli with ColE1-derived plasmids. In Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering (eds. H. W. Boyer and S. Nicosia); Mandel et al. (1970) J. Mol. Biol. 53:159; Taketo (1988) Biochim Biophys. Acta 949:318; Escherichia], [Chassy et al. (1987) FEMS Microbiol. Lett. 44:173 Lactobacillus]; [Fiedler et al. (1988) Anal. Biochem 170:38, Pseudomonas]; [Augustin et al. (1990) FEMS Microbiol. Lett. 66:203, Staphylococcus], [Barany et al. (1980) J. Bacteriol. 144:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: Streptococcal Genetics (ed. J. Ferretti and R. Curtiss III); Perry et al. (1981) Infect Immun. 32:1295; Powell et al. (1988) Appl. Environ. Microbiol. 54:655; Somkuti et al. (1987) Proc. 4th Evr. Cong. Biotechnology 1:412, Streptococcus].

[0101] v. Yeast Expression

[0102] Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (eg. structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

[0103] Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast PHO5 gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:1].

[0104] In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Pat. Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the ADH2, GAL4, GAL10, OR PHO5 genes, combined with the transcription activation with the transcription activation with the transcription of the regulatory sequences of either the ADH2, GAL4, GAL10, OR PHO5 genes, combined with the transcription activation activation the transcription activation with the transcription activation activation the transcription activation the transcription activation activation activation activation activation (U.S. Pat. Nos. 4,876,197 and 4,880,734).

scriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of nonyeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, inter alia, [Cohen et al. (1980) *Proc. Natl. Acad. Sci. USA* 77:1078; Henikoff et al. (1981) *Nature* 283:835; Hollenberg et al. (1981) *Curr. Topics Microbiol. Immunol.* 96:119; Hollenberg et al. (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast *Saccharomyces cerevisiae*," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K. N. Timmis and A. Puhler); Mercerau-Puigalon et al. (1980) *Gene* 11:163; Panthier et al. (1980) *Curr. Genet.* 2:109;].

[0105] A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by in vitro incubation with cyanogen bromide.

[0106] Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See eg. EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (eg. ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (eg. WO88/024066).

[0107] Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either in vivo or in vitro. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

[0108] DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EP-A-0 012 873; JPO. 62,096,086) and the A-factor gene (U.S. Pat. No. 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EP-A-0 060 057).

[0109] A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (U.S. Pat. Nos. 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (eg. see WO 89/02463.)

[0110] Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

[0111] Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (eg. plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeastbacteria shuttle vectors include YEp24 [Botstein et al. (1979) Gene 8:17-24], pCl/1 [Brake et al. (1984) Proc. Natl. Acad. Sci USA 81:4642-4646], and YRp17 [Stinchcomb et al. (1982) J. Mol. Biol. 158:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See eg. Brake et al., supra.

[0112] Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver et al. (1983) Methods in Enzymol. 101:228-245]. An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver et al., supra. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine et al. (1983) Proc. Natl. Acad. Sci. USA 80:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

[0113] Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as ADE2, HIS4, LEU2,

TRP1, and ALG7, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of CUP1 allows yeast to grow in the presence of copper ions [Butt et al. (1987) *Microbiol, Rev.* 51:351].

[0114] Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

[0115] Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, inter alia, the following yeasts: Candida albicans [Kurtz, et al. (1986) Mol. Cell. Biol. 6:142], Candida maltosa [Kunze, et al. (1985) J. Basic Microbiol. 25:141]. Hansenula polymorpha [Gleeson, et al. (1986) J. Gen. Microbiol. 132:3459; Roggenkamp et al. (1986) Mol. Gen. Genet. 202:302], Kluyveromyces fragilis [Das, et al. (1984) J. Bacteriol. 158:1165], Kluyveromyces lactis [De Louvencourt et al. (1983) J. Bacteriol. 154:737; Van den Berg et al. (1990) Bio/Technology 8:135], Pichia guillerimondii [Kunze et al. (1985) J. Basic Microbiol. 25:141], Pichia pastoris [Cregg, et al. (1985) Mol. Cell. Biol. 5:3376; U.S. Pat. Nos. 4,837, 148 and 4,929,555], Saccharomyces cerevisiae [Hinnen et al. (1978) Proc. Natl. Acad. Sci. USA 75:1929; Ito et al. (1983) J. Bacteriol. 153:163], Schizosaccharomyces pombe [Beach and Nurse (1981) Nature 300:706], and Yarrowia lipolytica [Davidow, et al. (1985) Curr. Genet. 10:380471 Gaillardin, et al (1985) Curr. Genet. 10:49].

[0116] Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See eg. [Kurtz et al. (1986) Mol. Cell. Biol. 6:142; Kunze et al. (1985) J. Basic Microbiol. 25:141; Candida]; [Gleeson et al. (1986) J. Gen. Microbiol. 132:3459; Roggenkamp et al. (1986) Mol. Gen. Genet. 202:302; Hansenula]; [Das et al. (1984) J. Bacteriol. 158:1165; De Louvencourt et al. (1983) J. Bacteriol. 154:1165; Van den Berg et al. (1990) Bio/ Technology 8:135; Kluyveromyces]; [Cregg et al. (1985) Mol. Cell. Biol. 5:3376; Kunze et al. (1985) J. Basic Microbiol. 25:141; U.S. Pat. Nos. 4,837,148 and 4,929,555; Pichia]; [Hinnen et al. (1978) Proc. Natl. Acad. Sci. USA 75;1929; Ito et al. (1983) J. Bacteriol. 153:163 Saccharomyces]; [Beach and Nurse (1981) Nature 300:706; Schizosaccharomyces]; [Davidow et al. (1985) Curr. Genet. 10:39; Gaillardin et al. (1985) Curr. Genet. 10:49; Yarrowia].

[0117] Antibodies

[0118] As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric

antibodies, humanised antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies. Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying Neisserial proteins.

[0119] Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 μ g/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the adt, which for the purposes of this invention is considered equivalent to in vivo immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25° C. for one hour, followed by incubating at 4° C. for 2-18 hours. The serum is recovered by centrifugation (eg. 1,000 g for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

[0120] Monoclonal antibodies are prepared using the standard method of Kohler & Milstein [Nature (1975) 256:495-96], or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (eg. hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either in vitro (eg. in tissue culture bottles or hollow fiber reactors), or in vivo (as ascites in mice).

[0121] If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ³²P and ¹²⁵I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ¹²⁵I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ¹²⁵I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

[0122] Pharmaceutical Compositions

[0123] Pharmaceutical compositions can comprise either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

[0124] The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

[0125] For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

[0126] A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

[0127] Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

[0128] Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

[0129] Delivery Methods

[0130] Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

[0131] Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (eg. see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

[0132] Vaccines

[0133] Vaccines according to the invention may either be prophylactic (ie. to prevent infection) or therapeutic (ie. to treat disease after infection).

[0134] Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, H. pylori, etc. pathogens.

[0135] Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59TM (WO 90/14837; Chapter 10 in Vaccine design: the subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, Mass.), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% plu-

ronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RibiTM adjuvant system (RAS), (Ribi Immunochem, Hamilton, Mont.) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+ CWS (Detox[™]); (3) saponin adjuvants, such as Stimulon[™] (Cambridge Bioscience, Worcester, Mass.) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (eg. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (eg. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59[™] are preferred.

[0136] As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)ethylamine (MTP-PE), etc.

[0137] The immunogenic compositions (eg. the immunising antigen/immunogen/polypeptide/protein/nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

[0138] Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

[0139] Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (eg. nonhuman primate, primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctors assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

[0140] The immunogenic compositions are conventionally administered parenterally, eg. by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (eg. WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

[0141] As an alternative to protein-based vaccines, DNA vaccination may be employed [eg. Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly et al. (1997) *Annu Rev Immunol* 15:617-648; see later herein].

[0142] Gene Delivery Vehicles

[0143] Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in in vivo or ex vivo modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

[0144] The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adenoassociated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parovvirus, picomavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

[0145] Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retrovirus eg. MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

[0146] Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

[0147] These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see U.S. Pat. No. 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

[0148] Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines

are made from human parent cells (eg. HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

[0149] Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Md. or isolated from known sources using commonly available techniques.

[0150] Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, U.S. Pat. No. 5,219,740, U.S. Pat. No. 4,405,712, U.S. Pat. No. 4,861,719, U.S. Pat. No. 4,980,289, U.S. Pat. No. 4,777,127, U.S. Pat. No. 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Rain (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

[0151] Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) Biotechniques 6:616 and Rosenfeld (1991) Science 252:431, and WO93/07283, WO93/ 06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/ 09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) Hum. Gene Ther. 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (ie. there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) Gene 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) J. Virol. 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in U.S. Pat. No. 5,478,745. Still other vectors are those disclosed in Carter U.S. Pat. No. 4,797,368 and Muzyczka U.S. Pat. No. 5,139,941, Chartejee U.S. Pat. No. 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) Human Gene Therapy 7:463-470. Additional AAV gene therapy vectors are described in U.S. Pat. No. 5,354, 678, U.S. Pat. No. 5,173,414, U.S. Pat. No. 5,139,941, and U.S. Pat. No. 5,252,479.

[0152] The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in U.S. Pat. No. 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

[0153] Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in U.S. Pat. Nos. 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in U.S. Ser. No. 08/405, 627, filed Mar. 15, 1995, WO94/21792, WO92/10578, WO95/07994, U.S. Pat. No. 5,091,309 and U.S. Pat. No. 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Md. or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see U.S. Ser. No. 08/679,640).

[0154] DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

[0155] Other viral vectors suitable for use in the present invention include those derived from poliovirus, for

example ATCC VR-58 and those described in Evans, Nature 339 (1989) 385 and Sabin (1973) J. Biol. Standardization 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) J Cell Biochem L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) Proc Natl Acad Sci 86:317; Flexner (1989) Ann NY Acad Sci 569:86, Flexner (1990) Vaccine 8:17; in U.S. Pat. No. 4,603,112 and U.S. Pat. No. 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) Nature 277:108 and Madzak (1992) J Gen Virol 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in U.S. Pat. No. 5,166,057 and in Enami (1990) Proc Nail Acad Sci 87:3802-3805; Enami & Palese (1991) J Virol 65:2711-2713 and Luytjes (1989) Cell 59:110, (see also McMichael (1983) NEJ Med 309:13, and Yap (1978) Nature 273:238 and Nature (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) J. Virol. 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) Proc Soc Exp Biol Med 121:190.

[0156] Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see U.S. Ser. No. 08/366,787, filed Dec. 30, 1994 and Curiel (1992) Hum Gene Ther 3:147-154 ligand linked DNA, for example see Wu (1989) J Biol Chem 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see U.S. Ser. No. 08/240,030, filed May 9, 1994, and U.S. Ser. No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in U.S. Pat. No. 5,149,655, ionizing radiation as described in U.S. Pat. No. 5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) Mol Cell Biol 14:2411-2418 and in Woffendin (1994) Proc Natl Acad Sci 91:1581-1585.

[0157] Particle mediated gene transfer may be employed, for example see U.S. Ser. No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level

expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) J. Biol. Chem. 262:4429-4432, insulin as described in Hucked (1990) Biochem Pharmacol 40:253-263, galactose as described in Plank (1992) Bioconjugate Chem 3:533-539, lactose or transferrin.

[0158] Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Pat. No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

[0159] Liposomes that can act as gene delivery vehicles are described in U.S. Pat. No. 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in U.S. S No. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al (1994) Proc. Natl. Acad. Sci. USA 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Pat. No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Pat. No. 5,206,152 and WO92/11033

[0160] Exemplary liposome and polycationic gene delivery vehicles are those described in U.S. Pat. Nos. 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697, and WO91/14445; in EP-0524968; and in Stryer, Biochemistry, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

[0161] A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

[0162] Delivery Methods

[0163] Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the

subject; (2) delivered ex vivo, to cells derived from the subject; or (3) in vitro for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

[0164] Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (eg. see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

[0165] Methods for the ex vivo delivery and reimplantation of transformed cells into a subject are known in the art and described in eg. WO93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells.

[0166] Generally, delivery of nucleic acids for both ex vivo and in vitro applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

[0167] Polynucleotide and Polypeptide Pharmaceutical Compositions

[0168] In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

[0169] A. Polypeptides

[0170] One example are polypeptides which include, without limitation: asioloorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of *plasmodium falciparum* known as RII.

[0171] B. Hormones, Vitamins, etc.

[0172] Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

[0173] C. Polyalkylenes, Polysaccharides, etc.

[0174] Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethlylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

[0175] D. Lipids, and Liposomes

[0176] The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

[0177] Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

[0178] Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

[0179] Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner supra). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, eg. Szoka (1978) *Proc. Nail. Acad. Sci. USA* 75:41944198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

[0180] Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

[0181] The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art See eg. Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) Cell 17:77); Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348); Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

[0182] E. Lipoproteins

[0183] In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, DL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

[0184] Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

[0185] A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

[0186] The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) Annu Rev. Biochem 54:699; Law (1986) Adv. Exp Med. Biol. 151:162; Chen (1986) J Biol Chem 261:12918; Kane (1980) Proc Natl Acad Sci USA 77:2465; and Utermann (1984) Hum Genet 65:232.

[0187] Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

[0188] Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol.* (supra); Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J. Clin. Invest* 64:743-750. Lipoproteins can also be produced by in vitro or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acia* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Techniologies, Inc., Stoughton, Mass., USA. Further description of lipoproteins can be found in Zuckermann et al. PCT/US97/14465.

[0189] F. Polycationic Agents

[0190] Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

[0191] Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of

neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

[0192] The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic aid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

[0193] Organic polycationic agents include: spermine, spermidine, and purtrescine.

[0194] The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

[0195] Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. LipofectinTM, and lipofectAMINETM are monomers that form polycationic complexes when combined with polynucleotides/ polypeptides.

[0196] Immunodiagnostic Assays

[0197] Neisserial antigens of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-Neisserial antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to Neisserial proteins within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

[0198] Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, etc.) required for the conduct of the assay, as well as suitable set of assay instructions.

[0199] Nucleic Acid Hybridisation

[0200] "Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions

that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook et al. [supra] Volume 2, chapter 9, pages 9.47 to 9.57.

[0201] "Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200° C. below the calculated Tm of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook et al. at page 9.50.

[0202] Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1 g for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 μ g of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/µg, resulting in an exposure time of 24 hours.

[0203] Several factors can affect the melting temperature (Tm) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

Tm—81+16.6(log₁₀ Ci)+0.4[% (G+C)]-0.6(% formamide)-600/n-1.5(% mismatch).

[0204] where Ci is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

[0205] In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (ie. stringency), it becomes less likely for hybrid-

ization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

[0206] In general, convenient hybridization temperatures in the presence of 50% formamide are 42° C. for a probe with is 95% to 100% homologous to the target fragment, 37° C. for 90% to 95% homology, and 32° C. for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

[0207] Nucleic Acid Probe Assays

[0208] Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

[0209] The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to the non-coding sequence.

[0210] The probe sequence need not be identical to the Neisserial sequence (or its complement)-some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

[0211] The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic

applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

[0212] Probes may be produced by synthetic procedures, such as the triester method of Matteucci et al. [*J. Am. Chem. Soc.* (1981) 103:3185], or according to Urdea et al. [*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461], or using commercially available automated oligonucleotide synthesizers.

[0213] The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated eg. backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase in vivo half-life, alter RNA affinity, increase nuclease resistance etc. [eg. see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387]; analogues such as peptide nucleic acids may also be used [eg. see Corey (1997) *TIBTECH* 15:224-229; Buchardt et al. (1993) *TIBTECH* 11:384-386].

[0214] Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis et al. [*Meth. Enzymol.* (1987) 155: 335-350]; U.S. Pat. Nos. 4,683,195 and 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

[0215] A thermostable polymerase creases copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labelled probe will hybridize to the Neisserial sequence (or its complement).

[0216] Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook et al [supra]. mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labelled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labelled with a radioactive moiety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0217] FIGS. 1-7 show biochemical data and sequence analysis pertaining to Examples 1, 2, 3, 7, 13, 16 and 19, respectively, with ORFs 40, 38, 44, 52, 114, 41 and 124. M1 and M2 are molecular weight markers. Arrows indicate the position of the main recombinant product or, in Western blots, the position of the main *N. meningitidis* immunoreactive band. TP indicates *N. meningitidis* total protein extract; OMV indicates *N. meningitidis* outer membrane vesicle preparation. In bactericidal assay results: a diamond (\blacklozenge) shows preimmune data; a triangle (\blacktriangle) shows GST control data; a circle (\bigcirc) shows data with recombinant *N*.

meningitidis protein. Computer analyses show a hydrophilicity plot (upper), an antigenic index plot (middle), and an AMPHI analysis (lower). The AMPHI program has been used to predict T-cell epitopes [Gao et al. (1989) *J. Immunol.* 143:3007; Roberts et al. (1996) *AIDS Res Hum Retrovir* 12:593; Quakyi et al. (1992) *Scand J Immunol* suppl. 11:9) and is available in the Protean package of DNASTAR, Inc. (1228 South Park Street, Madison, Wis. 53715 USA).

[0218] FIG. 8 shows an alignment comparison of amino acid sequences for ORF 40 for several strains of *Neisseria*. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. The Figure demonstrates a high degree of conservation among the various strains, further confirming its utility as an antigen for both vaccines and diagnostics.

EXAMPLES

[0219] The examples describe nucleic acid sequences which have been identified in *N. meningitidis*, along with their putative translation products. Not all of the nucleic acid sequences are complete ie. they encode less than the full-length wild-type protein. It is believed at present that none of the DNA sequences described herein have significant homologs in *N. gonorrhoeae*.

[0220] The examples are generally in the following format:

- **[0221]** a nucleotide sequence which has been identified in *N. meningitidis* (strain B)
- **[0222]** the putative translation product of this sequence
- **[0223]** a computer analysis of the translation product based on database comparisons
- [0224] a corresponding gene and protein sequence identified in *N. meningitidis* (strain A)
- **[0225]** a description of the characteristics of the proteins which indicates that they might be suitably antigenic
- **[0226]** results of biochemical analysis (expression, purification, ELISA, FACS etc.)

[0227] The examples typically include details of sequence homology between species and strains. Proteins that are similar in sequence are generally similar in both structure and function, and the homology often indicates a common evolutionary origin. Comparison with sequences of proteins of known function is widely used as a guide for the assignment of putative protein function to a new sequence and has proved particularly useful in whole-genome analyses.

[0228] Sequence comparisons were performed at NCBI (http://www.ncbi.nlm.nih.gov) using the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [eg. see also Altschul et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Searches were performed against the following databases: non-redundant GenBank+EMBL+ DDBJ+PDB sequences and non-redundant GenBank CDS translations+PDB+SwissProt+SPupdate+PIR sequences.

[0229] Dots within nucleotide sequences (eg. position 288 in Example 12) represent nucleotides which have been arbitrarily introduced in order to maintain a reading frame.

In the same way, double-underlined nucleotides were removed. Lower case letters (eg. position 589 in Example 12) represent ambiguities which arose during alignment of independent sequencing reactions (some of the nucleotide sequences in the examples are derived from combining the results of two or more experiments).

[0230] Nucleotide sequences were scanned in all six reading frames to predict the presence of hydrophobic domains using an algorithm based on the statistical studies of Esposti et al. [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. These domains represent potential transmembrane regions or hydrophobic leader sequences.

[0231] Open reading frames were predicted from fragmented nucleotide sequences using the program ORFFINDER (NCBI).

[0232] Underlined amino acid sequences indicate possible transmembrane domains or leader sequences in the ORFs, as predicted by the PSORT algorithm (http://www.psort.nib-b.ac.jp). Functional domains were also predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

[0233] Various tests can be used to assess the in vivo immunogenicity of the proteins identified in the examples. For example, the proteins can be expressed recombinantly and used to screen patient sera by immunoblot A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question ie. the protein is an immunogen. This method can also be used to identify immunodominant proteins.

[0234] The recombinant protein can also be conveniently used to prepare antibodies eg. in a mouse. These can be used for direct confirmation that a protein is located on the cell-surface. Labelled antibody (eg. fluorescent labelling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein.

[0235] In particular, the following methods (A) to (S) were used to express, purify and biochemically characterise the proteins of the invention:

[0236] A) Chromosomal DNA Preparation

[0237] N. meningitidis strain 2996 was grown to exponential phase in 100 ml of GC medium, harvested by centrifugation, and resuspended in 5 ml buffer (20% Sucrose, 50 mM Tris-HCl, 50 mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10 ml lysis solution (50 mM NaCl, 1% Na-Sarkosyl, 50 μ g/ml Proteinase K), and the suspension was incubated at 37° C. for 2 hours. Two phenol extractions (equilibrated to pH 8) and one ChCl₃/isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by central stratements.

trifugation. The pellet was washed once with 70% ethanol and redissolved in 4 ml buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

[0238] B) Oligonucleotide Design

[0239] Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus as necessary. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

[0240] The 5' primers included two restriction enzyme recognition sites (BamHI-NdeI, BamHI-NheI, or EcoRI-NheI, depending on the gene's own restriction pattern); the 3' primers included a XhoI restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either BamHI-XhoI or EcoRI-XhoI), and pET21b+ (using either NdeI-XhoI or NheI-XhoI).

5'end primer tail:	
CGC <u>GGATCCCATATG</u>	(BamHI-NdeI)
CGC <u>GGATCCGCTAGC</u>	(BamHI-NheI)
CCG <u>GAATTC</u> TA <u>GCTAGC</u>	(EcoRI-NheI)
3'-end primer tail:	
CCCG <u>CTCGAG</u>	(XhoI)

[0241] As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridised to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$T_{\rm m}$ =4 (G+C)+2 (A+T)	(tail excluded)
$T_{\rm m}$ =64.9+0.41 (% GC)-600/N	(whole primer)

[0242] The average melting temperature of the selected oligos were $65-70^{\circ}$ C. for the whole oligo and $50-55^{\circ}$ C. for the hybridising region alone.

[0243] Table I shows the forward and reverse primers used for each amplification. Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2 ml NH₄OH, 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 then centrifuged and the pellets resuspended in either 100 μ l or 1 ml of water. OD₂₆₀ was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10 pmol/ μ l.

TABLE I

PCR primers			
ORF	Primer	Sequence	Restriction sites
ORF 3	3 Forward	CGC <u>GGATCCCATATG</u> -TCGCCGCAAAATTCCGA <seq 112="" id=""></seq>	BamHI-NdeI
	Reverse	CCCG <u>CTCGAG</u> -TTTTGCCGCGTTAAAAGC <seq 113="" id=""></seq>	XhoI

TABLE I-continued

	PCR primers					
ORF	Primer	Sequence	Restriction sites			
ORF	40 Forward	CGC <u>GGATCCCATATG</u> -ACCGTGAAGACCGCC <seq 114="" id=""></seq>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -CCACTGATAACCGACAGA <seq 115="" id=""></seq>	XhoI			
ORF	41 Forward	CGC <u>GGATCCCATATG</u> -TATTTGAAACAGCTCCAAG <seo 116="" id=""></seo>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -TTCTGGGTGAATGTTA <seq 117="" id=""></seq>	XhoI			
ORF	44 Forward	GC <u>GGATCCCATATG</u> -GGCACGGACAACCCC <seq 118="" id=""></seq>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -ACGTGGGGGAACAGTCT <seq 119="" id=""></seq>	XhoI			
ORF	51 Forward	GC <u>GGATCCCATATG</u> -AAAAATATTCAAGTAGTTGC <seq 120="" id=""></seq>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -AAGTTTGATTAAACCCG <seq 121="" id=""></seq>	XhoI			
ORF	52 Forward	CGC <u>GGATCCCATATG</u> -TGCCAACCGCAATCCG <seq 122="" id=""></seq>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -TTTTTCCAGCTCCGGCA <seq 123="" id=""></seq>	XhoI			
ORF	56 Forward	GC <u>GGATCCCATATG</u> -GTTATCGGAATATTACTCG <seq 124="" id=""></seq>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -GGCTGCAGAAGCTGG <seq 125="" id=""></seq>	XhoI			
ORF	69 Forward	CGC <u>GGATCCCATATG</u> -CGGACGTGGTTGGTTTT <seq 126="" id=""></seq>	BamHI-NdeI			
	Reverse	CCCG <u>CTCGAG</u> -ATATCTTCCGTTTTTTCAC <seq 127="" id=""></seq>	XhoI			
ORF	82 Forward	CGC <u>GGATCCGCTAGC</u> -GTAAATTTATTATTTTAGAA <seq 128="" id=""></seq>	BamHI-NheI			
	Reverse	CCCG <u>CTCGAG</u> -TCCAACTCATTGAAGTA <seq 129="" id=""></seq>	XhoI			
ORF 114	Forward	CGC <u>GGATCCCATATG</u> -AATAAAGGTTTACATCGCAT	BamHI-NheI			
	Reverse	<seq 130="" id=""> CCCG<u>CTCGAG</u>-AATCGCTGCACCGGCT <seq 131="" id=""></seq></seq>	XhoI			
ORF 124	Forward	CGC <u>GGATCCCATATG</u> -ACTGCCTTTTCGACA	BamHI-NheI			
124	Reverse	<seq 132="" id=""> CCCG<u>CTCGAG</u>-GCGTGAAGCGTCAGGA <seq 133="" id=""></seq></seq>	XhoI			

[0244] C) Amplification

[0245] The standard PCR protocol was as follows: 50-200 ng of genomic DNA were used as a template in the presence of 20-40 μ M of each oligo, 400-8004M dNTs solution, 1×PCR buffer (including 1.5 mM MgCl₂), 2.5 units TaqI DNA polymerase (using Perkin-Elmer AmpliTaQ, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

perature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72° C.

5 cycles were performed using as the hybridization tem-

[0248] The standard cycles were as follows:

[0246] In some cases, PCR was optimised by the addition of 10 μ l DMSO or 50 μ l 2M betaine.

[0247] After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95° C.), each sample underwent a double-step amplification: the first

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds	30 seconds	30–60 seconds
	95° C.	50–55° C.	72° C.

-continued				
	Denaturation	Hybridisation	Elongation	
Last 30 cycles	30 seconds 95° C.	30 seconds 65–70° C.	30–60 seconds 72° C.	

[0249] The elongation time varied according to the length of the ORF to be amplified.

[0250] The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, $\frac{1}{10}$ of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

[0251] The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30 μ l or 500 of either water or 10 mM Tris, pH 8.5.

[0252] D) Digestion of PCR Fragments

[0253] The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

- [0254] NdeI/XhoI or NheI/XhoI for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion
- [0255] BamHI/XhoI or EcoRI/XhoI for cloning into pGEX-KG and further expression of the protein as N-terminus GST fusion.
- [0256] EcoRI/PstI, EcoRI/SalI, SalI/PstI for cloning into pGex-His and further expression of the protein as N-terminus His-tag fusion

[0257] Each purified DNA fragment was incubated (37° C. for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40 μ l final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 or 50 μ l of either water or 10 mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

[0258] E) Digestion of the Cloning Vectors (pET22B, pGEX-KG, pTRC-His A, and pGex-His)

[0259] 10 μ g plasmid was double-digested with 50 units of each restriction enzyme in 200 μ l reaction volume in the presence of appropriate buffer by overnight incubation at 37° C. After loading the whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick-Gel Extraction Kit and the DNA was eluted in 50 μ l of 10 mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD₂₆₀ of the sample, and adjusted to 50 μ g/ μ l. 1 μ l of plasmid was used for each cloning procedure.

[0260] The vector pGEX-His is a modified pGEX-2T vector carrying a region encoding six histidine residues upstream to the thrombin cleavage site and containing the multiple cloning site of the vector pTRC99 (Pharmacia).

[0261] F) Cloning

[0262] The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20 μ l, a molar ratio of 3:1 fragment/vector was ligated using 0.5 μ l of NEB T4 DNA ligase (400 units/ μ l), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

[0263] In order to introduce the recombinant plasmid in a suitable strain, 100 μ l *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37° C. for 3 minutes, then, after adding 800 μ l LB broth, again at 37° C. for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200 μ l of the supernatant. The suspension was then plated on LB ampicillin (100 mg/ml).

[0264] The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37° C. in either 2 ml (pGEX or pTC clones) or 5 ml (pET clones) LB broth+100 μ g/ml ampicillin. The cells were then pelletted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30 μ l. 5 μ l of each individual miniprep (approximately 1 g) were digested with either NdeI/XhoI or BamHI/XhoI and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1 Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

[0265] G) Expression

[0266] Each ORF cloned into the expression vector was transformed into the strain suitable for expression of the recombinant protein product. 1 μ l of each construct was used to transform 30 µl of E. coli BL21 (pGEX vector), E. coli TOP 10 (pTRC vector) or E. coli BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same E. coli strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2 ml LB+Amp (100 µg/ml), incubated at 37° C. overnight, then diluted 1:30 in 20 ml of LB+Amp (100 μ g/ml) in 100 ml flasks, making sure that the OD₆₀₀ ranged between 0.1 and 0.15. The flasks were incubated at 30° C. into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1 mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2 mM. After 3 hours incubation at 30° C., the final concentration of the sample was checked by OD. In order to check expression, 1 ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000 g and the pellet resuspended in PBS for further use.

[0267] H) GST-Fusion Proteins Large-Scale Purification.

[0268] A single colony was grown overnight at 37° C. on LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600 ml of fresh medium and allowed to grow at the optimal temperature (20-37° C.) to OD₅₅₀ 0.8-1. Protein expression was induced with 0.2 mM IPTG followed by three hours incubation. The culture was centrifuged at 800 rpm at 4° C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5 ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40 W using a Branson sonifier B-15, frozen and thawed twice and centrifuged again. The supernatant was collected and mixed with 150 µl Glutatione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4° C. The resin was washed twice with 10 ml cold PBS for 10 minutes, resuspended in 1 ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2 ml cold PBS until the flow-through reached OD_{280} of 0.02-0.06. The GST-fusion protein was eluted by addition of 70011 cold Glutathione elution buffer (10 mM reduced glutathione, 50 mM Tris-HCl) and fractions collected until the OD₂₈₀ was 0.1. 21 μ l of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M2) (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26 kDa, this value must be added to the MW of each GST-fusion protein.

[0269] I) His-Fusion Solubility Analysis

[0270] To analyse the solubility of the His-fusion expression products, pellets of 3 ml cultures were resuspended in buffer M1 [500 μ l PBS pH 7.2]. 25 μ l lysozyme (10 mg/ml) was added and the bacteria were incubated for 15 min at 4° C. The pellets were sonicated for 30 sec at 40 W using a Branson sonifier B-15, frozen and thawed twice and then separated again into pellet and supernatant by a centrifugation step. The supernatant was collected and the pellet was resuspended in buffer M2 [8M urea, 0.5M NaCl, 20 mM imidazole and 0.1M NaH₂ PO₄] and incubated for 3 to 4 hours at 4° C. After centrifugation, the supernatant was collected and the pellet was collected and the pellet was resuspended in buffer M3 [6M guanidinium-HCl, 0.5M NaCl, 20 mM imidazole and 0.1M NaH₂PO₄] overnight at 4° C. The supernatants from all steps were analysed by SDS-PAGE.

[0271] J) His-Fusion Large-Scale Purification.

[0272] A single colony was grown overnight at 37° C. on a LB+Amp agar plate. The bacteria were inoculated into 20 ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600 ml fresh medium and allowed to grow at the optimal temperature (20-37° C.) to OD₅₅₀ 0.6-0.8. Protein expression was induced by addition of 1 mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000 rpm at 4° C., the supernatant was discarded and 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) for soluble proteins or (ii) buffer B (urea 8M, 10 mM Tris-HCl, 100 mM phosphate buffer, pH 8.8) for insoluble proteins. **[0273]** The cells were disrupted by sonication on ice for 30 sec at 40 W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again.

[0274] For insoluble proteins, the supernatant was stored at -20° C., while the pellets were resuspended in 2 ml buffer C (6M guanidine hydrochloride, 100 mM phosphate buffer, 10 mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000 rpm for 40 minutes.

[0275] Supernatants were collected and mixed with 150 μ l Ni²⁺-resin (Pharmacia) (previously washed with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700 g for 5 minutes at 4° C. The resin was washed twice with 10 ml buffer A or B for 10 minutes, resuspended in 1 ml buffer A or B and loaded on a disposable column. The resin was washed at either (i) 4° C. with 2 ml cold buffer A or (ii) room temperature with 2 ml buffer B, until the flow-through reached OD₂₈₀ of 0.02-0.06.

[0276] The resin was washed with either (i) 2 ml cold 20 mM imidazole buffer (300 mM NaCl, 50 mM phosphate buffer, 20 mM imidazole, pH 8) or (ii) buffer D (urea 8M, 10 mM Tris-HCl, 100 mM phosphate buffer, pH 6.3) until the flow-through reached the O.D₂₈₀ of 0.02-0.06. The His-fusion protein was eluted by addition of 700 μ l of either (i) cold elution buffer A (300 mM NaCl, 50 mM phosphate buffer, 250 mM imidazole, pH 8) or (ii) elution buffer B (urea 8M, 10 mM Tris-HCl, 100 mM phosphate buffer, pH 4.5) and fractions collected until the O.D₂₈₀ was 0.1. 21 μ l of each fraction were loaded on a 12% SDS gel.

[0277] K) His-Fusion Proteins Renaturation

[0278] 10% glycerol was added to the denatured proteins. The proteins were then diluted to 20 μ g/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50 mM phosphate buffer, 5 mM reduced glutathione, 0.5 mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4° C. for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50 mM phosphate buffer, 5 mM reduced glutathione, 0.5 mM oxidised glutathione, pH 8.8) for 12-14 hours at 4° C. Protein concentration was evaluated using the formula:

Protein (mg/ml)=(1.55×OD₂₈₀)-(0.76×OD₂₆₀)

[0279] L) His-Fusion Large-Scale Purification

[0280] 500 ml of bacterial cultures were induced and the fusion proteins were obtained soluble in buffer M1, M2 or M3 using the procedure described above. The crude extract of the bacteria was loaded onto a Ni-NTA superflow column (Qiagen) equilibrated with buffer M1, M2 or M3 depending on the solubilization buffer of the fusion proteins. Unbound material was eluted by washing the column with the same buffer. The specific protein was eluted with the corresponding buffer containing 500 mM imidazole and dialysed against the corresponding buffer without imidazole. After each run the columns were sanitized by washing with at least two column volumes of 0.5 M sodium hydroxide and reequilibrated before the next use.

[0281] M) Mice Immunisations

[0282] 20 μ g of each purified protein were used to immunise mice intraperitoneally. In the case of ORF 44, CD1 mice were immunised with $Al(OH)_3$ as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For ORF 40, CD1 mice were immunised using Freund's adjuvant, rather than $Al(OH)_3$, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for ORF 38, CD1 mice were immunised with Freund's adjuvant, but the immune response was measured on day 49.

[0283] N) ELISA Assay (Sera Analysis)

[0284] The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37° C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7 ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD_{620} . The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000 rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4° C. with stirring. 100 μ l 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). 200 µl 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. 200 μ l of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to each well and the plates incubated for 90 minutes at 37° C. Wells were washed three times with PBT. 100 μ l 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. 100 μ l of substrate buffer for HRP (25 ml of citrate buffer pH5, 10 mg of O-phenildiamine and 10 μ l of H₂O) were added to each well and the plates were left at room temperature for 20 minutes. 100 μ l H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA was considered positive when OD₄₀₀ was 2.5 times the respective pre-immune sera.

[0285] O) FACScan Bacteria Binding Assay Procedure.

[0286] The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37° C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8 ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. 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 4000 rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN₃) and centrifuged for 5 minutes at 4000 rpm. Cells were resuspended in blocking buffer to reach OD_{620} of 0.07. 100 μ l bacterial cells were added to each well of a Costar 96 well plate. $100 \,\mu$ l of diluted (1:200) 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 4000 rpm, the supernatant aspirated and cells washed by addition of 200 μ l/well of blocking buffer in each well. 100 μ l of R-Phicoerytin conjugated F(ab)₂ goat antimouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4° C. Cells were spun down by centrifugation at 4000 rpm for 5 minutes and washed by addition of 200 μ l/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200 μ l/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H threshold: 92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539; compensation values: 0.

[0287] P) OMV Preparations

[0288] Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20 mM Tris-HCl. Heat inactivation was performed at 56° C. for 30 minutes and the bacteria disrupted by sonication for 10 minutes on ice (50% duty cycle, 50% output). Unbroken cells were removed by centrifugation at 5000 g for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000 g at 4° C. for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000 g for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000 g for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10 mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

[0289] Q) Whole Extracts Preparation

[0290] Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1 ml of 20 mM Tris-HCl. Heat inactivation was performed at 56° C. for 30 minutes.

[0291] R) Western Blotting

[0292] Purified proteins (500 ng/lane), outer membrane vesicles (5 μ g) and total cell extracts (25 μ g) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150 mA at 4° C., in transferring buffer (0.3% Tris base, 1.44% glycine, 20% methanol). 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.

[0293] S) Bactericidal Assay

[0294] MC58 strain was grown overnight at 37° C. on chocolate agar plates. 5-7 colonies were collected and used

to inoculate 7 ml Mueller-Hinton broth. The suspension was incubated at 37° C. on a nutator and let to grow until OD₆₂₀ was 0.5-0.8. The culture was aliquoted into sterile 1.5 ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD₆₂₀ of 0.5, diluted 1:20000 in Gey's buffer and stored at 25° C.

[0295] 50 μ l of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25 μ l of diluted mice sera (1:100 in Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4° C. 25 μ l of the previously described bacterial suspension were added to each well. 25 μ l of either heat-inactivated (56° C. waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22 μ l of each sample/well were plated on Mueller-Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37° C. with rotation and then 22 μ l of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1 hour were counted.

TABLE II

Cloning, expression and purification							
ORF	PCR/cloning	His-fusion expression	GST-fusion expression	Purification			
orf 38	+	+	+	His-fusion			
orf 40	+	+	+	His-fusion			
orf 41	+	n.d.	n.d.				
orf 44	+	+	+	His-fusion			
orf 51	+	n.d.	n.d.				
orf 52	+	n.d.	+	GST-fusion			
orf 56	+	n.d.	n.d.				
orf 69	+	n.d.	n.d.				
orf 82	+	n.d.	n.d.				
orf 114	+	n.d.	+	GST-fusion			
orf 124	+	n.d.	n.d.				

Example 1

[0297] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 1>:

1 ACACTGTTGT	TTGCAACGGT	TCAGGCAAGT	GCTAACCAAT	GAAGAGCAAG
51 AAGAAGATTT	ATATTTAGAC	CCCGTACAAC	GCACTGTTGC	CGTGTTGATA
101 GTCAATTCCG	ATAAAGAAGG	CACGGGAGAA	ааадааааад	TAGAAGAAAA
151 TTCAGATTGG	GCAGTATATT	TCAACGAGAA	AGGAGTACTA	ACAGCCAGAG
201 AAATCACCyT	CAAAGCCGGC	GACAACCTGA	ааатсаааса	AAACGGCACA
251 AACTTCACCT	ACTCGCTGAA	AAALGACCTC	ACAGATCTGA	CCAGTGTTGG
301 AACTGAAAAA	TTATCGTTTA	GCGCAAACGG	CAATAAAGTC	AACATCACAA
351 GCGACACCAA	AGGCTTGAAT	TTTGCGAAAG	AAACGGCTGG	sACGAACGgC
401 GACACCACGG	TTCATCTGAA	CGGTATTGGT	TCGACTTTGA	CCGATACGCT
451 GCTGAATACC	GGAGCGACCA	CAAACGTAAC	CAACGACAAC	GTTACCGATG
501 ACGAGAAAAA	ACGTGCGGCA	AGCGTTAAAG	ACGTATTAAA	CGCTGGCTGG
551 AACATTAAAG	GCGTTAAACC	CGGTACAACA	GCTTCCGATA	ACGTTGATTT
601 CGTCCGCACT	TACGACACAG	TCGAGTTCTT	GAGCGCAGAT	ACGAAAACAA
651 CGACTGTTAA	TGTGGAAAGC	AAAGACAACG	GCAAGAAAAC	CGAAGTTAAA
701 ATCGGTGCGA	AGACTTCTGT	TATTAAAGAA	AAAGAC	

[0296] Table II gives a summary of the cloning, expression and purification results.

[0298] This corresponds to the amino acid sequence <SEQ ID 2; ORF40>:

1..TLLFATVQAS ANQEEQEEDL YLDPVQRTVA VLIVNSDKEG TGEKEKVEEN
51 SDWAVYFNEK GVLTAREITX KAGDNLKIKQ NGTNFTYSLK KDLTDLTSVG
101 TEKLSFSANG NKVNITSDTK GLNFAKETAG TNGDTTVHLN GIGSTLTDTL
151 LNTGATTNVT NDNVTDDEKK RAASVKDVLN AGWNIKGVKP GTTASDNVDF
201 VRTYDTVEFL SADTKTITVN VESKDNGKKT EVKIGAXTSV IKEKD...

26

[0299] Further work revealed the complete DNA sequence <SEQ ID 3>:

1 ATGAACAAAA TATACCGCAT CATTTGGAAT AGTGCCCTCA ATGCCTGGGT 51 CGTCGTATCC GAGCTCACAC GCAACCACAC CAAACGCGCC TCCGCAACCG 101 TGAAGACCGC CGTATTGGCG ACACTGTTGT TTGCAACGGT TCAGGCAAGT 151 GCTAACAATG AAGAGCAAGA AGAAGATTTA TATTTAGACC CCGTACAACG 201 CACTGTTGCC GTGTTGATAG TCAATTCCGA TAAAGAAGGC ACGGGAGAAA 251 AAGAAAAAGT AGAAGAAAAT TCAGATTGGG CAGTATATTT CAACGAGAAA 301 GGAGTACTAA CAGCCAGAGA AATCACCCTC AAAGCCGGCG ACAACCTGAA 351 AATCAAACAA AACGGCACAA ACTTCACCTA CTCGCTGAAA AAAGACCTCA 401 CAGATCTGAC CAGTGTTGGA ACTGAAAAAT TATCGTTTAG CGCAAACGGC 451 AATAAAGTCA ACATCACAAG CGACACCAAA GGCTTGAATT TTGCGAAAGA 501 AACGGCTGGG ACGAACGGCG ACACCACGGT TCATCTGAAC GGTATTGGTT 551 CGACTTTGAC CGATACGCTG CTGAATACCG GAGCGACCAC AAACGTAACC 601 AACGACAACG TTACCGATGA CGAGAAAAAA CGTGCGGCAA GCGTTAAAGA 651 CGTATTAAAC GCTGGCTGGA ACATTAAAGG CGTTAAACCC GGTACAACAG 701 CTTCCGATAA CGTTGATTTC GTCCGCACTT ACGACACAGT CGAGTTCTTG 751 AGCGCAGATA CGAAAACAAC GACTGTTAAT GTGGAAAGCA AAGACAACGG 801 CAAGAAAACC GAAGTTAAAA TCGGTGCGAA GACTTCTGTT ATTAAAGAAA 851 AAGACGGTAA GTTGGTTACT GGTAAAGACA AAGGCGAGAA TGGTTCTTCT 901 ACAGACGAAG GCGAAGGCTT AGTGACTGCA AAAGAAGTGA TTGATGCAGT 951 AAACAAGGCT GGTTGGAGAA TGAAAACAAC AACCGCTAAT GGTCAAACAG 1001 GTCAAGCTGA CAAGTTTGAA ACCGTTACAT CAGGCACAAA TGTAACCTTT 1051 GCTAGTGGTA AAGGTACAAC TGCGACTGTA AGTAAAGATG ATCAAGGCAA 1101 CATCACTGTT ATGTATGATG TAAATGTCGG CGATGCCCTA AACGTCAATC 1151 AGCTGCAAAA CAGCGGTTGG AATTTGGATT CCAAAGCGGT TGCAGGTTCT 1201 TCGGGCAAAG TCATCAGCGG CAATGTTTCG CCGAGCAAGG GAAAGATGGA 1251 TGAAACCGTC AACATTAATG CCGGCAACAA CATCGAGATT ACCCGCAACG 1301 GTAAAAATAT CGACATCGCC ACTTCGATGA CCCCGCAGTT TTCCAGCGTT 1351 TCGCTCGGCG CGGGGGGGGGA TGCGCCCACT TTGAGCGTGG ATGGGGACGC 1401 ATTGAATGTC GGCAGCAAGA AGGACAACAA ACCCGTCCGC ATTACCAATG 1451 TCGCCCCGGG CGTTAAAGAG GGGGATGTTA CAAACGTCGC ACAACTTAAA 1501 GGCGTGGCGC AAAACTTGAA CAACCGCATC GACAATGTGG ACGGCAACGC 1551 GCGTGCGGGC ATCGCCCAAG CGATTGCAAC CGCAGGTCTG GTTCAGGCGT 1601 ATTTGCCCGG CAAGAGTATG ATGGCGATCG GCGGCGGCAC TTATCGCGGC 1651 GAAGCCGGTT ACGCCATCGG CTACTCCAGT ATTTCCGACG GCGGAAATTG

-continued 1701 GATTATCAAA GGCACGGCTT CCGGCAATTC GCGCGGCCAT TTCGGTGCTT

1751 CCGCATCTGT CGGTTATCAG TGGTAA

[0300] This corresponds to the amino acid sequence <SEQ ID 4; ORF40-1>:

1 MNKIYRIIWN SALNAWVVVS ELTRNHTKRA SATVKTAVLA TLLFATVQAS 51 ANNEEQEEDL YLDFVQRTVA VLIVNSDKEG TGEKEKVEEN SDWAVYFNEK 101 GVLTAREITL KAGDNLKIKQ NGTNFTYSLK KDLTDLTSVG TEKLSFSMIG 151 NKVNITSDTK GLNFAKETAG TNGDTTVHLN GIGSTLTDTL LNTGATTNVT 201 NDNVTDDEKK RAASVKDVLN AGWNIKGVKP GTTASDNVDF VRTYDTVEFL 251 SADTKTTTVN VESKDNGKKT EVKIGAKTSV IKEKDGKLVT GKDKGENGSS 301 TDEGEGLVTA KEVIDAYNKA GWRMKTTTAN GQTGQADKFE TVTSGTNVTF 351 ASGKGTTATV SKDDQGNITV NYDVNVGDAL NVNQLQNSGW NLDSKAVAGS 401 SGKVISGNVS PSKGKMDETV NINAGNNIEI TRNGKNIDIA TSHTPOFSSV 451 SLGAGADAPT LSVDGDALNV GSKKDNKPVR TTNVAPGVKE GOVTNVAOLK 501 GVAONLNNRI DNVDGNARAG ZAOAIATAGL VOAYLPGKSM MAIGGGTYRG 551 EAGYAIGYSS ISDGGNWIIK GTASGNSRGH FGASASVGYQ W*

[0301] Further work identified the corresponding gene in strain A of N. meningitidis <SEQ ID 5>:

1 ATGAACAAAA TATACCGCAT CATTTGGAAT AGTGCCCTCA ATGCCTGNGT 51 CGCCGTATCC GAGCTCACAC GCAACCACAC CAAACGCGCC TCCGCAACCG 101 TGAAGACCGC CGTATTGGCG ACACTGTTGT TTGCAACGGT TCAGGCGAAT 151 GCTACCGATG AAGATGAAGA AGAAGAGTTA GAATCCGTAC AACGCTCTGT 201 CGTAGGGAGC ATTCAAGCCA GTATGGAAGG CAGCGGCGAA TTGGAAACGA 251 TATCATTATC AATGACTAAC GACAGCAAGG AATTTGTAGA CCCATACATA 301 GTAGTTACCC TCAAAGCCGG CGACAACCTG AAAATCAAAC AAAACACCAA 351 TGAAAACACC AATGCCAGTA GCTTCACCTA CTCGCTGAAA AAAGACCTCA 401 CAGGCCTGAT CAATGTTGAN ACTGAAAAAT TATCGTTTGG CGCAAACGGC 451 AAGAAAGTCA ACATCATAAG CGACACCAAA GGCTTGAATT TCGCGAAAGA 501 AACGGCTGGG ACGAACGGCG ACACCACGGT TCATCTGAAC GGTATCGGTT 551 CGACTTTGAC CGATACGCTT GCGGGTTCTT CTGCTTCTCA CGTTGATGCG 601 GGTAACCNAA GTACACATTA CACTCGTGCA GCAAGTATTA AGGATGTGTT 651 GAATGCGGGT TGGAATATTA AGGGTGTTAA ANNNGGCTCA ACAACTGGTC 701 AATCAGAAAA TGTCGATTTC GTCCGCACTT ACGACACAGT CGAGTTCTTG 751 AGCGCAGATA CGNAAACAAC GACNGTTAAT GTGGAAAGCA AAGACAACGG 801 CAAGAGAACC GAAGTTAAAA TCGGTGCGAA GACTTCTGTT ATTAAAGAAA 851 AAGACGGTAA GTTGGTTACT GGTAAAGGCA AAGGCGAGAA TGGTTCTTCT 28

-continued 901 ACAGACGAAG GCGAAGGCTT AGTGACTGCA AAAGAAGTGA TTGATGCAGT 951 AAACAAGGCT GGTTGGAGAA TGAAAACAAC AACCGCTAAT GGTCAAACAG 1001 GTCAAGCTGA CAAGTTTGAA ACCGTTACAT CAGGCACAAA TGTAACCTTT 1051 GCTAGTGGTA AAGGTACAAC TGCGACTGTA AGTAAAGATG ATCAAGGCAA 1101 CATCACTGTT ATGTATGATG TAAATGTCGG CGATGCCCTA AACGTCAATC 1151 AGCTGCAAAA CAGCGGTTGG AATTTGGATT CCAAAGCGGT TGCAGGTTCT 1201 TCGGGCAAAG TCATCAGCGG CAATGTTTCG CCGAGCAAGG GAAAGATGGA 1251 TGAAACCGTC AACATTAATG CCGGCAACAA CATCGACATT AGCCGCAACG 1301 GTAAAAATAT CGACATCGCC ACTTCGATGG CGCCGCAGTT TTCCAGCGTT 1351 TCGCTCGGCG CGGGGGCAGA TGCGCCCACT TTAAGCGTGG ATGACGAGGG 1401 CGCGTTGAAT GTCGGCAGCA AGGATGCCAA CAAACCCGTC CGCATTACCA 1451 ATGTCGCCCC GGGCGTTAAA GANGGGGATG TTACAAACGT CNCACAACTT 1501 AAAGGCGTGG CGCAAAACTT GAACAACCGC ATCGACAATG TGGACGGCAA 1551 CGCGCGTGCN GGCATCGCCC AAGCGATTGC AACCGCAGGT CTGGTTCAGG 1601 CGTATCTGCC CGGCAAGAGT ATGATGGCGA TCGGCGGCGG CACTTATCGC 1651 GGCGAAGCCG GTTACGCCAT CGGCTACTCC AGTATTTCCG ACGGCGGAAA 1701 TTGGATTATC AAAGGCACGG CTTCCGGCAA TTCGCGCGGC CATTTCGGTG 1751 CTTCCGCATC TGTCGGTTAT CAGTGGTAA

[0302] This encodes a protein having amino acid sequence <SEQ ID 6; ORF40a>:

1MNKIYRIIWNSALNPXVAVSELTRNHTKRASATVKTAVLATLLFATVQAN51ATDEDEKEELESVQRSVVGSIQASMEGSGELETISLSHTNDSKEFVDPYI101VVTLKAGDNLKIKONTHENTNASSFTYSLKKDLTGLINVXTEKLSFGANG151KKVNIISDTKGLNFAXETAGTNGDTTVHLNGIGSTLTDTLAGSSASHVDA201GNXSTHYTRAASIKDVLNAGWNIKGVKXGSTTGQSENVDFVRTYDTVEFL251SADTXTTVNVESKDNGKRTEVXIGAXTSVIKEKDGKLVTGKGKGENGSS301TDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTF351ASGKGTTATVSXDDQGNITVMYDVNVGDALNVNQLONSGWNLDSKAVAGS401SGKVISGNVSPSKGKMDETVNINAGNNIEISRNGKNIDIATSMAPQFSSV451SLGAGADAPTLSVDDEGALNVGSKDANKPVRITNVAPGVKXGDVTNVXQL501GEAGYAIGYSSISDGGNWIIKGTASGNSGHFGASASVGYQW

[0303] The originally-identified partial strain B sequence (ORF40) shows 65.7% identity over a 254 aa overlap with ORF40a:

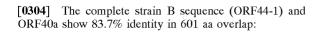
 10
 20
 30

 orf40.pep
 TLLFATVQASANQEEQEEDLYLDPVQRTVA

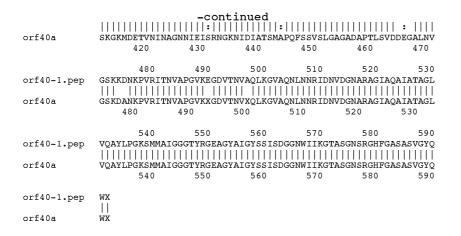
 ||||||||||:|:::::::::|::|::|::|::|::|

 orf40a
 SALNAXVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL--ESVQRSV

-continued						
	20	30	40	50	60	
	40	50	60	70	80	
orf40.pep					LKIKQNGT	
orf40a					:: ILKIKQNTNENTNAS	
011404	70 80			110	120	
640	90	100		.20 13		
ort40.pep			SANGNKVNITSD : :	TKGLNFAKETA	GTNGDTTVHLNGIG	
orf40a	:		• • GANGKKVNIISE			
OII40u	130	140		.60 17	0 180	
	150	160		.80 19		
orf40.pep	STLTDTLLNTGAT	TNVTNDNVTD	DEKKRAASVKDV	'LNAGWNIKGVF	PGTTASDNVDFV	
	::: :		: :		: : :	
orf40a					XGSTTGQSENVDFV	
	190	200	210 2	20 23	0 240	
	210	220	230	240		
orf40.pep	RTYDTVEFLSADI					
			:			
orf40a	RTYDTVEFLSADI	XTTTVNVESK	DNGKRTEVKIGA	KTSVIKEKDGF	LVTGKGKGENGSST	
	250	260	270	280	290 300	



orf40-1.pep orf40a	10 MNKIYRIIWNSALN MNKIYRIIWNSALN 10	:			Î : : :	: :.̃ :
orf40-1.pep orf40a	70 YLDPVQRTVAVLIV : : : ESVQRSV-VGSI 70	::: :	: :::	: : :	: :	 AGDNLKIK
1 orf40-1.pep orf40a	20 QNGTNFTY ::: QNTNENTNASSFTY 120	:	:	:		
orf40-1.pep orf40a	180 DTTVHLNGIGSTLT DTTVHLNGIGSTLT 180	::: :	: :	: :		:
orf40-1.pep orf40a	240 ASDNVDFVRTYD : : TGQSENVDFVRTYD 240			:		
orf40-1.pep orf40a	300 KDKGENGSSTDEGE KGKGENGSSTDEGE 300			Î Î	Ĩ	
orf40-1.pep orf40a	360 SGKGTTATVSKDDQ SGKGTTATVSKDDQ 360		IIIIIII	1Î		
orf40-1.pep	420 SKGKMDETVNINAG	430 NNIEITRNGK	440 NIDIATSMT	450 PQFSSVSLGA	460 GADAPTLSVI	470 DGD-ALNV



[0305] Computer analysis of these amino acid sequences gave the following results:

[0306] Homology with Hsf Protein Encoded by the Type b Surface Fibrils Locus of *H. influenzae* (Accession Number U41852)

[0307] ORF40 and Hsf protein show 54% as identity in 251 as overlap:

```
Orf40
       1 TLLFATVQASANQEEQEEDLYLDPVQRTVAVLVINSDXXXXXXXXXXXSDWAVYFNEK 60
         TLLFATVQA+A E++E LDPV RT VL +SD
                                                     NS+W +YF+ K
       41 TLLFATVQANATDEDEE----LDPVVRTAPVLSFHSDKEGTGEKEVTE-NSNWGIYFDNK 95
Hsf
      61 GVLTAREITXKAGDNLKIKQN-----GTNFTYSLKKDLTDLTSVGTEKLSFSANGNKVN 114
Orf40
         GVL A IT KAGDNLKIKON
                                  ++FTYSLKKDLTDLTSV TEKLSF ANG+KV+
Hsf
      96 GVLKAGAITLKAGDNLKIKONTDESTNASSFTYSLKKDLTDLTSVATEKLSFGANGDKVD 155
ITSD GL AK
                        G+ VHLNG+ STL D + NTG
                                                         EK RAA+
     156 ITSDANGLKLAK----TGNGNVHLNGLDSTLPDAVTNTGVLSSSSFTPNDV-EKTRAAT 209
Hsf
orf40 175 VKDVLNAGWNIKGVKPGTTASDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKI 234
                           ++VD V Y+ VEF++ D T V ++K+NGK TEVK
         VKDVLNAGWNIKG K
     210 VKDVLNAGWNIKGAKTAGGNVESVDLVSAYNNVEFITGDKNTLDVVLTAKENGKTTEVKF 269
Hsf
Orf40 235 GAKTSVIKEKD 245
           KTSVIKEKD
     270 TPKTSVIKEKD 280
Hsf
```

[0308] ORF40a also shows homology to Hsf:

```
gi|1666683 (U41852) hsf gene product [Haemophilus influenzae] Length = 2353
Score = 153 (67.7 bits), Expect = 1.5-116, Sum P(11) = 1.5e-116
Identities = 33/36 (91%), Positives = 34/36 (94%)
        16 VAVSELTRNHTKRASATVKTAVLATLLFATVQANAT 51
Query:
            V VSELTR HTKRASATV+TAVLATLLFATVONAT
Sbjct:
        17 VVVSELTRTHTKRASATVETAVLATLLFATVQANAT 52
Score = 161 (71.2 bits), Expect = 1.5e-116, Sum P(11) 1.5e-116
Identities = 32/38 (84%), Positives = 36/38 (94%)
Query: 101 VTLKAGDNLKIKQNTNENTNASSFTYSLKKDLTGLINV 138
            +TLAGDNLKIKQNT+E+TNASSFTYSLKKDLT L +V
Sbjct: 103 ITLKAGDNLKIKQNTDESTNASSFFYSLKKDLTDLTSV 140
Score = 110 (48.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
Identities = 21/29 (72%), Positives = 25/29 (86%)
Query: 138 VTEKLSFGANGKKVNIISDTKGLNFAKET 166
            V++KLS G NG KVNI SDTKGLNFAK++
```

-continued sbjct: 1439 VSDKLSLGTNGNKVNITSDTXGLNFAKDS 1467 Score = 85 (37.6 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116 Identities = 18/32 (56%), Positives = 20/32 (62%) Query: 169 TNGDTTVHLNGIGSTLTDTLAGSSASHVDAGN 200 T D +HLNGI STLTDTL S A+ GN Sbjct: 1469 TGDDANIHLNGIASTLTDTLLNSGATTNLGGN 1500 Score = 92 (40.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116 Identities = 16/19 (84%), Positives = 19/19 (100%) Query: 206 RAASIKOVLNAGWNIKGVK 224 RAAS+KDVLNAGWN++GVK Sbjct: 1509 RAASVKDVLNAGWNVRGVK 1527 Score = 90 (39.8 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116 Identities = 17/28 (60%), Positives 20/28 (71%) Query: 226 STTGQSENVDFVRTYDTVEFLSADTTTT 253 S Q EN+DFV TYDTV+F+S D TT Sbjct: 1530 SANNQVENIDFVATYDTVDFVSGDKDTT 1557

[0309] Based on homology with Hsf, it was predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

[0310] ORF40-1 (61 kDa) was cloned in pET and pGex vectors and expressed in *E. coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. **FIG. 1A** shows the results of affinity purification of the His-fusion protein, and **FIG. 1B** shows the results of expression of the GST-fusion in *E. coli*.

bactericidal assay (**FIG. 1D**), and ELISA (positive result). These experiments confirm that ORF40-1 is a surfaceexposed protein, and that it is a useful immunogen.

[0311] FIG. 1E shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF40-1.

Example 2

[0312] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 7>

1 ATGTFACGTT TGACTGCTTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC

51 GTGTT[]GCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GaACAG-GCGG

101 TTTCCGCCGC ACAAACCGAA GgCGCGTCCG TTACCGTCAA AACCGCGCGC

- 151 GGCGACGTTC AAATACCGCA AAACCCCGAA CGCATCGCCG TTTACGATTT
- 201 GGGTATGCTC GACACCTTGA GCAAACTGGG CGTGAAAACC GGTTTGTCCG
- 251 TCGATAAAAA CCGCCTGCCG TATTTAGAGG AATATTTCAA AACGACAAAA
- 301 CCTGCcGGCA CTTTGTTCGA GCCGGATTAC GAAACGCTCA ACGCTTACAA
- 351 ACCGCAGCTC ATCATCATCG GCAGCCGCGC CgCCAAGGCG TTTGACAAAT
- 401 TGAAcGAAAT CGCGCCGACC ATCGrmwTGA CCGCCGATAC CGCCAACCTC
- 451 AAAGAAAGTG CCAArGAGGC ATCGACGCTG GCGCAAATCT TC..

Purified His-fusion protein was used to immunise mice, whose sera were used for FACS analysis (FIG. 1C), a

[0313] This corresponds to the amino acid sequence <SEQ ID 8; ORF38>:

1 MLRLTAL<u>AVC TALALGAC</u>SP QNSDSAPOAK EQAVSAAQTE GASVTVKTAR 51 GDVQIPQNPE RIAVYDLGHL DTLSKLGVKT GLSVDKNRLP YLEEYFKTTK 101 PAGTLFEPDY ETLNAYKPQL IIIGSRAAKA FDKLNEIAPT IXXTADTANL 151 KESAKEASTL AQIF.. **[0314]** Further work revealed the complete nucleotide sequence <SEQ ID 9>:

1 ATGTTACGTT TGACTGCTTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC 51 GTGTTCGCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GAACAGGCGG 101 TTTCCGCCGC ACAAACCGAA GGCGCGTCCG TTACCGTCAA AACCGCGCGC 151 GGCGACGTTC AAATACCGCA AAACCCCCGAA CGCATCGCCG TTTACGATTT 201 GGGTATGCTC GACACCTTGA GCAAACTGGG CGTGAAAACC GGTTTGTCCG 251 TCGATAAAAA CCGCCTGCCG TATTTAGAGG AATATTTCAA AACGACAAAA 301 CCTGCCGGCA CTTTGTTCGA GCCGGATTAC GAAACGCTCA ACGCTTACAA 351 ACCGCAGCTC ATCATCATCG GCAGCCGCGC CGCCAAGGCG TTTGACAAAT 401 TGAACGAAAT CGCGCCGACC ATCGAAATGA CCGCCGATAC CGCCAACCTC 451 AAAGAAAGTG CCAAAGAGCG CATCGACGCG CTGGCGCAAA TCTTCGGCAA 501 ACAGGCGGAA GCCGACAAGC TGAAGGCGGA AATCGACGCG TCTTTTGAAG 551 CCGCGAAAAC TGCCGCACAA GGTAAGGGCA AAGGTTTGGT GATTTTGGTC 601 AACGGCGGCA AGATGTCGGC TTTCGGCCCG TCTTCACGCT TGGGCGGCTG 651 GCTGCACAAA GACATCGGCG TTCCCGCTGT CGATGAATCA ATTAAAGAAG 701 GCAGCCACGG TCAGCCTATC AGCTTTGAAT ACCTGAAAGA GAAAAATCCC 751 GACTGGCTGT TTGTCCTTGA CCGAAGCGCG GCCATCGGCG AAGAGGGTCA 801 GGCGGCGAAA GACGTGTTGG ATAATCCGCT GGTTGCCGAA ACAACCGCTT 851 GGAAAAAAGG ACAGGTCGTG TACCTCGTTC CTGAAACTTA TTTGGCAGCC 901 GGTGGCGCGC AAGAGCTGCT GAATGCAAGC AAACAGGTTG CCGACGCTTT 951 TAACGCGGCA AAATAA

[0315] This corresponds to the amino acid sequence <SEQ ID 10; ORF38-1>:

1MLRLTALAVC TALALGACSPQNSDSAPQAKEQAVSAAQTEGASVTVKTAR51GDVQIPQNPERIAVYDLQILDTLSXLGVKTGLSVDKNRLPYLEEYFKTTK101PAGTLFEPDYETLNAYKPQLIIIGSRAAKAFDKLNEIAPTIENTADTANL151KESAKERIDALAQIFGKQAEADKLKAEIDASFEAAKTAAQGKGKGLVILV201NGGKMSAFGPSSRLGGWLKKDIGVPAVDESIKEGSHGQPISFEYLKEKNP251DWLFVLDRSAAIGEEGQAAKDVLDNPLVAETTAWKKGQVVYLVETYLAA301GGAQELLNASKQVADAFNAAK*

[0316] Computer analysis of this amino acid sequence reveals a putative prokaryotic membrane lipoprotein lipid attachment site (underlined).

[0317] Further work identified the corresponding gene in strain A of *N. meningitidis* <SEQ ID 11>:

1 ATGTTACGTT TGACTGCTTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC

51 GTGTTCGCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GAACAGGCGG

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-conti 101 TTTCCGCCGC ACAATCCGAA GGCGT	
151 GGCGATGTTC AAATACCGCA AAACC	CCGAA CGTATCGCCG TTTACGATTT
201 GGGTATGCTC GACACCTTGA GCAAA	ACTGGG CGTGAAAACC GGTTTGTCCG
251 TCGATAAAAA CCGCCTGCCG TATTI	AGAGG AATATTTCAA AACGACAAAA
301 CCTGCCGGAA CTTTGTTCGA GCCGG	ATTAC GAAACGCTCA ACGCTTACAA
351 ACCGCAGCTC ATCATCATCG GCAGC	CGCGC AGCCAAAGCG TTTGACAAAT
401 TGAACGAAAT CGCGCCGACC ATCGA	AATGA CCGCCGATAC CGCCAACCTC
451 AAAGAAAGTG CCAAAGAGCG TATCG	ACGCG CTGGCGCAAA TCTTCGGCAA
501 AAAGGCGGAA GCCGACAAGC TGAAG	GCGGA AATCGACGCG TCTTTTGAAG
551 CCGCGAAAAC TGCCGCGCAA GGCAA	AGGCA AGGGTTTGGT GATTTTGGTC
601 AACGGCGGCA AGATGTCCGC CTTCG	GCCCG TCTTCACGAC TGGGCGGCTG
651 GCTGCACAAA GACATCGGCG TTCCC	GCTGT TGACGAAGCC ATCAAAGAAG
701 GCAGCCACGG TCAGCCTATC AGCTT	TGAAT ACCTGAAAGA GAAAAATCCC
751 GACTGGCTGT TTGTCCTTGA CCGCA	AGCGCG GCCATCGGCG AAAAGGGTCA
601 GGCGGCGAAA GACGTGTTGA ACAAT	CCGCT GGTTGCCGAA ACAACCGCTT
851 GGAAAAATGG ACAAGTCGTT TACCI	TGTTC CTGAAACTTA TTTGGCAGCC
901 GGTGGCGCGC AAGAGCTACT GAATG	CAAGC AAACAGGTTG CCGACGCTTT
951 TAACGCGGCA AAATAA	

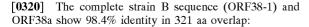
[0318] This encodes a protein having amino acid sequence <SEQ ID 12; ORF38a>:

1MLRLTALAVCTALALGACSPQNSDSAPOAKEQAVSAAQSEGVSVTVKTAR51GDVQIPQNPERIAVYDLGHLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTK101PAGTLFEPDYETLNAYKPQLIIIGSRAAKAFDKLNEIAPTIENTADTANL151KESAKERIDALAOIFGKKAEADKLKAEIDASFEAAKTAAQGKGKGLVILV201NGGKMSAFGPSSRLGGWLHKDIGVPAVDEAIKEGSHGQPISFEYLKEKNP251DWLFVLDRSAAIGEEGQAAKDVLNNPLVAETTAWKKGQVVYLVPETYLAA301GGAQELLNASKQVAOAFWAAK*IIIGANAIIIGANA

[0319] The originally-identified partial strain B sequence (ORF38) shows 95.2% identity over a 165 aa overlap with ORF38a:

	10	20	30	40	50	60
orf38.pep	MLRLTALAVCT	ALALGACSPQNS	DSAPQAKEQAV	SAAQTEGAS	VTVKTARGDVÇ	IPQNPE
				: :		
orf38a	MLRLTALAVCT	ALALGACSPQNS	DSAPQAKEQAV	SAAQSEGVS	VTVKTARGDVQ	IPQNPE
	10	20	30	40	50	60
	70	80	90	100	110	120
orf38.pep	RIAVYDLGMLD	TLSKLGVKTGLS	VDKNRLPYLEE	YFKTTKPAG	FLFEPDYETLN	AYKPQL
orf38a	RIAVYDLGMLD	TLSKLGVKTGLS	VDKNRLPYLEE	YFKTTKPAG	FLFEPDYETLN	AYKPQL
	70	80	90	100	110	120

-continued						
	130	140	150	160		
orf38.pep	IIIGSRAAKAFDKI	NEIAPTIXXI	ADTANLKESA	KE-ASTLAQI	F	
			1111111111	::		
orf39a	IIIGSRAAKAFDKI	NEIAPTIEMI	ADTANLKESA	KERIDALAQI	FGKKAEADKI	KAEIDA
	130	140	150	160		
orf38a	SFEAAKTAAQGKGH	GLVILVNGGK	MSAFGPSSRI	GGWLHKDIGV	PAVDEAIKE	SHGQPI
	190	200	210	220	230	240



orf38a.pep	MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQSEGVSVTVKTARGDVQIPQNPE
orf38-1	MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQTEGASVTVKTARGDVQIPQNPE
orf38a.pep	RIAVYDLGMLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTKPAGTLFEPDYETLNAYKPOL
orf38-1	RIAVYDLGMLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTKPAGTLFEPDYETLNAYKPQL
orf38a.pep	IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKKAEADKLKAEIDA
orf38-1	IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKQAEADKLKAEIDA
orf38a.pep	SFEAAKTAAQGKGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDEAIKEGSHGQPI
orf38-1	SFEAAKTAAQGKGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDESIKEGSHGQPI
orf38a.pep	SFEYLKEKNPDWLFVLDRSAAIGEEGQAAKDVLNNPLVAETTAWKKGQVVYLVPETYLAA
orf38-1	SFEYLKEKNPDWLFVLDRSAAIGEEGQAAKDVLDNPLVAETTAWKKGQVVYLVPETYLAA
orf38a.pep	GGAQELLNASKQVADAFNAAK
orf38-1	

[0321] Computer analysis of these sequences revealed the following:

[0322] Homology with a Lipoprotein (lipo) of *C. jejuni* (Accession Number X82427)

[0323] ORF38 and lipo show 38% as identity in 96 as overlap:

lyzed by SDS-PAGE. **FIG. 2A** shows the results of affinity purification of the His-fusion protein, and **FIG. 2B** shows the results of expression of the GST-fusion in *E. coli*. Purified His-fusion protein was used to immunise mice, whose sera were used for Western blot analysis (**FIG. 2C**) and FACS analysis (**FIG. 2D**). These experiments confirm that ORF38-1 is a surface-exposed protein, and that it is a useful immunogen.

```
Orf38: 40 EGASVTVKTARGDVQIPQNPERIAVYDLGMLDTLSKLGVKTGLS-VKDNRLPYLEEYFKT 98
EG S VK + G+ + P+NP ++ + DLG+LDT L + ++ V LP + FK
Lipo: 51 EGDSFLVKDSLGENKTPKNPSKVVILDLGILDTFDALKLNDKVAGVPAKNLPKYLQQFKN 110
Orf38: 99 TKPAGTLFEPDYETLNAYKPQLIIIGSRAAKAFDKL 134
G + + D+E +NA KP LIII R +K +DKL
Lipo: 111 KPSVGGVQQVDFEAINALKPDLIIISGRQSKFYDKL 146
```

[0324] Based on this analysis, it was predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

[0325] ORF38-1 (32 kDa) was cloned in pET and pGex vectors and expressed in *E. coli*, as described above. The products of protein expression and purification were ana-

[0326] FIG. 2E shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF38-1.

Example 3

[0327] The following *N. meningitidis* DNA sequence was identified <SEQ ID 13>:

1 ATGAAACTTC TGACCACCGC AATCCTGTCT TCCGCAATCG CGCTCAGCAG

51 TATGGCTGCC GCCGCTGGCA CGGACAACCC CACTGTTGCA AAAAAAACCG

101 TCAGCTACGT CTGCCAGCAA GGTAAAAAAG TCAAAGTAAC CTACGGCTTC

151 AACAAACAGG GTCTGACCAC ATACGCTTCC GCCGTCATCA ACGGCAAACG

201 CGTGCAAATG CCTGTCAATT TGGACAAATC CGACAATGTG GAAACATTCT

251 ACGGCAAAGA AGGCGGTTAT GTTTTGGGTA CCGGCGTGAT GGATGGCAAA

301 TCCTACCGCA AACAGCCCAT TATGATTACC GCACCTGACA ACCAAATCGT

351 CTTCAAAGAC TGTTCCCCAC GTTAA

[0328] This corresponds to the amino acid sequence <SEQ ID 14; ORF44>:

1 <u>MKLLTTAILS SAIALSSMAA AA</u>GTDWPTVA KKTVSYVCQQ GKKVKVTYGF

51 NKQGLTTYAS AVINGKRVQH PVNLDKSDNV ETFYGKEGGY VLGTGVMDGK

101 SYRKQPIHIT APDNQIVFKD CSPR*

[0329] Computer analysis of this amino acid sequence predicted the leader peptide shown underlined.

[0330] Further work identified the corresponding gene in strain A of *N. meningitidis* <SEQ ID 15>:

1 ATGAAACTTC TGACCACCGC AATCCTGTCT TCCGCAATCG CGCTCAGCAG

51 TATGGCTGCT GCTGCCGGCA CGAACAACCC CACCGTTGCC AAAAAAAACCG

101 TCAGCTACGT CTGCCAGCAA GGTAAAAAAG TCAAAGTAAC CTACGGCTTT

151 AACAAACAGG GCCTGACCAC ATACGCTTCC GCCGTCATCA ACGGCAAACG

201 TGTGCAAATG CCTGTCAATT TGGACAAATC CGACAATGTG GAAACATTCT

251 ACGGCAAAGA AGGCGGTTAT GTTTTGGGTA CCGGCGTGAT GGATGGCAAA

301 TCCTATCGCA AACAGCCTAT TATGATTACC GCACCTGACA ACCAAATCGT

351 CTTCAAAGAC TGTTCCCCAC GTTAA

[0331] This encodes a protein having amino acid sequence <SEQ ID 16; ORF44a>:

1 MKLLTTAILS SAIALSSMAA AAGTNNPTVA KKTVSYVCQQ GKKVKVTYGF

51 NKQGLTTYAS AVINGKRVQM PVNLDKSDNV ETFYGKEGGY VLGTGVMDGK

101 SYRKQPIMIT APDNQIVFKD CSPR*

[0332] The strain B sequence (ORF44) shows 99.2% identity over a 124 aa overlap with ORF44a:

	10	20	30	40	50	60
orf44.pep	MKLLTTAILSSAI	ALSSMAAAAGT	DNPTVAKKTV	SYVCQQGKK\	/KVTYGFNKQGI	LTTYAS
			:			
orf44a	<u>MKLLTTAILSSAI</u>	ALSSMAAAAGT	NNPTVAKKTV	/SYVCQQGKK\	/KVTYGFNKQGI	LTTYAS
	10	20	30	40	50	60

-continued							
7	D	80	90	100	110	120	
orf44.pep	AVINGKR					IMITAPDNQIVF	'KD
	1111111						
orf44a	AVINGKR	VQMPVNLDKS	DNVETFYGKI	EGGYVLGTGVI	IDGKSYRKQP	IMITAPDNQIVF	'KD
		70	80	90	100	110 1	.20
orf44.pep	CSPRX						
orf44a	CSPRX						

[0333] Computer analysis gave the following results:

[0334] Homology with the LecA Adhesin of *Eikenella* corrodens (Accession Number D78153)

[0335] ORF44 and LecA protein show 45% as identity in 91 as overlap:

purification of the His-fusion protein, and **FIG. 3B** shows the results of expression of the GST-fusion in E-coli. Purified His-fusion protein was used to immunise mice, whose sera were used for ELISA, which gave positive results, and for a bactericidal assay (**FIG. 3C**). These experiments confirm that ORF44-1 is a surface-exposed protein, and that it is a useful immunogen.

```
Orf44 33 TVSYVCQQGKKVKVTYGFNKQGLTTYASAVINGKRVQMPVNLDKSDNVETFYGKEGGYVL 92
+V+YVCQQG+++ V Y FN G+ T A +N + +++P NL SDNV+T + GY L
LecA 135 SVAYVCQQGRRLNVNYRFNSAGVPTSAELRVNNRNLRLPYNLSASDNVDTVF-SANGYRL 193
```

```
Orf44 93 GTGVHDGKSYRKQPIHITAPDNQIVFKDCSP 123
T MD +YR Q I+++AP+ Q+++KDCSP
LecA 194 TTNAMDSANYRSQDIIVSAPNGQNLYKDCSP 224
```

[0336] Based on homology with the adhesin, it was predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

[0337] ORF44-1 (11.2 kDa) was cloned in pET and pGex vectors and expressed in *E. coli*, as described above. The

[0338] FIG. 3D shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF44-1.

Example 4

[0339] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 17>

1GGCACCGAATTCAAAACCACCCTTTCCGGAGCCGACATACAGGCAGGGGT51GGGTGAAAAAGCCCGAGCCGATGCGAAAATTATCCTAAAAGGCATCGTAA101ACCGCATCCAAACCGAAGAAAAGCTGGAATCCAACTCGACCGTATGGCAA151AAGCAGGCCGGAACGGCAGCACGGTTGAAACGCTGAAGCTACCGAGCTT201TGAAGGGCCGGCACTGCCTAAGCTGACCGTCCCGGCGGCTATATCGCCG251ACATCCCCAAAGGCAACCTCAAAACCGAAATCGAAAAAGCGGCCAAACAG351GAACCAAGTACAGCTGCTTACGACAAATGGGACTATAAACAGGAAGCC401TAACCGGAGCGGAACCGGAGCCTTATCGGATTAANACGNGTGGCCGCCG451TCAGGCGCAGGAACCGGAGCCGTATTGGGATTAANACGNGTGGCCGCCG501CGCAACCGATGCAGCATTT...CCCC

products of protein expression and purification were analyzed by SDS-PAGE. **FIG. 3A** shows the results of affinity

[0340] This corresponds to the amino acid sequence <SEQ ID 18; ORF49>:

1 GTEFKTTLSG ADIQAGVGEK ARADPKIILK GIVNRIQTEE KLESNSTVWQ

51 KQAGSGSTVE TLKLPSFEGP ALPKLTAPGG YIADIPKGNL KTEIEKLAKQ

101 PEYAYLKOLO TVKDVNWNQV QLAYDKWDYK QEGLTCAGAA IXALAVTVVT

151 SGAGTGAVLG LXRVAAAATD AAF..

[0341] Further work revealed the complete nucleotide sequence <SEQ ID 19>:

1 ATGCAACTGC TGGCAGCCGA AGGCATTCAC CAACACCAAT TGAATGTTCA 51 GAAAAGTACC CGTTTCATCG GCATCAAAGT GGGTAAAAGC AATTACAGCA 101 AAAACGAGCT GAACGAAACC AAACTGCCCG TACGCGTTAT CGCCCAAACA 151 GCCAAAACCC GTTCCGGCTG GGATACCGTA CTCGAAGGCA CCGAATTCAA 201 AACCACCCTT TCCGGAGCCG ACATACAGGC AGGGGTGGGT GAAAAAGCCC 251 GAGCCGATGC GAAAATTATC CTAAAAGGCA TCGTTAACCG CATCCAAACC 301 GAAGAAAAGC TGGAATCCAA CTCGACCGTA TGGCAAAAGC AGGCCGGAAG 351 CGGCAGCACG GTTGAAACGC TGAAGCTACC GAGCTTTGAA GGGCCGGCAC 401 TGCCTAAGCT GACCGCTCCC GGCGGCTATA TCGCCGACAT CCCCAAAGGC 451 AACCTCAAAA CCGAAATCGA AAAGCTGGCC AAACAGCCCG AATATGCCTA 501 TCTGAAACAG CTTCAGACGG TCAAGGACGT GAACTGGAAC CAAGTACAGC 551 TCGCTTACGA CAAATGGGAC TATAAACAGG AAGGCCTAAC CGGAGCCGGA 601 GCCGCAATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG GCGCAGGAAC 651 CGGAGCCGTA TTGGGATTAA ACGGTGCGGC CGCCGCCGCA ACCGATGCAG 701 CATTTGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTFCAT CAACAACAAA 751 CGCAATATCG GTAACACCCT GAAAGAGCTG GGCAGAAGCA GCACGGTGAA 801 AAATCTGATG GTTGCCGTCG CTACCGCAGG CGTAGCCGAC AAAATCGGTG 851 CTTCGGCACT GAACAATGTC AGCGATAAGC AGTGGATCAA CAACCTGACC 901 GTCAACCTGG CCAATGCGGG CAGTGCCGCA CTGATTAATA CCGCTGTCAA 951 CGGCGGCAGC CTGAAAGACA ATCTGGAAGC GAATATCCTT GCGGCTTTGG 1001 TGAATACTGC GCATGGAGAG GCAGCAAGTA AAATCAAACA GTTGGATCAG 1051 CACTACATTG CCCATAAGAT TGCCCATGCC ATAGCGGGGCT GTGCGGCAGC 1101 GGCGGCGAAT AAGGGCAAGT GTCAAGATGG TGCGATCGGT GCGGCGGTCG 1151 GTGAAATCCT TGGCGAAACC CTACTGGACG GCAGAGACCC TGGCAGCCTG 1201 AATGTGAAGG ACAGGGCAAA AATCATTGCT AAGGCGAAGC TGGCAGCAGG 1251 GGCGGTTGCG GCGTTGAGTA AGGGGGGATGT GAGTACGGCG GCGAATGCGG 1301 CTGCTGTGGC GGTAGAGAAT AATTCTTTAA ATOATATACA GGATCGTTTG 1351 TTGAGTGGAA ATTATGCTTT ATGTATGAGT GCAGGAGGAG CAGAAAGCTT 1401 TTGTGAGTCT TATCGACCAC TGGGCTTGCC ACACTTTGTA AGTGTTTCAG 1451 GAGAAATGAA ATTACCTAAT AAATTCGGGA ATCGTATGGT TAATGGAAAA 1531 TTAATTATTA ACACTAGAAA TGGCAATGTA TATTTCTCTG TAGGTAAAAT 1551 ATGGAGTACT GTAAAATCAA CAAAATCAAA TATAAGTGGG GTATCTGTCG 1601 GTTGGGTTTT AAATGTTTCC CCTAATGATT ATTTAAAAGA AGCATTTATG

1651 AATGATTTCAGAAATAGTAATCAAAATAAAGCCTATGCAGAATGATTTC1701 CCAGACTTGGTAGGTGAGAGTGTTGGTGGTAGTCTTGTCTGACAAGAG1751 CCTGCTTTCGGTAAGTTCAACAATATCTAAATCTAAAATTCCTTTTAAA1801 GATTCAAAAATTATTGGGGAAATCGGTTTGGGAAGTGGTGTTGCTGCAGG1851 AGTAGAAAAAACAATATACATAGGTAACATAAAAGATATTGATAAATTTA1901 TTAGTGCAAACATAAAAAATAG

[0342] This corresponds to the amino acid sequence <SEQ ID 20; ORF49-1>:

1MQLLAAEGIHQHQLNVQKSTRFIGIKVGKSNYSKNELNETKLPVRVIAQT51AKTRSGWDTVLEGTEFKTTLSGADIQAGVGEKARADAKIILKGIVNRIQT101EEKLESNSTVWQKQAGSGSTVETLKLPSFEGPALPKLTAPGGYIADIPKG151NLKTEIEKLAKQPEYAYLKQLQTVKDVNWQVQLAYDKWDYKQEGLTGAG201AAIIALAVTVVTSGAGTGAVLGLNGAAAAATDAAFASLASQASVSFINNK251GNIGNTLKELGRSSTVKNLMVAVATAGVADKIGASALNNVSDKQWINNLT301VNLANAGSAALINTAVNGSLKDNLEANIAAUVATAGVNSLNQLQQ351HYIAHKIAHAIAGCAAAAANKGKCQDGAIGAAVGEILGETLLDGRDPGSL401NVKDRAKIIAKAKLAAGAVAALSKGDVSTAANAAAVAVENNSLNDLQDRL451LSGNYALCNSAGGAESFCESYRPLGLPHFVSVSGENKLPNKFGNRNVNGK501LIINTRNGNVYFSVGKIWSTVKSTKSNISGVSVGWLNNSPNDYLKEASM501DSKIIGEIGLGSGVAAGVEKTIYIGNIKDIDKFISANIKK*

[0343] Computer analysis predicts a transmembrane domain and also indicates that ORF49 has no significant amino acid homology with known proteins. A corresponding ORF from *N. meningitidis* strain A was, however, identified:

[0344] ORF49 shows 86.1% identity over a 173 as overlap with an ORF (ORF49a) from strain A of *N. meningitidis*:

orf49.pep			G T 	10 EFKTTLSGAI	20 DIQAGVGEKAN	30 RADAKIILK
orf49a	SKNELNETKLPVR	VVAQXAATRS	GWDTVLEGTE	11111111111	QAGVXEKAR	VDAKIILK
	40	50	60	70	80	90
	40	50	60	70	80	90
orf49.pep	GIVNRIQTEEKLE	SNSTVWQKQA			KLTAPGGY12	ADIPKGNL
		:			:	:
orf49a	GIVNRIQSEEKLE					
	100	110	120	130	140	150
	100	110	120	130	140	150
orf49.pep	KTEIEKLAKQPEY.	AYLKQLQTVK	DVNWNQVQLA	YDKWDYKQEC	LTGAGAAIXA	ALAVTVVT
			· • •	:		
orf49a	KTEIEKLSKQPEY.	AYLKQLQVAK	NINWNQVQLA	YDRWDYKQEC	LTEAGAAII	ALAVTVVT
	160	170	180	190	200	210
	160	170				

- 38

-continued						
orf49.pep	SGAGTGAVLGLXF	VAAAATDA	AF			
		:				
orf49a	SGAGTGAVLGLNG	AXAAATDAA	AFASLASQAS	SFINNKGDV0	GKTLKELGRS	STVKNLVVA
	220	230	240	250	260	270

[0345]	ORF49-1 and	l ORF49a s	show 83.2%	identity in 457
aa overl	ap:			

orf49a.pep	XQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNELNETKLPVRVVAQXAATRSGWDTV : ::
orf49-1	MQLLAAEGIHQHQLNVQKSTRFIGIKVGKSNYSKNELNETKLPVRVIAQTAKTRSGWDTV
orf49a.pep	LEGTEFKTTLAGADIQAGVXEKARVDAKIILKGIVNRIQSEEKLETNSTVWQKQAGRGST
orf49-1	LEGTEFKTTLSGADIQAGVGEKARADAXIILKGIVNRIQTEEKLESNSTVWQKQAGSGST
orf49a.pep	IETLKLPSFESPTPPKLSAPGGYIVDIPKGNLKTEIEKLSKQPEYAYLKQLQVAKNINWN : ::::::::::::::::::::::::::::
orf49-1	VETLKLPSFEGPALPKLTAPGGYIADIPKGNLKTEIEKLAKQPEYAYLKQLQTVKDVNWN
orf49a.pep	QVQLAYDRWDYKQEGLTEAGAAIIALAVTVVTSGAGTGAVLGLNGAXAAATDAAFASLAS
orf49-1	QVQLAYDKWDYKQEGLTGAGAAIIALAVTVVTSGAGTGAVLGLNGAAAAATDAAFASLAS
orf49a.pep	QASVSFINNKGDVGKTLKELGRSSTVKNLVVAAATAGVADKIGASALXNVSDKQWINNLT
orf49-1	eq:QASVSFINNKGNIGNTLKELGRSSTVKNLMVAVATAGVADKIGASALNNVSDKQWINNLT
orf49a.pep	VNLANAGSAALINTAVNGGSLKDXLEANILAALVNTAHGEAASKIKQLDQHYIVHKIAHA
orf49-1	$\texttt{VNLANAGSAALINTAVNGGSLKDNLEANILAALVNTAHGEAASKIKQLDQH\texttt{YIAHKIAHA}$
orf49a.pep	IAGCAAAAANKGKCQDGAIGAAVGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVS
orf49-1	IAGCAAAAANKGKCQDGAIGAAVGEILGETLLDGRDPGSLNVKDRAKIIAKAKLAAGAVA
orf49a.pep	GVVGGDVNAAANAAEVAVKNNQLSDXEGREFDNEMTACAKQNXPQLCRKNTVKKYQNVAD :: :: : ::::::
orf49-1	ALSKGDVSTAANAAAVAVENNSLNDIQDRLLSGNYALCMSAGGAESFCESYRPLGLPHFV
orf49a.pep	${\tt KRLAASIAICTDISRSTECRTIRKQHLIDSRSLHSSWEAGLIGKDDEWYKLFSKSYTQAD$
orf49-1	SVSGEMKLPNKFGNRMVNGKLIINTRNGNVYFSVGKIWSTVKSTKSNISGVSVGWVLNVS

[0346] The complete length ORF49a nucleotide sequence <SEQ ID 21> is:

1	NTGCAACTGC	TGGCAGAAGA	AGGCATCCAC	AAGCACGAGT	TGGATGTCCA
51	AAAAAGCCGC	CGCTTTATCG	GCATCAAGGT	AGGTNAGAGC	AATTACAGTA
101	AAAACGAACT	GAACGAAACC	AAATTGCCTG	TCCGCGTCGT	CGCCCAAANT
151	GCAGCCACCC	GTTCAGGCTG	GGATACCGTG	CTCGAAGGTA	CCGAATTCAA
201	AACCACGCTG	GCCGGTGCCG	ACATTCAGGC	AGGTGTANGC	GAAAAAGCCC
251	GTGTCGATGC	GAAAATTATC	CTCAAAGGCA	TTGTGAACCG	TATCCAGTCG
301	GAAGAAAAAT	TAGAAACCAA	CTCAACCGTA	TGGCAGAAAC	AGGCCGGACG
351	CGGCAGCACT	ATCGAAACGC	TAAAACTGCC	CAGCTTCGAA	AGCCCTACTC
401	CGCCCAAATT	GTCCGCACCC	GGCGGNTATA	TCGTCGACAT	TCCGAAAGGC
451	ААТСТБАААА	CCGAAATCGA	AAAGCTGTCC	AAACAGCCCG	AGTATGCCTA

501 TCTGAAACAG CTCCAAGTAG CGAAAAACAT CAACTGGAAT CAGGTGCAGC 551 TTGCTTACGA CAGATGGGAC TACAAACAGG AGGGCTTAAC CGAAGCAGGT 601 GCGGCGATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG GCGCAGGAAC 651 CGGAGCCGTA TTGGGATTAA ACGGTGCGNC CGCCGCCGCA ACCGATGCAG 701 CATTCGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTTCAT CAACAACAAA 751 GGCGATGTCG GCAAAAACCCT GAAAGAGCTG GGCAGAAGCA GCACGGTGAA 801 AAATCTGGTG GTTGCCGCCG CTACCGCAGG CGTAGCCGAC AAAATCGGCG 851 CTTCGGCACT GANCAATGTC AGCGATAAGC AGTGGATCAA CAACCTGACC 901 GTCAACCTAG CCAATGCGGG CAGTGCCGCA CTGATTAATA CCGCTGTCAA 951 CGGCGGCAGC CTGAAAGACA NTCTGGAAGC GAATATCCTT GCGGCTTTGG 1001 TCAATACCGC GCATGGAGAA GCAGCCAGTA AAATCAAACA GTTGGATCAG 1051 CACTACATAG TCCACAAGAT TGCCCATGCC ATAGCGGGCT GTGCGGCAGC 1101 GGCGGCGAAT AAGGGCAAGT GTCAGGATGG TGCGATAGGT GCGGCTGTGG 1151 GCGAGATAGT CGGGGAGGCT TTGACAAACG GCAAAAATCC TGACACTTTG 1201 ACAGCTAAAG AACGCGAACA GATTTTGGCA TACAGCAAAC TGGTTGCCGG 1251 TACGGTAAGC GGTGTGGTCG GCGGCGATGT AAATGCGGCG GCGAATGCGG 1301 CTGAGGTAGC GGTGAAAAAT AATCAGCTTA GCGACTAAGA GGGTAGAGAA 1351 TTTGATAACG AAATGACTGC ATGCGCCAAA CAGAATANTC CTCAACTGTG 1401 CAGAAAAAAT ACTGTAAAAA AGTATCAAAA TGTTGCTGAT AAAAGACTTG 1451 CTGCTTCGAT TGCAATATGT ACGGATATAT CCCGTAGTAC TGAATGTAGA 1501 ACAATCAGAA AACAACATTT GATCGATAGT AGAAGCCTTC ATTCATCTTG 1551 GGAAGCAGGT CTAATTGGTA AAGATGATGA ATGGTATAAA TTATTCAGCA 1601 AATCTTACAC CCAAGCAGAT TTGGCTTTAC AGTCTTATCA TTTGAATACT 1651 GCTGCTAAAT CTTGGCTTCA ATCGGGGCAAT ACAAAGCCTT TATCCGAATG 1701 GATGTCCGAC CAAGGTTATA CACTTATTTC AGGAGTTAAT CCTAGATTCA 1751 TTCCAATACC AAGAGGGITF GTAAAACAAA ATACACCTAT TACTAATGTC 1801 AAATACCCGG AAGGCATCAG TTTCGATACA AACCTANAAA GACATCTGGC 1851 AAATGCTGAT GGTTTTAGTC AAGAACAGGG CATTAAAGGA GCCCATAACC 1901 GCACCAATNT TATGGCAGAA CTAAATTCAC GAGGAGGANG NGTAAAATCT 1951 GAAACCCANA CTGATATTGA AGGCATTACC CGAATTAAAT ATGAGATTCC 2001 TACACTAGAC AGGACAGGTA AACCTGATGG TGGATTTAAG GAAATTTCAA 2051 GTATAAAAAC TGTTTATAAT CCTAAAAANT TTTNNGATGA TAAAATACTT 2101 CAAATGGCTC AANATGCTGN TTCACAAGGA TATTCAAAAG CCTCTAAAAT 2151 TGCTCAAAAT GAAAGAACTA AATCAATATC GGAAAGAAAA AATGTCATTC 2201 AATTCTCAGA AACCTTTGAC GGAATCAAAT TTAGANNNTA TNTNGATGTA 2251 AATACAGGAA GAATTACAAA CATTCACCCA GAATAATTTA A

[0347] This encodes a protein having amino acid sequence <SEQ ID 22>:

1 XQLLAEEGIH KHELDVQKSR RFIGIKVGXS NYSKNELNET KLPVRVVAQX 51 AATRSGWDTV LEGTEFKTTL AGADIOAGVX EKARVOAKII LKGIVNRIOS 101 EEKLETNSTV WQKQAGRGST IETLKLPSFE SPTPPKLSAP GGYIVDIPKG 151 NLKTEIEKLS KOPEYAYLKO LOVAKNINWN OVOLAYDRWD YKOEGLTEAG 201 AAIIALAVTV VTSGAGTGAV LGLNGAXAAA TORAFASLAS QASVSFINNK 251 GDVGKTLKEL GRSSTVKNLV VAAATAGVAD KIGASALXNV SDKQNINNLT 301 VNLANAGSAA LINTAVNGGS LKDXLEANIL AALVNTAHGE AASKIKQLDQ 351 HYIVHKIAHA IAGCAAAAAN KGKCODGAIG AAVGEIVGEA LTNGKNPDTL 401 TAKEREQILA YSKLVAGTVS GVVGGDVNAA ANAAEVAVKN NQLSDXEGRE 451 FDWEHTACAK QNXPQLCRXN TVKKYQNVAD KRLAASIAIC TDISRSTECR 501 TIRKQHLIDS RSLHSSWEAG LIGKDDEWYK LFSKSYTQAD LALOSYHLNT 551 AAKSWLQSGN TKPLSEWNSD QGYTLISGVN PRFIPIPRGF VKQNTPITNV 601 KYPEGISFDT NLXRHLATAD GFSQEQGIKG AHNRTNXMAE LNSRGGXVKS 651 ETXTDIEGIT RIKYEIPTLD RTGKPDGGFK EISSIKTVYN FKXFKDDKIL 701 QMAQXAXSQG YSKASKIAQN ERTKSISERK NVIQFSETFD GIKFRXYXDV 751 NTGRITNIHP E

[0348] Based on the presence of a putative transmembrane domain, it is predicted that these proteins from *N. menin-gitidis*, and their epitopes, could be useful antigens for vaccines or diagnostics.

Example 5

[0349] The following partial DNA sequence was identified in *N. meningitidis* SEQ ID 23>

51AAGTATAACCCAAGGCTTTGTCTTCGCCTTTCATTCCGATAAGGGATATG101ACGCTTTGGTCGGTATAGCCGTCTTGGGAACCTTTGTCCACCCAACGCAT151ATCTGCCTGCGGATTCTCATTGCCGCTTCTTGGCTGCTGATTTTTCTGCC201TTCGCGTTTTTCAACTTCGCGCTTGAGGGCTTCGGCATATTTGTCGGCCA251ACGCCATTCTTTCGGGATGCAGCTGCCTATTGTTCCAATCTACATCGCA

1..CGGATCGTTG TAGGTTTGCG GATTTCTTGC GCCGTAGTCA CCGTAGTCCC

301 CCCACCACAG CACCACCACT ACCACCAGTT GCATAG

[0350] This corresponds to the amino acid sequence <SEQ ID 24; ORF50>:

1...RIVVGLRISC AVVTVVPSIT QGFVFAFHSD KGYDALVGIA VLGTFVHPTH

51<u>ICLRILIAAS WLLIFLP</u>SRF STSRLRASAY LSANAISFGC SCLLFQSTFA

101PTTAPPLPPV A*

[0351] Computer analysis predicts two transmembrane domains and also indicates that ORF50 has no significant amino acid homology with known proteins.

[0352] Based on the presence of a putative transmembrane domain, it is predicted that this protein from *N. meningitidis*,

and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 6

[0353] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 25>

1..AAGTTTGACT TTACCTGGTT TATTCCGGCG GTAATCAAAT ACCGCCGGTT 51 GTTTTTTGAA GTATTGGTGG TGTCGGTGGT GTTGCAGCTG TTTGCGCTGA 101 TTACGCCTCT GTTTTTCCAA GTGGTGATGG ACAAGGTGCT GGTACATCGG 151 GGATTCTCTA CTTTGGATGT GGTGTCGGTG GCTTTGTTGG TGGTGTCGCT 201 GTTTGAGATT GTGTTGGGCG GTTTGCGGAC GTATCTGTTT GCACATACGA 251 CTTCACGTAT TGATGTGGAA TTGGGCGCGC GTTTGTTCCG GCATCTGCTT TCCCTGCCTT TATCCTATTT CGAGCACAGA CGAGTGGGTG ATACGGTGGC 301 351 TCGGGTGCGG GAATTGGAGC AGATTCGCAA TTTCTTGACC GGTCAGGCGC 401 TGACTTCGGT GTTGGATTTG GCGTTTTCGT TTATCTTTCT GGCGGTGATG TGGTATTACA GCTCCACTCT GACTTGGGTG GTATTGGCTT CGTTG..... 451 11 1451 1501 1551 CAACCGGACG GTGCTGATTA TCGCCCACCG TCTGTCCACT GTTAAAACGG 1601 CACACCGGAT CATTGCCATG GATAAAGGCA GGATTGTGGA AGCGGGAACA 1651 CAGCAGGAAT TGCTGGCGAA CG..AACGGA TATTACCGCT ATCTGTATGA

1701 TTTACAGAAC GGGTAG

[0354] This corresponds to the amino acid sequence <SEQ ID 26; ORF39>:

1 ... KFDFTWFIPA VIKYRRLFFE VLVVSVVLQL FALITPLFFQ VVMDKVLVHR

51 GFSTLDVVSV ALLVVSLFEI VLGGLRTYLF AHTTSRIDVE LGARLFRHLL

101 SLPLSYFEHP RVGDTVARVR ELEQIRNFLT GQALTSVLDL AFSFIFLAVM

151 WYYSSTLTWV VLASL.....

- 11

501 ICANRT VLIIAHRLST VKTAHRIIAH DKGRIVEAGT

551 QQELLANXNG YYRYLYDLQN G*

[0355] Further work revealed the complete nucleotide sequence <SEQ ID 27>:

1 ATGTCTATCG TATCCGCACC GCTCCCCGCC CTTTCCGCCC TCATCATCCT 51 CGCCCATTAC CACGGCATTG CCGCCAATCC TGCCGATATA CAGCATGAAT 101 TTTGTACTTC CGCACAGAGC GATTTAAATG AAACGCAATG GCTGTTAGCC

-continued 151 GCCAAATCTT TGGGATTGAA GGCAAAGGTA GTCCGCCAGC CTATTAAACG 201 TTTGGCTATG GCGACTTTAC CCGCATTGGT ATGGTGTGAT GACGGCAACC 251 ATTTCATTTT GGCCAAAACA GACGGTGAGG GTGAGCATGC CCAATTTTFG 301 ATACAGGATT TGGTTACGAA TAAGTCTGCG GTATTGTCTT TTGCCGAATT 351 TTCTAACAGA TATTCGGGCA AACTGATATT GGTTGCTTCC CGCGCTTCGG 401 TATTGGGCAG TTTGGCAAAG TTTGACTTTA CCTGGTTTAT TCCGGCGGTA 451 ATCAAATACC GCCGGTTGTT TTTTGAAGTA TTGGTGGTGT CGGTGGTGTT 501 GCAGCTGTTT GCGCTGATTA CGCCTCTGTT TTTCCAAGTG GTGATGGACA 551 AGGTGCTGGT ACATCGGGGA TTCTCTACTT TGGATGTGGT GTCGGTGGCT 601 TTGTTGGTGG TGTCGCTGTT TGAGATTGTG TTGGGCGGTT TGCGGACGTA 651 TCTGTTTGCA CATACGACTT CACGTATTGA TGTGGAATTG GGCGCGCGTT 701 TGTTCCGGCA TCTGCTTTCC CTGCCTTTAT CCTATTTCGA GCACAGACGA 751 GTGGGTGATA CGGTGGCTCG GGTGCGGGAA TTGGAGCAGA TTCGCAATTT 801 CTTGACCGGT CAGGCGCTGA CTTCGGTGTT GGATTTGGCG TTTTCGTTTA 951 TCTTTCTGGC GGTGATGTGG TATTACAGCT CCACTCTGAC TTGGGTGGTA 901 TTGGCTTCGT TGCCTGCCTA TGCGTTTTGG TCGGCATTTA TCAGTCCGAT 951 ACTGCGGACG CGTCTGAACG ATAAGTTCGC GCGCAATGCA GACAACCAGT 1001 CGTTTTTAGT AGAAAGCATC ACTGCGGTGG GTACGGTAAA GGCGATGGCG 1051 GTGGAGCCGC AGATGACGCA GCGTTGGGAC AATCAGTTGG CGGCTTATGT 1101 GGCTTCGGGA TTTCGGGTAA CGAAGTTGGC GGTGGTCGGC CAGCAGGGGG 1151 TGCAGCTGAT TCAGAAGCTG GTGACGGTGG CGACGTTGTG GATTGGCGCA 1201 CGGCTGGTAA TTGAGAGCAA GCTGACGGTG GGGCAGCTGA TTGCGTTTAA 1251 TATGCTCTCG GGACACGTGG CGGCGCCTGT TATCCGTTTG GCGCAGTTGT 1301 GGCAGGATTT CCAGCAGGTG GGGATTTCGG TGGCGCGTTT GGGGGATATT 1351 CTGAATGCGC CGACCGAGAA TGCGTCTTCG CATTTGGCTT TGCCCGATAT 1401 CCGGGGGGGAG ATTACGTTCG AACATGTCGA TTTCCGCTAT AAGGCGGACG 1451 GCAGGCTGAT TTTGCAGGAT TTGAACCTGC GGATTCCGGC GGGGGAAGTG 1501 CTGGGGATTG TGGGACGTTC GGGGTCGGGC AAATCCACAC TCACCAAATT 1551 GGTGCAGCGT CTGTATGTAC CGGAGCAGGG ACGGGTGTTG GTGGACGGCA 1601 ACGATTTGGC TTTGGCCGCT CCTGCCTGGC TGCGGCGGCA GGTCGGCGTG 1651 GTCTTGCAGG AGAATGTGCT GCTCAACCGC AGCATACGCG ACAATATCGC 1701 GCTGACGGAT ACGGGTATGC CGCTGGAACG CATTATCGAA GCAGCCAAAC 1751 TGGCGGGCGC ACACGAGTTT ATTATGGAGC TGCCGGAAGG CTACGGCACC 1801 GTGGTGGGCG AACAAGGGGC CGGCTTGTCG GGCGGACAGC GGCAGCGTAT 1951 TGCGATTGCC CGCGCGTTAA TCACCAATCC GCGCATTCTG ATTTTTGATG 1901 AAGCCACCAG CGCGCTGGAT TATGAAAGTG AACGAGCGAT TATGCAGAAC 1951 ATGCAGGCCA TTTGCGCCAA CCGGACGGTG CTGATTATCG CCCACCGTCT

2001 GTCCACTGTT AAAACGGCAC ACCGGATCAT TGCCATGGAT AAAGGCAGGA

2051 TTGTGGAAGC GGGAACACAG CAGGAATTGC TGGCGAAGCC GAACGGATAT

2101 TACCGCTATC TGTATGATTT ACAGAACGGG TAG

[0356] This corresponds to the amino acid sequence <SEQ ID 28; ORF39-1>:

1MSIVSAPLPA LSALIILAMY HGIAANPADI OHEFCTSAOSDLNETQWLLA51AKSLGLKAKV VRQPIKRLAMATLPALVWCDDGNHFILAKTDGEGEHAQFL101IQDLVTNKSA VLSFAEFSNRYSGKLILVASRASVLGSLAKFDFTWFIPAV151IKYRRLFFEV LVVSVVLQLFALITPLFFQVVMDKVLVHRGFSTLDVVSVA201LLVVSLFEIV LGGLRTYLFAHTTSRIDVELGARLFRHLSLPLSYFEHPA251VGDTVARVRELEQIRNFLTGQALTSVLDLAFSFIFLAVMYSSTLTWVV301LASLPAYAFW SAFISPILRTRLNDKFAPNADNQSFLVESITAVGTVKAMA351VEPQHTQRWDNQLAAYVASGFRVTKLAVGQQGVQLIQKLVTVATLWIGA401RLVIESRLTVGQLIAFNMLSGQVAAPVIRLAQLWQDFQQGISVARLGDI501LGIVGRSGSGKSTLTKLVQRLYVPEQGRVLVDGNDLALAAPAWLRQVGVG551VLOENVLLNRSIRDNIALTDTGNPLERIIEAAKLAGAHEFIMELPEGYGT651MQAICANRTVLIIAHRLSTVKTAHRIIANDKGRIVEAGTQELLAKPNGY701YRYLYDLQNG *****

[0357] Computer analysis of this amino acid sequence gave the following results:

[0358] Homology with a Predicted ORF from *N. menin-gitidis* (Strain A)

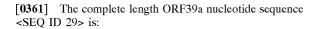
[0359] ORF39 shows 100% identity over a 165 as overlap with an ORF (ORF39a) from strain A of *N. meningitidis*:

					10	20	30
orf39.pep				KFDFT	WFIPAVIKY	RRLFFEVLVV	SVVLQL
				11111			
orf39a	AVLSF	AEFSNRYSG	LILVASRAS	VLGSLAKFDFI	WFIPAVIKY	RRLFFEVLVV	SVVLQL
	110	120	130	140	150	160	
		40	50	60	70	80	90
orf39.pep	FALIT	PLFFQVVMDF	(VLVHRGFST)	LDVVSVALLVV	SLFEIVLGG	LRTYLFAHTT	SRIDVE
		1111111111					
orf39a	FALIT	PLFFQVVMDF	VLVHRGFST	LDVVSVALLVV	SLFEIVLGG	LRTYLFAHTT	SRIDVE
	170	180	190	200	210	220	
		100	110	120	130	140	150
orf39.pep	LGARL	FRHLLSLPLS	SYFEHRRVGD'	FVARVRELEQI	RNFLTGQAL	TSVLDLAFSF	IFLAVM
orf39a	LGARL	FRHLLSLPLS	SYFEHRRVGD'	FVARVRELEQI	RNFLTGQAL	TSVLDLAFSF	IFLAVM
	230	240	250	260	270	280	

-continued							
		160	170	180	190	200	210
orf39.pep	WYYSS	FLTWVVLAS	LXXXXXXXXX	XXXXXXXXXXX	XXXXXXXXI	CANRTVLIIA	HRLSTV
orf39a	WYYSS	FLTWVVLAS	LPAYAFWSAF	ISPILRTRLN	DKFARNADNQ	SFLVESITAV	GTVKAM
	290	300	310	320	330	340	

[0360] ORF39-1 and ORF39a show 99.4%	6 identity in 710
aa overlap:	

orf39-1.pep MSIVSAPLPALSALIILAHYHGIAANPADIQHEFCTSAQSDLNETQWLLAAKSLGLKAKV
orf39-1.pep VRQPIKRLAMATLPALVWCDDGNHFILAKTDGEGEHAQFLIQDLVTNKSAVLSFAEFSNR
orf39-1.pep YSGKLILVASRASVLGSLAKFDFTWFIPAVIKYRRLFFEVLVVSVVLQLFALITPLFFQV
orf39-1.pep VMDKVLVHRGFSTLDVVSVALLVVSLFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLS
orf39-1.pep LPLSYFEHRRVGDTVARVRELEQIRNFLTGQALTSVLDLAFSFIFLAVMWYYSSTLTWVV
orf39-1.pep LASLPAYAFWSAFISPILRTRLNDKFARNADNQSFLVESITAVGTVKAMAVEPQMTQRWD
orf39-1.pep NQLAAQVASGFRVTKLAVVGQQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLS
orf39-1.pep GQVAAPVIRLAQLWQDFQQVGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRY
orf39-1.pep KADGRLILQDLNLRIRAGEVLGIVGRSGSGKSTLTKLVQRLYVPEQGRVLVDGNDLALAA
orf39-1.pep PAWLRRQVGVVLQENVLLNRSIRDNIALTDTGMPLERIIEAAKLAGAHEFIHELPEGYGT
orf39-1.pep VVGEQGAGLSGGQRQRIAIARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTV
orf39-1.pep LIIAHRLSTVKTAHRIIAMDKGRIVEAGTQQELLAKPNGYYRYLYDLQNGX



1ATGTCTATCGTATCCGCACCGCTCCCCGCCCTTTCCGCCCTCATCATCCT51CGCCATYACCACGGCATGCCGCCAATCCTGCCGATATACAGCATGAAT101TTTGTACTTCCGCACAGAGCGATTTAAATGAAACGCAATGGCTGTTAGCC151GCCAAATCTTTGGGATTGAAGGCAAAGGTAGTCCGCCAGCCTATTAAACG201TTTGGCTATGGCGACTTTACCCGCATTGGTATGGTGTGATGACGGCAACC

-continued 251 ATTTTATTTT GGCTAAAACA GACGGTGGGG GTGAGCATGC CCAATATCTA 301 ATACAGGATT TAACTACGAA TAAGTCTGCG GTATTGTCTT TTGCCGAATT 351 TTCTAACAGA TATTCGGGCA AACTGATATT GGTTGCTTCC CGCGCTTCGG 401 TATTGGGCAG TTTGGCAAAG TTTGACTTTA CCTGGTTTAT TCCGGCGGTA 451 ATCAAATACC GCCGGTTGTT TTTTGAAGTA TTGGTGGTGT CGGTGGTGTT 501 GCAGCTGTTT GCGCTGATTA CGCCTCTGTT TTTCCAAGTG GTGATGGACA 551 AGGTGCTGGT ACATCGGGGA TTCTCTATTT TGGATGTGGT GTCGGTGGCT 601 TTGTTGGTGG TGTCGCTGTT TGAGATTGTG TTGGGCGGTT TGCGGACGTA 651 TCTGTTTGCA CATACGACTT CACGTATTGA TGTGGAATTG GGCGCGCGTT 701 TGTTCCGGCA TCTGCTTTCC CTGCCTTTAT CCTATTTCGA GCACAGACGA 751 GTGGGTGATA CGGTGGCTCG GGTGCGGGAA TTGGAGCAGA TTCGCAATTT 801 CTTGACCGGT CAGGCGCTGA CTTCGGTGTT GGATTTGGCG TTTTCGTTTA 951 TCTTTCTGGC GGTGATGTGG TATTACAGCT CCACTCTGAC TTGGGTGGTA 901 TTGGCTTCGT TGCCTGCCTA TGCGTTTTGG TCGGCATTTA TCAGTCCGAT 951 ACTGCGGACG CGTCTGAACG ATAAGTTCGC GCGCAATGCA GACAACCAGT 1001 CGTTTTTAGT AGAAAGCATC ACTGCGGTGG GTACGGTAAA GGCGATGGCG 1051 GTGGAGCCGC AGATGACGCA GCGTTGGGAC AATCAGTTGG CGGCTTATGT 1101 GGCTTCGGGA TTTCGGGTAA CGAAGTTGGC GGTGGTCGGC CAGCAGGGGG 1151 TGCAGCTGAT TCAGAAGCTG GTGACGGTGG CGACGTTGTG GATTGGCGCA 1201 CGGCTGGTAA TTGAGAGCAA GCTGACGGTG GGGCAGCTGA TTGCGTTTAA 1251 TATGCTCTCG GGACAGGTGG CGGCGCCTGT TATCCGTTTG GCGCAGTTGT 1301 GGCAGGATTT CCAGCAGGTG GGGATTTCGG TGGCGCGTTT CGGGGATATT 1351 CTGAATGCGC CGACCGAGAA TGCGTCTTCG CATTTGGCTT TGCCCGATAT 1401 CCGGGGGGGAG ATTACGTTCG AACATGTCGA TTTCCGCTAT AAGGCGGACG 1451 GCAGGCTGAT TTTGCAGGAT TTGAACCTGC GGATTCGGGC GGGGGAAGTG 1501 CTGGGGATTG TGGGACGTTC GGGGTCGGGC AAATCCACAC TCACCAAATT 1551 GGTGCAGCGT CTGTATGTAC CGGCGCAGGG ACGGGTGTTG GTGGACGGCA 1601 ACGATTTGGC TTTTGGCCGCT CCTGCTTGGC TGCGGCGGCA GGTCGGCGTG 1651 GTCTTGCAGG AGAATGTGCT GCTCAACCGC AGCATACGCG ACAATATCGC 1701 GCTGACGGAT ACGGGTATGC CGCTGGAACG CATTATCGAA GCAGCCAAAC 1751 TGGCGGGCGC ACACGAGTTT ATTATGGAGC TGCCGGAAGG CTACGGCACC 1801 GTGGTGGGCG AACAAGGGGC CGGCTTGTCG GGCGGACAGC GGCAGCGTAT 1851 TGCGATTGCC CGCGCGTTAA TCACCAATCC GCGCATTCTG ATTTTTGATG 1901 AAGCCACCAG CGCGCTGGAT TATGAAAGTG AACGAGCGAT TATGCAGAAC 1951 ATGCAGGCCA TTTGCGCCAA CCGGACGGTG CTGATTATCG CCCACCGTCT 2001 GTCCACTGTT AAAACGGCAC ACCGGATCAT TGCCATGGAT AAAGGCAGGA 2051 TTGTGGAAGC GGGAACACAG CAGGAATTGC TGGCGAAGCC GAACGGATAT 2101 TACCGCTATC TGTATGATTT ACAGAACGGG TAG

[0362] This encodes a protein having amino acid sequence <SEQ ID 30>:

1 MSIVSAPLPA LSALIILAHY HGIAANPADI QHEFCTSAQS DLNETQWLLA

[0363] ORF39a is homologous to a cytolysin from *A*. *pleuropneumoniae*:

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sp|P26760|RT1B_ACTPL RTX-I TOXIN DETERMINANT B (TOXIN RTX-I SECRETION ATP-
BINDING PROTEIN) (APX-IB) (HLY-IB) (CYTOLYSIN IB) (CLY-IB)
>gi|97137|pir||043599 cytolysin IB - Actinobacillus pleuropneumoniae
(serotype 9) >gi|36944 (X6112) ClyI-B protein [Actinobacillus
pleuropneumoniae] Length = 707 Score = 931 bits (2379), Expect =0.0
Identities = 472/690 (68%), Positives = 540/690 (77%),
Gaps = 3/690 (0%)
Query: 20 YHGIAANPADIQHEFCTSAQSDLNETQWXXXXXXXXXXVVRQPIKRLAMATLPALVWC 79
           YH IA NP +++H+F
                            + L+ T W
                                                    V++ I RLA LPALVW
sbjct: 20 YHNIAVNPEELKHKFDLEGKG-LDLTAWLLAAKSLELKAKQVKKAIDRLAFIALPALVWR 78
Query: 80 DDGNHFILAKTDGGGEHAQYLIQDLTTNKSAVLSFAEFSNRYSGKLILVASRASVLGSLA 139
           +DG HFIL K D E +YLI DL T+ +L AEF + Y GKLILVASRAS++G LA
sbjct: 79 EDGKHFILTKIDN--EAKKYLIFDLETHNPRILEQAEFESLYQGKLILVASRASIVGKLA 136
Query: 140 KFDFTWFIPAVIKYRRXXXXXXXXXXXXXXXXITPLFFQVVMDKVLVHRGFXXXXXXXX 199
                                             ITPLFFQVVMDKVLVHRGF
           KFDFTWFIPAVIKYR+
Sbjct: 137 KFDFTWFIPAVIKYRKIFIETLIVSIFLQIFALITPLFFQVVMDKVLVHRGFSTLNVITV 196
Query: 200 XXXXXXFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLSLPLSYFEHRRVGDTVARVR 259
                  FEIVL GLRTY+FAH+TSRIDVELGARLFRHLL+LP+SYFE+RRVGDTVARVR
Sbjct: 197 ALAIVVLFEIVLNGLRTYIFAHSTSRIDVELGARLFRHLLALPISYFENRRVGDTVARVR 256
Ouerv: 260 ELEOIRNFLTGOALTSVLDLAFSFIFLAVMWYYSSTLTWVVLASLPAYAFWSAFISPILR 319
           EL+QIRNFLTGQALTSVLDL FSFIF AVMWYYS LT V+L SLP Y WS FISPILR
Sbjct: 257 ELDQIRNFLTGQALTSVLDIMFSFIFFAVMWYYSPKLTLVILGSLPFYNGWSIFISPILR 316
Query: 320 TRLNDKFARNADNQSFLVESITAVGTVKAMAVEPQNTQRWDNQLAAYVASGFRVTKLAVV 379
            RL++KFAR ADNQSFLVES+TA+ T+KA+AV PQMT WD QLA+YV++GFRVT LA -
Sbjct: 317 RRLDEKFARGADNQSFLVESVTAINTIKALAVTPQMTNTWDKQLASYVSAGFRVTTLATI 376
Query: 380 GQQGVQLIQKLVTVATLWIGARLVIESKLTVGOLIAFNNLSGQVAAPVIRLAQLWQDFQQ 439
           GQQGVQ IQK+V V TLW+GA LVI L++GQLIAFNNLSGQV APVIRLAQLWQDFQQ
sbjct: 377 GQQGVQFIQKVVNVITLWLGAMLVISGDLSIGQLIAFNNLSGQVIAPVIRLAQLWQDFQQ 436
Query: 440 VGISVAPLGDILNAPTENASSHLALPDIRGEITFEHVDFRYKADGRLILQDLNLRIRAGE 499
```

Query: 440 VGISVAPLGDILNAPTENASSHLALPDIRGEITFEHVDFRYKADGRLILQDLNLRIRAGE 499 VGISV RLGD+LN+PTE+ LALP+I+G+ITF ++ FRYX D +IL D+NL I+ GE

		-continued	
Sbjct:	437	VGISVTRLGDVLNSPTESYQGKLALPEIKGDITFRNIRFRYXPDAPVILNDVNLSIQQGE	496
Quer y:	500	VLGIVGRSGSGKSTLTKLVQRLYVPAOGRVLVDGNDLALAAPAWLRRQVGVVLQENVLLN V+GIVGRSGSGKSTLTKL+QR Y+P G+VL+DG+DLALA P WLRRQVGVVLQ+NVLLN	559
Chiat.	107	VIGIVGRSGSGRSTLIKLIORFYIPENGOVLIDGHDLALADPNWLRRQVGVVLQHNVLLN	556
abjet:	497	A 101A0426264211141164411155M60AF1DGUDTWTWDEM#TWK0AGAAAF0DMAFFW	220
Quer y:	560	RSIRDNIALTDTGMPLERIIEAAKLAGAIIEFINELPEGYGTVVGEQGNLSGGQRQRIAI	619
		RSIRDNIAL D GMP+E+I+ AAKLAGAHEFI EL EGY T+VGEQGAGLSGGQRQRIAI	
Sbjct:	557	${\tt RSIRDNIALADPGMPMEKIV} HAAKLAGAHEFISELREGYNTIVGEOGAGLSGGQRQRIAI$	616
0	c		670
Query:	620	ARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTVLIIAHRLSTVKTAHRIIAM ARAL+ NP+ILIFDEATSALDYESE IM+NM IC RTV+IIAHRLSTVK A RII M	6/9
ah dari s	617		676
Sbjct:	617	ARALVNNPKILIFDEATSALDYESEHIIMRNMHQICKGRTVIIIAHRLSTVKNADRIIVM	676
Ouerv:	680	DKGRIVEAGTQQELLAKPNGYYRYLYDLQN 709	
~1 -		+KG+IVE G +ELLA PNG Y YL+ LQ+	
Sbjct:	677	EKGQIVEQGKHKELLADPNGLYHYLHQLQS 706	

[0364] Homology with the HlyB Leucotoxin Secretion ATP-Binding Protein of *Haemophilus actinomycetemcomitans* (Accession Number X53955)

[0365] ORF39 and HlyB protein show 71% and 69% amino acid identity in 167 and 55 overlap at the N- and C-terminal regions, respectively:

```
Orf39
        KFDFTWFTPAVTKYR+
                                        ITPLFFOVVMDKVLVHRGF
     137 KFDFTWFIPAVIKYRKIFIETLIVSIFLQIFALITPLFFQVVMDKVLVHRGFSTLNVITV 196
HlyB
      61 XXXXXXXFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLSLPLSYFEHRRVGDTVARVR 120
Orf39
                FEI+LGGLRTY+FAH+TSRIDVELGARLFMLL+LP+SYFE RRVGDTVARVR
HlyB 197 ALAIVVLFEIILGGLRTYVFAHSTSRIDVELGARLFRHLLALPISYFEARRVGDTVARVR 256
Orf39 121 ELEQIRNFLTGQALTSVLDLAFSFIFLAVMWYYSSTLTWVVLASLIC 167
         EL+QIRNFLTGQALTS+LDL FSFIF AVMWYYS LT VVL SL C
     257 ELDQIRNFLTGQALTSILDLLFSFIFFAVMWYYSPKLTLVVLGSLPC 303
HlyB
                                   11
Orf39 166 ICANRTVLIIAHRLSTVKTAHRIIAMDKGRIVEAGTQQELLANXNGYYRYLYDLQ 220
   IC
NRTV-
LII-
AHRL-
STVK A
RII
MDKG
I + E
G QELL
+ G Y
YL+ LQ
Hlyb 651 ICQNRTVLIIAHRLSTVKNADRIIVMDKGEIIEQGKHQELLKDEKGLYSYLHQLQ 705
```

[0366] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 7

[0367] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 31>

1 ATGAAATACT TGATCCGCAC CGCCTTACTC GCAGTCGCAG CCGCCGGCAT

51 CTACGCCTGC CAACCGCAAT CCGAAGCCGC AGTGCAAGTC AAGGCTGAAA

-continued 101 ACAGCCTGAC CGCTATGCGC TTAGCCGTCG CCGACAAACA GGCAGAGATT

151 GACGGGTTGA ACGCCCAAAk sGACGCCGAA ATCAGA...

[0368] This corresponds to the amino acid sequence SEQ ID 32; ORF52>:

1 MKYLIRTALL AVAAAGIYAC OPOSEAAVQV KAZNSLTANR LAVADKQAEI

51 DGLNAQXDAE IR..

[0369] Further work revealed the complete nucleotide sequence <SEQ ID 33>:

1 ATGAAATACT TGATCCGCAC CGCCTTACTC GCAGTCGCAG CCGCCGGCAT

51 CTACGCCTGC CAACCGCAAT CCGAAGCCGC AGTGCAAGTC AAGGCTGAAA

101 ACAGCCTGAC CGCTATGCGC TTAGCCGTCG CCGACAAACA GGCAGAGATT

151 GACGGGTTGA ACGCCCAAAT CGACGCCGAA ATCAGACAAC GCGAAGCCGA

201 AGAATTGAAA GACTACCGAT GGATACACGG CGACGCGGAA GTGCCGGAGC

251 TGGAAAAATG A

[0370] This corresponds to the amino acid sequence <SEQ ID 34; ORF52-1>:

1 <u>MKYLIRTALL AVAAAGIYA</u>C QPQSEAAVQV KAENSLTAMR LAVADKQAEI

51 DGLNAQIDAE IRQREAEELK DThWIHGDAE VPELEK

[0371] Computer analysis of this amino acid sequence predicts a prokaryotic membrane lipoprotein lipid attachment site (underlined).

[0372] ORF52-1 (7 kDa) was cloned in the pGex vectors and expressed in *E. coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. FIG. 4A shows the results of affinity purification of the GST-fusion. FIG. 4B shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF52-1. **[0373]** Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 8

[0374] The following DNA sequence was identified in *N. meningitidis* <SEQ ID 35>

1 ATGGTTATCG GAATATTACT CGCATCAAGC AAGCATGCTC TTGTCATTAC

51 TCTATTGTTA AATCCCGTCT TCCATGCATC CAGTTGCGTA TCGCGTTSGG

101 CAATACGGAA TAAAATCTGC TGTTCTGCTT TGGCTAAATT TGCCAAATTG

151 TTTATTGTTT CTTTAGGAGC AGCTTGCTTA GCCGCCTTCG CTTTCGACAA

201 CGCCCCACA GGCGCTTCCC AAGCGTTGCC TTCCGTTACC GCACCCGTGG

251 CGATTCCCGC GCCCGCTTCG GCAGCCTGA

[0375] This corresponds to the amino acid sequence <SEQ ID 36; ORF56>:

1 MVIGILLASS KHALVITLLL NPVFHASSCV SRXAIRNKIC CSALAKFAKL

51 <u>FIVSLG</u>AACL AAFAFDNAPT GASQALPTVT APVAIPAPAS AA*

[0376] Further work revealed the complete nucleotide sequence <SEQ ID 37>:

1 ATGGCTTGTA CAGGTTTGAT GGTTTTTCCG TTAATGGTYA TCGGAATATT

51 ACTTGCATCA AGCAAGCCTG CTCCTTTCCT TACTCTATTG TTAAATCCCG

101 TCTTCCATGC ATCCAGTTGC GTATCGCGTT GGGCAATACG GAATAAAATC

151 TGCTGTTCTG CTTTGGCTAA ATTTGCCAAA TTGTTTATTG TTTCTTTAGG

201 AGCAGCTTGC TTAGCCGCCT TCGCTTTCGA CAACGCCCCC ACAGGCGCTT

251 CCCAAGCGTT GCCTACCGTT ACCGCACCCG TGGCGATTCC CGCGCCCGCT

301 TCGGCAGCCT GA

[0377] This corresponds to the amino acid sequence <SEQ ID 38; ORF56-1>:

- 1 MACTGLMVFP LNVZGILLAS SKPAPFLTLL LNPVFHASSC VSRWAIRNKI
- 51 CCSALAKFAK LFIVSLGAAC LAAFAFDNAP TGASQALPTV TAPVAIPAPA
- 101 SAA*

[0378] Computer analysis of this amino acid sequence predicts a leader peptide (underlined) and suggests that ORF56 might be a membrane or periplasmic protein.

[0379] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 9

[0380] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 39>

1 ATGTTCAGTA TTTTAAATGT GTTTCTTCAT TGTATTCTGG CTTGTGTAGT

51 CTCTGGTGAG ACGCCTACTA TATTTGGTAT CCTTGCTCTT TTTTACTTAT

101 TGTATCTTTC TTATCTTGCT GTTTTTAAGA TTTTCTTTC TTTTTCTTA

151 GACAGAGTTT CACTCCGGTC TCCCAGGCTG GAGTGCAAAT GGCATGACCC

201 TTTGGCTCAC TGGCTCACGG CCACTTCTGC TATTCTGCCG CCTCAGCCTC

251 CAGGG...

[0381] This corresponds to the amino acid sequence <SEQ ID 40; ORF63>:

1 MFSILNVFLR CILACVVSGE TPTIFGILAL FYLLYLSYLA VFKIFFSFFL

51 DRVSLRSPRL ECKWNDPLAH WLTATSAILP PQPPG...

[0382] Computer analysis of this amino acid sequence predicts a transmembrane region.

[0383] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 10

[0384] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 41>

1...GTGCGGACGT GGTTGGTTTT TTGGTTGCAG CGTTTGAAAT ACCCGTTGTT

51 GCTTTGGATT GCGGATATGT TGCTGTACCG GTTGTTGGGC GGCGCGGAAA

101 TCGAATGCGG CCGTTGCCCT GTGCCGCCGA TGACGGATTG GCAGCATTTT

151 TTGCCGGCGA TGGGAACGGT GTCGGCTTGG GTGGCGGTGA TTTGGGCATA

201 CCTGATGATT GAAAGTGAAA AAAACGGAAG ATATTGA

[0385] This corresponds to the amino acid sequence <SEQ ID 42; ORF69>:

1 ..VRTWLVFWLQ RLKYPLLLWI ADNLLYRLLG GAE1ECGRCP VPPMTDWQHF

51 LPANGTVSAW VAVIWAYLMI ESEKNGRY*

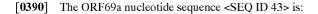
[0386] Computer analysis of this amino acid sequence predicts a transmembrane region.

[0387] A corresponding ORF from strain A of *N. menin-gitidis* was also identified:

[0388] Homology with a Predicted ORF from *N. menin-gitidis* (Strain A)

[0389] ORF69 shows 96.2% identity over a 78 as overlap with an ORF (ORF69a) from strain A of *N. meningitidis*:





1 GTGCGGACGT GGTTGGTTTT TTGGTTGCAG CGTTTGAAAT ACCCGTTGTT

51 GCTTTGTATT GCGGATATGC TGCTGTACCG GTTGTTGGGC GGCGCGGAAA

101 TCGAATGCGG CCGTTGCCCT GTACCGCCGA TGACGGATTG GCAGCATTTT

151 TTGCCGACGA TGGGAACGGT GGCGGCTTGG GTGGCGGTGA TTTGGGCATA

201 CCTGATGATT GAAAGTGAAA AAAACGGAAG ATATTGA

[0391] This encodes a protein having amino acid sequence <SEQ ID 44>:

1 VRTWLVFWLQ RLKYPLLLCI ADMLLYRLLG GAEIECGRCP VPPNTDWQHF

51 LPTMGTVAAW VAVIWAYLMI ESEKNGRY*

[0392] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 11

[0393] The following DNA sequence was identified in *N. meningitidis* <SEQ ID 45>

1ATGTTTCAAAATTTTGATTGGCGTGTTCCTGCTTGCCGTCCTCCCCGT51GCTGCCCTCATTACCGTCCGCACGTGCGCGCGCTATACGGCGCGCT101ACTGGGGAGACAACACTGCGAACAATACGGCAGGCTGACACTGAACCCC151CTGCCCCATATCGATTGGTCGGCACAATCACTGTACCGTGCTTACTTT201GATGTTCACGCCCTTCCTGTTCGGCTGGCGCGTCCGATTCCTATCGATT251CGCGCAACTTCCGCAACCCGCGCTGCCTGGCGTGCGGTGCTCACGGTG301GGCCCGCTGTCGAATCTACGGATGCGTGTGTGGTTTGGT351GCTGACTCGTATGTCGACGGGCGTATCAGATGCCGTT401CAAACTACGGTATTCTGATCAATGCGATTCATGCACACT451CCCATCCTGCCTTGGGACGGCGGCATTTCATGCACACT501GAAATATCGCAAGCGTCCSGGGTTTGGGTCCTTAT601aTGCGGMTGCGTGATTGCTTTGTGCAGATGTGCTTCA651GACGGCATAAGACGATAAGGCGATAAGTGCGTTAA

[0394] This corresponds to the amino acid sequence <SEQ ID 46; ORF77>:

1 <u>MFONFDLGVF LLAVLPVLPS ITVSNVA</u>RGY TARYWGDNTA EQYGRLTLNP 51 LP<u>HIDLVGTI IVPLLTLMF</u>T PFLFGWPRPI PIDSRNFRNP RLAWRCVAAS 101 GP<u>LSNLAMAV LWGVVLVLT</u>P YVGGAYQMPL A<u>QMANYGILI NAILFPLNII</u> 151 PILPWDGGIF IDTFLSAKYS QAFRKIEPY<u>G TWIILLLMLT XVLGAF</u>IAPI 201 XRXRDCXCAD VRLTGFQTA*

[0395] Further work revealed the complete nucleotide sequence <SEQ ID 47>:

1 ATGTTTCAAA ATTTTGATTT GGGCGTGTTT CTGCTTGCCG TCCTGCCCGT

51 GCTGCTCTCC ATTACCGTCA GGGAGGTGGC GCGCGGCTAT ACGGCGCGCGC

101 ACTGGGGAGA CAACACTGCC GAACAATACG GCAGGCTGAC ACTGAACCCC

151CTGCCCCATA TCGATTGGTCGGCACAATCATCGTACCGCTGCTTACTTT201GATGTTCACGCCCTTCCTGTCGGCTGGCGCGTCCGATCCTATCGATT251GGCCGCAGTCGGAACCTGGATGCCGTGTGGCGTGGCGTGGCTGGCGTG301GGCCGCCTGTCGAATCTAGCGATGCCGTTCTGTGGGGCGGGCTTTGGT351GCTGACTCCGTATGTCGGCGGGGCGTATCAGATGCCGTTGCAACATCAGG401CAAACTACGGTATTCTGATCAATGCGATCTGTTCGCGCTCAACATCAGC451CCCATCCTGCCTTGGGACGGCGGCATTTCATCGAACCTTCCTGTCGGC501GAAATATCGCAAGCTGACCGGGGTTTGGGTGCGTTAATGCACCGATT601GTGCGCTGGTGATTGCGTTTGTGCAGAGGTCCTCTGA

[0396] This corresponds to the amino acid sequence <SEQ ID 48; ORF77-1>:

1 MFONFDLGVF LLAVLPVLLS ITVREVARGY TARYWGDNTA EQYGRLTLNP

51 <u>LPHIDLVGTI IV</u>PLLTLMFT PFLFGWARPI PIDSRNFRNP RLAWRCVAAS

101 GPLSNLAMAV LWGVVLVLTP YVGGAYQMPL AQMANYGILI NAILFALNII

151 <u>PIL</u>PWDGGIF IDTFLSAXYS QAYRRIEPY<u>G TWIILLLNLT GVLGAF</u>IAPI

201 VRLVIAFVQH FV*

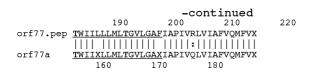
[0397] Computer analysis of this amino acid sequence reveals a putative leader sequence and several transmembrane domains.

[0398] A corresponding ORF from strain A of *N. menin-gitidis* was also identified:

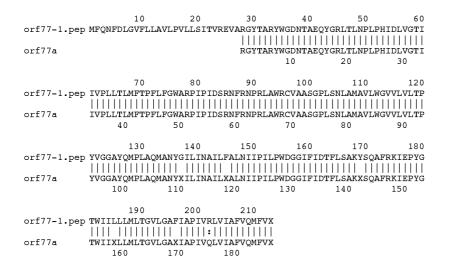
[0399] Homology with a Predicted ORF from *N. menin-gitidis* (Strain A)

[0400] ORF77 shows 96.5% identity over a 173 as overlap with an ORF (ORF77a) from strain A of *N. meningitidis:*

	10	20	30	40	50	60
ort77.pep	MFQNFDLGVFLI	AVLPVLPSIT	SHVARGYTAR	YWGDNTAEQ	YGR <u>LTLNPLPH</u>	IDLVGTI
orf77a			RGYTAR	WGDNTAEQ	YGR <u>LTLNPLPH</u>	IDLVGTI
				10	20	30
	70	80	90	100	110	120
orf77.pep	<u>IV</u> PLLTLMFTPF	LFGWARPIPII	OSRNFRNPRLA	WRCVAASGPI	LSN <u>LAMAVLWG</u>	VVLVLTP
orf77a	<u>IV</u> PLLTLMFTPF	LFGWARPIPI	OSRNFRNPRLAN	WRCVAASGPI	LSN <u>LAMAVLWG</u>	VVLVLTP
	40	50	60	70	80	90
	130	140	150	160	170	180
orf77.pep	<u>YV</u> GGAYQMPLAQ	MANYGILINA	ILFALNIIPIL	WDGGIFID	FFLSAKYSQAF	RKIEPY <u>G</u>
orf77a	<u>YV</u> GGAYQMPLAQ	MANYXILINA	ILXALNIIPIL	WDGGIFID	FFLSAKXSQAF	RKIEPY <u>G</u>
	100	110	120	130	140	150



[0401] ORF77-1 and ORF77a show 96.8% identity in 185 aa overlap:



[0402] A partial ORF77a nucleotide sequence <SEQ ID 49> was identified:

1..CGCGGCTATA CAGCGCGCTA CTGGGGTGAC AACACTGCCG AACAATACGG 51 CAGGCTGACA CTGAACCCCC TGCCCCATAT CGATTTGGTC GGCACAATCA 101 TCGTACCGCT GCTTACTTTG ATGTTTACGC CCTTCCTGTT CGGCTGGGCG CGTCCGATTC CTATCGATTC GCGCAACTTC CGCAACCCGC GCCTTGCCTG 151 201 GCGTTGCGTT GCCGCGTCCG GCCCGCTGTC GAATCTGGCG ATGGCTGTTC 251 TGTGGGGCGT GGTTTTGGTG CTGACTCCGT ATGTCGGTGG GGCGTATCAG ATGCCGTTGG CNCAAATGGC AAACTACNNN ATTCTGATCA ATGCGATTCT 301 GTNCGCGCTC AACATCATCC CCATCCTGCC TTGGGACGGC GGCATTTTCA 351 401 TCGACACCTT CCTGTCGGCN AAATANTCGC AAGCGTTCCG CAAAATCGAA 451 CCTTATGGGA CGTGGATTAT CCNGCTGCTT ATGCTGACCG GGGTTTTGGG 501 TGCGTNTATT GCACCGATTG TGCAGCTGGT GATTGCGTTT GTGCAGATGT 551 TCGTCTGA

[0403] This encodes a protein having amino acid sequence <SEQ ID 50>:

1 ...RGYTARYWGD NTAEQYGR<u>LT LNPLPHIDLV GTIIV</u>PLLTL MFTPFLFGWA

51 RPIPIDSRNF RNPRLAWRCV AASGFLSN<u>LA MAVLWGVVLV LTPYV</u>GGAYQ

101 MPLAQNANYX ILINAILXAL NIIPILPWDG GIFIDTFLSA KXSQAFRKIE

151 PY<u>GTWIIXLL MLTGVLGAX</u>I APIVQLVIAF VQNFV*

[0404] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 12

[0405] The following partial DNA sequence was identified in *N. meningitidis* SEQ ID 51>

1 ATGAACCTGA TTTCACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT

51TTACGCGCTCCTTGCCTTCCTCGCTTTGTACAGCTTTTTGAAATCCTGT101ACGAAACCGGCAACCTCGGCAAAGGCAGTTACGGCATATGGGAAATGCTG151GGCTACAACGCCCTCAAAAATGCCCGCCCGCGCCTACGAACTGATTCCCCT201CGCCGTCCTTATCGGCGGACTGGTCTCCCTCAGCCAGCCTGCCGCCGGCA251GCGAACTGACCGTCATCAAAGCCAGCGGCATGAGCACCAAAAAGCTGCTG301TTGATTCTGTCGCAGTCGGTTTTATTTTGCTATTGCCACCGTCGCGCT351CGGCGAATGGGTTGCGCCCACACTGAGCCAAAAAGCCGAAAACATCAAAG401CCGCCGCCATCAACGGCAAAATCAACACCGGCAATACCGGCCTTTGGCTG451AAAGAAAAAACAGCGTGATCAATGTGCGCGAAATGTTGCCCGACCAT.

[0406] This corresponds to the amino acid sequence SEQ ID 52; ORF112>:

- 1 HNLISRYIIR QMAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEML
- 51 GYTALIQPAR AYE<u>LIPLAVL IGGLVSLSOL</u> AAGSELTVIK ASGNSTKK<u>LL</u>
- 101 <u>LILSOFGFIF AIATV</u>PLGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL
- 151 KEKNSVINVR EHLPDH...

[0407] Further work revealed further partial nucleotide sequence <SEQ ID 53>:

ATGAACCTGATTTCACGTTACATCATCCGTCAAATGGCGGTTATGGCGGT51TTACGCGCCCCTTGCCTTCCTCGCTTTGTACAGCTTTTTGAAATCCTGT101ACGAAACCGGCAACCTCGGCAAAGGCAGTTACGGCATATGGGAAATGCTG151GGCTACACCGCCCTCAAAAATGCCCGCCCGCGCCTACGAACTGATTCCCCT201CGCCGTCCTTATCGGCGGCATGGTCTCCCTCAGCCAGCTAGCCGCCGCCA251GCGAACTGACCGTCATCAAAGCCAGCGGCATGAGCACCAAAAAGCTGCTG301TTGATTCTGTCGCAGTCCGCACTGAGCCAAAAAGCCGAAAACATCAAAG401CCGCCGCCATCAACGGCAAAATCAGCACCGGCAATACCGGCCTTGCTCTG

451 AAAGAAAAA ACAGCTTAAT CAATGTGCCC GAAATGTTGC CCGACCATAC
501 GCTTTTGGCC ATCAAAATT GGGCGCGCAA CGATAAAAC GAATGGCGAG
551 AGGCAGTGGA AGCCGATTCC GCCGTTTGA ACAGCGACGG CAGTGGCAG
601 TTGAAAAACA TCCGCCGCAG CACGCTTGCC GAAGACAAAG TCGAGGTCTC
651 TATTGCGGCT GAAGAAACT GGCCGATTC CGTCAAACC ACCGCAGAG
701 ACGTATTGCT CGTCAAACC GACCAAATGT CCGTCGGCGA ACTGACCACC
751 TACATCCGCC ACCTCCAAAA CAACAGCCAA AACACCCGAA TCTACGCCAG
601 CGCATGGTGG CGCAAATGG TTTACCCCGC CGCAGCCTGG CAATAGGGCG
851 TCGTCGCCTT TGCCTTTACC CCGCAAACCA CCCGCCACGG AATAGGGCG
901 TTAAAACTC TCGGCGGCAT CTGTSTCGGA TTGCTGTCC ACCTGCCGG
951 ACGGCTCTTT GGGTTTACCA GCCAACTGG...

[0408] This corresponds to the amino acid sequence <SEQ ID 54; ORF112-1>:

1 MNLISRYIIR QMAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEHL

51 GYTALKMPAR AYELIPLAVL IGGLVSLSQL AAGSELTVIK ASGMSTKKLL

101 <u>LILSOFGFIF AIATV</u>ALGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL

151 KEKNSXINVR EHLPDHTLLG IKIWARNDKN ELAEAVEADS AVLNSDGSWQ

201 LKNIRRSTLG EDKVEVSIAA EENWPISVKR NLTDVLLVKP DQMSVGELTT

251 YIRHLONNSQ NTRIYAIAWW RKLVYPAAAW VMALVAFAFT PQTTRHGNMG

301 <u>LKLFGGICXG LLFHL</u>AGRLF GFTSQL...

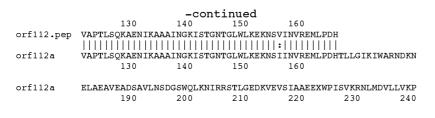
[0409] Computer analysis of this amino acid sequence predicts two transmembrane domains.

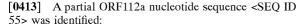
[0410] A corresponding ORF from strain A of *N. menin-gitidis* was also identified:

[0411] Homology with a Predicted ORF from *N. meningitidis* (Strain A)

[0412] ORF112 shows 96.4% identity over a 166 aa overlap with an ORF (ORF112a) from strain A of N. *meningitidis*:

	10	20	30	40	50	60
orf112.pep	MNLISRYIIRQMAVM	AVYALLAFL	ALYSFFEILY	ETGNLGKGSY	GIWEMLGYTA	LKMPAR
orf112a	MNLISRYIIRQMAVM	AVYALLAFL	ALYSFFEILY	ETGNLGKGSY	GIWEMXGYTA	LKMXAR
	10	20	30	40	50	60
	70	80	90	100	110	120
orf112.pep	AYELIPLAVLIGGLV	SLSQLAAGS	ELTVIKASGM	STKKLLLILS	QFGFIFAIAT	VALGEW
	:	$[\ []]] []] []]$:			
orf112a	AYELMPLAVLIGGLV	SXSQLAAGS	ELTVIKASGM	SXKKLLLILS	QFGFIFAIAT	VALGEW
	70	80	90	100	110	120





1 ATGAACCTGA TTTCACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT 51 TTACGCGCTC CTTGCCTTCC TCGCTTTGTA CAGCTTTTTT GAAATCCTGT 101 ACGAAACCGG CAACCTCGGC AAAGGCAGTT ACGGCATATG GGAAATGNTG 151 GGNTACACCG CCCTCAAAAT GNCCGCCCGC GCCTACGAAC TGATGCCCCT 201 CGCCGTCCTT ATCGGCGGAC TGGTCTCTNT CAGCCAGCTT GCCGCCGGCA 251 GCGAACTGAN CGTCATCAAA GCCAGCGGCA TGAGCACCAA AAAGCTGCTG 301 TTGATTCTGT CGCAGTTCGG TTTTATTTTT GCTATTGCCA CCGTCGCGCT 351 CGGCGAATGG GTTGCGCCCA CACTGAGCCA AAAAGCCGAA AACATCAAAG 401 CCGCGGCCAT CAACGGCAAA ATCAGTACCG GCAATACCGG CCTTTGGCTG 451 AAAGAAAAAA ACAGCATTAT CAATGTGCGC GAAATGTTGC CCGACCATAC 501 CCTGCTGGGC ATTAAAATCT GGGCCCGCAA CGATAAAAAC GAACTGGCAG 551 AGGCAGTGGA AGCCGATTCC GCCGTTTTGA ACAGCGACGG CAGTTGGCAG 601 TTGAAAAACA TCCGCCGCAG CACGCTTGGC GAAGACAAAG TCGAGGTCTC 651 TATTGCGGCT GAAGAAAANT GGCCGATTTC CGTCAAACGC AACCTGATGG 701 ACGTATTGCT CGTCAAACCC GACCAAATGT CCGTCGGCGA ACTGACCACC 751 TACATCCGCC ACCTCCAAAN NNACAGCCAA AACACCCGAA TCTACGCCAT 801 CGCATGGTGG CGCAAATTGG TTTACCCCGC CGCAGCCTGG GTGATGGCGC 851 TCGTCGCCTT TGCCTTTACC CCGCAAACCA CCCGCCACGG CAATATGGGC 901 TTAAAANTCT TCGGCGGCAT CTGTCTCGGP TTGCTGTTCC ACCTTGCCGG 951 NCGGCTCTTC NGGTTTACCA GCCAACTCTA CGGCATCCCG CCCTTCCTCG 1001 NCGGCGCACT ACCTACCATA GCCTTCGCCT TGCTCGCCGT TTGGCTGATA 1051 CGCAAACAGG AAAAACGCTA A

[0414] This encodes a protein having amino acid sequence <SEQ ID 56>:

 1
 MNLISRYIIR OMAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEMK

 51
 GYTALKMXAR AYELMPLAVL IGGLVSXSOL
 AAGSELXVIX
 ASGNSTKKLL

 101
 LILSOFGFIF AIATVALGEV
 VAPTLSQKAE
 NIKAAAINGK
 ISTGNTGLWL

 151
 KEKNSIINVR
 EMLPDHTLLG
 IKIWAIWDKN
 ELAEAVFADS
 AVLNSDGSWQ

 201
 LKNIRRSTLG
 EDKVEVSIAA
 EEXWPISVKR
 NLMDVLLVKP
 DQMSVGELTT

 251
 YIRHLQXXSQ
 NTRIYAIAWW
 RKLVYPAAAW
 VMALVAFAFT
 PQTTRHGNMG

301 <u>LKXFGGICLG LLFHL</u>AGRLF XFTSQLYGIP PFLXGALPTI AFALLAVWLI

351 RKQEKR*

[0415] ORF112a and ORF112-1 show 96.3% identity in 326 aa overlap:

orf112a.pep	MNLISRYIIRQMAVMAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMXGYTALKMXAR
orf112-1	MNLISRYIIRQMAVMAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMLGYTALKMXAR
orf112a.pep	AYEIIPLAVLIGGLVSXSQLAAGSELXVIKASGHSTKKLLLILSQFGFIFAIATVALGEW
orf112-1	AYEIIPLAVLIGGLVSLSQLAAGSELTVIKASGHSTKKLLLILSQFGFIFAIATVALGEW
orf112a.pep	VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSIINVREMLPDHTLLGIKIWARNDKN
orf112-1	VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSXINVREMLPDHTLLGIKIWARNDKN
orf112a.pep	ELAEAVEADSAVLNSDGSWQLKNIRRSTLGEDKVEVSIAAEEXWPISVKRNLMDVLLVKP
orf112-1	ELAEAVEADSAVLNSDGSWQLKNIRRSTLGEDKVEVSIAAEENWPISVKRNLMDVLLVKP
orf112a.pep	DQMSVGELTTYIRHLQXXSQNTRIYAIAWWRKLVYPAAAWVMALVAFAFTPQTTRHGNMG
orf112-1	DQMSVGELTTYIRHLQXXSQNTRIYAIAWWRKLVYPAAAWVMALVAFAFTPQTTRHGNMG
orf112a.pep	LKXFGGICLGLLFHLAGRLFXFTSQLYGIPPFLXGALPTIAFALLAVWLIRKQEXRX
orf112-1	

[0416] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 13

[0417] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 57>

1..GCAGTAGCCG AAACTGCCAA CAGCCAGGGC AAAGGTAAAC AGGCAGGCAG

51	TTCGGTTTCT	GTTTCACTGA	AAACTTCAGG	CGACCTTTGC	GGCAAACTCA
101	AAACCACCCT	TAAAACTTTG	GTCTGCTCTT	TGGTTTCCCT	GAGTATGGTA
151	TTGCCTGCCC	ATGCCCAAAT	TACCACCGAC	AAATCACCAC	CTAAAAACCA
201	GCAGGTCGTT	ATCCTTAAAA	CCAACACTGG	TGCCCCCTTG	GTGAATATCC
251	AAACTCCGAA	TGGACGCGGA	TTGAGCCACA	ACCGCTA.TA	CGCATTTGAT
301	GTTGACAACA	AAGGGGCAGT	GTTAAACAAC	GACCGTAACA	ATAATCCGTT
351	TGTGGTCAAA	GGCAGTGCGC	AATTGATTTT	GAACGAGGTA	CGCGGTACGG
401	CTAGCAAACT	CAACGGCATC	GTTACCGTAG	GCGGTCAAAA	GGCCGACGTG
451	ATTATTGCCA	ACCCCAACGG	CATTACCGTT	AATGGCGGCG	GCTTTAAAAA
501	TGTCGGTCGG	GGCATCTTAA	CTACCGGTGC	GCCCCAAATC	GGCAAAGACG
551	GTGCACTGAC	AGGATTTGAT	GTGCGTCAAG	GCACATTGGA	CCGTAGTAGC
601	AGCAGGTTGG	AATGATAAAG	GCGGAGCmrm	YTACACCGGG	GTACTTGCTC
651	GTGCAGTTGC	TTTGCAGGGG	AAATTwmmGG	GTAAA.AACT	GGCGGTTTCT

-continued 701 ACCGGTCCTC AGAAAGTAGA TTACGCCAGC GGCGAAATCA GTGCAGGTAC

751 GGCAGCGGGT ACGAAACCGA CTATTGCCCT TGATACTGCC GCACTGGGCG

901 GTATGTACGC CGACAGCATC ACACTGATTG CCAATGAAAA AGGCGTAGGC

951 GTCTAA

[0418] This corresponds to the amino acid sequence <SEQ ID 58; ORF114>:

1...AVAETANSQG KGKQAGSSVS VSLKTSGDLC GKLKTT<u>LKTL VCSLVSLSHV</u>

51 LPAHAQITTD KSAPKNQQVV ILKTNTGAPL VNIQTPNGRG LSHNRXYAFD
 101 VDNKGAVLNN DRNNNPFVVK GSAQLILNEV RGTASKLNGI VTVGGQKADV
 151 IIANPNGITV NGGGFXNVGR GILTTGAPQI GKDGALTGFD VVKAHVTVXA
 201 AGWNDKGGAX YTGVLAPAVA LQGKXXGLXL AVSTGPQKVD YASGEISAGT

251 AAGTKPTIAL DTAALGGNYA DSITLIANEX GVGV*

[0419] Further work revealed the complete nucleotide sequence SEQ ID 59>:

1 ATGAATAAAG GTTTACATCG CATTATCTTT AGTAAAAAGC ACAGCACCAT 51 GGTTGCAGTA GCCGAAACTG CCAACAGCCA GGGCAAAGGT AAACAGGCAG 101 GCAGTTCGGT TTCTGTTTCA CTGAAAACTT CAGGCGACCT TTGCGGCAAA 151 CTCAAAACCA CCCTTAAAAC TTTGGTCTGC TTTTTGGTTT CCCTGAGTAT 201 GGTATTGCCT GCCCATGCCC AAATTACCAC CGACAAATCA GCACCTAAAA 251 ACCAGCAGGT CGTTATCCTT AAAACCAACA CTGGTGCCCC CTTGGTGAAT 301 ATCCAAACTC CGAATGGACG CGGATTGAGC CACAACCGCT ATACGCAGTT 351 TGATGTTGAC AACAAAGGGG CAGTGTTAAA CAACGACCGT AACAATAATC 401 CGTTTGTGGT CAAAGGCAGT GCGCAATTGA TTTTGAACGA GGTACGCGGT 451 ACGGCTAGCA AACTCAACGG CATCGTTACC GTAGGCGGTC AAAAGGCCGA 501 CGTGATTATT GCCAACCCCA ACGGCATTAC CGTTAATGGC GGCGGCTTTA 551 AAAATGTCGG TCGGGGCATC TTAACTACCG GTGCGCCCCA AATCGGCAAA 601 GACGGTGCAC TGACAGGATT TGATGTGCGT CAAGGCACAT TGACCGTAGG 651 AGCAGCAGGT TGGAATGATA AAGGCGGAGC CGACTACACC GGGGTACTTG 701 CTCGTGCAGT TGCTTTGCAG GGGAAATTAC AGGGTAAAAA CCTGGCGGTT 751 TCThCCGGTC CTCAGAAAGT AGATTACGCC AGCGGCGAAA TCAGTGCAGG 801 TACGGCAGCG GGTACGAAAC CGACTATTGC CCTTGATACT GCCGCACTGG 951 GCGGTATGTA CGCCGACACC ATCACACTGA TTGCCAATGA AAAAGGCGTA 901 GGCGTCAAAA ATGCCGGCAC ACTCGAAGCG GCCAAGCAAT TGATTGTGAC 951 TTCGTCAGGC CGCATTGAAA ACAGCGGCCG CATCGCCACC ACTGCCGACG 1001 GCACCGAAGC TTCACCGACT TATCTCTCCA TCGAAAACCAC CGAAAAAGGA 1051 GCGGCAGGCA CATTTATCTC CAATGGTGGT CGGATCGAGA GCAAAGGCTT -continued 1101 ATTGGTTATT GAGACGGGAG AAGATATCAG CTTGCGTAAC GGAGCCGTGG 1151 TGCAGAATAA CGGCAGTCTC CCAGCTACCA CGGTATTAAA TGCTGGTCAT 1201 AATTTGGTGA TTGAGAGCAA AACTAATGTG AACAATGCCA AAGGCCCGGC 1251 TACTCTGTCG GCCGACGGCC GTACCGTCAT CAAGGAGGCC AGTATTCAGA 1301 CTGGCACTAC CGTATACAGT TCCAGCAAAG GCAACGCCGA ATTAGGCAAT 1351 AACACACGCA TTACCGGGGC AGATGTTACC GTATTATCCA ACGGCACCAT 1401 CAGCAGTTCC GCCGTAATAG ATGCCAAAGA CACCGCACAC ATCGAAGCAG 1451 GCAAACCGCT TTCTTTGGAA GCTTCAACAG TTACCTCCGA TATCCGCTTA 1501 AACGGAGGCA GTATCAAGGG CGGCAAGCAG CTTGCTTTAC TGGCAGACGA 1551 TAACATTACT GCCAAAACTA CCAATCTGAA TACTCCCGGC AATCTGTATG 1601 TTCATACAGG TAAAGATCTG AATTIGAATG TTGATAAAGA TTTGTCIGCC 1651 GCCAGCATCC ATTTGAAATC GGATAACGCT GCCCATATTA CCGGCACCAG 1701 TAAAACCCTC ACTGCCTCAA AAGACATGGG TGTGGAGGCA GGCTCGCTGA 1751 ATGTTACCAA TACCAATCTG CGTACCAACT CGGGTAATCT GCACATTCAG 1801 GCAGCCAAAG GCAATATTCA GCTTCGCAAT ACCAAGCTGA ACGCAGCCAA 1851 GGCTCTCGAA ACCACCGCAT TGCAGGGCAA TATCGTTTCA GACGGCCTTC 1901 ATGCTGTTTC TGCAGACGGT CATGTATCCT TATCGGCCAA CGGTAATGCC 1951 GACTTTACCG GTCACAATAC CCTGACAGCC AAGGCCGATG TCAATGCAGG 2001 ATCGGTTGGT AAAGGCCGTC TGAAAGCAGA CAATACCAAT ATCACTTCAT 2051 CTTCAGGAGA TATTACGTTG GTTGCCGGCA ACGGTATTCA GCTTGGTGAC 2101 GGAAAACAAC GCAATTCAAT CAACGGAAAA CACATCAGCA TCAAAAACAA 2151 CGGTGGTAAT GCCGACTTAA AAAACCTTAA CGTCCATGCC AAAAGCGGGG 2201 CATTGAACAT TCATTCCGAC CGGGCATTGA GCATAGAAAA TACCAAGCTG 2251 GAGTCTACCC ATAATACGCA TCTTAATGCA CAACACGAAG GGGTAACGCT 2301 CAACCAAGTA GATGCCTACG CACACCGTCA TCTAAGCATT ACCGGCAGCC 2351 AGATTTGGCA AAACGACAAA CTGCCTTCTG CCAACAAGCT GGTGGCTAAC 2401 GGTGTATTGG CACTCAATGC GCGCTATTCC CAAATTGCCG ACAACACCAC 2451 GCTGAGAGCG GGTGCAATCA ACCTTATTGC CGGTACCGCC CTAGTCAAGC 2501 GCGGCAACAT CAATTGGAGT ACCGTTGCGA CCAAAACTTT GGAAGATAAT 2551 GCCGAATTAA AACCATTGGC CGGACGGCTG AATATTGAAG CAGGTAGCGG 2601 CACATTAACC ATCGAACCTG CCAACCGCAT CAGTGCGCAT ACCGACCTGA 2651 GCATCAAAAC AGGCGGAAAA TTGCTGTTGT CTGCAAAAGG AGGAAATGCA 2701 GGTGCGCCTA GTGCTCAAGT TTCCTCATTG GAAGCAAAAG GCAATATCCG 2751 TCTGGTTACA GGAGAAACAG ATTTAAGAGG TTCTAAAATT ACAGCCGGTA 2801 AAAACTTGGT TGTCGCCACC ACCAAAGGCA AGTTGAATAT CGAAGCCGTA 2951 AACAACTCAT TCAGCAATTA TTTTCCTACA CAAAAAGCGG CTGAACTCAA 2901 CCAAAAATCC AAAGAATTGG AACAGCAGAT TGCGCAGTTG AAAAAAAGCT

2951 CGCCTAAAAG CAAGCTGATT CCAACCCTGC AAGAAGAACG CGACCGTCTC 3001 GCTTTCTATA TTCAAGCCAT CAACAAGGAA GTTAAAGGTA AAAAACCCAA

3051 AGGCAAAGAA TACCTGCAAG CCAAGCTTTC TGCACAAAAT ATTGACTTGA 3101 TTTCCGCACA AGGCATCGAA ATCAGCGGTT CCGATATTAC CGCTTCCAAA 3151 AAACTGAACC TTCACGCCGC AGGCGTATTG CCAAAGGCAG CAGATTCAGA 3201 GGCGGCTGCT ATTCTGATTG ACGGCATAAC CGACCAATAT GAAATTGGCA 3251 AGCCCACCTA CAAGAGTCAC TACGACAAAG CTGCTCTGAA CAAGCCTTCA 3301 CGTTTGACCG GACGTACAGG GGTAAGTATT CATGCAGCTG CGGCACTCGA 3351 TGATGCACGT ATTATTATCG GTGCATCCGA AATCAAAGCT CCCTCAGGCA 3401 GCATAGACAT CAAAGCCCAT AGTGATATTG TACTGGAGGC TGGACAAAAC 3451 GATGCCTATA CCTTCTTAAA AACCAAAGGT AAAAGCGGCA AAATCATCAG 3501 AAAAACCAAG TTTACCAGCA CCCGCGACCA CCTGATTATG CCAGCCCCCG 3551 TCGAGCTGAC CGCCAACGGC ATAACGCTTC AGGCAGGCGG CAACATCGAA 3601 GCTAATACCA CCCGCTTCAA TGCCCCTGCA GGTAAAGTTA CCCTGGTTGC 3651 GGGTGAAGAG CTGCAACTGC TGGCAGAASA AGGCATCCAC AAGCACGAGT 3701 TGGATGTCCA AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGCAAGAGC 3751 AATTACAGTA AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT 3801 CGCCCAAACT GCAGCCACCC GTTCAGGCTG GGATACAGTG CTCGAAGGTA 3051 CCGAATTCAA AACCACGCTG GCCGGTGCGG ACATTCAGGC AGGTGTAGGC 3901 GAAAAAGCCC GTGCCGATGC GAAAATTATC CTCAAAGGCA TTGTGAACCG 3951 TATCCAGTCG GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC 4001 AGGCCGGACG CGGCAGCACT ATCGAAACGC TGAAACTGCC CAGCTTCGAA 4051 AGCCCTACTC CGCCCAAACT GACCGCCCCC GGTGGCTATA TCGTCGACAT 4101 TCCGAAAGGC AATTTGAAAA CCGAAATCGA AAAGCTGGCC AAACAGCCCG 4151 AGTATGCCTA TCTGAAACAG CTCCAAGTAG CGAAAAACGT CAACTGGAAC 4201 CAGGTGCAAC TGGCTTACGA TAAATGGGAC TATAAGCAGG AAGGCTTAAC 4251 CAGAGCCGGT GCAGCGATTG TTACCATAAT CGTAACCGCA CTGACTTATG 4301 GATACGGCGC AACCGCAGCG GGCGGTGTAG CCGCTTCAGG AAGTAGTACA 4351 GCCGCAGCTG CCGGAACAGC CGCCACAACG ACAGCAGCAG CTACTACCGT 4401 TTCTACAGCG ACTGCCATGC AAACCGCTGC TTTAGCCTCC TTGTATAGCC 4451 AAGCAGCTGT ATCCATCATC AATAATAAAG GTGATGTCGG CAAAGCGTTG 4501 AAAGATCTCG GCACCAGTGA TACGGTCAAG CAGATTGTCA CTTCTGCCCT 4551 GACGGCGGGT GCATTAAATC AGATGGGCGC AGATATIGCC CAATTGAACA 4601 GCAAGGTAAG AACCGAACTG TTCAGCAGTA CGGGCAATCA AACTATTGCC 4651 AACCTTGGAG GCAGATTGGC TACCAATCTC AGTAATGCAG GTATCTCAGC 4701 TGGTATCAAT ACCGCCGTCA ACGGCGGCAG CCTGAAAGAC AACTTAGGCA 4751 ATGCCGCATT AGGAGCATTG GTTAATAGCT TCCAAGGAGA AGCCGCCAGC 4801 AAAATCAAAA CAACCTTCAG CGACGATTAT GTTGCCAAAC AGTTCGCCCA 4851 CGCTTTGGCT GGGTGTGTTA GCGGATTGGT ACAAGGAAAA TGTAAAGACG 4901 GGGCAATTGG CGCAGCAGTT GGGGAAATCG TAGCCGACTC CATGCTTGGC

-continued 4951 GGCAGAAACC CTGCTACACT CAGCGATGCG GAAAAGCATA AGGTTATCAG 5001 TTACTCGAAG ATTATTGCCG GCAGCGTGGC GGCACTCAAC GGCGGCGATG 5051 TGAATACTGC GGCGAATGCG GCTGAGGTGG CGGTAGTGAA TAATGCTTTG 5101 AATTTTGACA GTACCCCTAC CAATGCGAAA AAGCATCAAC CGCAGAAGCC 5151 CGACAAAACC GCACTGGAAA AAATTATCCA AGGTATTATG CCTGCACATG 5201 CAGCAGGTGC GATGACTAAT CCGCAGGATA AGGATGCTGC CATTTGGATA 5251 AGCAATATCC GTAATGGCAT CACAGGCCCG ATTGTGATTA CCAGCTATGG 5301 GGTTTATGCT GCAGGTTGGA CAGCTCCGCT GATCGGTACA GCGGGTAAAT 5351 TAGCTATCAG CACCTGCATG GCTAATCCTT CTGGTTGTAC TGTCATGGTC 5401 ACTCAGGCTG CCGAAGCGGG CGCGGGAATC GCCACGGGTG CGGTAACGGT 5451 AGGCAACGCT TGGGAAGCGC CTGTGGGGGGC GTTGTCGAAA GCGAAGGCGG 5501 CCAAGCAGGC TATACCAACC CAGACAGTTA AAGAACTTGA TGGCTTACTA 5551 CAAGAATCAA AAAATATAGG TGGTGTAAAT ACACGAATAA ATATAGCGAA 5601 TAGTACTACT CGATATACAC CAATGAGACA AACGGGACAA CCGCTATCTG 5651 CTGGCTTTGA GCATGTFCTT GAGGGGGGACT TCCATAGGCC TATTGCGAAT 5701 AACCGTTCAG TTTTTACCAT CTCCCCAAAT GAATTGAAGG TTATACTTCA 5751 AAGTAATAAA GTAGTTTCTT CTCCCGTATC GATGACTCCT GATGGCCAAT 5801 ATATGCGGAC TGTCGATGTA GGAAAAGTTA TTGGTACTAC TTCTATTAAA 5651 GAAGGTGGAC AACCCACAAC TACAATTAAA GTATTTACAG ATAAGTCAGG 5901 AAATTTGATT ACTACATACC CAGTAAAAGG AAACTAA

[0420] This corresponds to the amino acid sequence <SEQ ID 60; ORF114-1>:

1HNKGLHRIIFSXKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDLCGK51LKTTLKTLVCSLVSLSHVLPAHAQITTDKSAPKNQQVVILKFNTGAPLVN101IQTPNGRGLSHNRYTQFDVDNKGAVLNNDRNNNPFVVKGSAQLILNEVRG151TASKLNGIVTVGGQKADVIIANPNGITVNGGGFKNVGRGILTTGAPQIGK201DGALTGFDVRQGTLTVGAAGWNDKGGADYTGVLAAAVALQGKLQGKLLAV251STGFQKVDYASGEISAGTAAGTKPTIALDTAALGGHYADSITLIANEKGV301GVKNAGTLKAAXQLIVTSSGRIENSGRIATTADGTFASPTYLSIETTEKG301AAGTFISNGGRIESKGLLVIETGEDISLRNGAVVQINGSRPATTVLNAGH401HLVIESKTNVNNAKGFATLSADGRTVIKEASIQTGTTVSSSKGTAELGN401NTRZTGADVTVLSNGTISSSAVIDAKOTANIEAGKPLSLTASTVTSDIRL501NGGSIKGGKQLALLADDNITAXTTNLNTGNLVHTGKDLNIMVDKDLSA511AAKGNIQLRNTKLNAAKALETTALQGNIVSGCLHAVSADGHVSLLANGNA651DFTGHNTLTAKADVNAGSVGKGRLKADNTNITSSGDITLVAGNGIQLG701GKQRNSINGKHISIKNNGGNADLIQLNVHAKSGALNIHSDRALSIENTKL

63

-continued 751 ESTRNTHLNA QHERVTLNQV DAYAHRHLSI TGSQIWQNOK LPSANKLVTN 801 GVLALNARYS QIADNTTLRA GAINLTAGTA LVKRGNINWS TVSTKTLEDN 851 AELKPLAGRL NIEAGSGTLT IEPANRISAH TDLSIKTGGK LLLSAKGGNA 901 GAFSAQVSSL EAKGNIRLVT GETDLRGSKI TAGKNLVVAT TKGKLNIEAV 951 NNSFSNYFPT QKAAELNQKS KELEQQIAQL KKSSPKSKLI PTLQEERDRL 1001 AFYIQAINKE VKGKKPKGKE YLQAKLSAQN IDLISAQGIE ISGSDITASK 1051 KLNLHAAGVL PKAADSEAAA ILIDGITDQY EIGKPTYKSH YOKAALNKPS 1101 RLTGRTGVSI HAAAALDDAR IIIGASEIKA FSGSIDIKAH SDIVLEAGON 1151 DAYTFLKTKG KSGKIIRXTK FTSTRDHLIM PAPVELTANG ITLQAGGNIE 1201 ANTTRFNAPA GKVTLVAGEE LQLLAEEGIH KHELDVQKSR RFIGIKVGKS 1251 NYSKNELNET KLPVRVVAQT AATRSGWDTV LEGTEFKTTL AGADIQAGVG 1301 EKAPADAKII LKGIVNRIQS EEKLETNSTV WQKQAGRGST IETLKLPSFE 1351 SPTPPKLTAP GGYIVDIPKG NLKTEIEKLA KQPEPEYLKO LOVAKNVNWN 1401 QVQLAYDKWD YKQEGLTRAG AAIVTIIVTA LTYGYGATAA GGVAASGSST 1451 AAAAGTAATT TAAATTVSTA TANQTAALAS LYSQAAVSII NNKGDVGKAL 1501 KDLGTSDTVK QIVISALTAG ALNQMGADIA QLNSKVRTEL FSSTGVQTIA 1551 NLGGRLATNL SNAGISAGIN TAVNGGSLKD NLGNAALGAL VNSFQGEAAS 1601 KIKTTFSDDY VAKQFAHALA GCVSGLVQGK CKDGAIGAAV GEIVADSNLG 1651 GRNPATLSDA EKHKVISYSK IIAGSVAALN GGDVNTAANA AEVAVVNNAL 1701 NFDSTPTNAK KNQPQKPDKT ALEKIIQGIM PAHAAGAMTN PQDKDAAIWI 1751 SNIRNGITGP IVITSYGVYA AGWTAPLIGT AGKLAISTCM ANPSGCTVNV 1801 TQAAEAGAGI ATGAVTVGNA WEAPVGALSK AKAAKQAIPT QTVKELDGLL 1851 OESKNIGAVN TRINIANSTT RYTPNROTGO PVSAGFENVL EGHFHRPIAN 1901 NRSVFTXSPN ELKVILQSNK VVSSPVSMTP DGQYNRTVDV GKVIGTTSIK 1951 EGGQPTTTIK VFTDKSGNLI TTYPVKGN*

[0421] Computer analysis of this amino acid sequence predicts a transmembrane region and also gives the following results:

[0422] Homology with a Predicted ORF from *N. meningitidis* (Strain A)

[0423] ORF114 shows 91.9% identity over a 284 aa overlap with an ORF (ORF114a) from strain A of N. *meningitidis*:

			10	20	30	40
orf114.pep				GSSVSVSLKT		
orf114a	MNKGLHRIIFSKKH	ISTMVAVAETA	NSQGKGKQA	GSSVSVSLKT	SGDLCGKLKI	TLKTLVC
	10	20	30	40	50	60
	50	60	70	80	90	100
orf114.pep	SLVSLSMVLPAHAÇ	ITTDKSAPKN	IQQVVILKTN	FGAPLVNIQT	PNGRGLSHNF	XYAFDVD
		1111111111				
orf114a	SLVSLSMXXXXXX	ITTDKSAPKN	XQVVILKTN	IGAPLVNIQT:	PNGRGLSHNF	YTQFDVD
	70	80	90	100	110	120

-continued						
orf114.pep orf114a	110 NKGAVLNNDRNNN NKGAVLNNDRNNN 130	:			ĨIIIIIII	
orf114.pep orf114a	170 GGFKNVGRGILTI GGFKNVGRGILTI 190	Ĩ ::	1			~
orf114.pep orf114a	230 GKXXGKXLAVSTO GKLQGKNLAVSTO 250	1Ť111111	11111111111	1111111111		
orf114.pep orf114a	GVX GVKNAGTLEAAKQ 310	LIVTSSGRIE 320	NSGRIATTAD 330	GTEASPTYLX 340	IETTEKGAXG 350	TFISNGG 360

[0424] The complete length ORF114a nucleotide sequence <SEQ ID 61> is:

1 ATGAATAAAG GTTTACATCG CATTATCTTT AGTAAAAAGC ACAGCACCAT 51 GGTTGCAGTA GCCGAAACTG CCAACAGCCA GGGCAAAGGT AAACAGGCAG 101 GCAGTTCGGT TTCTGTTTCA CTGAAAACTT CAGGCGACCT TTGCGGCAAA 151 CTCAAAACCA CCCTTAAAAC CTTGGTCTGC TCTTTGGTTT CCCTGAGTAT 201 GGNATTNCNN NNCNNTNCCC AAATTACCAC CGACAAATCA GCACCTAAAA 251 ACCANCAGGT CGTTATCCTT AAAACCAACA CTGGTGCCCC CTTGGTGAAT 301 ATCCAAACTC CGAATGGACG CGGATTGAGC CACAACCGCT ATACGCAGTT 351 TGATGTTGAC AACAAAGGGG CAGTGTTAAA CAACGACCGT AACAATAATC 401 CGTTTCTGGT CAAAGGCAGT GCGCAATTGA TTTTGAACGA GGTACGCGGT 451 ACGGCTAGCA AACTCAACGG CATCGTTACC GTAGGCGGTC AAAAGGCCGA 501 CGTGATTATT GCCAACCCCA ACGGCATTAC TGTTAATGGC GGCGGCTTTA 551 AAAATGTCGG TCGGGGGCATC TTAACTATCG GTGCGCCCCA AATCGGCAAA 601 GACGGTGCAC TGACAGGATT TGATGTGCGT CAAGGCACAT TGACCGTAGG 651 AGCAGCAGGT TGGAATGATA AAGGCGGAGC CGACTACACC GGGGTACTTG 701 CTCGTGCAGT TGCTTTGCAG GGGAAATTAC AAGGTAAAAA CCTGGCGGTT 751 TCTACCGGTC CTCAGAAAGT AGATTACGCC AGCGGCGAAA TCAGTGCAGG 801 TACGGCAGCG GGTACGAAAC CGACTATTGC CCTTGATACT GCCGCACTGG 951 GCGGTATGTA CGCCGAGAAA ATCACACTGA TTGCCAATGA AAAAGGCGTA 901 GGcGTCAAAA ATGCCGGCAC ACTCGAAGCG GCCAAGCAAT TGATTGTGAC 951 TTCGTCAGGC CGCATTGAAA ACAGCGGCCG CATCGCCACC ACTGCCGACG 1001 GCACCGAAGC TTCACCGACT TATCTNNCNA TCGAAACCAC CGAAAAAGGA 1051 GCNNCAGGCA CATTTATCTC CAATGGTGGT CGGATCGAGA GCAAAGGCTT 1101 ATTGGTTATT GAGACGGGAG AAGATATCAT CTTGCGTAAC GGAGCCGTGG

-continued 1151 TGCAGAATAA CGGCAGTCGC CCAGCTACCA CGGTATTAAA TGCTGGTCAT 1201 AATTTGGTGA TTGAGAGTAA AACTAATGTG AACAATGCCA AAGGCTCGNC 1251 TAATCTGTCG GCCGGCGGTC GTACTACGAT CAATGATGCT ACTATTCAAG 1301 CGGGCAGTTC CGTGTACAGC TCCACCAAAG GCGATACTGA NTTGGGTGAA 1351 AATACCCGTA TTATTGCTGA AAACGTAACC GTATTATCTA ACGGTAGTAT 1401 TGGCAGTGCT GCTGTAATTG AGGCTAAAGA CACTGCACAC ATTGAATCGG 1451 GCAAACCGCT TTCTTTAGAA ACCTCGACCG TTGCCTCCAA CATCCGTTTG 1501 AACAACGGTA ACATTAAAGG CGGAAAGCAG CTTGCTTTAC TGGCAGACGA 1551 TAACATTACT GCCAAAACTA CCAATCTGAA TACTCCCGGC AATCTGTATG 1601 TTCATACAGG TAAAGATCTG AATTTGAATG TTGATAAAGA TTTGTCTGCC 1651 GCCAGCATCC ATTTGAAATC GGATAACGCT GCCCATATTA CCGGCACCAG 1701 TAAAACCCTC ACTGCCTCAA AAGACATGGG TGTGGAGGCA GGCTTGCTGA 1751 ATGTTACCAA TACCAATCTG CGTACCAACT CGGGTAATCT GCACATTCAG 1801 GCAGCCAAAG GCAATATTCA GCTTCGCAAT ACCAAGCTGA ACGCAGCCAA 1851 GGCTCTCGAA ACCACCGCAT TGCAGGGCAA TATCGTTTCA GACGGCCTTC 1901 ATGCTGTTTC TGCAGACGGT CATGTATCCT TATTGGCCAA CGGTAATGCC 1951 GACTTTACCG GTCACAATAC CCTGACAGCC AAGGCCGATG TCNATGCAGG 2001 ATCGGTTGGT AAAGGCCGTC TGAAAGCAGA CAATACCAAT ATCACTTCAT 2051 CTTCAGGAGA TATTACGTTG GTTGCCGNNN NCGGTATTCA GCTTGGTGAC 2101 GGAAAACAAC GCAATTCAAT CAACGGAAAA CACATCAGCA TCAAAAAACAA 2151 CGGTGGTAAT GCCGACTTAA AAAACCTTAA CGTCCATGCC AAAAGCGGGG 2201 CATTGAACAT TCATTCCGAC CGGGCATTGA GCATAGAAAA TACNAAGCTG 2251 GAGTCTACCC ATAATACGCA TCTTAATGCA CAACACGAGC GGGTAACGCT 2301 CAACCAAGTA GATGCCTACG CACACCGTCA TCTAAGCATT ANCGGCAGCC 2351 AGATTTGGCA AAACGACAAA CTGCCTTCTG CCAACAAGCT GGTGGCTAAC 2401 GGTGTATTGG CAATCAATGC GCGCTATTCC CAAATTGCCG ACAACACCAC 2451 GCTGAGAGCG GGTGCAATCA ACCTTACTGC CGGTACCGCC CTAGTCAAGC 2501 GCGGCAACAT CAATTGGATT ACCGTTTCGA CCAAGACTTT GGAAGATAAT 2551 GCCGAATTAA AACCATTGGC CGGACGGCTG AATATTGAAG CAGGTAGCGG 2601 CACATTAACC ATCGAACCTG CCAACCGCAT CAGTGCGCAT ACCGACCTGA 2651 GCATCAAAAC AGGCGGAAAA TTGCTGTTGT CTGCAAAAGG AGGAAATGCA 2701 GGTGCGCNTA GTGCTCAAGT TTCCTCATTG GAAGCAAAAG GCAATATCCG 2751 TCTGGTTACA GGAGNAACAG ATTTAAGAGG TTCTAAAATT ACAGCCGGTA 2901 AAAACTTGGT TGTCGCCACC ACCAAAGGCA AGTTGAATAT CGAAGCCGTA 2951 AACAACTCAT TCAGCAATTA TTTTCNTACA CAAAAAGNGN NNGNNCTCAA 2901 CCAAAAATCC AAAGAATTGG AACAACAGAT TGCGCAGTIG AAAAAAAGCT 2951 CGCNTAAAAG CAAGCTGATT CCAACCCTGC AAGAAGAACG CGACCGTCTC 3001 GCTTTCTATA TTCAAGCCAT CAACAAGGAA GTTAAAGGTA AAAAACCCAA 3051 AGGCAAAGAA TACCTGCAAG CCAAGCTTTC TGCACAAAAT ATTGACTTGA

3101 TTTCCGCACA AGGCATCGAA ATCAGCGGTT CCGATATTAC CGCTTCCAAA 3151 AAACTGAACC TTCACGCCGC AGGCGTATTG CCAAAGGCAG CAGATTCAGA 3201 GGCGGCTGCT ATTCTGATTG ACGGCATAAC CGACCAATAT GAAATTGGCA 3251 AGCCCACCTA CAAGAGTCAC TACGACAAAG CTGCTCTGAA CAAGCCTTCA 3301 CGTTTGACCG GACGTACGGG GGTAAGTATT CATGCAGCTG CGGCACTCGA 3351 TGATGCACGT ATTATTATCG GTGCATCCGA AATCAAAGCT CCCTCAGGCA 3401 GCATAGACAT CAAAGCCCAT AGTGATATTG TACTGGAGGC TGGACAAAAC 3451 GATGCCTATA CCTTCTTAAA AACCAAAGGT AAAAGCGGCA NAATNATCAG 3501 AAAAACNAAG TTTACCAGCA CCNGCGANCA CCTGATTATG CCAGCCCCNG 3551 TCGAGCTGAC CGCCAACGGT ATCACGCTTC ACGCAGGCGG CAACATCGAA 3601 GCTAATACCA CCCGCTTCAA TGCCCCTGCA GGTAAAGTIA CCCTGGTTGC 3651 GGGTGAANAG NTGCAACTGC TGGCAGAAGA AGGCATCCAC AAGCACGAGT 3701 TGGATGTCCA AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGTNAGAGC 3751 AATTACAGTA AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT 3801 CGCCCAAAAT GCAGCCACCC GTTCAGGCTG GGAThCCGTG CTCGAAGGTA 3851 CCGAATTCAA ATCCACGCTG GCCGGTGCCG ACATTCAGGC AGGTGTANGC 3901 GAAAAAGCCC GTGTCGATGC GAAAATCATC CTCAAAGGCA TTGTGAACCG 3951 TATCCAGTCG GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC 4001 AGGCCGGACG CGGCAGCACT ATCGAAACGC TAAAACTGCC CAGCTTCGAA 4051 AGCCCTACTC CGCCCAAATT GTCCGCACCC GGCGGNTATA TCGTCGACAT 4101 TCCGAAAGGC AATCTGAAAA CCGAAATCGA AAAGCTGTCC AAACAGCCCG 4151 AGTATGCCta TCTGAAACAG CTCCAAGTAG CGAAAAACAT CAACTGGAAT 4201 CAGGTGCAGC TTGCTTACGA CAGATGGGAC TACAAACAGG AGGGCTTAAC 4251 CGAAGCAGGT GCGGCGATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG 4301 GCGCAGGAAC CGGAGCCGTA TTGGGATTAA ACGGTGCGNC CGCCGCCGCA 4351 ACCGATGCAG CATTCGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTTCAT 4401 CAACAACAAA GGCGATGTCG GCAAAACCCT GAAAGAGCTG GGCAGAAGCA 4451 GCACGGTGAA AAATCTGGTG GTTGCCGCCG CTACCGCAGG CGTAGCCGAC 4501 AAAATCGGCG CTTCGGCACT GANCAATGTC AGCGATAAGC AGTGGATCAA 4551 CAACCTGACC GTCAACCTAG CCAATGNCGG GCAGTGCCGC ACTGAttaa

[0425] This encodes a protein having amino acid sequence <SEQ ID 62>:

1MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDLCGK51LKTTLKTLVCSLVSLSMXXXXXXQITTDKSAPIDXQVVILKTNTGAPLVN101IQTPNGRGLSHNRYTQFDVDNKGAVLNNDRNNNPFLVKGSAQLILNEVRG151TASKLNGIVTVGGQKADVIIANPNGITVNGGGFKNVGRGILTIGAPQIGK201DGALTGFDVRQGTLTVGAAGWNDKGGADYTGVLARAVALQGKLQGKNLAV

67

251 STGPOKVDYA SGEISAGTAA GTKPTIALDT AALGGMYADS ITLTAXEKGV 301 GVKNAGTLEA AKQLIVTSSG RIENSGRIAT TADGTEASPT YLXIETTEKG 351 AXGTFISNGG RIESKGLLVI ETGEDIXLPA GAVVQNNGSR PATTVLNAGH 401 NLVIESKTNV NNAXGSXNLS AGGRTTINDA TIOAGSSVYS STKGDTXLGE 451 NTRIIAENVT VLSNGSIGSA AVIEAKDTAN IESGKPLSLE TSTVASNIRL 501 NNGNIKGGKO LALLADDNIT AKTTNLNTPG NLYVHTGKDL NLNVDKDLSA 551 ASIHLKSDNA AHITGTSKTL TASKDNGVEA GLLNVTNTNL RTNSGNLHIQ 601 AAKGNZQLRH TKLNAAKALE TTALQGNIVS DGLHAVSADG HVSLLANGNA 651 DFTGHNTLTA KADVXAGSVG KGRLKADNTN ITSSSGDITL VAXXGIQLGD 701 GKQRNSINGK HISIKNNGGN ADLKNLNVHA KSGALNIHSO RALSIENTKL 751 ESTHNTHLNA QHERVTLNQV DAYAHRHLSI XGSQIWQNDK LPSANKLVAN 801 GVLAXNARYS QIADNTTLRA CAINLTAGTA LVKRGNINWS TVSTKTLEDN 851 AELKPLAGRL NIEAGSGTLT IEFANRISAH TDLSIKTGGK LLLSAXGGNA 901 GAXSAQVSSL EAKGNIRLVT GXTDLRGSKI TAGKNLVVAT TKGKLNIEAV 951 NNSFSNYFXT QKXXXLNQKS KELEOQIAQL KKSSXKSKLI PTLQEERDRL 1001 AFYIQAINKE VKGKKPKGKE YLQAXLSAQN IDLISAQGIE ISGSDITASK 1051 KLNLHAAGVL PKAADSEAAA ILIDGITOQY EIGKPTYKSH YDKAALNKPS 1101 RLTGRTGVSI HAAAALDDAR IIIGASEIKA PSGSIDIKAR SDIVLEAGQN 1151 DAYTFLXTKG KSGXXIRKTK FTSTXXHLIM PAPVELTANG ITLQAGGNIE 1201 ANTTRFHAPA GKVTLVAGEK XQLLAEEGIK KHELDVQKSR RFIGIKVGXS 1251 NYSINELNET KLPVRVVAQX AATRSGWDTV LEGTEFKTTL AGADIQAGVX 1301 EKARVQAXII LKGIVNRIQS EEKLETNSTV WQKQAGRGST IETLKLPSFE 1351 SPTPPKLSAP GGYIVDIPKG NLKTEIEKLS KQPEYAYLKO LOVAKNINWN 1401 QVQLAYQRWD YKQEGLTEAG AAIIALAVTV VTSGAGTGAV LGLNGAXAAA 1451 TDAAFASLAS OASVSFINNK GDVGKTLKEL GRSSTVKNLV VAAATAGVAD 1501 KIGASALXNV SDKQWINNLT VNLANXGQCR TD*

[0426] ORF114-1 and ORF114a show 89.8% identity in 1564 aa overlap

orf114a.pep orf114-1	MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDLCGKLKTTLKTLVC
	~ ~
orf114a.pep	> SLVSLSMXXXXXQITTDKSAPKNXQVVILKTNTGAPLVNIQTPNGRGLSHNRYTQFDVD
orf114-1	SLVSLSMXXXXXQITTDKSAPKNQQVVILKTNTGAPLVNIQTPNGRGLSHNRYTQFDVD
orf114a.pep	> NKGAVLNNDRNNNPFLVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG
orf114-1	
orf114a.pep	GGFKNVGRGILTIGAPQIGKDGALTGFDVRQGTLTVGAAGWNDKGGADYTGVLARAVALQ
orf114-1	

orf114a.pep GKLQGKNLAVSTGFQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEKGV

-continued orf114-1 GKLOGKNLAVSTGPOKVDYASGEISAGTAAGTKPTIALDTAALGGMYADS ITLIANEKGW orf114a.pep GVKNAGTLEAAXQLIVTSSGRIENSGRIATTADGTLASPTYLXIETTEKGAXGTFISNGG orf114-1 GVKNAGTI.EAAXOLTVTSSGRIENSGRIATTADGTLASPTYLSIETTEKGAAGTFISNGG orf114a.pep RIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKTNVNNAKGSXNLS orf114-1 RIESKGLLVIETGEDISLRNGAVVQNNGSRPATTVLNAGHNLVIESKTNVNNAKGPANLS $orf 114 \verb"a.pep" AGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVTVLSNGSIGSAAVIEAKDTAH$ orf114-1 ADGRTVIKEASIOTGTTVYSSSKGNAELGNNTRITGADVTVLSNGTISSSAVIDAKDTAH orf114a.pep IESGKPLSLETSTVASNIRLNNGNIKGGKQLALLADDNITAKTTNLNTPGNLYVHTGKDL orf114-1 IEAGKPLSLEASTVTSDIRLNGGSIKGGKQLALLADDNITAKTTNLNTPGNLYVHTGKDL orf114a.pep NLNVDKDLSAASIHLKSDNAAHITGTSKTLTASKDMGVEAGLLNVTNTNLRTNSGNLHIQ orf114-1 NLNVDKDLSAASIHLKSDNAAHITGTSKTLTASKDMGVEAGSLNVTNTNLRTNSGNLHIQ orf114a.pep AAKGNIQLRNTKLNAAKALETTALQGNIVSDGLHAVSADGHVSLLANGNADFTGHNTLTA AAKGNIQLRNTKLNAAKALETTALQGNIVSDGLHAVSADGHVSLLANGNADFTGHNTLTA orf114-1 orf114a.pep KADVXAGSVGKGRLKADNTNITSSSGDITLVAXXGIQLGDGKQRNSINGKHISIKNNGGN orf114-1 orf114a.pep ADLKNLNVHAKSGALNIHSDRALSIENTKLESTHNTHLNAQHERVTLNQVDAYAHRHLSI ADLKNLNVHAKSGALNIHSDRALSIENTKLESTHNTHLNAQHERVTLNQVDAYAHRHLSI orf114-1 orf114a.pep XGSQIWQNDKLPSANKLVANGVLAXNARYSQIADNTTLRAGAINLTAGTALVKRGNINWS TGSQIWQNDKLPSANKLVANGVLALNARYSQIADNTTLRAGAINLTAGTALVKRGNINWS orf114-1 orf114a.pep TVSTKTLEDNAELKPLAGRLNIEAGSGTLTIEPANRISAHTDLSIKTGGKLLLSAKGGNA orf114-1 TVSTKTLEDNAELKPLAGRLNIEAGSGTLTIEPANRISAHTDLSIKTGGKLLLSAKGGNA orf114a.pep GAXSAQVSSLEAKGNIRLVTGXTDLRGSKITAGKNLVVATTKGKLNIEAVNNSFSNYFXT GAPSAQVSSLEAKGNIRLVTGETDLRGSKITAGKNLVVATTKGKLNIEAVNNSFSNYFPT orf114-1 orf114a.pep QKXXXLNQKSKELEQQIAQLKKSSXKSKLIPTLQEERDRLAFYIQAINKEVKGKKPKGKE QKAAELNQKSKELEQQIAQLKKSSPKSKLIPTLQEERDRLAFYIQAINKEVKGKKPKGKE orf114-1 orf114a.pep YLQAKLSAQNIDLISAQGIEISGSDITASKKLNLHAAGVLPKAADSEAAAILIDGITDQY orf114-1 YLOAKLSAONIDLISAOGIEISGSDITASKKLNLHAAGVLPKAADSEAAAILIDGITDOY orf114a.pep EIGKPTYKSHYDKAALNKPSRLTGRTGVSIHAAAALDDARIIIGASEIKAPSGSIDIKAH orf114-1 EIGKPTYKSHYDKAALNKPSRLTGRTGVSIHAAAALDDARIIIGASEIKAPSGSIDIKAH orf114a.pep SDIVLEAGQNDAYTFLXTKGKSGXXIRKTKFTSTXXHLIMPAPVELTANGITLQAGGNIE orf114-1 ${\tt SDIVLEAGQNDAYTFLKTKGKSGKIIRKTKFTSTRDHLIMPAPVELTANGITLQAGGNIESPACE (Construction) and the second statement of the second s$ orf114a.pep ANTTRFNAPAGKVTLVAGEXXQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNELNET orf114-1 ANTTRFNAPAGKVTLVAGEELQLLAEEGIHKHELDVQKSRRFIGIKVGKSNYSKNELNET orf114a.pep KLPVRVVAQXAATRSGWDTVLEGTEFKTTLAGADIQAGVXEKARVDAKIILKGIVNRIQS orf114-1 KLPVRVVAQTAATRSGWDTVLEGTEFKTTLAGADIQAGVGEKARADAKIILKGIVNRIQS orf114a.pep EEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLSAPGGY1VDIPKGNLKTEIEKLS EEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLSTPGGYIVDIPKGNLKTEIEKLA orf114-1 orf114a.pep KQPEYAYLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAAIIALAVTVVTSGAGTGAV

orf114 - 1	-continued KQPEYAYLKQLQVAKNVNWQVQLAYDKWDYKQEGLTRAGAAIVTIIVTALTYGYGATAA	
orf114a.pep	LGLNGAXAATDAAFASLASQASVSFINNKGDVGKTL : :: : : : : : : : : : :	1477
orf114-1	${\tt GGVAASGSSTAAAAGTAATTTAAATTVSTATAMQTAALASLYSQAAVSIINNKGDVGKAL$	1500
orf114a.pep	KELGRSSTVKNLVVAAATAGVADKIGASALXNVSDKQWINNLTVNL : :: : :: ::::	1523
orf114-1	${\tt KDLGTSDTVKQIVTSALTAGALNQMGADIAQLNSKVRTELFSSTGNQTIANLGGRLATNL}$	1560
orf114a.pep	ANXGQCRTDX :	
orf114-1	SNAGISAGINTAVN	

[0427] Homology with pspA Putative Secreted Protein of *N. meningitidis* (Accession Number AF030941)

[0428] ORF114 and pspA protein show 36% aa identity in 302 aa overlap:

Orf114:	1 AVAETANSQGKGKQAGSSVSVSLKTSGDXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	56
pspA:	19 AVAENVHRDGKSMQDSEAASVRVTGAASVSSARAAFGFRMAAFSVMLALGVAAFSPAPAS	78
Orf114:	57-ITTDKSAPKNQQVVILKTNTGAPLVNIQTPNGRGLSHNRXYAFDVDNKGAVLNNDRNN- I DKSAPKNQQ VIL+T G P VNIQTP+ +G+S NR FDVD KG +LNN R+N	114
pspA:	79 GIIADKSAPKNQQAVILQTANGLPQVNIQTPSSQGVSVNRFKQFDVDEKGVILNNSRSNT	138
Orf114:	115NPFVVKGSAQLILNEV-RGTASKLNGIVTVGGQKADVIIANPNGITVNGG NP + +G A++I+N++ S LNG + VGG++A+V++ANP+GI VNGG	163
pspA:	$139 \underline{\texttt{QTQLGGWIQGNPHLARGEARVIVNQIDSSNPSLLNGYIEVGGKRAEVVVANPSGIRVNGG}$	198
Orf114:	164GFKNVGRGILTTGAPQIGKDGALTGFDVVKAHWTVXAAGWNDKGGAXYTGVLARAVALQG G N LT+G P + +G LTGFDV + G D A YT +L+RA +	223
papA:	199GLINAASVTLTSGVPVL-NNGNLTGFDVSSGKVVIGGKGL-DTSDADYTRILSRAAEINA	256
Orf114:	224KXXGKXLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIANE GK + V +G K+D+ +A + PT+A+DTA LGGMYAD ITLI+ +	279
pspA:	$257{\tt GVWGKDVKVVSGKNKLDFDGSLAKTASAPSSSDSVTPTVAIDTATLGGMYAQKITLISTD$	316
Orf114:	280KG 291 G	
papA:	317NG 318	

[0429] ORF114a is also homologous to pspA:

gil2623258 (AF030941) putative secreted protein (<i>Neisseria meningitidis</i>) Length = 2273 Score +32 261 bits (659), Expect +32 3e-69 Identities = 203/663 (30%), Positives 314/663 (46%), Gaps 76/663 (11%)	
Query: 1 MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDXXXXXXXX 55 MNK +++IF+KK S M+AVAE + GK 0 + SV + +S	
Sbjct: 1 MNKRCYKVIFNKKRSCMMAVAENVHRDGKSMQDSEAASVRVTGAASVSSARAAFGFRMAA 60	
Query: 56 XXXXXXXXXXXXXXXXXQITTKDSAPKNXQVVILKTNTGAPLVNIQTPNGRGLSHNRYT 115 I DKSAPKN O VIL+T G P VNIOTP+ +G+S NR+	5
Sbjct: 61 FSVMLALGVAAFSPAPASGIIADKSAPKNQQAVILQTANGLPQVNIQTPSSQGVSVNRFK 120	C
Query: 116 QFDVDNKGAVLNNDRNNNPFLVKGSAQLILNEV-RGTASKLNGIVTVGG 163 QFDVD KG +LNN R+NNP L +G A++I+N++ S LNG + VGG	3
Sbjct: 121 QFDVDEKGVILNNSRSNTQTQLGGWIQGNPHLARGEARVIVNQIDSSNPSLLNGYIEVGG 180	C
Query: 164 QKADVIIANPNGITVNGGGFKNVGRGILTIGAPQIGKDGALTGFDVRQGTLTVGAAGWND 223 ++A+V++ANP+GI VNGGG N LT G P + +G LTGFDV G + +G G D	3
Sbjct: 181 KRAEVVVANPSGIRVNGGGLINAASVTLTSGVPVL-NNGNLTGFDVSSGKVVIGGKGL-D 238	3

Query:	224	KGGADYTGVLARAVALQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALD ADYT +L+RA + + GK++ V +G K+D+ +A + PT+A+D	279
Sbjct:	239	$\verb"TSDADYTRILSRAAEINAGVWGKDVKVVSGKNKLDFDGSLAKTASAPSSSDSVTPTVAID"$	298
Query:	280	TAALGGMYADSITLIAXEKGVGVKNAGTLEAAK-QLIVTSSGRIENSGRIATTADGTEAS TA LGGMYAD ITLI+ + G ++N G + AA + +++ G++ NSG I	338
Sbjct:	299	TATLGGMYADKITLISTDNGAVIRNKGRIFAATGGVTLSADGKLSNSGSIDAA	351
Query:	339	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	398
Sbjct:	352	EITISAQTVDRQGFIRSGKGSVLKVSDGINNQAGLIGSAGLLDIRDT	399
Query:	399	GHNLVIESKTNVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRI G +S ++NN G+ ++S ++ ND + A V S + D G+	454
Sbjct:	400	GKSSLHINNTDGTIIAGKDVSLQAKSLDNDGILTAARDV-SVSLHDDFAGKRDIE	453
Query:	455	$\label{eq:linear} \begin{array}{rrrr} \textbf{IAENVTVLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASNIRLNNGNIKGGKQLALL} \\ +T &+ \texttt{G} &+ + + \texttt{I+A} \ \texttt{DT} &+ + + + + + + \texttt{S} \ \texttt{R} & \texttt{G} & \texttt{L+} \end{array}$	514
Sbjct:	454	AGRTLTFSTQGRLKNTRIIQAGDTVSLTAAQIDNTVSGKIQSGNRTGLNGKNGITNRGLI	513
Query:	515	eq:addnitakttnlntpgnlyvhtgkdlnlnvdkdlsaasihlksdnaahitgtskt + it ak+ n t g + y g + + d l+ aa	569
Sbjct:	514	NSNGITLLQTEAKSDNAGT-GRIYGSRVAVEADTLLNREETVNGETKAAV	562
Query:	570	eq:ltaskdmgveagxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx	625
Sbjct:	563	${\tt IAARERLDIGAREIENREAALLSSSGDLHIGSALNGSRQVQGANTSLHNRSAAIESS{}$	619
Query:	626	GNI 628 GNI	
Sbjct:	620	GNI 622	
Casha	32 3	$7 = b_1 + a_2 (55) = F_{TTDOA} + 0 = 53$	
		7.5 bits (65), Expect = 0.53 = 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14	18)
Identit	ies	<pre>= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK</pre>	
Identit Query:	ies 239	= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14	298
Identit Quer y: Sbjct:	239 1023	<pre>= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF</pre>	298 1072
Identit Query: Sbjct: Query:	ies - 239 1023 299	= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE	298 1072 355
Identit Query: Sbjct: Query: Sbjct:	ies 239 1023 299 1073	<pre>= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14) LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT</pre>	298 1072 355 1120
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Identit Query: Sbjct: Query: Sbjct: Query: Sbjct:	ies 239 1023 299 1073 356 1121	<pre>= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14) LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT</pre>	298 1072 355 1120 408 1179
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query:	ies 239 1023 299 1073 356 1121 409	<pre>= 87/432 (20%), Positives +32 159/432 (36%), Gaps = 62/432 (14) LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA</pre>	298 1072 355 1120 408 1179 460
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct:	ies 239 1023 299 1073 356 1121 409 1180	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASWIRLNNGNIKGGKQLALLADDNIT	298 1072 355 1120 408 1179 460 1234
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query:	ies , 239 1023 299 1073 356 1121 409 1180 461	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I Å Å K Å + +S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + Å L +G + + I TÅ E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG	298 1072 355 1120 408 1179 460 1234 520
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Sbjct:	ies - 239 1023 299 1073 356 1121 409 1180 461 1235	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASWIRLNNGNIKGGKQLALLADDNIT +G++ +I +G + + T+ S NN +K + A+ N QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILSAKNNIVLKAAETRSRSAEMNKK AKTTNLNTPG-NLYVHTGKDLNLNVDKDLSAASIHLKSDNAAHITGTSKTLTA	298 1072 355 1120 408 1179 460 1234 520 1292
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query:	ies - 239 1023 299 1073 356 1121 409 1180 461 1235 521	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN+ + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASWIRLNNGNIKGGKQLALLADDNIT +G++ +I +G + + T+ S NN +K + A+ N QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILSAKNNIVLKAAETRSRSAEMNKK	298 1072 355 1120 408 1179 460 1234 520 1292 572
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct:	ies - 239 1023 299 1073 356 1121 409 1180 461 1235 521 1293	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN + + G + + G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASWIRLNNGNKGGKQLALLADDNIT +G++ +I +G + + T+ S NN +K + A+ N QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILS-AKNNIVLKAAETRSRSAEMNKK AKTTNLNTPG-NLYVHTGKDLNLNVDKDLSAASIHLKSDNAAHITGTSKTLTA K+ ++ G + KD N ++S + S N H T T T+++ EKSGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISS SK-DMGVEAGXXXXXXXXXXXXXXXSGNLHIQAAKGNIQLRNTKLNAAKALETTALQG	298 1072 355 1120 408 1179 460 1234 520 1292 572 1352
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct:	ies - 239 1023 299 1073 356 1121 409 1180 461 1235 521 1293 573	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN + + G + +G I A A K A + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLLKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + A L +G + + I TA E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASWIRLNNGNKGGKQLALLADDNIT +G++ +I +G + +T+ S NN +K + A+ N QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILSAKNNIVLKAAETRSRSAEMNKK AKTTNLNTPG-NLYVHTGKDLNLNVDKDLSAASIHLKSDNAHHITGTSKTLTA K+ ++ G + KD N ++S + S N H T T T+++ EKSGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISS	298 1072 355 1120 408 1179 460 1234 520 1292 572 1352 626
Identit Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct: Query: Sbjct:	ies - 239 1023 2999 1073 356 1121 409 1180 461 1235 521 1293 573 1353	= $87/432$ (20%), Positives +32 159/432 (36%), Gaps = $62/432$ (14 LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEK LQG LQGKN + + G + + G I Å Å K Å + + S T + LQGDLQGKNIFAAAGSDITNTGSIGAENALLIKASNNIESRSETRSNQNE GVGVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXG-TF V+N G + Å L +G + + I TÅ E T + G T QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTASELTNQSEDGQTV ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKT ++ GG I S + I + V++ + +T+ G NL + +K LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIPAA NVNNAKGSXNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVT V + +G L+AG D ++AG + Y+ G + TR + EVGSEQGRLKLAAGRDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASWIRLNNGNIKGGKQLALLADDNIT +G++ +I +G + + T+ S NN +K + A + N QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILSAKNNIVLKAAETRSRSAEMNKK AKTTNLNTPG-NLYVHTGKDLNLNVDKDLSAASIHLKSDNAHHITGTSKTLTA K+ + +G + KD N + +S + S N H T T T+++ EKSGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISS SK-DMGVEAGXXXXXXXXXXXXXSGNLHIQAAKGNIQLRNTKLNAAKALETTALQG + D+G+ +G + KD + KG ++ + NT + A A++ G	298 1072 355 1120 408 1179 460 1234 520 1292 572 1352 626

[0430] Amino acids 1-1423 of ORF114-1 were cloned in the pGex vector and expressed in *E. coli*, as described above. GST-fusion expression was visible using SDS-PAGE, and **FIG. 5** shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF114-1.

[0431] Based on these results, including the homology with the putative secreted protein of *N. meningitidis* and on

the presence of a transmembrane domain, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 14

[0432] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 63>

1 CGCTTCATTC	ATGATGAAGC	AGTCGGCAGC	AACATCGGCG	GCGGCAAAAT
51 GATTGTTGCA	GCCGGGCAGG	ATATCAATGT	ACGCGGCAnA	AGCCTTATTT
101 CTGATAAGGG	CATTGTTTTA	AAAGCAGGAC	ACGACATCGA	TATTTCTACT
151 gcccataatc	GCTATACCGG	CAATGAATAC	CACGAGAGCA	WAAAWTCAGG
201 CGTCATGGGT	ACTGGCGGAT	TGGGCTTTAC	TATCGGTAAC	CGGAAAACTA
251 CCGATGACAC	TGATCGTACC	AATATTGTsC	ATACAGGCAG	CATTATAGGC
301 AGCCTGAaTG	GAGACACCGT	TACAGTTGCA	GGAAACCGCT	ACCGACAAAC
351 CGGCAGTACC	GTCTCCAGCC	CCGACGGGGCG	CAATACCGTC	ACAGCCAAAw
401 GCATAGATGT	AGAGTTCGCA	AACAACCGGT	ATGCCACTGA	CTACGcCCAT
451 ACCCAGGGAA	CAAAAAGGCC	TTACCGTCGC	CCTCAATGTC	CCGGTTGTCC
501 AAGCTGCACA	AAACTTCATA	CAAGCAGCCC	AAAATGTGGG	СААААБТААА
551 AATAAACGCG	TTAATGCCAT	GGCTGCAGCC	AATGCTGCAT	GGCAGAGTTA
601 TCAAGCAACC	CAACAAATGC	AACAATTTGC	TCCAAGCAGC	AGTGCGGGAC
651 AAGGTCAAAA	СТАСААТСАА	AGCCCCAGTA	TCAGTGTGTC	CATTAC.TAC
701 GGCGAACAGA	AAAGTCGTAA	CGAGCAAAAA	AGACATTACA	CCGAAgCGGC
751 AgCAAGTCAA	ATTATCGGCA	AAGGGCAAAC	CACACTTGCG	GCAACAGGAA
801 GTGGGGAGCA	GTCCAATATC	AATATTACAG	GTTCCGATGT	CATCGGCCAT
951 GCAGGTACTC	C.CTCATTGC			
	0.010111100	AAGCAACCAT	ATCAGACTCC	AATCTGCCAA
901 ACAGGACGGC				
901 ACAGGACGGC 951 GCGTACGTnn	AGCGAGCAAA	GCAAAAACAA	AAGCAGTGGT	TGGAATGCAG
	AGCGAGCAAA CAAAATAGGC	GCAAAAACAA AAcGGCATCA	AAGCAGTGGT GGTTTGGAAT	TGGAATGCAG TACCGCCGGA
951 GCGTACGTnn	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA	GCAAAAAACAA AACGGCATCA AGAGCAAGGG	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA	TGGAATGCAG TACCGCCGGA CCCACCGCCA
951 GCGTACGTnn 1001 GGAAATATCG	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA	GCAAAAACAA AAcGGCATCA AGAGCAAGGG CCGGCAAAAC	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA	TGGAATGCAG TACCGCCGGA CCCACCGCCA AGCGGCGGG <u>G</u>
951 GCGTACGTnn 1001 GGAAATATCG 1051 CACCCATGTC	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA	TGGAATGCAG TACCGCCGGA CCCACCGCCA AGCGGCGGG <u>G</u> TACAGGCAGA
951 GCGTACGTnn 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA	TGGAATGCAG TACCGCCGGA CCCACCGCCA AGCGGCGGG <u>G</u> TACAGGCAGA ACCTATCAGA
951 GCGTACGTnn 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u>	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT	TGGAATGCAG TACCGCCGGA CCCACCGCCA AGCGGCGGG <u>G</u> TACAGGCAGA ACCTATCAGA ACGGATTCAG
951 GCGTACGTNN 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT AGTTACCGCC	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>±</u> AAAGCAAAGT	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC	TGGAATGCAG TACCGCCGGA CCCACCGCCA AGCGGCGGGG TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG
951 GCGTACGTnn 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA 1251 TGCAAGCGGC	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT AGTTACCGCC AAgCGGTATT	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u> AAAGCAAAGT	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC AAGACGGCTA	TGGAATGCAG TACCGCCGGA AGCGGCGGG <u>G</u> TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG TCAAATYAAA
951 GCGTACGTNN 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA 1251 TGCAAGCGGC 1301 TAACCGGGCA	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT AGTTACCGCC AAgCGGTATT ACACAGACCT	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u> AAAGCAAAGT TATGCCGGAG yAAGGGCGGT	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC AAGACGGCTA ATCATCACGT	TGGAATGCAG TACCGCCGGA AGCGGCGGG <u>G</u> TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG TCAAATYAAA CTAGCCAAAG
951 GCGTACGTNN 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA 1251 TGCAAGCGGC 1301 TAACCGGGCA 1351 GTYAGAGACA	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT AGTTACCGCC AAgCGGTATT ACACAGACCT AAGGGCAAAA	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u> AAAGCAAAGT TATGCCGGAG yAAGGGCGGT ACCTTTTTCA	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC AAGACGGCTA ATCATCACGT GACGGCCACC	TGGAATGCAG TACCGCCGGA AGCGGCGGGG TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG TCAAATYAAA CTAGCCAAAG CTTACTGCCA
951 GCGTACGTNN 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA 1251 TGCAAGCGGC 1301 TAACCGGGCA 1351 GTYAGAGACA 1401 CGCAGAAGAT	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT AGTTACCGCC AAgCGGTATT ACACAGACCT AAGGGCAAAA AAACCACAGAC	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u> AAAGCAAAGT TATGCCGGAG yAAGGGCGGT ACCTTTTTCA CGCTACGAAG	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC AAGACGGCTA ATCATCACGT GACGGCCACC GCAGAAGCTT	TGGAATGCAG TACCGCCGGA AGCGGCGGG <u>G</u> TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG TCAAATYAAA CTAGCCAAAG CTTACTGCCA
951 GCGTACGTNN 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA 1251 TGCAAGCGGC 1301 TAACCGGGCA 1351 GTYAGAGACA 1401 CGCAGAAGAT 1451 GCGACATTCA	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTATA CTGCATATAG AAACGGCAAT AGTTACCGCC AAGCGGTATT ACACAGACCT AAGGGCAAAA AAACCACAGC	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u> AAAGCAAAGT TATGCCGGAG yAAGGGCGGT ACCTTTTTCA CGCTACGAAG	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC AAGACGGCTA ATCATCACGT GACGGCCACC GCAGAAGCTT GGCACGGTTA	TGGAATGCAG TACCGCCGGA AGCGGCGGGG TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG TCAAATYAAA CTAGCCAAAG CTTACTGCCA CGGCATAGGC
951 GCGTACGTNN 1001 GGAAATATCG 1051 CACCCATGTC 1101 GATACCACCC 1151 TACGCGCAAC 1201 GCAAACAGCA 1251 TGCAAGCGGC 1301 TAACCGGGCA 1351 GTYAGAGACA 1401 CGCAGAAGAT 1451 GCGACATTCA 1501 GGCAGTTTCG	AGCGAGCAAA CAAAATAGGC GTAAAGGTAA GGCAGCACAA TCAAAGGTGT CTGCATATAG AAACGGCAAT AGTTACCGCC AAGCGGTATT ACACAGACCT AAGGGCAAAA AAACCACAGC ACCTGAACGG	GCAAAAACAA AACGGCATCA AGAGCAAGGG CCGGCAAAAC GCAGCTCATC AAAGTGTTCA GTCCAAGTT <u>t</u> AAAGCAAAGT TATGCCGGAG yAAGGCCGGAG CGCTACGAAG CGGCTGGGAC TAAGCCCGGC	AAGCAGTGGT GGTTTGGAAT GGAAGTACTA TACCATCCGA GGCAAAGGCA AGATACTGAA ACTGTCGGTT CAAAGCAGAC AAGACGGCTA GACGGCCACC GCAGAAGCTT GGCACGGTTA AGCCGGCTAC	TGGAATGCAG TACCGCCGGA AGCGGCGGG <u>G</u> TACAGGCAGA ACCTATCAGA ACGGATTCAG CATGCCTCCG TCAAATYAAA CTAGCCAAAG CTTACTGCCA CGGCATAGGC CCGACAAACA

1701 AGAAACCGAA GCGCGTATCT ACACCGGCAT CGACACCGAA ACTGCGGATC

1751 AACACTCAGG CCATCTGAAA AACAGCTTCG AC...

[0433] This corresponds to the amino acid sequence <SEQ ID 64; ORF116>:

1...RFIHDEAVGS NIGGGKNIVA AGQDINVRGX SLISDKGIVL KAGADIDIST

51 AHNRYTGNEY HESXXSGVMG TGGLGFTIGN RKTTDDTDRT NIVHTGSIIG

101 SLNGDTVTVA GNRYRQTGST VSSPEGRNTV TAKXIDVEFA NNRYATDYAH

151 TQEQKGLTVA LNVPVVQAAQ NFIQAAQNVG KSKNKRVNAM AAANAAWQSY

201 QATQQMQQFA PSSSAGQGQN YNQSPSISVS IXYGEQKSRN EQKRNYTEAA

251 ASQIIGKGQT TLAATGSGEQ SNINITGSDV IGHAGTXLIA DNHIRLQSAX

301 QDGSEQSKNK SSGWNAGVRX KIGNGIRFGI TAGGNIGKGK EQGGSTTHRH

351 THVGSTTGKT TIRSGGDTTL KGVQLIGXGI QADTRNLHIE SVQDTETYQS

401 KOONGNVOVT VGYGFSASGS YROSKVKADH ASVTGOSGIY AGEDGYOIKV

451 RDNTDLKGGI ITSSQSAEDK GKNLFQTATL TASDIQNHSR YEGRSFGIGG

501 SFDLNGGWDG TVTDKQGRPT DRISPAAGYG SDGDSKNSTT RSGVNTHNIH

551 ITDEAGQLAR TGRTAKETEA RIYTGIDTET ADQHSGHLKN SFD...

[0434] Computer analysis of this amino acid sequence gave the following results:

[0435] Homology with pspA Putative Secreted Protein of *N. meningitidis* (Accession Number AF030941)

[0436] ORF116 and pspA protein show 38% as identity in 502 as overlap:

Orf116: 6	EAVGSNIGGGKMIVAAGQDINVRGXSLISDKGIVLKAGHDIDISTAHNRYTGNEYHESXX +AV + G ++I+ +G+DI V G ++I+D +L A ++I + A R E ++	65
PspA: 1235	QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILSAKNNIVLKAAETRSRSAEMNKKEK	1294
Orf116: 66	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	125
PspA: 1295	${\tt SGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISSPQ$	1354
Orf116: 126	GRNTVTAKXIDVEFANNRYATDYAHTQEQKGLTVALNVPXXXXXXXXXXXXXXKKS G +++ I ++ A NRY+ + EOKG+TVA++VP GKS	182
PspA: 1355	GDVGISSGKISIDAAQNRYSQESKQVYEQKGVTVAISVPVVNTVMGAVDAVKAVQTVGKS	1414
Orf116: 183	KNKRVXXXXXXXWQSYQATQQMQQFAPSSSAGQGQNYNQSPSISVSIXYGEQKSRN KN RV + + + A P +AGOG ISVS+YGEOK+ +	240
PspA: 1415	KNSRVNAMAAANALNKGVDSGVALYNAARNPKKAAGQGISVSVTYGEQKNTS	1466
Orf116: 241	EQKRHYTEAAASQIIGKGQTTLAATGSGEQSNINITGSDVIGHAGTXLIADNHIRLQSAK	300
PspA: 1467	E + T+ +I G G+ +L A+G+G+ S I ITGSDV G GT L A+N ++++A+ ESRIKGTQVQEGKITGGGKVSLTASGAGKDSRITITGSDVYGGKGTRLKAENAVQIEAAR	1526
Orf116: 301	QDGSEQSKNKSSGWNAGVRXKIGNGIRFGITAXXXXXXXXXXXTHRHTHVGSTTGKT	360
PspA: 1527	Q E+S+NKS+G+NAGV I GI FG TA T +R++H+GS +T QTHQERSENKSAGFNAGVAIAINKGISFGFTAGANYGKGYGNGDETAYRNSHIGSKDSQT	1586
Orf116: 361	TIRSGGDTTLKGVQLIGKGIQADTRNLHIESVQDTETYQSKQQNGNVQVTVGYGFSASGS I SGGDT +KG QL GKG+ +LHIES+QDT ++ KQ+N + QVTVGYGFS GS	420
PspA: 1587	AIESGGDTVIKGGQLKGKGVGVTAESLHIESLQDTAVFKGKQENVSAQVTVGYGFSVGGS	1646

-continued Orf116: 421 YRQSKVKADHASVTGQSGIYAGEDGYQIKVRDNTDLKGGIITSSQSAEDKGKNLFQTATL 480 Y +SK +D+ASV QSGI+AG DGY+I+V T L G + S DK KNL +T+ + PspA: 1647 YNRSKSSSDYASVNEQSGIFAGGDGYRIRVNGKTGLVGAAVVSD---ADKSKNLLKTSEI 1703

Orf116:	481	TASDIQNHSRYEGRSFGIGGSF	502
		DIQNH+ + G+ G F	
PspA:	1704	WHKDIQNHASAAASALGLSGGF	1725

[0437] Based on homology with pspA, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 15

[0438] The following partial DNA sequence was identified in *N. meningitidis* SEQ ID 65>

1..ACGACCGGCA GCCTCGGCGG CATACTGGCC GGCGGCGGCA CTTCCCTTGC

51 CGCACCGTAT TTGGACAAAG CGGCGGAAAA CCTCGGTCCG GCGGGCAAAG

101 CGGCGGTCAA CGCACTGGGC GGTGCGGCCA TCGGCTATGC AACTGGTGGT

151 AGTGGTGGTG CTGTGGTGGG TGCGAATGTA GATTGGAACA ATAGGCAGCT

201 GCATCCGAAA GAAATGGCGT TGGCCGACAA ATATGCCGAA GCCCTCAAGC

251 GCGAAGTTGA AAAACGCGAA GGCAGAAAAA TCAGCAGCCA AGAAGCGGCA

301 ATGAGAATCC GCAGGCAGAT ATGCGTTGGG TGGACAAAGG TTCCCAAGAC

351 GGCTATACCG ACCAAAGCGT CATATCCCTT ATCGGAATGA

[0439] This corresponds to the amino acid sequence <SEQ ID 66; ORF118>:

1...TTGSLGGILA GGGTSLAAPY LDKAAENLGP AGKAAVNALG GAAIGYATGG

51 <u>SGGAVVGA</u>NV DWNNRQLHPK EMALADKYAE ALKREVEKRE GRKISSQEAA

101 MRIRRQICVG WTKVPKTAIP TKASYPLSE*

[0440] Computer analysis of this amino acid sequence reveals two putative transmembrane domains.

[0441] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 16

[0442] The following partial DNA sequence was identified in *N. meningitidis* SEQ ID 67>

1..CAATGCCGTC TGAAAAGCTC ACAATYTTAC AGACGGCATT TGTTATGCAA

51 GTACATATAC AGATTCCCTA TATACTGCCC AGrkGCGTGC GTgGCTGAAG

101 ACACCCCCTA CGCTTGCTAT TTGrAACAGC TCCAAGTCAC CAAAGACGTC

151 AACTGGAACC AGGTACWACT GGCGTACGAC AAATGGGACT ATAAACAGGA

201 AGGCTTAACC GGAGCCGGAG CAGCGATTAT TGCGCTGGCT GTTACCGTGG

-continuedresolution251TTACTGCGGGCGCGGGAGCCGGAGCCGCACTGGGCTTAAACGGCGGGGCA301GCAGCGGCAACCGATGCCGCATTCGCCTCGCTGGCCAGCCAGGGTTCCGT351ATCGCTCATCAaCAACAAAGGCAATATCGGTAaCACCCTGAAAGAGCTGG401GCAGAAGCAGCACGGTGAAAAATCTGATGGTTGCCGTCGCtACCGCAGGC451GTagCcgaCAAAATCGGTGCTTCGGCACTGAAATAGCGGCAGCGATAAGCA501GTGGATCAACAACCTGACCTCAACCTGGCCAATGCGGGCAGTGCCGCACC501GTGGATCAACCGCTGTCACGGCGGCACCtgAAAGACAATCTGGAAGCG601AATATCCTTGCGGCTTTGGTGAATACTGCCACACAAGATGCCCATGCAC601AATCAAACAGTTGGATCAGCACTACATTACCACACAAGATGCCCATGCAC701TAGCGGGCTGTGCGGCTGTGGGCGAGAAAATAGGGCAACAATTTGGCAT751GCGATAGGCGCGCTGTGGGCGAGTAAGCAGTGTGGCGGCGGCGATGTA851ACAGCAAACTGCTTGCCGCACGGTAAGCAGTGTGGCTGGCGCGATGTAG951CGACAAtGACGACAACGATCACGAACACTCACGCTAGAACCGCAACAA

[0443] This corresponds to the amino acid sequence <SEQ ID 68; ORF41>:

1..QCRLKSSQFY RRHLLCKYIY RFPIYCPXAC VAEDTPYACY LXQLQVTKDV
51 HWNQVXLAYD KWDYKQEG<u>LT GAGAAIIALA VTVVT</u>AGAGA GAALGLNGAA
101 AAATDAAFAS LASQASVSLI NNKGNIGNTL KELGRSSTVK NU4VAVATAG
151 VADKIGASAL NNVSDKQWIN NLTVNLANAG SAALINTAVN GGSLKDNLEA
201 NILAALVNTA HGEAASKIKQ LDQHYITHKI AHAIAGCAAA AANKGKCQDG
251 AIGAAVGEIV GEALTNGKNP DTLTAKEREQ ILAYSKLVAG TVSGVVGGDV
301 NAAANAAEVA VKNNQLSDK*

[0444] Further work revealed the complete nucleotide sequence <SEQ ID 69>:

1ATGCAAGTAAATATTCAGATTCCCTATATACTGCCCAGATGCGTGCGTGC51TGAAGACACCCCTACGCTGCTATTTGAAACAGCTCCAAGTCACCAAAG101ACGTCAACTGGAACCAGGTACAACTGGCGTACGACAAATGGGACTATAAA151CAGGAAGCCTAACCGGAGCCGGAGCAGCGATTATTGCCGTGGCTGTTAC201CGTGGTTACTGCGGGCCGCGGAGCCGGAGCCGCACTGGCCTTAAACGGCG251CGGCCGCAGCGGCAACCGATGCCGCTAGCCCAGCCAGGCT301TCCGTATCGCTCATCAACAACAAAGGCAATATCGGTAACA351GCTGGGCAGAAGCAGACGGTGACAAAATCGATGGTTGCC451AAGCAGTGGATCAACAACCTGACCGTCACCCTGGCCAATG

-continued 501 CGCACTGATT AATACCGCTG TCAACGGCGG CAGCCTGAAA GACAATCTGG 551 AAGCGAATAT CCTTGCGGCT TTGGTGAATA CTGCGCATGG AGAAGCAGCC 601 AGTAAAATCA AACAGTTGGA TCAGCACTAC ATTACCCACA AGATTGCCCA 651 TGCCATAGCG GGCTGTGCGG CTGCGGCGGC GAATAAGGGC AAGTGTCAGG 701 ATGGTGCGAT AGGTGCGGCT GTGGGCGAGA TAGTCGGGGA GGCTTTGACA 751 AACGGCAAAA ATCCTGACAC TTTGACAGCT AAAGAACGCG AACAGATTTT 801 GGCATACAGC AAACTGGTTG CCGGTACGGT AAGCGGTGTG GTCGGCGGCG 851 ATGTAAATGC GGCGGCGAAT GCGGCTGAGG TAGCGGTGAA AAATAATCAG 901 CTTAGCGACA AAGAGGGTAG AGAATTTGAT AACGAAATGA CTGCATGCGC 951 CAAACAGAAT AATCCTCAAC TGTGCAGAAA AAATACTGTA AAAAAGTATC 1001 AAAATGTTGC TGATAAAAGA CTTGCTGCTT CGATTGCAAT ATGTACGGAT 1051 ATATCCCGTA GTACTGAATG TAGAACAATC AGAAAACAAC ATTTGATCGA 1101 TAGTAGAAGC CTTCATTCAT CTTGGGAAGC AGGTCTAATT GGTAAAGATG 1151 ATGAATGGTA TAAATTATTC AGCAAATCTT ACACCCAAGC AGATTTGGCT 1201 TTACAGTCTT ATCATTTGAA TACTGCTGCT AAATCTTGGC TTCAATCGGG 1251 CAATACAAAG CCTTTATCCG AATGGATGTC CGACCAAGGT TATACACTTA 1301 TTTCAGGAGT TAATCCTAGA TTCATTCCAA TACCAAGAGG GTTTGTAAAA 1351 CAAAATACAC CTATTACTAA TGTCAAATAC CCGGAAGGCA TCAGTTTCGA 1401 TACAAACCTA AAAAGACATC TGGCAAATGC TGATGGTTTT AGTCAAAAAC 1451 AGGGCATTAA AGGAGCCCAT AACCGCACCA ATTTTATGGC AGAACTAAAT 1501 TCACGAGGAG GACGCGTAAA ATCTGAAACC CAAACIGATA TTGAAGGCAT 1551 TACCCGAATT AAATATGAGA TTCCTACACT AGACAGGACA GGTAAACCTG 1601 ATGGTGGATT TAAGGAAATT TCAAGTATAA AAACTGTTTA TAATCCTAAA 1651 AAATTTTCTG ATGATAAAAT ACTTCAAATG GCTCAAAATG CTGCTTCACA 1701 AGGATATTCA AAAGCCTCTA AAATTGCTCA AAATGAAAGA ACTAAATCAA 1751 TATCGGAAAG AAAAAATGTC ATTCAATTCT CAGAAACCTT TGACGGAATC 1801 AAATTTAGAT CATATTTTGA TGTAAATACA GGAAGAATTA CAAACATTCA 1851 CCCAGAATAA

[0445] This corresponds to the amino acid sequence <SEQ ID 70; ORF41-1>:

1 MOVNIQIPYI LPRCVRAEDT PYACYLKOLO VTKDVNWNOV QLAYOKWDYK 51 QEGLTG<u>AGAA IIALAVTVVT AGA</u>GAGAALG LNGAAAAATD AAFASLASQA 101 SVSLINNKGN IGNTLKELGR SSTVKNLHVA VATAGVADKI GASALNNVSD 151 KOWINNLTVN LANAGSAALI NTAVNGGSLX DNLEANILAA LVNTAHGEAA 201 SKIKQLDQHY ITHKIAHAIA GCAAAAANKG KCQDGAIGAA VGEIVGEALT 251 NGKNPDTLTA KEREQILAYS KLVAGTVSGV VGGDVNAAAN AAEVAVKNNQ 301 LSDKLGREFD NEMTACAKON NPOLCRKNTV KKYONVADKR LAASIAICTD

-continued 351 ISRSTECRTI RKQHLIDSRS LHSSWEAGLX GKDDEWYKLF SKSYTQADLA 401 LQSYHLNTAA KSWLOSGNTK PLSEWNSDQG YTLISGVNPR FIPIPRGFVK 451 QHTFITNVKY PEGISFDTNL KRMLANADGF SQKQGIKGAH NRTNFNAELN 501 SRGGRVKSET QTDIEGITRI KYEIPTLDRT GKPDGGFKEI SSIKTVYNPK 551 KFSDDKILQH AQNAASQGYS KASKIAQNER TKSISERKNV IQFSETFDGI 601 KFRSYFDVNT GRITNIHPE*

[0446] Computer analysis of this amino acid sequence predicts a transmembrane domain, and homology with an ORF from N. meningitidis (strain A) was also found.

[0447] ORF41 shows 92.8% identity over a 279 aa overlap with an ORF (ORF41a) from strain A of N. meningitidis:

orf41.pep orf41a	10 YRRHLL	20 CKYIYRFPIY	30 CPXACVAEDI	11 111	1:1::11111	60 XLAYDKWDYKÇ 1111:1111 QLAYDRWDYKÇ 20	1111
orf41.pep orf41a	70 TG <u>AGAA</u> TE <u>AGAA</u>		<u>: </u> : :	1111 11111		120 SVSLINNKGNI : :: SVSFINNKGDV 80	: :
orf41.pep orf41a			:		1111111	180 LANAGSAALIN LANAGSAALIN 140	
orf41.pep orf41a				1111111111	:	240 GCAAAAANKGH GCAAAAANKGH 200	IIÎI
orf41.pep orf41a				1111111111		300 VGGDVNAAANA VGGDVNAAANA 260	
orf41.pep orf41a	310 AVKNNQ AVKNNQ		NENTACAKQN 290	XPQLCRXWIV	KKYQNVADKR 310	LAASIAICTDI 320	ISRS 330

[0448]	A partial	ORF41a	nucleotide	sequence	<seq< th=""><th>ID</th></seq<>	ID
71> is:						

1 TATCTGAAAC AGCTCCAAGT AGCGAAAAAC ATCAACTGGA ATCAGGTGCA 51 GCTTGCTTAC GACAGATGGG ACTACAAACA GGAGGGCTTA ACCGAAGCAG 101 GTGCGGCGAT TATCGCACTG GCCGTTACCG TGGTCACCTC AGGCGCAGGA 151 ACCGGAGCCG TATTGGGATT AAACGGTGCG NCCGCCGCCG CAACCGATGC 201 AGCATTCGCC TCTTTGGCCA GCCAGGCTTC CGTATCGTTC ATCAACAACA 251 AAGGCGATGT CGGCAAAACC CTGAAAGAGC TGGGCAGAAG CAGCACGGTG 77

-continued

301 AAAAATCTGG TGGTTGCCGC CGCTACCGCA GGCGTAGCCG ACAAAATCGG 351 CGCTTCGGCA CTGANCAATG TCAGCGATAA GCAGTGGATC AACAACCTGA 401 CCGTCAACCT AGCCAATGCG GGCAGTGCCG CACTGATTAA TACCGCTGTC 451 AACGGCGGCA GCCTGAAAGA CANTCTGGAA GCGAATATCC TTGCGGCTTT 501 GGTCAATACC GCGCATGGAG AAGCAGCCAG TAAAATCAAA CAGTTGGATC 551 AGCACTACAT AGTCCACAAG ATTGCCCATG CCATAGCGGG CTGTGCGGCA 601 GCGGCGGCGA ATAAGGGCAA GTGTCAGGAT GGTGCGATAG GTGCGGCTGT 651 GGGCGAGATA GTCGGGGAGG CTTTGACAAA CGGCAAAAAT CCTGACACTT 701 TGACAGCTAA AGAACGCGAA CAGATTTTGG CATACAGCAA ACTGGTTGCC 751 GGTACGGTAA GCGGTGTGGT CGGCGGCGAT GTAAATGCGG CGGCGAATGC 801 GGCTGAGGTA GCGGTGAAAA ATAATCAGCT TAGCGACNAA GAGGGTAGAG 851 AATTTGATAA CGAAATGACT GCATGCGCCA AACAGAATAN TCCTCAACTG 901 TGCAGAAAAA ATACTGTAAA AAAGTATCAA AATGTTGCTG ATAAAAGACT 951 TGCTGCTTCG ATTGCAATAT GTACGGATAT ATCCCGTAGT ACTGAATGTA 1001 GAACAATCAG AAAACAACAT TTGATCGATA GTAGAAGCCT TCATTCATCT 1051 TGGGAAGCAG GTCTAATTGG TAAAGATGAT GAATGGTATA AATTATTCAG 1101 CAAATCTTAC ACCCAAGCAG ATTTGGCTTT ACAGTCTTAT CATTTGAATA 1151 CTGCTGCTAA ATCTTGGCTT CAATCGGGCA ATACAAAGCC TTTATCCGAA 1201 TGGATGTCCG ACCAAGGTTA TACACTTATT TCAGGAGTTA ATCCTAGATT 1251 CATTCCAATA CCAAGAGGGT TTGTAAAACA AAATACACCT ATTACTAATG 1301 TCAAATACCC GGAAGGCATC AGTTTCGATA CAAACCTANA AAGACATCTG 1351 GCAAATGCTG ATGGTTTTAG TCAAGAACAG GGCATTAAAG GAGCCCATAA 1401 CCGCACCAAT NTTATGGCAG AACTAAATTC ACGAGGAGGA NGNGTAAAAT 1451 CTGAAACCCA NACTGATATT GAAGGCATTA CCCGAATTAA ATATGATATT 1501 CCTACACTAG ACAGGACAGG TAAACCTGAT GGTGGATTTA AGGAAATTTC 1551 AAGTATAAAA ACTGTTTATA ATCCTAAAAA NTTTTNNGAT GATAAAATAC 1601 TTCAAATGGC TCAANATGCT GNTTCACAAG GATATTCAAA AGCCTCTAAA 1651 ATTGCTCAAA ATGAAAGAAC TAAATCAATA TCGGAAAGAA AAAATGTCAT 1701 TCAATTCTCA GAAACCTTTG ACGGAATCAA ATTTAGANNN TATNTNGATG 1751 TAAATACAGG AAGAATTACA AACATTCACC CAGAATAA

[0449] This encodes a protein having the partial amino acid sequence <SEQ ID 72>:

1 YLKQLQVAKN INWNQVQLAY DRWDYKQEGL TE<u>AGAAIIAL AVTVVTSGA</u>G 51 TGAVLGLNGA XAAATDAAFA SLASQASVSF INNKGDVGKT LKELGRSSTV 101 KNLVVAAATA GVADKIGASA LXNVSDKQWI NNLTVNLANA GSAALINTAV 151 NGGSLKDXLE ANILAALVNT AHGEAASKIK QLDQHYIVRK IAHAIAGCAA 201 AAANKGKCQD GAIGAAVGEI VGEALTNGKN PDTLTAKERE QILAYSKLVA

251	GTVSGVVGGD	VNAAANAAEV	AVKNNQLSDX	EGREFONENT	ACAKQNXPQL
301	CRKNTVKKYQ	NVADKRLAAS	IAICTDISRS	TECRTIRKQH	LIDSRSLHSS
351	WEAGLIGKDD	EWYKLFSKSY	TQADLALQSY	BLNTAAKSWL	QSGNTKPLSE
401	VNSDQGYTLI	SGVNPRFIFI	PRGFVKQNTP	ITNVKYPEGI	SFDTNLXRHL
451	ANADGFSQEQ	GIKGAHNRTN	XMAELNSRGG	XVKSETXTDI	EGITRIKYEI
501	PTLDRTGKPD	GGFKEISSIK	TVYNPKXFXD	DKILQMAQXA	XSQGYSKASK
551	IAQNERTKSI	SERKNVIQFS	ETFDGIKFRX	YXDVNTGRIT	NIHPE*

[0450] ORF41a and ORF41-1 show 94.8% identity in 595 aa overlap:

orf41a.pep orf41-1	MQVNIQIPYI: 10	LPRCVRAEDT 20	PYACYLKQLÇ	: :: VTKDVNWNQV	: QLAYDKWDYK	QEGLTGAGAA
orf41a.pep orf41-1	40 IIALAVTVVT: IIALAVTVVT: 70	: : :	 LNGAAAAATI	 AAFASLASQA	: : SVSLINNKGN	: : IGNTLKELGR
orf41a.pep orf41-1	100 SSTVKNLVVA : SSTVKNLMVA 130	:	 GASALNNVSI			 NTAVNGGSLK
orf41a.pep orf41-1	160 DXLEANILAA DNLEANILAA 190		SKIKQLDQHY	: THKIAHAI?	 GCAAAAANKG	
orf41a.pep orf41-1	220 VGEIVGEALT VGEIVGEALT 250		KEREQILAYS		 VGGDVNAAAN	 AAEVAVKNNQ
orf41a.pep orf41-1	280 LSDXEGREFDI LSDKEGREFDI 310	HHHHĨI		 KKYQNVADKF	 RLAASIAICTE	 ISRSTECRTI
orf41a.pep orf41-1	340 RKQHLIDSRS: RKQHLIDSRS: 370					Î XSWLOSGNTK
orf41a.pep orf41-1	400 PLSEWMSDQG ⁻ PLSEWMSDQG 430		 FIPIPRGFVP		 PEGISFDTNL	 KRHLANADGF
orf41a.pep orf41-1	460 SQEQGIKGAHI : SQKQGIKGAHI 490 520		 ISRGGRVKSEI	 QTDIEGITRI	 KYEIPTLDRT	 GKPDGGFKEI

		-con	tinued			
orf41a.pep	SSIKTVYNPKXFX	DDKILQMAQX	AXSQGYSKASK	IAQNERTKS	ISERKNVIQFS	ETFDGI
		IIIII III III				
orf41-1	SSIKTVYNPKKFS	DDKILQMAQN	AASQGYSKASK	IAQNERTKS	ISERKNVIQFS	ETFDGI
	550	560	570	580	590	600
	580 59	0				
orf41a.pep	KFRXYXDVNTGRI	INIHPEX				
orf41-1	KFRSYFDVNTGRI	INIHPEX				
	610	620				

[0451] Amino acids 25-619 of ORF41-1 were amplified as described above. **FIG. 6** shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF41-1.

[0452] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 17

[0453] The following DNA sequence was identified in *N. meningitidis* <SEQ ID 73>

1 ATGGCAATCA TTACATTGTA TTATTCTGTC AATGGTATTT TAAATGTATG

51TGCAAAAGCAAAAAATATTCAAGTAGTTGCCAATAATAAGAATATGGTTC101TTTTTGGGTTTTTGGSmrGCATCATCGGCGGTTCAACCAATGCCATGTCT151CCCATATTGTTAATATTTTGCTTAGCGAAACAGAAAATAAAAATcgTAT201CGTAAAATCAAGCAATCTATGCTATCTTTGGCGAAAATTGTTCAAATAT251ATATGCTAAGAGACCAGTATTGGTTATTAAATAAGAGTGAATACGdTTTA301ATATTTTTACTGTCGTATTGTCTGTTATTGGATTGTATGTTGGAATTCG

351 GTTAAGGACT AAGATTAGCC CAAATTTTTT TAAAATGTTA ATTTTTATTG

401 tTTTATTGGT ATTGGCtCTG AAAATCGGGC AttCGGGTTT AAtCAAACTT

451 TAA

[0454] This corresponds to the amino acid sequence <SEQ ID 74; ORF51>:

1 HAIITLYYSV NGILNVCAKA KNIQVVANNK NMVLFG<u>FLXX IICGSTNANS</u> 51 <u>PIL</u>LIFLLSE TENKNRIVKS SNLCYLLAKI VQIYMLRDQY WLLNKS<u>EYXL</u> 101 <u>IFLLSVLSVI GLY</u>VGIRLRT KI<u>SPNFFKML IFIVLLVLA</u>L KIGHSGLIKL 151 *

[0455] Further work revealed the complete nucleotide sequence <SEQ ID 75>:

1 ATGCAAGAAA TAATGCAATCTATCGTTTTTGTTGCTGCCGCAATACTGCA51 CGGAATTACA GGCATGGGATTTCCGATGCTCGGTACAACCGCATGGCTT101 TTATCATGCCATTGTCTAAGGTTGTTGCCTTGGTGGCATTACCAAGCCTG151 TTAATGAGCTTGTTGGTTCTATGCAGCAATAACAAAAAGGGTTTTTGGCA

80

-continued

[0456] This corresponds to the amino acid sequence <SEQ ID 76; ORF51-1>:

1 MOEIMOSIVF VAAAILHGIT GMGFPMLGTT ALAFIMPLSK VVALVALPSL

51 <u>LMSLLVL</u>CSN NKKGFWQEIV YYLKTYKLLA IGSVVGSILG VK<u>LLLILPVS</u>

101 <u>WLLLLMAII</u>T LYYSVNGILN VCAKAKNIQV VANNKNNVLF GFLAG<u>IIGGS</u>

151 <u>TNAMSPILLI FL</u>LSETENKN RIVKSSNLCY LLAKIVQIYN LRDQYWLLNK

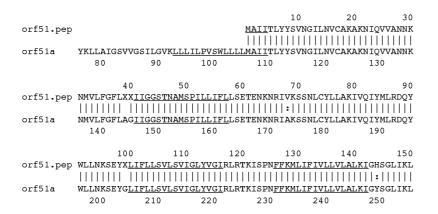
201 SEYGLIFLLS VLSVIGLYVG IRLRTKISPN FFKMLIFIVL LVLALKIGHS

251 GLIKL*

[0457] Computer analysis of this amino acid sequence reveals three putative transmembrane domains. A corresponding ORF from strain A of *N. meningitidis* was also identified:

[0458] Homology with a Predicted ORF from *N. menin-gitidis* (Strain A)

[0459] ORF51 shows 96.7% identity over a 150 as overlap with an ORF (ORF51a) from strain A of *N. meningitidis*:



[0460]	ORF51-1 and ORF51a show 99.2% identity in 255
aa overl	ap:

orf51a.pep	MQEIMQSIVFVAAAILHGITGMGFPMLGTTALAFIMPLSKVVALVALPSLLMSLLVLCSN
orf51-1	MQEIMQSIVFVAAAILHGITGMGFPMLGTTALAFIMPLSKVVALVALPSLLMSLLVLCSN
orf51a.pep	NKKGFWQEIVYYLKTYKLLAIGSVVGSILGVKLLLILPVSWLLLLMAIITLYYSVNGILN
orf51-1	NKKGFWQEIVYYLKTYKLLAIGSVVGSILGVKLLLILPVSWLLLLMAIITLYYSVNGILN
orf51a.pep	VCAKAKNIQVVANNKNMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIAKSSNLCY
orf51-1	VCAKAKNIQVVANNKNMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIVKSSNLCY
orf51a.pep	LLAKIVQIYMLRDQYWLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNFFKMLIFIVL
orf51-1	LLAKIVQIYMLRDQYWLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNFFKMLIFIVL
orf51a.pep : orf51-1	LVLALKIGYSGLIKLX LVLALKIGHSGLIKLX
01101-1	HARMONDOLLINA

[0461] The complete length ORF51a nucleotide sequence SEQ D 77> is:

1 ATGCAAGAAA TAATGCAATC TATCGTTTTT GTTGCTGCCG CAATACTGCA 51 CGGAATTACA GGCATGGGAT TTCCGATGCT CGGTACAACC GCATTGGCTT 101 TTATCATGCC ATTGTCTAAG GTTGTTGCCT TGGTGGCATT ACCAAGCCTG 151 TTAATGAGCT TGTTGGTTCT ATGCAGCAAT AACAAAAAGG GTTTTTGGCA 201 AGAGATTGTT TATTATTTAA AAACCTATAA ATTGCTTGCT ATCGGCAGCG 251 TCGTTGGCAG CATTTTGGGG GTGAAGTTGC TTTTGATACT TCCAGTGTCT 301 TGGCTGCTTT TACTGATGGC AATCATTACA TTGTATTATT CTGTCAATGG 351 TATTTTAAAT GTATGTGCAA AAGCAAAAAA TATTCAAGTA GTTGCCAATA 401 ATAAGAATAT GGTTCTTTTT GGGTTTTTGG CAGGCATCAT CGGCGGTTCA 451 ACCAATGCCA TGTCTCCCAT ATTGTTAATA TTTTTGCTTA GCGAAACAGA 501 GAATAAAAAT CGTATCGCAA AATCAAGCAA TCTATGCTAT CTTTTGGCAA 551 AAATTGTTCA AATATATATG CTAAGAGACC AGTATTGGTT ATThAATAAG 601 AGTGAATACG GTTTAATATT TTTACTGTCC GTATTGTCTG TTATTGGATT 651 GTATGTTGGA ATTCGGTTAA GGACTAAGAT TAGCCCAAAT TTTTTTAAAA 701 TGTTAATTTT TATTGTTTTA TTGGTATTGG CTCTGAAAAT CGGGTATTCA 751 GGTTTAATCA AACTTTAA

[0462] This encodes a protein having amino acid sequence <SEQ ID 78>:

1 MOEIMOSIVF VAAAILHGIT GMGFPNLGTT ALAFIMPLSK VVALVALPSL

51 <u>LMSLLVL</u>CSN NKKGFWQEIV YYLKTYKLLA IGSVVGSILG VK<u>LLLILPVS</u> 101 <u>WLLLLMAII</u>T LYYSVNGILN VCAKAKNIQV VANNKNMVLF GFLAG<u>IIGGS</u> 151 <u>TNAMSFILLI FL</u>LSETENKN RIAXSSNLCY LLAKIVQIYM LRDQYWLLNK

-continued 201 SEYG<u>LIFLLS VLSVIGLYVG I</u>RLRTKISPN <u>FFKMLIFIVL LVLALKI</u>GYS

251 GLIKL*

[0463] Based on this analysis, it is predicted that this protein from N. meningitidis, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 18

[0464] The following partial DNA sequence was identified in N. meningitidis <SEQ ID 79>

1 ATGAGACATA TGAAAATACA AAATTATTTA CTAGTATTTA TAGTTTTACA

51 TATAGCCTTG ATAGTAATTA ATATAGTGTT TGGTTATTTT GTTTTTCTAT 101 TTGATTTTTT TGCGTTTTTG TTTTTTGCAA ACGTCTTTCT TGCTGTAAAT 151 TTATTATTTT TAGAAAAAAA CATAAAAAAC AAATTATTGT TTTTATTGCC 201 GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA 251 AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT 301 ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA 351 TGGATATGCT AAATTAAAAG ATAATCATAG ATATGGTAGG GTAATTAGAG 401 AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA 451 AGATTAAGCT TGGTTTGTGG TATTCATTCA TATGCTCCAT GTGCCAATTT 501 TATAAAATTT GTCAGG..

[0465] This corresponds to the amino acid sequence <SEQ ID 80; ORF82>:

1 MRHMKIQNYL LVFIVLHIAL IVINIVFGYF VFLFDFFAFL FFANVFLAVN

51 LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI

- 101 TGVIKPNDSY NYVYDSNGYA KLKDWHRYGR VIRETPYIDV VASDVKNKSI
- 151 RLSLVCGIHS YAPCANFIKF VR..

[0466] Further work revealed the complete nucleotide sequence SEQ ID 81>:

1 ΑΤGAGACATA ΤGAAAAATAA AAATTATTTA CTAGTATTTA TAGTTTTACA 51 ТАТАСССТТС АТАСТААТТА АТАТАСТСТТ ТССТТАТТТ СТТТТСТАТ 101 TTGATTTTTT TGCGTTTTTG TTTTTTGCAA ACGTCTTTCT TGCTGTAAAT 151 TTATTATTTT TAAAAAAAAA CATAAAAAAC AAATTATTGT TTTTATTGCC 201 GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA 251 AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT 301 ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA 351 TGGATATGCT AAATTAAAAG ATAATCATAG ATATGGTAAG GTAATTAGAG -continued 401 AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA

401 AAACACCITA TATIGATGIA GITGGATCIO ATGITAARA TARACCATA
451 AGATTAAGCT TGGTTTGTGG TATFCATTCA TATGCFCCAT GTGCCAATTT
501 TATAAAATTT GCAAAAAAAC CTGTTAAAAT TTATTTTAT AATCAACCTC
551 AAGGAGATTT TATAGATAAT GTAATATTG AAATTAATGA TGGAAACAAA
601 AGTTTGTACT TGTTAGATAA GTATAAAACA TTTTTTCTTA TTGAAAACAG
651 TGTTTGTATC GTATTAATTA TTTTATATTT AAAATTTAAT TTGCTTTTAT
701 ATAGGACTTA CTTCAATGAG TTGGAATAG

[0467] This corresponds to the amino acid sequence <SEQ ID 82; ORF82-1>:

1 MRHMKNKNYL LVFIVLHIAL IVINIVFGYF VFLFDFFAFL FFANVFLAVN

51 LLFLEKNIKH KLLFLLPISI IIWMVIHISH INIKFYKFEH QIKEQNISSI

101 TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVKNKSI

151 RLSLVCGIHS YAPCANFIKF AKKPVKIYFY NQPQGDFIDN VIFEINDGNK

201 SLYLLDKYKT FFLIENSVCI VLIILYLKFN LLLYRTYFNE LE*

[0468] Computer analysis of this amino acid sequence reveals a predicted leader peptide.

[0469] A corresponding ORF from strain A of *N. menin-gitidis* was also identified:

[0470] Homology with a Predicted ORF from *N. menin-gitidis* (Strain A)

[0471] ORF82 shows 97.1% identity over a 172 as overlap with an ORF (ORF82a) from strain A of *N. meningitidis*:

orf82 pep orf82a	10 <u>MRHMKIONYLLVFIVLH</u> : <u>MRHMKIKNYLLVFIVLH</u>	:			50 60 <u>A</u> VNLLFLEKNIKN <u>AV</u> NLLFLEKNIKN
	10	20	30	40	50 60
orf82 pep orf82a	70 K <u>LLFLLPISIIIWMVIH</u> K <u>LLFLLPISIIIWMVIH</u> 70	<u>-</u>	FEHQIKEQNI FEHQIKEQNI	SSITGVIKPH	
130 orf82 pep orf82a	140 15 KLKDNHRYGRVIRETPY KLKDNHRYGRVIRETPY 130	IDVVASDVKNY IDVVASDVKNY	KSIRLSLVCG	IHSYAPCANF	IKFVR :: IKFAKKPVKIYFY 170
orf82a.pep orf82-1	MRHMKNKNYLLVFIVLH 	:::::::::::::::::::::::::::::::::::::::			
orf82a.pep orf82-1	KLLFLLPISIIIWMVIH 		Î Î		
orf82a.pep orf82-1	KLKDNHRYGRVIRETPY KLKDNHRYGRVIRETPY				

orf82a.pep orf82-1	-continued NQPQGDFIDNVIFEINKGKKSLYLLDKYKTFFLIENSVCIVLIILYLKFNLLLYRTYFNE
orf82a.pep orf82-1	LEX LEX

[0472] ORF82a and ORF82-1 show 99.2% identity in 242 aa overlap:

orf82a.pep orf82-1	MRHMKNKNYLLVFIVLHITLIVINIVFGYGVFLFDFFAFLFFANVFLAVNLLFLEKNIKN
orf82a.pep	KLLFLLPISIIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
orf82-1	KLLFLLPISIIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
orf82a.pep	KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY
orf82-1	KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY
orf82a.pep	NQPQGDFIDNVIFEINDGKKSLYLLDKYKTFFLIENSVCIVLIILYLKFNLLLYRTYFNE
orf82-1	NQPQGDFIDNVIFEINDGNKSLYLLDKYKTFFLIENSVCIVLIILYLKFNLLLYRTYFNE
orf82a.pep	LEX
orf82-1	LEX

[0473] The complete length ORF82a nucleotide sequence <SEQ D 83> is:

[0474] This encodes a protein having amino acid sequence <SEQ ID 84>:

1 MRHMKNKNYL LVFIVLHITL IVINIVFGYF VFLFDFFAFL FFANVFLAVN

51 LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI

101 TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVIQKSI

151 RLSLVCGIHS YAPCANFIKF AXKPVKIYFY NQPQGDFXDN VIFEINDGKK

201 SLYLLDKYKT <u>FFLIENSVCI VLIILYL</u>KFN LLLYRTYFNE LE*

[0475] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 19

[0476] The following partial DNA sequence was identified in *N. meningitidis* <SEQ ID 85>

1..ACCCCCAACA GCGTGACCGT CTTGCCGTCT TTCGGCGGAT TCGGGCGTAC
51 CGGCGGACC ATCAATGCAG CAGGCGGGGT CGGCATGACT GCCTTTTCGA
101 CAACCTTAAT TTCCGTAGCC GAGGGCGCGG TTGTAGAGCT GCAGGCCGTG
151 AGAGCCAAAG CCGTCAATGC AACCGCCGCT TGCATTTTA CGGTCTTGAG
201 TAAGGACATT TTCGATTCC TTTTTATTTT CCGTTTTCAG ACGGCTGACT
251 TCCGCCTGTA TTTTCGCCAA AGCCATGCCG ACAGCGTGCG CCTTGACTTC
301 ATATTTAAAA GCTTCCGCGC GTGCCAGTTC CAGTTCGCGC GCATAGTTTT
351 GAGCCGACAA CAGCAGGCCT TGCCGCTTGT CGCGCTCCAT CTTGTCGATG
401 ACCGCCTGCA GCTTCGCAAA TGCCGACTG TAGCCTTGAT GGTGCGACAC
451 AGCCAAGCCC GTGCCGACAA CGCGGATAAT GGCAATCGGT TGCCAGTAAT
501 TCGCCAGCAG TTTCACGAGA TTCATTCTC ACCTCTGAC GCTTCACGCT

[0477] This corresponds to the amino acid sequence <SEQ ID 86; ORF124>:

1..TPNSVTVLPS FGGFGRTGAT INAAGGVGMT AFSTTLISVA EGAVVELQAV

- 51 RAKAVNATAA <u>CIFTVLSKDI FDFLFIF</u>RFQ TADFRLYFRQ SHADSVRLDF
- 101 IFKSFRACQF QFARIVLSRQ QQGLRLVALH LVDORLQLRX CRLVALMVRH
- 151 SQARADKRDN GNRLPVIRQQ FHEIHSRPPD ASR*

[0478] Computer analysis of this amino acid sequence predicts a transmembrane domain.

[0479] Further work revealed the complete nucleotide sequence SEQ ID 87>:

1 ATGACTGCCT TTTCGACAAC CTTAATTTCC GTAGCCGAGG GCGCGGTTGT

-continued 51 AGAGCTGCAG GCCGTGAGAG CCAAAGCCGT CAATGCAACC GCCGCTTGCA

101 TTTTTACGGT CTTGAGTAAG GACATTTCG ATTTCCTTTT TATTTTCCGT
151 TTTCAGACGG CTGACTTCCG CCTGTTTTT CGCCAAAGCC ATGCCGACAG
201 CGTGCGCCTT GACTTCATAT TTTTTAGCTT CCGCGGCGTGC CAGTTCCAGT
251 TCGCGCGCAT AGTTTTGAGC CGACAACAGC AGGGCTTGCG CCTTGTCGCG
301 CTCCATCTTG TCGATGACCG CCTGCTGCTT CGCAAATGCC GACTTGTAGC
351 CTTGATGGTG CGACACAGCC AAGCCCGTGC CGACAAGCGC GATAATGGCA
401 ATCGGTTGCC AGTTATTCGC CAGCAGTTC ACGAGATTCA TTCTCGACCT
451 CCTGACGCTT CACGCTGA

[0480] This corresponds to the amino acid sequence SEQ ID 88; ORF124-1>:

1 MTAFSTTLIS VAEGAVVELQ AVRAKAVNAT AACIFTVLSK DIFDFLFIFR

51 FQTADFRLFF RQSHADSVRL DFIFFSFRAC QFQFARIVLS RQQQGLRLVA

101 LHLVDDRLLL RKCRLVAIMV RHSQARADKR DNGNRLPVIR QQFHEIHSRP

151 PDASR*

[0481] A corresponding ORF from strain A of *N. menin-gitidis* was also identified:

[0482] Homology with a Predicted ORF from *N. meningitidis* (Strain A)

[0483] ORF124 shows 87.5% identity over a 152 aa overlap with an ORF (ORF124a) from strain A of *N. meningitidis*:

	10	20	30	40	50	60
orf124.pep	TPNSVTVLPSFGGF	GRTGATINAA	GGVGMTAFST	TLISVAEGA		AVNATAA
orf124a			MTAFST		• LVELQAVMAK	AVNTTAA
				10	20	30
	70	80	90	100	110	120
orf124.pep	CIFTVLSKDIFDFL	FIFRFQTADE				RIVLSRQ
orf124a	CIFTVLSKDIFDFL	 FIFRFOTADF		: GVRLDFIFF	: SFRTRLFOFA	GVVLSRO
	40	50	60	70	80 ~	90
	130	140	150	160	170	180
orf124.pep	QQGLRLVALHLVDI	RLQLRKCRLV		ADKRDNGNR	LPVIRQQFHE	IHSRPPD
orf124a	::: OOGLRLVALHFLNE				LEVIROOFHE	
0111240	100	110	120	130	140	150
orf124.pep	ASRX					
orriz4.pep	:					
orf124a	VX					

[0484] ORF124a and ORF124-1 show 89.5% identity in 152 aa overlap:

orf124a	-continued MTAFSTTLISVAEGALVELQAVMAKAVNTTAACIFTVLSKDIFDFLFIFRFQTADFRLFF
orf124-1.pep	RQSHADSVRLDFIFFSFRACQFQFARIVLSRQQQGLRLVALHLVDDRLLLRKCRLVALMV
orf124a	RQSHADGVRLDFIFFSFRTRLFQFAGVVLSRQQQGLRLVALHFLNDRLLLRKSRLVALMV
orf124-1.pep	RHSQARADKRDNGNRLPVIRQQFHEIHSRPPDASRX : : :
orf124a	RHRQTRADKRDDGNRLPVIRQQFHEIHSRPPDVX

[0485] The complete length ORF124a nucleotide sequence <SEQ ID 89> is:

1 ATGACCGCCT TTTCGACAAC CTTAATTTCC GTAGCCGAGG GCGCGCTTGT

51 AGAGCTGCAA GCCGTGATGG CCAAAGCCGT CAATACAACC GCCGCCTGCA

101 TTTTTACGGT CTTGAGTAAG GACATTTTCG ATTTCCTTTT TATTTTCCGT

151 TTTCAGACGG CTGACTTCCG CCTGTTTTTT CGCCAAAGCC ATGCCGACGG

201 CGTGCGCCTT GACTTCATAT TTTTTAGCTT CCGCACGCGC CTGTTCCAGT

251 TCGCGGGCGT AGTTTTGAGC CGACAACAGC AGGGCTTGCG CCTTGTCGCG

301 CTTCATTTTC TCAATGACCG CCTGCTGCTT CGCAAAAGCC GACTTGTAGC

351 CTTGATGGTG CGACACCGCC AAACCCGTGC CGACAAGCGC GATGATGGCA

401 ATCGGTTGCC AGTTATTCGC CAGCAGTTTC ACGAGATTCA TTCTCGACCT

451 CCTGACGTTT GA

[0486] This encodes a protein having amino acid sequence <SEQ ID 90>:

- 1 MTAFSTTLIS VAEGALVELQ AVNAXAVNTT AACIFTVLSK DIFDFLFIFR
- 51 FQTADFRLFF RQSHADGVRL DFIFFSFRTR LFQFAGVVLS RQQQGLRLVA
- 101 LHFLNDELLL RKSRLVALHV RHRQTRADKR DDGNRLPVIR QQFHEIHSRP
- 151 PDV*

[0487] ORF124-1 was amplified as described above. **FIG.** 7 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF124-1.

[0488] Based on this analysis, it is predicted that this protein from *N. meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 20

[0489] Table III lists several *Neisseria* strains which were used to assess the conservation of the sequence of ORF 40 among different strains.

TABLE II

List of Neis	seria Strains V	Used for Gene Variability Study of ORF 40	
Identification number	Strains	Source/reference	
Group B			
zn02_1 zn03_1	BZ198 NG3/88	R. Moxon/Seiler et al., 1996 R. Moxon/Seiler et al., 1996	

TABLE III-continued

List of Neisseria Strains Used for Gene Variability Study of ORF 40

Identification		
number	Strains	Source/reference
zn04_1	297-0	R. Moxon/Seiler et al., 1996
zn06_1	BZ147	R. Moxon/Seiler et al., 1996
zn07_1	BZ169	R. Moxon/Seiler et al., 1996
zn08_1	528	R. Moxon/Seiler et al., 1996
zn 10_1	BZ133	R. Moxon/Seiler et al., 1996
zn11_1ass	NGE31	R. Moxon/Seiler et al., 1996
zn14_1	NGH38	R. Moxon/Seiler et al., 1996
zn16_1	NGH15	R. Moxon/Seiler et al., 1996
zn18_1	BZ232	R. Moxon/Seiler et al., 1996
zn19_1	BZ83	R. Moxon/Seiler et al., 1996
zn20_1	44/76	R. Moxon/Seiler et al., 1996
zn21_1	MC58	R. Moxon

TABLE III-continued

List of Nei	sseria Strains U	Jsed for Gene Variability Study of ORF 40
Identification number	Strains	Source/reference
		Group A
zn22_1	205900	R. Moxon
zn23_1	F6124	R. Moxon
z2491_1	Z2491	R. Moxon/Maiden et al., 1998
		Group C
zn24_1	90/18311	R. Moxon
zn25_1ass	93/4286	R. Moxon

TABLE III-continued

List of Neis	sseria Strains Used for	Gene Variability Study of ORF 40	
Identification number	Strains	Source/reference	
Others			
zn28_1ass zn29_1ass	860800 (group Y) E32 (group Z)	R. Moxon/Maiden et al., 1998 R. Moxon/Maiden et al., 1998	
References:			

Seiler A. et al., Mol. Microbiol., 1996, 19(4): 841-856. Maiden et al., Proc. Natl. Acad. Sci. USA, 1998, 95: 3140-3145.

[0490] The amino acid sequences for each listed strain are as follows:

>Z2491 <SEQ ID 91>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT NASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKISGNVSPSKADEMDETV NINAGNNIEISRNGKNIDIATSNAPQFSSVSLGAGADAPTLSVDDEGALNVGSKDANKPV RINVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVWDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW

>ZN02_1 <SEQ ID 92>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVATAVLATLLFATVQANATDDDDLYLE PVQRTAVLSFRSDKEGTGEKEGTEDSHGGAVYFDEKRVLKAGAITLKAGDNLKIKQNTNE NTNDSSFTYSLKKDLTDLTSVETEKLSFGAAGNKVNITSDTKGLNFAKETAGTAGDPTVH LNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNV DFVRTYDTVEFLSADTKTTTNVSKDNGKKTEVICIGAIVTSVIKEKDGKLVTGKGKDENG SSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTA TVSKODQGNITVRYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGRMDE TVN INAGNNIEITRNGKNIDIATSMAPOFSSVSLGAGADAPTLSVDDEGALNVGSKDTNK PVRITNVAPGVKEGDVTNVAOLKGVAONLNNRIDNVDGNARAGIAOAIATAGLVOAYLPG KSMMAIGGDTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW*

>ZN03 1 <SEO ID 93>

MNKTYRTTWNSALNAWVAVSELTRNHTKRASATVATAVLATLIFATVOASTTDDDDLYLF PVORTAPVLSFHADSEGTGEKEVTEDSNWGVYFDKKGVLTAGTITLKAGDNLKIKONTDE NTNDSSFTYSLKKDLTDLTSVETEKLSFGANGNKVNITSDTKGLNFAKETAGTNGDPTVH LNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNV DFVRTYDTVEFLSADTRTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGKGKDENG SSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTA TVSKDDOGNITVKYDVNVGDALNVNOLONSGWNLDSKAVAGSSGKVISGNVSPSKGKMDE ${\tt TVNINAGNNIEITRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGALNVGSKDTNK$ ${\tt PVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPG}$ ${\tt KSMMAIGGDTYRGEAGYAIGYSSISDGGNMIIKGTASGNSRGHFGASASVGYQW*}$

>ZN04 1 <SEO ID 94>

 ${\tt MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVATAVLATLLFATVQASTTDDDDLYLE}$ PVORTAPVLSFHADSEGTGEKEVTEDSNWGVYFDKKGVLTAGTITLKAGDNLKIKONTDE ${\tt NTNDSSFTYSLKKDLTDLTSVETEKLSFGANGNKVNITSDTKGLNFAKETAGTNGDPTVH$ LNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNV ${\tt DFVRTYDTVEFLSADTRTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGKGKDENG$ SSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTA TVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDE TVNINAGNNIEITRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGALNVGSKDTNK PVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPG KSMMAIGGDTYRGEAGYAIGYSSISDGGNMIIKGTASGNSRGHFGASASVGYQW*

>ZN06_1 <SEO ID 95>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ${\tt ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT}$ NASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKISGNVSPSKADEMDETV

NINAGNNIEISRNGKNIDIATSNAPQFSSVSLGAGADAPTLSVDDEGALNVGSKDANKPV RINVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVWDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW*

>ZN07_1 <SEQ ID 96>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT NASFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTCKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKISGNVSPSKADEMDETV NINACNNIEISRNGKNIDIATSNAPQFSVSLGAGADAPTLSVDDEGALNVGSKDANKPV RINVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVWDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGTYRCEAGYAIGYSSISDGGWNIKGTASGNSGHFGASASVGYOW*

>ZN08_1 <SEQ ID 97>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVETAVLATLLFATVQANATDTDEDDEL EPVVRSALVLQFMIDKEGNGEIESTGDIGWSIYYDDHNTLHGATVTLKAGDNLKIKQNTD ENTNASSFTYSLKKDLTDLTSVGTELSFGANGNKVNITSDTKGLNFAKKAGTMGDTTV HLNGIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTGQSEN VDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGEN GSSTEDGEGELVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTT ATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKISGNAVSPSKGKMD ETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPLTLSVDDEALNVCSKDAN KPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIATAGLVQAYLP GKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASCNSGHFGASASVGYQM*

>ZN10_1 <SEQ ID 98>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT NASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETV NINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPILSVDDEGALNVGSKDANKPV RITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGYTRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW*

>ZN11_1 ASS <SEQ ID 99>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVATAVLATLLFATVQASTTDDDDLYLE PVQRTAPVLSFHADSEGTGEKEVTEDSNWGVYFDKKGVLTAGTITLKAGDNLKIKQNTDE NTNDSSFTYSLKKDLTDLTSVETEKLSFGANGNKVNITSDTKGLNFAKETAGTNGDPTVH LNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNV DFVRTYDTVEFLSADTRTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGKGKDENG SSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTA TVSKDDQGNITVKYDVNVGDALNVNQLQNSGWLDSKAVAGSSGKVISGNVSPSKGKMDE TVNINAGNNIEITRNGKNIDIATSMAPQFSSVSLGAGADAPLSVDGALNVGSKDTNK PVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVGGNARAGIAQAIATAGLVQAYLPG KSMMAIGGDTYRGEAGYAIGYSSISDGGNMIIKGTASGNSGRHFGASASVGYQW*

>ZN14_1 <SEQ ID 100>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVETAVLATLLFATVQANATDTDEDDEL EPVVRSALVLQFMIDKEGNGEIESTGDIGWSIYYDDHNTLHGATVTLKAGNLKIKQNTD ENTNASSFTYSLKKDLTDLTSVGTEELSFGANGNKVNITSDTKGLNFAKKTAGTNGDTV HLNGIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSEN VDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKCKGEN GSSTEDGEGELVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTT ATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKISGNAVSPSKGKMD ETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPLTLSVDDEALNVGSKDAN KPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIATAGLVQAYLP GKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASCNSRGHFGASASVGYOW*

>ZN16_1 <SEQ ID 101>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVATAVLATLLFATVQANATDDDDLYLE PVQRTAVVLSFRSDKEGTEGEKEGTEDSNWAVYFDEKRVLKAGATTLKAGDNLKIKQNTNE NTNENTNDSSFTYSLKKDLTDLTSVETEKLSFGANGNKVNITSDTKGGNFAKETAGTNGD PTVHLNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRASVKDVLNAGWNIKGVKPGTTA SDNVDFVRTVDTVEFLSADTKTTTVNVESKDNGKKTEVNIGAKTSVIKEKDGKLVTCKGK DENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKVTFASGN GTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKG KMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGALNVGSK DANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLAQA YLPGKSMMAIGGGTYRGEAGYAIGYSSISDTGNWVIKGTASONSRGHFGASASVGYQW*

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MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVATAVLATLLFATVQASTTDDDDLYLE PVQRTAPVLSFHADSEGTGEKEVTEDSNWGVYFDKKGVLTAGTITLKAGDNLKIKQNTDE NTNDSSFTYSLKKDLTDLTSVETEKLSFGANGNKVNITSDTKGLNFAKETAGTNGPFVH LNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRASVKDVLNAGWNIKGVKPGTTASDNV DFVRTYDTVEFLSADTRTTTVNVESKDNGKKTEVKIGATSVIKEKDGKLVTGKGKDENG SSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTA TVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDE TVNINAGNNIEITRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGALNVGSKDTNK PVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPG KSMMAIGGDTYRGEAGYAIGYSSISDGGMUIIKGTAGSNGSRFGASASVGYOW*

>ZN19_1 <SEQ ID 103>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVKTAVLATLLFATVQASANNEEQEEDL YLDPVQRTVAVLIVNSDKEGTGEKEKVEENSDWAVYFNEKGVLTAREITLKAGDNLKIKQ NGTNFTYSLKKDLTDLTSVGTEKLSFSANGNKVNITSDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNVDF VRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGKDKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVOBALNVNQLQNSGWNLDSKAVAGSSGKVISGNAVSPSKGKMDETV NINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGDALVSGSKKDNKPVR ITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKSM

>ZN20_1 <SEQ ID 104>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVKTAVLATLLFATVQASANNEEQEEDL YLDPVQRTVAVLIVNSDKEGTGEKEKVEENSDWAVYFNEKGVLTAREITLKAGDNLKIKQ NGTNFTYSLKKDLTDLTSVGTEKLSFSANGNKVNITSDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTASDNVDF VRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTCKDKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETV NINAGNNIEITRNGKNIDIATSMTPQFSVSLGAGADAPTLSVDGDAUNSGSKKDNKPVR ITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKSM MAIGGGTYRCEAGYAIGYSSISDGGNWIKGTASGNSCHFGASASVGYQW*

>ZN21_1 <SEQ ID 105>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVKTAVLATLLFATVQASANNEEQEEDL YLDPVQRTVAVLIVNSDKEGTGEKEKVEENSDWAVYFNEKGVLTAREITLKAGDNLKIKQ NGTNFTYSLKKDLTDLTSVGTEKLSFSANGNKVNITSDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTASDNVDF VRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGKDKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETV NINACMNIEITRNGKNIDIATSMTPQFSVSLGAGADAPTLSVDGDALNVGSKKDNKPVR ITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKSM MAIGGGTYRGEAGYAIGYSSISDGGNWIKGTASGNSGHFGASASVGYOW*

>ZN22_1 <SEQ ID 106>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT NASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKENDGKLVTGKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETV NINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGALNVGSKDANKPV RITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGYTRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW*

>ZN23_1 <SEQ ID 107>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT NASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETV NINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDGAIANVGSKDANKPV RITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGYTRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW*

>ZN24_1 <SEQ ID 108>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVATAVLATLLSATVQANATDTDEDEEL ESVVRSALVLQFMIDKEGNGEIESTGDIGWSIYYDDHNTLHGATVTLKAGNLKIKQSGK DFYYSLKKELKDLTSVETEKLSFGANGNKVNITSDTKGLNFAKETAGTNGDPTVHLNGIG STLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDFVRT YDTVEFLSADTKTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSSTDE

GEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKD DQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETVNIN AGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGALNVGSKDANKPVRIT NVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLAQAYLPGKSMAA IGGGTYRGEAGYAIGYSSISDTCNWVIKGTASGNSRGHFGTSASVGYOW*

>ZN25_ASS <SEQ ID 109>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVATAVLATLLSATVQANATDTDEDEEL ESVVRSALVLQFMIDKEGNGEIESTGDIGWSIYYDDHNTLHGATVTLKAGNLKIKQSGK DFYYSLKKELKDLTSVETEKLSFGANGNKVNITSDTKGLNFAKETAGTNGDPTVHLNGIG STLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDFVRT YDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSSTDE GEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKD DQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSSKGKMDETVNIN AGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGALNVGSKDANKPVRIT NVAPGYKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLAQAYLPGKSMMA IGGGTYRGEAGYAIGYSSISDTGNWVIKGTASGNSGHFGTSASVGYQW*

>ZN28_ASS <SEQ ID 110>

MNKIYRIIWNSALNAWVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL ESVQRSVVGSIQASMEGSGELETISLSMTNDSKEFVDPYIVVTLKAGDNLKIKQNTNENT NASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLN GIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTTGQSENVDF VRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSS TDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFASGKGTTATV SKDDQGNITVMYDVNVODALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETV NINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGIAVNGSKDANKPV RITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKS MMAIGGGYTRGEAGYAIGYSSISDGGWNIKGTASGNSRGHFGASASVGYQW*

>ZN29_ASS <SEQ ID 111>

MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVETAVLATLLFATVQANATDTDEDDEL EPVVRTAPVLSFHSDKEGTGEKEEVGASSNLTVYFDKNRVLKAGTITLKAGDNLKIKQNT NENTNENTNASSFTYSLKKDLTGLINVETEKLSFGANGKKVNIISDTKGLNFAKETAGTN GDPTVHLNGIGSTLTDTLAGSSASHVDAGNQSTHYTRAASIKDVLNAGWNIKGVKTGSTT GQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEGDKLVTGK GKGENGSSTDEGEGLVTAKEV IDAVNKAGWRMKTTANQGTQADKFETVTSGTKVTFAS GNGTTATVSKDDQGNITVKYDVNGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPS KGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVVEAGALNVG SKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLV QAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW

[0491] FIG. 8 shows the results of aligning the sequences of each of these strains. Dark shading indicates regions of homology, and gray shading indicates the conservation of amino acids with similar characteristics. As is readily discernible, there is significant conservation among the various strains of ORF 40, further confirming its utility as an antigen for both vaccines and diagnostics.

[0492] It will be appreciated that the invention has been described by means of example only, and that modifications may be made whilst remaining within the spirit and scope of the invention.

Appendix 1

[0493]

Scarlato, Continuation of U.S. App. Ser. No. 10/695,499, filed herewith	Ruelle, U.S. Pat. No. 6,780,419
 18. (New) An isolated polypeptide comprising a member selected from the group consisting of (a) the amino acid sequence of SEQ ID NO: 4; and (b) an immunogenic fragment of at least 15 contiguous amino acids of SEQ ID NO: 4, wherein the immunogenic fragment, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell meditated immune response that recognizes the isolated polypeptide SEQ ID NO: 4. 19. (New) The isolated polypeptide of claim 18, wherein the polypeptide is according to (a). 20. (New) The isolated polypeptide of claim 18, wherein the polypeptide is according to (b). 	 An isolated polypeptide comprising a member selected from the group consisting of (a) the amino acid sequence of SEQ ID NO: 2; (b) an immunogenic fragment of at least 15 contiguous amino acids of SEQ ID NO: 2; wherein the immunogenic fragment, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell meditated immune response that recognizes the isolated polypeptide SEQ ID NO: 2. The isolated polypeptide of claim 1, wherein the polypeptide is according to (a). The isolated polypeptide is claim 1, wherein the polypeptide is according to (b).

Scarlato, Continuation of U.S. App. Ser. No. 10/695,499, filed herewith	Ruelle, U.S. Pat. No. 6,780,419
 (New) The isolated polypeptide of claim 18, wherein the immunogenic fragment of (b) comprises at least 20 contiguous amino acids of SEQ ID NO: 4; wherein the immunogenic fragment, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell meditated immune response that recognizes the isolated polypeptide SEQ ID NO: 4. (New) The isolated polypeptide of claim (New) A fusion protein comprising the isolated polypeptide of claim 18. (New) An immunogenic composition comprising the polypeptide. (New) An immunogenic composition comprising the polypeptide. (New) The isolated polypeptide of claim wherein the isolated polypeptide of claim wherein the isolated polypeptide is a recombinant polypeptide. (New) The isolated polypeptide of claim wherein the isolated polypeptide is a recombinant polypeptide. (New) The isolated polypeptide of claim wherein the isolated polypeptide of claim the wolypeptide of claim the woly	 4. The isolated polypeptide of claim 1, wherein the immunogenic fragment of (b) comprises at least 20 contiguous amino acids of SEQ ID NO: 2; wherein the immunogenic fragment, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell meditated immune response that recognizes the isolated polypeptide SEQ ID NO: 2. 5. The isolated polypeptide of claim 1, wherein the isolated polypeptide of claim 1, wherein the isolated polypeptide of claim 1. 7. An immunogenic composition comprising the polypeptide of claim 1, and a pharmaceutically acceptable carrier. 9. The isolated polypeptide of claim 1, wherein the isolated polypeptide is a recombinant polypeptide. 10. The isolated polypeptide is a recombinant polypeptide. 11. The isolated polypeptide of claim 3, wherein the isolated polypeptide is a recombinant polypeptide. 12. An immunogenic composition comprising the isolated polypeptide. 13. An immunogenic composition comprising the isolated polypeptide. 14. A fusion protein comprising the isolated polypeptide of claim 3. 14. A fusion protein comprising the isolated polypeptide of claim 3. 15. A fusion protein comprising the isolated polypeptide of claim 3.

Appendix 2

[0494]

Added Claim #	Written Description Support in the Current Application (Continuation of Application No. 10/695,499)	Written Description Support in Application No. PCT/IB99/00103
Claims 18–31	Throughout the application and at least at the following citations: Page 3, lines 2–24; Page 31, line 7 to page 34, line 17; Page 52, lines 10–18; Page 65, line 3 to page 70, line 3.	Throughout the application and at least at the following citations: Page 2, line 29 to page 3, line 20; Page 30, line 6 to page 33, line 11; Page 50, lines 12–20; Page 61, line 11 to page 66, line 6.
Claims 23, 30, and 31	Throughout the application and at least at the following citations: Page 3, lines 24-27; Page 9, line 26 to page 10, line 4; Page 21, lines 1-22.	Throughout the application and at least at the following citations: Page 3, lines 21–24; Page 9, lines 11–18; Page 20, line 6 to page 21, line 4.
Claims 25–27	Throughout the application and at least at the following citations: Page 3, lines 24–27;	Throughout the application and at least at the following citations: Page 3, lines 17–20;

-continued

Added Claim #	Written Description Support in the Current Application (Continuation of Application No. 10/695,499)	Written Description Support in Application No. PCT/IB99/00103
	Page 8, line 15 to page 28, line 23.	Page 8, line 1 to page 27, line 25.

Appendix 3

Disclosure of Constructive Reductions to Practice within the Scope of the Interfering Subject Matter in Application No. GB 9800760.2, filed Jan. 14, 1998

[0495]

	Location in ORF40 of Application No. GB 9800760.2	Location in SEQ 2 of '419 Patent
25	Residues 85–109	Residues 127–151
16	Residues 111–126	Residues 153–168
98	Residues 131–228	Residues 173–270
16	Residues 230–245	Residues 272–287

SEQUENCE LISTING

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145					150					155					160		
Leu	Ala	Gln	Ile	Phe 165	Gly	Lys	Gln	Ala	Glu 170	Ala	Asp	Lys	Leu	L y s 175	Ala		
Glu	Ile	Asp	Ala 180	Ser	Phe	Glu	Ala	Ala 185	Lys	Thr	Ala	Ala	Gln 190	Gly	Lys		
Gly	Lys	Gly 195	Leu	Val	Ile	Leu	Val 200	Asn	Gly	Gly	Lys	Met 205	Ser	Ala	Phe		
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Pro 225	Ala	Val	Asp	Glu	Ser 230	Ile	Lys	Glu	Gly	Ser 235	His	Gly	Gln	Pro	Ile 240		
Ser	Phe	Glu	Tyr	Leu 245	Lys	Glu	Lys	Asn	Pro 250	Asp	Trp	Leu	Phe	Val 255	Leu		
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Leu	Asp	Asn 275	Pro	Leu	Val	Ala	Glu 280	Thr	Thr	Ala	Trp	L y s 285	Lys	Gly	Gln		
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ggc	gtgt	ccg ·	ttac	cgtca	aa a	acggo	cgcgo	c ggo	gate	gttc	aaa	tacc	gca (aaaco	ccga	a :	180
cgt	atcg	ccg ·	ttta	cgati	tt g	ggtai	tgcto	c gao	cacci	tga	gca	aact	aaa	cgtga	aaac	c 2	240
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gac	atcg	gcg .	ttcc	cgct	gt te	gacga	aagco	c ato	caaa	gaag	gca	gcca	cdd .	tcago	ctat	c î	720
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aca	accg	ctt (ggaa	aaaa	gg a	caag	tcgt	t tao	cctt	gttc	ctg	aaac	tta ·	tttg	gcagc	c S	900
ggt	ggcgo	cgc i	aaga	gcta	ct ga	aatgo	caago	c aaa	acag	gttg	ccg	acgc [.]	ttt ·	taaco	ledde	a S	960
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gccgtcatca acggcaaacg cgtgcaaatg cctgtcaatt tggacaaatc cgacaatgtg	240
gaaacattot acggcaaaga aggcggttat gttttgggta coggogtgat ggatggcaaa	300
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Thr Val Ser Tyr Val Cys Gln Gln Gly Lys Lys Val Lys Val Thr Tyr 35 40 45	
Gly Phe Asn Lys Gln Gly Leu Thr Thr Tyr Ala Ser Ala Val Ile Asn 50 55 60	
Gly Lys Arg Val Gln Met Pro Val Asn Leu Asp Lys Ser Asp Asn Val 65 70 75 80	
Glu Thr Phe Tyr Gly Lys Glu Gly Gly Tyr Val Leu Gly Thr Gly Val 85 90 95	
Met Asp Gly Lys Ser Tyr Arg Lys Gln Pro Ile Met Ile Thr Ala Pro 100 105 110	
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ggtaaaaaag tcaaagtaac ctacggcttt aacaaacagg gcctgaccac atacgcttcc	180
gccgtcatca acggcaaacg tgtgcaaatg cctgtcaatt tggacaaatc cgacaatgtg	240
gaaacattot acggcaaaga aggcggttat gttttgggta coggogtgat ggatggcaaa	300
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tgttccccac gttaa	375
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- <213> ORGANISM: Neisseria meningitidis

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Ser Met Ala Ala Ala Gly Thr Asn Asn Pro Thr Val Ala Lys Lys 20 25 30	
Thr Val Ser Tyr Val Cys Gln Gln Gly Lys Lys Val Lys Val Thr Tyr 35 40 45	
Gly Phe Asn Lys Gln Gly Leu Thr Thr Tyr Ala Ser Ala Val Ile Asn 50 55 60	
Gly Lys Arg Val Gln Met Pro Val Asn Leu Asp Lys Ser Asp Asn Val 65 70 75 80	
Glu Thr Phe Tyr Gly Lys Glu Gly Gly Tyr Val Leu Gly Thr Gly Val 85 90 95	
Met Asp Gly Lys Ser Tyr Arg Lys Gln Pro Ile Met Ile Thr Ala Pro 100 105 110	
Asp Asn Gln Ile Val Phe Lys Asp Cys Ser Pro Arg 115 120	
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aagctggaat ccaactcgac cgtatggcaa aagcaggccg gaagcggcag cacggttgaa	180
acgotgaago tacogagott tgaagggoog goactgoota agotgacogo tocoggoggo	240 300
tatatcgccg acatccccaa aggcaacctc aaaaccgaaa tcgaaaagct ggccaaacag	300
cagctcgctt acgacaaatg ggactataaa caggaaggcc taaccggagc cggagccgca	420
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Trp Gln Lys Gln Ala Gly Ser Gly Ser Thr Val Glu Thr Leu Lys Leu 50 55 60	
Pro Ser Phe Glu Gly Pro Ala Leu Pro Lys Leu Thr Ala Pro Gly Gly 65 70 75 80	
Tyr Ile Ala Asp Ile Pro Lys Gly Asn Leu Lys Thr Glu Ile Glu Lys 85 90 95	
Leu Ala Lys Gln Pro Glu Tyr Ala Tyr Leu Lys Gln Leu Gln Thr Val 100 105 110	
Lys Asp Val Asn Trp Asn Gln Val Gln Leu Ala Tyr Asp Lys Trp Asp	
115 120 125	
Tyr Lys Gln Glu Gly Leu Thr Gly Ala Gly Ala Ala Ile Xaa Ala Leu 130 135 140	
Ala Val Thr Val Val Thr Ser Gly Ala Gly Thr Gly Ala Val Leu Gly 145 150 155 160	
Leu Xaa Arg Val Ala Ala Ala Ala Thr Asp Ala Ala Phe 165 170	
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caggetteeg tategtteat caacaacaaa ggeaatateg gtaacaecet gaaagagetg	780
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gcctatgcag aaat	gatttc ccagacttt	g gtaggtgaga	gtgttggtgg tagtctttgt
ctgacaagag cctg	cttttc ggtaagttc	a acaatatcta	aatctaaatc tccttttaaa
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Ser Lys Asn Glu 35	Leu Asn Glu Thr 40	Lys Leu Pro	Val Arg Val Ile Ala 45
Gln Thr Ala Lys 50	Thr Arg Ser Gly 55	Trp Asp Thr	Val Leu Glu Gly Thr 60
Glu Phe Lys Thr 65	Thr Leu Ser Gly 70	Ala Asp Ile 75	Gln Ala Gly Val Gly 80
Glu Lys Ala Arg	Ala Asp Ala Lys 85	Ile Ile Leu 90	Lys Gly Ile Val Asn 95
Arg Ile Gln Thr 100	-	Glu Ser Asn 105	Ser Thr Val Trp Gln 110
Lys Gln Ala Gly 115	Ser Gly Ser Thr 120	Val Glu Thr	Leu Lys Leu Pro Ser 125
Phe Glu Gly Pro 130	Ala Leu Pro Lys 135	Leu Thr Ala	Pro Gly Gly Tyr Ile 140
Ala Asp Ile Pro 145	Lys Gly Asn Leu 150	Lys Thr Glu 155	Ile Glu Lys Leu Ala 160
Lys Gln Pro Glu		-	Gln Thr Val Lys Asp
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275 280 285 Asn Val Ser Asp Lys Gln Trp Ile Asn Asn Leu Thr Val Asn Leu Ala 300 Asn Ala Gly Ser Ala Ala Leu Ile Asn Thr Ala Val Asn Gly Gly Ser 320 Leu Lys Asp Asn Leu Glu Ala Asn Ile Leu Ala Ala Ala Leu Val Asn Thr Ala His Gly Glu Ala Ala Ser Lys Ile Lys Gln Leu Asp Gln His Tyr
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325 330 335 Ala His Gly Glu Ala Ala Ser Lys Ile Lys Gln Leu Asp Gln His Tyr
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Ile Ala His Lys Ile Ala His Ala Ile Ala Gly Cys Ala Ala Ala Ala 355 360 365
Ala Asn Lys Gly Lys Cys Gln Asp Gly Ala Ile Gly Ala Ala Val Gly 370 375 380
Glu Ile Leu Gly Glu Thr Leu Leu Asp Gly Arg Asp Pro Gly Ser Leu 385 390 395 400
Asn Val Lys Asp Arg Ala Lys Ile Ile Ala Lys Ala Lys Leu Ala Ala 405 410 415
Gly Ala Val Ala Ala Leu Ser Lys Gly Asp Val Ser Thr Ala Ala Asn 420 425 430
Ala Ala Val Ala Val Glu Asn Asn Ser Leu Asn Asp Ile Gln Asp 435 440 445
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Glu Ser Phe Cys Glu Ser Tyr Arg Pro Leu Gly Leu Pro His Phe Val 465 470 475 480
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Leu Ala Ala Lys Ser Leu 50	u Gly Leu Lys Ala Lys 55	Val Val Arg Gln Pro 60	
Ile Lys Arg Leu Ala Met 65 70		Leu Val Trp Cys Asp 80	
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Pro Ile Asp Ser Arg Asn Phe Arg Asn Pro Arg Leu Ala Trp Arg Cys 85 90 95	
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let Pro Leu Ala Val Leu 65 70	ı Ile Gly Gly Leu Val	Ser Xaa Ser Gln Leu	
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140

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Asp	A rg 130	Asn	Asn	Asn	Pro	Phe 135	Val	Val	Lys	Gly	Ser 140	Ala	Gln	Leu	Ile	
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995 1000 1005 Lys Glu Val Lys Gly Lys Gly Lys Pro Lys Gly Lys Glu Tyr Leu Gln Ala 1010 1015 1010 1015 1020 1020 Lys Leu Ser Ala Gln Asn Ile Asp Leu Ile Ser Ala Gln Gly Ile Glu 1035 1040 1025 1030 1035 1040 1025 1045 1050 1055 Ala Gly Val Leu Pro Lys Ala Ala Ser Lys Lys Leu Asn Leu His Ala 1055 Ala Gly Val Leu Pro Lys Ala Ala Asp Ser Glu Ala Ala Ala Ile Leu 1065 1060 1065 1070 Ile Asp Gly Ile Thr Asp Gln Tyr Glu Ile Gly Lys Pro Thr Tyr Lys 1070 1075 1080 1085 Ser His Tyr Asp Lys Ala Ala Leu Asn Lys Pro Ser Arg Leu Thr Gly 1095 1090 1095 1100 Arg Thr Gly Val Ser Ile His Ala Ala Ala Ala Ala Leu Asp Asp Ala Arg 1120 1105 1110 1115 1120 1110 1115 1121 1125 1130 1122 1110 1145 1125 1130 1135 1120 1121 1145 1125 1130 1155 <	Ile	Ala	Gln		Lys	Lys	Ser	Ser		Lys	Ser	Lys	Leu		Pro	Thr
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Arg Phe Ile His Asp Glu Ala Val Gly Ser Asn Ile Gly Gly Lys

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Met	Ile	Val	Ala 20	Ala	Gly	Gln	Asp	Ile 25	Asn	Val	Arg	Gly	Xaa 30	Ser	Leu
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Ser	Thr 50	Ala	His	Asn	Arg	Ty r 55	Thr	Gly	Asn	Glu	Tyr 60	His	Glu	Ser	Xaa
Xaa 65	Ser	Gly	Val	Met	Gly 70	Thr	Gly	Gly	Leu	Gly 75	Phe	Thr	Ile	Gly	Asn 80
Arg	Lys	Thr	Thr	Asp 85	Asp	Thr	Asp	Arg	Thr 90	Asn	Ile	Val	His	Thr 95	Gly
Ser	Ile	Ile	Gly 100	Ser	Leu	Asn	Gly	A sp 105	Thr	Val	Thr	Val	Ala 110	Gly	Asn
Arg	Tyr	Arg 115	Gln	Thr	Gly	Ser	Thr 120	Val	Ser	Ser	Pro	Glu 125	Gly	Arg	Asn
Thr	Val 130	Thr	Ala	Lys	Xaa	Ile 135	Asp	Val	Glu	Phe	Ala 140	Asn	Asn	Arg	Tyr
Ala 145	Thr	Asp	Tyr	Ala	His 150	Thr	Gln	Glu	Gln	L y s 155	Gly	Leu	Thr	Val	Ala 160
Leu	Asn	Val	Pro	Val 165	Val	Gln	Ala	Ala	Gln 170	Asn	Phe	Ile	Gln	Ala 175	Ala
Gln	Asn	Val	Gly 180	Lys	Ser	Lys	Asn	L y s 185	Arg	Val	Asn	Ala	Met 190	Ala	Ala
Ala	Asn	Ala 195	Ala	Trp	Gln	Ser	Ty r 200	Gln	Ala	Thr	Gln	Gln 205	Met	Gln	Gln
Phe	Ala 210	Pro	Ser	Ser	Ser	Ala 215	Gly	Gln	Gly	Gln	Asn 220	Tyr	Asn	Gln	Ser
Pro 225	Ser	Ile	Ser	Val	Ser 230	Ile	Xaa	Tyr	Gly	Glu 235	Gln	Lys	Ser	Arg	Asn 240
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Glu 305	Gln	Ser	Lys	Asn	L y s 310	Ser	Ser	Gly	Trp	Asn 315	Ala	Gly	Val	Arg	Xaa 320
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Thr	Leu 370	Lys	Gly	Val	Gln	Leu 375	Ile	Gly	Lys	Gly	Ile 380	Gln	Ala	Asp	Thr
A rg 385	Asn	Leu	His	Ile	Glu 390	Ser	Val	Gln	Asp	Thr 395	Glu	Thr	Tyr	Gln	Ser 400
Lys	Gln	Gln	Asn	Gly 405	Asn	Val	Gln	Val	Thr 410	Val	Gly	Tyr	Gly	Phe 415	Ser

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n Ala Leu Gly Gly Ala Ala Ile Gly Tyr Ala Th
r35 40 45 Gly Gly Ser Gly Gly Ala Val Val Gly Ala Asn Val Asp Trp Asn Asn

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Arg Gln Leu H. 65	His Pro L y s Glu Met A 70	Ala Leu Ala Asp Lys 75	Tyr Ala Glu 80		
	Arq Glu Val Glu Lys i				
ma nea nyo m	85	90	95		
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	ys Thr Ala Ile Pro 1				
115	120	125			
Glu					
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	tacattac ccacaagatt			720	
	gggcaagtg tcaggatggt			780	
	Jacaaacgg caaaaatcct			840	
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Lys Tyr Ile Tyr Arg Phe Pro Ile Tyr Cys Pro Xaa Ala Cys Val Ala 20 25 30
Glu Asp Thr Pro Tyr Ala Cys Tyr Leu Xaa Gln Leu Gln Val Thr Lys 35 40 45
Asp Val Asn Trp Asn Gln Val Xaa Leu Ala Tyr Asp Lys Trp Asp Tyr 50 55 60
Lys Gln Glu Gly Leu Thr Gly Ala Gly Ala Ala Ile Ile Ala Leu Ala 65 70 75 80
Val Thr Val Val Thr Ala Gly Ala Gly Ala Gly Ala Ala Leu Gly Leu 85 90 95
Asn Gly Ala Ala Ala Ala Thr Asp Ala Ala Phe Ala Ser Leu Ala 100 105 110
Ser Gln Ala Ser Val Ser Leu Ile Asn Asn Lys Gly Asn Ile Gly Asn 115 120 125
Thr Leu Lys Glu Leu Gly Arg Ser Ser Thr Val Lys Asn Leu Met Val 130 135 140
Ala Val Ala Thr Ala Gly Val Ala Asp Lys Ile Gly Ala Ser Ala Leu 145 150 155 160
Asn Asn Val Ser Asp Lys Gln Trp Ile Asn Asn Leu Thr Val Asn Leu 165 170 175
Ala Asn Ala Gly Ser Ala Ala Leu Ile Asn Thr Ala Val Asn Gly Gly 180 185 190
Ser Leu Lys Asp Asn Leu Glu Ala Asn Ile Leu Ala Ala Leu Val Asn 195 200 205
Thr Ala His Gly Glu Ala Ala Ser Lys Ile Lys Gln Leu Asp Gln His 210 215 220
Tyr Ile Thr His Lys Ile Ala His Ala Ile Ala Gly Cys Ala Ala Ala 225 230 235 240
Ala Ala Asn Lys Gly Lys Cys Gln Asp Gly Ala Ile Gly Ala Ala Val 245 250 255
Gly Glu Ile Val Gly Glu Ala Leu Thr Asn Gly Lys Asn Pro Asp Thr 260 265 270
Leu Thr Ala Lys Glu Arg Glu Gln Ile Leu Ala Tyr Ser Lys Leu Val 275 280 285
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Tyr Lys Gln Glu Gly Leu Thr Gly Ala Gly Ala Ala Ile Ile Ala Leu 50 55 60	
Ala Val Thr Val Val Thr Ala Gly Ala Gly Ala Gly Ala Ala Leu Gly 65 70 75 80	

Leu	Asn	Gly	Ala	Ala 85	Ala	Ala	Ala	Thr	Asp 90	Ala	Ala	Phe	Ala	Ser 95	Leu
Ala	Ser	Gln	Ala 100	Ser	Val	Ser	Leu	Ile 105	Asn	Asn	Lys	Gly	Asn 110	Ile	Gly
Asn	Thr	Leu 115	Lys	Glu	Leu	Gly	Arg 120	Ser	Ser	Thr	Val	Lys 125	Asn	Leu	Met
Val	Ala 130	Val	Ala	Thr	Ala	Gly 135	Val	Ala	Asp	Lys	Ile 140	Gly	Ala	Ser	Ala
Leu 145	Asn	Asn	Val	Ser	Asp 150	Lys	Gln	Trp	Ile	Asn 155	Asn	Leu	Thr	Val	Asn 160
Leu	Ala	Asn	Ala	Gly 165	Ser	Ala	Ala	Leu	Ile 170	Asn	Thr	Ala	Val	Asn 175	Gly
Gly	Ser	Leu	L <b>y</b> s 180	Asp	Asn	Leu	Glu	Ala 185	Asn	Ile	Leu	Ala	Ala 190	Leu	Val
Asn	Thr	Ala 195	His	Gly	Glu	Ala	Ala 200	Ser	Lys	Ile	Lys	Gln 205	Leu	Asp	Gln
His	<b>Tyr</b> 210	Ile	Thr	His	Lys	Ile 215	Ala	His	Ala	Ile	Ala 220	Gly	Cys	Ala	Ala
Ala 225	Ala	Ala	Asn	Lys	Gly 230	Lys	Cys	Gln	Asp	Gly 235	Ala	Ile	Gly	Ala	Ala 240
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Thr	Leu	Thr	Ala 260	Lys	Glu	Arg	Glu	Gln 265	Ile	Leu	Ala	Tyr	Ser 270	Lys	Leu
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Asn	Pro	Gln	Leu	C <b>y</b> s 325	Arg	Lys	Asn	Thr	Val 330	Lys	Lys	Tyr	Gln	Asn 335	Val
Ala	Asp	Lys	Arg 340	Leu	Ala	Ala	Ser	Ile 345	Ala	Ile	Сув	Thr	Asp 350	Ile	Ser
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Arg	Ser 370	Leu	His	Ser	Ser	Trp 375	Glu	Ala	Gly	Leu	Ile 380	Gly	Lys	Asp	Asp
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Val	Lys 450	Gln	Asn	Thr	Pro	Ile 455	Thr	Asn	Val	Lys	<b>Ty</b> r 460	Pro	Glu	Gly	Ile
Ser 465	Phe	Asp	Thr	Asn	Leu 470	Lys	Arg	His	Leu	Ala 475	Asn	Ala	Asp	Gly	Phe 480

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Asp	Ile	Glu 515		Ile	Thr	Arg	Ile 520		Tyr	Glu	Ile	Pro 525	Thr	Leu	Asp	
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Thr 545	Val	Tyr	Asn	Pro	L <b>y</b> s 550	Lys	Phe	Ser	Asp	Asp 555	Lys	Ile	Leu	Gln	Met 560	
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aaca	aaaa	agg g	gttt	ttgg	ca aç	gagat	tgti	t tai	tati	taa	aaa	ccta	taa a	attgo	cttgct	240
atcg	gcag	gog t	cgt	tggca	ag ca	attti	aggg	g gto	gaagt	tgc	ttt	tgata	act 1	tcca	gtgtct	300
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Leu Phe Asp Phe Phe Ala Phe Leu Phe Phe Ala Asn Val Phe Leu Ala 35 40 45	
Val Asn Leu Leu Phe Leu Glu Lys Asn Ile Lys Asn Lys Leu Leu Phe 50 55 60	
Leu Leu Pro Ile Ser Ile Ile Ile Trp Met Val Ile His Ile Ser Met 65 70 75 80	
Ile Asn Ile Lys Phe Tyr Lys Phe Glu His Gln Ile Lys Glu Gln Asn 85 90 95	
Ile Ser Ser Ile Thr Gly Val Ile Lys Pro His Asp Ser Tyr Asn Tyr 100 105 110	
Val Tyr Asp Ser Asn Gly Tyr Ala Lys Leu Lys Asp Asn His Arg Tyr 115 120 125	
Gly Arg Val Ile Arg Glu Thr Pro Tyr Ile Asp Val Val Ala Ser Asp 130 135 140	
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agtttgtact tgttagataa gtataaaaca ttttttctta ttgaaaacag tgtttgtatc	660
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Leu Phe Asp Phe Phe Ala Phe Leu Phe Phe Ala Asn Val Phe Leu Ala 35 40 45	
Val Asn Leu Leu Phe Leu Glu Lys Asn Ile Lys Asn Lys Leu Leu Phe 50 55 60	
Leu Leu Pro Ile Ser Ile Ile Ile Trp Met Val Ile His Ile Ser Met 65 70 75 80	
Ile Asn Ile Lys Phe Tyr Lys Phe Glu His Gln Ile Lys Glu Gln Asn 85 90 95	
Ile Ser Ser Ile Thr Gly Val Ile Lys Pro His Asp Ser Tyr Asn Tyr 100 105 110	
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Tyr Ala Pro Cys Ala Asn Phe Ile Lys Phe Ala Lys Lys Pro Val Lys 165 170 175	
Ile Tyr Phe Tyr Asn Gln Pro Gln Gly Asp Phe Ile Asp Asn Val Ile 180 185 190	
Phe Glu Ile Asn Asp Gly Asn Lys Ser Leu Tyr Leu Leu Asp Lys Tyr 195 200 205	
Lys Thr Phe Phe Leu Ile Glu Asn Ser Val Cys Ile Val Leu Ile Ile 210 215 220	
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Cys Ile	Phe 35	Thr	Val	Leu	Ser	Lys 40	Asp	Ile	Phe	Asp	Phe 45	Leu	Phe	Ile	
Phe Arg 50	Phe	Gln	Thr	Ala	Asp 55	Phe	Arg	Leu	Phe	Phe 60	Arg	Gln	Ser	His	
Ala Asp 65	Gly	Val	Arg	Leu 70	Asp	Phe	Ile	Phe	Phe 75	Ser	Phe	Arg	Thr	Arg 80	
Leu Phe	Gln	Phe	Ala 85	Gly	Val	Val	Leu	Ser 90	Arg	Gln	Gln	Gln	Gly 95	Leu	
Arg Leu	Val	Ala 100	Leu	His	Phe	Leu	Asn 105	Asp	Arg	Leu	Leu	Leu 110	Arg	Lys	
Ser Arg	Leu 115	Val	Ala	Leu	Met	Val 120	Arg	His	Arg	Gln	Thr 125	Arg	Ala	Asp	
Lys Arg 130	Asp	Asp	Gly	Asn	Arg 135	Leu	Pro	Val	Ile	Arg 140	Gln	Gln	Phe	His	
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1			5					10					15		
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Ala Asn 50	Ala	Thr	Asp	Glu	Asp 55	Glu	Glu	Glu	Glu	Leu 60	Glu	Ser	Val	Gln	
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Leu Glu	Thr	Ile	Ser 85	Leu	Ser	Met	Thr	Asn 90	Asp	Ser	Lys	Glu	Phe 95	Val	
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Ser 145	Phe	Gly	Ala	Asn	Gly 150	Lys	Lys	Val	Asn	Ile 155	Ile	Ser	Asp	Thr	Lys 160
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Gl <b>y</b> 225	Val	Lys	Thr	Gly	Ser 230	Thr	Thr	Gly	Gln	Ser 235	Glu	Asn	Val	Asp	Phe 240
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Lys	Ile	Gl <b>y</b> 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	<b>As</b> p 285	Gly	Lys	Leu
Val	Thr 290	Gly	Lys	Gly	Lys	Gly 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly
Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320
Gly	Trp	Arg	Met	L <b>y</b> s 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala
Asp	Lys	Phe	Glu 340	Thr	Val	Thr	Ser	Gl <b>y</b> 345	Thr	Asn	Val	Thr	Phe 350	Ala	Ser
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Leu 385	Gln	Asn	Ser	Gly	Trp 390	Asn	Leu	Asp	Ser	L <b>y</b> s 395	Ala	Val	Ala	Gly	Ser 400
Ser	Gly	Lys	Val	Ile 405	Ser	Gly	Asn	Val	Ser 410	Pro	Ser	Lys	Gly	L <b>y</b> s 415	Met
Asp	Glu	Thr	Val 420	Asn	Ile	Asn	Ala	Gl <b>y</b> 425	Asn	Asn	Ile	Glu	Ile 430	Ser	Arg
Asn	Gly	L <b>y</b> s 435	Asn	Ile	Asp	Ile	Ala 440	Thr	Ser	Met	Ala	Pro 445	Gln	Phe	Ser
Ser	Val 450	Ser	Leu	Gly	Ala	Gly 455	Ala	Asp	Ala	Pro	Thr 460	Leu	Ser	Val	Asp
Asp 465	Glu	Gly	Ala	Leu	Asn 470	Val	Gly	Ser	Lys	Asp 475	Ala	Asn	Lys	Pro	Val 480
Arg	Ile	Thr	Asn	Val 485	Ala	Pro	Gly	Val	Lys 490	Glu	Gly	Asp	Val	Thr 495	Asn
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Ala	Gly 530	Leu	Val	Gln	Ala	<b>Ty</b> r 535	Leu	Pro	Gly	Lys	Ser 540	Met	Met	Ala	Ile					
Gl <b>y</b> 545	Gly	Gly	Thr	Tyr	Arg 550	Gly	Glu	Ala	Gly	<b>Ty</b> r 555	Ala	Ile	Gly	Tyr	Ser 560					
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Thr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln					
Ala	Asn 50	Ala	Thr	Asp	Asp	Asp 55	Asp	Leu	Tyr	Leu	Glu 60	Pro	Val	Gln	Arg					
Thr 65	Ala	Val	Val	Leu	Ser 70	Phe	Arg	Ser	Asp	L <b>y</b> s 75	Glu	Gly	Thr	Gly	Glu 80					
Lys	Glu	Gly	Thr	Glu 85	Asp	Ser	Asn	Trp	Ala 90	Val	Tyr	Phe	Asp	Glu 95	Lys					
Arg	Val	Leu	Lys 100	Ala	Gly	Ala	Ile	Thr 105	Leu	Lys	Ala	Gly	Asp 110	Asn	Leu					
Lys	Ile	Lys 115	Gln	Asn	Thr	Asn	Glu 120	Asn	Thr	Asn	Asp	Ser 125	Ser	Phe	Thr					
Tyr	Ser 130	Leu	Lys	Lys	Asp	Leu 135	Thr	Asp	Leu	Thr	Ser 140	Val	Glu	Thr	Glu					
L <b>y</b> s 145	Leu	Ser	Phe	Gly	Ala 150	Asn	Gly	Asn	Lys	Val 155	Asn	Ile	Thr	Ser	Asp 160					
Thr	Lys	Gly		Asn 165				Glu			Gly	Thr	Asn	Gly 175	Asp					
Pro	Thr	Val	His 180	Leu	Asn	Gly	Ile	Gl <b>y</b> 185	Ser	Thr	Leu	Thr	Asp 190	Thr	Leu					
Leu	Asn	Thr 195	Gly	Ala	Thr	Thr	Asn 200	Val	Thr	Asn	Asp	Asn 205	Val	Thr	Asp					
Asp	Glu 210	Lys	Lys	Arg	Ala	Ala 215	Ser	Val	Lys	Asp	Val 220	Leu	Asn	Ala	Gly					
Trp 225	Asn	Ile	Lys	Gly	Val 230	Lys	Pro	Gly	Thr	Thr 235	Ala	Ser	Asp	Asn	Val 240					
Asp	Phe	Val	Arg	Thr 245	Tyr	Asp	Thr	Val	Glu 250	Phe	Leu	Ser	Ala	Asp 255	Thr					
Lys	Thr	Thr	Thr 260	Val	Asn	Val	Glu	Ser 265	Lys	Asp	Asn	Gly	L <b>y</b> s 270	Lys	Thr					
									1	- 1	-	- 1	-	Asp	<b>a</b> 1					

Lys       Leu       Val       Th       Gly       Lys       Gly       Sap       Glu       An       Gly       Sap       An																
305       310       315       320         Lys Ala Gly Try Arg Met Lys Thr Thr Thr Ala Asn Gly Gln Thr Gly       335       330         Gln Ala Asp Lys Phe Glu Thr Val Thr Ser Gly Thr Asn Val Thr Phe       345       360         Ala Ser Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly       365         Ala Ser Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Cln Gly       365         Asn Ile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val       380         Ass Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala       390         390       390       390         Gly Ser Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly       410         410       405       410         420       405       410         420       405       410         420       405       410         435       400         Gly Ser Ser Gly Lys Val Ile Ser Gly Ala Cly Asn Asn Ile Glu Ile         420       455         Asn Ala Pro Thr Leu Ser       445         440       450         110       Asn Ala Cly Asp Ala Pro Thr Leu Ser         445       450         111       Asn Ala Cly Lys Asp Thr Asn Lys         445       470         111       Asp Ala Cly Asp Thr Asn Lys <td>Lys</td> <td></td> <td>Val</td> <td>Thr</td> <td>Gly</td> <td>Lys</td> <td></td> <td>Lys</td> <td>Asp</td> <td>Glu</td> <td>Asn</td> <td></td> <td>Ser</td> <td>Ser</td> <td>Thr</td> <td>Asp</td>	Lys		Val	Thr	Gly	Lys		Lys	Asp	Glu	Asn		Ser	Ser	Thr	Asp
325       330       335         Gln Ala Asp Lys Phe Glu Thr Val Thr Ser Gly Thr Asn Val Thr Phe 340       345         Ala Ser Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly 375         Asn Ile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 370         Asn Ile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 375         Asn Ile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 380         Asn Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala 395         Gly Ser Ser Gly Lys Val Ile Ser Gly Asn Nal Er Dro Ser Lys Gly 405         Thr Arg Asn Gly Lys Asn Ile Asp Tle Ala Thr Ser Met Ala Pro Gln 420         Thr Arg Asn Gly Lys Asn Ile Asp Tle Ala Thr Ser Met Ala Pro Gln 445         Phe Ser Ser Val Ser Leu Gly Ala Cly Ala Asp Ala Pro Thr Leu Ser 455         Val Asp Asp Glu Gly Ala Leu Asn Val Gly Ser Lys Asp Thr Asn Lys 465         Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 465         Yan Ash Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asp Arg 510         Thr Asn Val Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met 530         Sta Si Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met 530         Sta Si Ala Ser Arg Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala 565         Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 580         Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 580         Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 580		Gly	Glu	Gly	Leu		Thr	Ala	Lys	Glu		Ile	Asp	Ala	Val	
Alo Ser Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly Asn Tile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 355 Asn Tile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 370 Asn Tile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 395 Cln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala 395 Cln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala 395 Cln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala 395 Cln Leu Gln Asn Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly 405 Val Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Tile Glu Ile 420 Thr Arg Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Ala Pro Gln 435 Cln Chr Asp Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser 455 Cln Val Ser Leu Gly Ala Cly Ala Asp Ala Pro Thr Leu Ser 456 Asp Asp Glu Gly Ala Leu Asn Val Gly Ser Lys Asp Thr Asn Lys 475 Cln Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 475 Cln Asp Asp Clu Asp Gly Asn Ala Arg Ala Gln Asn Leu Asn Arg 510 Cln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg 510 Cln Asp Asp Nel Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile 515 Cln Asp Asp Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile 515 Cln Asp Asp Clu Gly Asp Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly 546 Cln Cly Asp Thr Tyr Arg Gly Glu Ala Cly Tyr Ala Ile Gly 545 Cln DNO 93 520 Cln No 93 520 Cln No 93 520 Cln No 93 520 Cln No 93 520 SEQUENCE: 93 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 Clo SEQUENCE: 93 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn His Thr Lys Arg Ala Ser Ala 20 Clu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 30 Cr Ala Clu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 30 Cr Ala Clu Leu Arn Ala Thr Leu Leu Phe Ala Thr Val Gln	Lys	Ala	Gly	Trp		Met	Lys	Thr	Thr		Ala	Asn	Gly	Gln		Gly
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370 375 380 Asn Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala 395 395 395 395 395 395 395 395 395 395	Ala	Ser		Lys	Gly	Thr	Thr		Thr	Val	Ser	Lys		Asp	Gln	Gly
385 390 390 395 400 Gly Ser Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly 405 415 Lys Met Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile 420 440 440 Thr Arg Asn Gly Lys Asn Ile Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile 435 Phe Ser Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser 455 Val Asp Asp Glu Gly Ala Leu Asn Val Gly Ser Lys Asp Thr Asn Lys 465 Asn Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 480 Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 485 Thr Asn Val Ala Gln Leu Lys Gly Val Ala Gly Ile Ala Gln Ala Ile 500 Thr Asn Val Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met 530 Ala Thr Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met 530 Ala Ile Gly Gly Asp Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly 545 Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Thr Ala 580 Gln Trp 2110 SEQUENCE: 93 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 2100 SEQUENCE: 93 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 2110 SEQUENCE: 93 Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln	Asn		Thr	Val	Lys	Tyr		Val	Asn	Val	Gly		Ala	Leu	Asn	Val
$\frac{405}{420}$ $\frac{410}{415}$ $\frac{415}{420}$ $\frac{415}{440}$ $\frac{41}{450}$ $\frac{41}{40}$ $\frac{41}{450}$ $\frac{41}{4$		Gln	Leu	Gln	Asn		Gly	Trp	Asn	Leu		Ser	Lys	Ala	Val	
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435440445Phe Ser Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser 450455Aa Gly Ala Asp Ala Pro Thr Leu Ser 460Ser Lys Asp Thr Asn Lys 480Val Asp Asp Glu Gly Ala Leu Asn Val Gly Ser Lys Asp Thr Asn Lys 465Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 480Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 485Asn Asn Asn Arg 500Thr Asn Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg 500Ile Asp Asn Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile 515Ala Thr Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met 530Ala Ile Gly Gly Asp Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly 545Ser Gly Asn Ser Arg Gly His Phe Gly Asn Trp Ile Ile Lys Gly Thr Ala 580Seq Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 580Seq UENCE: 93Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1Val Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln	Lys	Met	Asp		Thr	Val	Asn	Ile		Ala	Gly	Asn	Asn		Glu	Ile
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465 470 475 480 Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val 485 485 480 Val Ala Pro Gly Val Lys Glu Gly Asp Val 485 490 495 Thr Asn Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg 500 505 505 505 505 505 505 505 505 505	Phe		Ser	Val	Ser	Leu		Ala	Gly	Ala	Asp		Pro	Thr	Leu	Ser
Thr Asn Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg 500 Thr Asn Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg 500 Ile Asp Asn Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile 515 Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile 515 Son Val Asp Gly Asn Ala Tyr Leu Pro Gly Lys Ser Met Met 530 Ala Thr Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met 530 Ala Ile Gly Gly Asp Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly 545 Ser Ser Ile Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala 566 Tyr Ser Ser Ile Ser Asp Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 587 Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr 588 Seq ID NO 93 2112 LENGTH: 594 2122 TYPE: PRT 2133 ORGANISM: Neisseria meningitidis <400> SEQUENCE: 93 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 5 Nal Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20 Charter Ala Charter Ala Charter Val Gln Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln		Asp	Asp	Glu	Gly		Leu	Asn	Val	Gly		Lys	Asp	Thr	Asn	-
500 = 505 = 510 Ile Asp Asn Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile $515$ S = 0 Asn Val Gly Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile $525$ Gly Ash Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met $530$ Ala Ile Gly Gly Asp Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly $545$ S = 0 F = 1 E Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala $560$ Tyr Ser Ser Ile Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala $575$ S = Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr $580$ G = $585$ S = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F = 0 F =	Pro	Val	Arg	Ile		Asn	Val	Ala	Pro	_	Val	Lys	Glu	Gly	-	Val
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Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 5 10 15 Val Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20 25 30 Thr Val Ala Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln	<211 <212	l> LE 2> TY	NGTH	I: 59 PRT	94	seri	La me	ening	ritid	lis						
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	Val	Ala	Val		Glu	Leu	Thr	Arg		His	Thr	Lys	Arg		Ser	Ala
	Thr	Val		Thr	Ala	Val	Leu		Thr	Leu	Leu	Phe		Thr	Val	Gln

Ala	Ser 50	Thr	Thr	Asp	Asp	Asp 55	Asp	Leu	Tyr	Leu	Glu 60	Pro	Val	Gln	Arg
Thr 65	Ala	Pro	Val	Leu	Ser 70	Phe	His	Ala	Asp	Ser 75	Glu	Gly	Thr	Gly	Glu 80
Lys	Glu	Val	Thr	Glu 85	Asp	Ser	Asn	Trp	Gly 90	Val	Tyr	Phe	Asp	Lys 95	Lys
Gly	Val	Leu	Thr 100	Ala	Gly	Thr	Ile	Thr 105	Leu	Lys	Ala	Gly	Asp 110	Asn	Leu
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L <b>y</b> s 145	Leu	Ser	Phe	Gly	Ala 150	Asn	Gly	Lys	Lys	Val 155	Asn	Ile	Thr	Ser	Asp 160
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Thr	Thr	Val	His 180	Leu	Asn	Gly	Ile	Gl <b>y</b> 185	Ser	Thr	Leu	Thr	Asp 190	Thr	Leu
Leu	Asn	Thr 195	Gly	Ala	Thr	Thr	Asn 200	Val	Thr	Asn	Asp	Asn 205	Val	Thr	Asp
Asp	Glu 210	Lys	Lys	Arg	Ala	Ala 215	Ser	Val	Lys	Asp	Val 220	Leu	Asn	Ala	Gly
Trp 225	Asn	Ile	Lys	Gly	Val 230	Lys	Pro	Gly	Thr	Thr 235	Ala	Ser	Asp	Asn	Val 240
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Lys	Leu 290	Val	Thr	Gly	Lys	Asp 295	Lys	Gly	Glu	Asn	Gl <b>y</b> 300	Ser	Ser	Thr	Asp
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Gly	Ser	Ser	Gly	L <b>y</b> s 405	Val	Ile	Ser	Gly	Asn 410	Val	Ser	Pro	Ser	Lys 415	Gly
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Thr	Arg	Asn 435	Gly	Lys	Asn	Ile	Asp 440	Ile	Ala	Thr	Ser	Met 445	Thr	Pro	Gln

-continued

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Val 465	Asp	Asp	Glu	Gly	Ala 470	Leu	Asn	Val	Gly	Ser 475	Lys	Asp	Ala	Asn	Lys 480	
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Tyr	Ser	Ser	Ile	Ser 565	Asp	Gly	Gly	Asn	Trp 570	Ile	Ile	Lys	Gly	Thr 575	Ala	
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Lys	Glu	Gly	Thr	Glu 85	Asp	Ser	Asn	Trp	Ala 90	Val	Tyr	Phe	Asp	Glu 95	Lys	
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L <b>y</b> s 145	Leu	Ser	Phe	Gly	Ala 150	Asn	Gly	Asn	Lys	Val 155	Asn	Ile	Thr	Ser	Asp 160	
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Pro	Thr	Val	His 180	Leu	Asn	Gly	Ile	Gly 185	Ser	Thr	Leu	Thr	Asp 190	Thr	Leu	
Leu	Asn	Thr 195	Gly	Ala	Thr	Thr	Asn 200	Val	Thr	Asn	Asp	Asn 205	Val	Thr	Asp	

cont i	nued

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re The The The Val Aan Val Gu See Lys Aap Aan Gly Lys Lys The 270 yes The 275 He Gly Lys Lys The 285 yes that Let $V_{285}$ and
260265270uu Val Lys Ile Gly Ala Lys Thr Ser Val Ile Lys Glu Lys Asp Gly 27578 Leu Val Thr Gly Lys Gly Lys Asp Glu Asn Gly Ser Ser Thr Asp 29010 Gly Glu Gly Leu Val Thr Ala Lys Glu Val Ile Asp Ala Val Asn 31511 Gly Trp Arg Met Lys Thr Thr Thr Ala Asn Gly Gln Thr Gly 32512 sc Ala Gly Trp Arg Met Lys Thr Thr Thr Ala Asn Gly Gln Thr Gly 32513 as Cly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gl Gly 35514 as p Lys Fhe Glu Thr Val Thr Ser Gly Thr Asn Val Thr Fhe 36015 as Cly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gl Gly 37516 m Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala 39017 of Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val 39018 Ser Glu Thr Val Lys Tyr Asp Val Asn Val Ser Pro Ser Lys Glu 40019 Ser Ser Glu Lys Asn Ile Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile 42010 Asn Ser Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile 42011 Asp Asp Glu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser 45012 Asp Asp Glu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser 45013 Asp Asp Glu Gly Ala Gly Val Iles Glu Gly Asp Val 45514 Asp Asp Glu Gly Ala Gly Ala Asp Ala Glu Asn Lys 45015 Asp Asp Glu Gly Ala Asp Ala Gli Jee Ann Asp 51016 Asp Glu Soli Soli Soli Soli Soli Soli Soli Soli
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1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
340 $345$ $350$ Ia SerGly Lys Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly $355$ $355$ Ser Us Asp Asp Gln Gly $365$ in The Tval Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val $370$ $375$ Ser Us Asp Ser Lys Ala Val Ala $400$ in Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala $400$ $400$ iy Ser Ser Gly Lys Val IIe $420$ Ser Gly Asn Val Ser Pro Ser Lys Gly $410$ ir Asp Glu Lys Val IIe $420$ Ser Gly Asn Nal Gly Asn Asn Ie Glu IIe $420$ ir Arg Asn Gly Lys Asn IIe Asp IIe Ala Thr Ser Met Ala Pro Gln $445$ ie Ser Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser $450$ ich Asp Glu Gly Ala Leu Asn Val Gly $475$ ich Asp Glu Gly Ala Cu Lys Gly Val Ala Ser Lys Asp Thr Asn Lys $460$ ich Asp Asp Glu Gly Ala Gly Ala Cu Ser Lys Glu Gly Asp Val $490$ ich Asn Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg $510$ ich Asp Su Val Asp Gly Asn Ala Arg Ala Gly IIe Ala Gln Ala IIe $520$ ich Asp Man Val Gly Asn Ala Gly IIe Ala Gln Asn Leu Asn Asn Arg $510$ ich Asp Val Asp Gly Asn Ala Arg Ala Gly IIe Ala Gln Ala IIe $520$ ich Asp Man Val Asp Gly Asn Ala Arg Ala Gly IIe Ala Gln Ala IIe $520$ ich Asp Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met $540$ ich Gly Gly Asp Thr Tyr Arg Gly Glu Ala Gly Tyr Ala IIe Gly $550$ ich Ser Ser IIe Ser Asp Gly Gly Asn Trp IIe IIe Lys Gly Thr Ala $570$ ich Ser Ser IIe Ser Asp Gly Gly Asn Trp IIe IIe Lys Gly Thr Ala $570$
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450 450 450 450 450 450 450 450
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495    495 $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$ $495$
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15     550     555     560       7r Ser Ser Ile Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala       565     570     575       er Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr
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n Trp

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Phe	Asn	Glu	L <b>y</b> s 100	Gly	Val	Leu	Thr	Ala 105	Arg	Glu	Ile	Thr	Leu 110	Lys	Ala		
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Val	His	Leu	Asn 180	Gly	Ile	Gly	Ser	Thr 185	Leu	Thr	Asp	Thr	Leu 190	Leu	Asn		
Thr	Gly	Ala 195	Thr	Thr	Asn	Val	Thr 200	Asn	Asp	Asn	Val	Thr 205	Asp	Asp	Glu		
Lys	L <b>y</b> s 210	Arg	Ala	Ala	Ser	Val 215	Lys	Asp	Val	Leu	Asn 220	Ala	Gly	Trp	Asn		
Ile 225	Lys	Gly	Val	Lys	Pro 230	Gly	Thr	Thr	Ala	Ser 235	Asp	Asn	Val	Asp	Phe 240		
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Val	Thr 290	Gly	Lys	Asp	Lys	Gly 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly		
Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320		
Gly	Trp	Arg	Met	L <b>y</b> s 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala		
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Asp	Glu	Thr	Val 420	Asn	Ile	Asn	Ala	Gly 425	Asn	Asn	Ile	Glu	Ile 430	Thr	Arg				
Asn	Gly	L <b>y</b> s 435	Asn	Ile	Asp	Ile	Ala 440	Thr	Ser	Met	Thr	Pro 445	Gln	Phe	Ser				
Ser	Val 450	Ser	Leu	Gly	Ala	Gly 455	Ala	Asp	Ala	Pro	Thr 460	Leu	Ser	Val	Asp				
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Ala	Gln	Leu	L <b>y</b> s 500	Gly	Val	Ala	Gln	Asn 505	Leu	Asn	Asn	Arg	Ile 510	Asp	Asn				
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Gly	Leu 530	Val	Gln	Ala	Tyr	Leu 535	Pro	Gly	Lys	Ser	Met 540	Met	Ala	Ile	Gly				
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Thr	Gly	Glu	Lys	Glu 85	_	Val	Glu	Glu	Asn 90	Ser	Asp	Trp	Ala	Val 95	Tyr				
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Leu	L <b>y</b> s 130	Lys	Asp	Leu	Thr	Asp 135	Leu	Thr	Ser	Val	Gly 140	Thr	Glu	Lys	Leu				

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Lys	L <b>y</b> s 210	Arg	Ala	Ala	Ser	Val 215	Lys	Asp	Val	Leu	Asn 220	Ala	Gly	Trp	Asn
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Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys	<b>A</b> sp 265	Asn	Gly	Lys	Lys	Thr 270	Glu	Val
Lys	Ile	Gly 275		Lys	Thr	Ser	Val 280		Lys	Glu	Lys	Asp 285		Lys	Leu
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Glv	Lvs	Glv	340 Thr	Thr	Ala	Thr	Val	345 Ser	Lvs	Asp	Asp	Gln	350 Glv	Asn	Ile
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Asn	Gly	L <b>y</b> s 435	Asn	Ile	Asp	Ile	Ala 440	Thr	Ser	Met	Thr	Pro 445	Gln	Phe	Ser
Ser	Val 450	Ser	Leu	Gly	Ala	Gly 455		Asp	Ala	Pro	Thr 460	Leu	Ser	Val	Asp
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Ile	Thr	Asn	Val	Ala 485	Pro	Gly	Val	Lys	Glu 490	Gly	Asp	Val	Thr	Asn 495	Val
Ala	Gln	Leu	L <b>y</b> s 500	Gly	Val	Ala	Gln	Asn 505	Leu	Asn	Asn	Arg	Ile 510	Asp	Asn
Val	Asp	Gly 515	Asn	Ala	Arg	Ala	Gly 520	Ile	Ala	Gln	Ala	Ile 525	Ala	Thr	Ala
Gly	Leu 530		Gln	Ala	Tyr	Leu 535		Gly	Lys	Ser	Met 540		Ala	Ile	Gly
Gly		Thr	Tyr	Arg	Gly		Ala	Gly	Tyr	Ala		Gly	Tyr	Ser	Ser

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Gln	Phe 450	Ser	Ser	Val	Ser	Leu 455		Ala	Gly	Ala	Asp 460	Ala	Pro	Thr	eu	
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Lys	Pro	Val	Arg	Ile 485	Thr	Asn	Val	Ala	Pro 490	Gly	Val	Lys	Glu	Gly 495	sp	
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Ala	Asn 50	Ala	Thr	Asp	Glu	Asp 55	Glu	Glu	Glu	Glu	Leu 60	Glu	Ser	Val	ln	
Arg 65	Ser	Val	Val	Gly	Ser 70	Ile	Gln	Ala	Ser	Met 75	Glu	Gly	Ser	Gly	lu 80	

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Gl <b>y</b> 225	Val	Lys	Thr	Gly	Ser 230	Thr	Thr	Gly	Gln	Ser 235	Glu	Asn	Val	Asp	Phe 240
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L <b>y</b> s 145	Leu	Ser	Phe	Gly	Ala 150	Asn	Gly	Lys	Lys	Val 155	Asn	Ile	Thr	Ser	Asp 160	-					
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Ala	Asn 50	Ala	Thr	Asp	Glu	Asp 55	Glu	Glu	Glu	Glu	Leu 60	Glu	Pro	Val	Val
Arg 65	Ser	Ala	Leu	Val	Leu 70	Gln	Phe	Met	Ile	Asp 75	Lys	Glu	Gly	Asn	Gly 80
Glu	Asn	Glu	Ser	Thr 85	Gly	Asn	Ile	Gly	Trp 90	Ser	Ile	Tyr	Tyr	Asp 95	Asn
His	Asn	Thr	Leu 100	His	Gly	Ala	Thr	Val 105	Thr	Leu	Lys	Ala	Gly 110	Asp	Asn
Leu	Lys	Ile 115	Lys	Gln	Asn	Thr	Asn 120	Lys	Asn	Thr	Asn	Glu 125	Asn	Thr	Asn
Asp	Ser 130	Ser	Phe	Thr	Tyr	Ser 135	Leu	Lys	Lys	Asp	Leu 140	Thr	Asp	Leu	Thr
Ser 145	Val	Glu	Thr	Glu	L <b>y</b> s 150	Leu	Ser	Phe	Gly	Ala 155	Asn	Gly	Asn	Lys	Val 160
Asn	Ile	Thr	Ser	Asp 165	Thr	Lys	Gly	Leu	Asn 170	Phe	Ala	Lys	Glu	Thr 175	Ala
Gly	Thr	Asn	Gly 180	Asp	Thr	Thr	Val	His 185	Leu	Asn	Gly	Ile	Gly 190	Ser	Thr
		195			Leu		200	_				205			
Asp	Asn 210	Val	Thr	Asp	Asp	L <b>y</b> s 215	Lys	Lys	Arg	Ala	Ala 220	Ser	Val	Lys	Asp
Val 225	Leu	Asn	Ala	Gly	Trp 230	Asn	Ile	Lys	Gly	Val 235	Lys	Pro	Gly	Thr	Thr 240
Ala	Ser	Asp	Asn	Val 245	Asp	Phe	Val	His	Thr 250	Tyr	Asp	Thr	Val	Glu 255	Phe
Leu	Ser	Ala	Asp 260	Thr	Lys	Thr	Thr	Thr 265	Val	Asn	Val	Glu	Ser 270	Lys	Asp
Asn	Gly	L <b>y</b> s 275	Arg	Thr	Glu	Val	L <b>y</b> s 280	Ile	Gly	Ala	Lys	Thr 285	Ser	Val	Ile
Lys	Glu 290	Lys	Asp	Gly	Lys	Leu 295	Val	Thr	Gly	Lys	Gly 300	Lys	Gly	Glu	Asn
Gly 305	Ser	Ser	Thr	Asp	Glu 310	Gly	Glu	Gly	Leu	Val 315	Thr	Ala	Lys	Glu	Val 320
Ile	Asp	Ala	Val	Asn 325	Lys	Ala	Gly	Trp	Arg 330	Met	Lys	Thr	Thr	Thr 335	Ala
Asn	Gly	Gln	Thr 340	Gly	Gln	Ala	Asp	Lys 345	Phe	Glu	Thr	Val	Thr 350	Ser	Gly
Thr	Asn	Val 355	Thr	Phe	Ala	Ser	Gly 360	Lys	Gly	Thr	Thr	Ala 365	Thr	Val	Ser
Lys	Asp 370	Asp	Gln	Gly	Asn	Ile 375	Thr	Val	Lys	Tyr	Asp 380	Val	Asn	Val	Gly
Asp 385	Ala	Leu	Asn	Val	Asn 390	Gln	Leu	Gln	Asn	Ser 395	Gly	Trp	Asn	Leu	Asp 400
Ser	Lys	Ala	Val	Ala 405	Gly	Ser	Ser	Gly	Lys 410	Val	Ile	Ser	Gly	Asn 415	Val

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Ser	Pro	Ser	L <b>y</b> s 420	Gly	Lys	Met	Asp	Glu 425	Thr	Val	Asn	Ile	Asn 430	Ala	Gly
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Ser	Met 450	Thr	Pro	Gln	Phe	Ser 455	Ser	Val	Ser	Leu	Gly 460	Ala	Gly	Ala	Asp
Ala 465	Pro	Thr	Leu	Ser	Val 470	Asp	Asp	Lys	Gly	Ala 475	Leu	Asn	Val	Gly	Ser 480
Lys	Asp	Ala	Asn	L <b>y</b> s 485	Pro	Val	Arg	Ile	Thr 490	Asn	Val	Ala	Pro	Gly 495	Val
Lys	Glu	Gly	Asp 500		Thr	Asn	Val	Ala 505		Leu	Lys	Gly	Val 510		Gln
Asn	Leu	Asn 515		Arg	Ile	Asp	Asn 520		Asp	Gly	Asn	Ala 525		Ala	Gly
Ile	Ala 530		Ala	Ile	Ala	Thr 535		Gly	Leu	Val	Gln 540		Tyr	Leu	Pro
_	Lys	Ser	Met	Met			Gly	Gly	Gly			Arg	Gly	Glu	
545 Gly	Tyr	Ala	Ile		550 Tyr	Ser	Ser	Ile		555 Asp	Gly	Gly	Asn		560 Ile
Ile	Lys	Gly		565 Ala	Ser	Gly	Asn		570 Arg	Gly	His	Phe	-	575 Ala	Ser
		Val	580 Glv	Tyr	Gln	Trp		585					590		
Ala	Ser	VUII													
Ala	Ser	595	1												
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Thr	Asp	Thr 195	Leu	Leu	Asn	Thr	Gly 200	Ala	Thr	Thr	Asn	Val 205	Thr	Asn	Asp	
Asn	Val 210	Thr	Asp	Asp	Glu	L <b>y</b> s 215	Lys	Arg	Ala	Ala	Ser 220	Val	Lys	Asp	Val	
Leu 225	Asn	Ala	Gly	Trp	Asn 230	Ile	Lys	Gly	Val	L <b>y</b> s 235	Pro	Gly	Thr	Thr	Ala 240	
Ser	Asp	Asn	Val	Asp 245	Phe	Val	Arg	Thr	<b>Ty</b> r 250	Asp	Thr	Val	Glu	Phe 255	Leu	
Ser	Ala	Asp	Thr 260	Lys	Thr	Thr	Thr	Val 265	Asn	Val	Glu	Ser	L <b>y</b> s 270	Asp	Asn	
Gly	Lys	L <b>y</b> s 275	Thr	Glu	Val	Lys	Ile 280	Gly	Ala	Lys	Thr	Ser 285	Val	Ile	Lys	
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Ser 305	Ser	Thr	Asp	Glu	Gly 310	Glu	Gly	Leu	Val	Thr 315	Ala	Lys	Glu	Val	Ile 320	
Asp	Ala	Val	Asn	L <b>y</b> s 325	Ala	Gly	Trp	Arg	Met 330	Lys	Thr	Thr	Thr	Ala 335	Asn	
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Lys	Val	Thr 355	Phe	Ala	Ser	Gly	Asn 360	Gly	Thr	Thr	Ala	Thr 365	Val	Ser	Lys	
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Ala 385	Leu	Asn	Val	Asn	Gln 390	Leu	Gln	Asn	Ser	Gly 395	Trp	Asn	Leu	Asp	Ser 400	
Lys	Ala	Val	Ala	Gly 405	Ser	Ser	Gly	Lys	Val 410	Ile	Ser	Gly	Asn	Val 415	Ser	
Pro	Ser	Lys	Gly 420	Lys	Met	Asp	Glu	Thr 425	Val	Asn	Ile	Asn	Ala 430	Gly	Asn	
Asn	Ile	Glu 435	Ile	Thr	Arg	Asn	Gly 440	Lys	Asn	Ile	Asp	Ile 445	Ala	Thr	Ser	
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Pro 465	Thr	Leu	Ser	Val	Asp 470	Asp	Glu	Gly	Ala	Leu 475	Asn	Val	Gly	Ser	Lys 480	
Asp	Ala	Asn	Lys	Pro 485	Val	Arg	Ile	Thr	Asn 490	Val	Ala	Pro	Gly	Val 495	Lys	
Glu	Gly	Asp	Val 500	Thr	Asn	Val	Ala	Gln 505	Leu	Lys	Gly	Val	Ala 510	Gln	Asn	
Leu	Asn	Asn 515	Arg	Ile	Asp	Asn	Val 520	Asp	Gly	Asn	Ala	Arg 525	Ala	Gly	Ile	
Ala	Gln 530	Ala	Ile	Ala	Thr	Ala 535	Gly	Leu	Ala	Gln	Ala 540	Tyr	Leu	Pro	Gly	
L <b>y</b> s 545	Ser	Met	Met	Ala	Ile 550	Gly	Gly	Gly	Thr	<b>Ty</b> r 555	Arg	Gly	Glu	Ala	Gl <b>y</b> 560	
Tyr	Ala	Ile	Gly	<b>Ty</b> r 565	Ser	Ser	Ile	Ser	Asp 570	Thr	Gly	Asn	Trp	Val 575	Ile	

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Asn Ile Thr 370	Val Lys	Tyr Asp 375		Asn	Val	Gly	Asp 380	Ala	Leu	Asn	Val
Asn Gln Leu 385	ı Gln Asn	Ser Gly 390	' Trp	Asn	Leu	Asp 395	Ser	Lys	Ala	Val	Ala 400
Gly Ser Ser	Gly Lys 405		e Ser	Gly	Asn 410	Val	Ser	Pro	Ser	Lys 415	Gly
Lys Met Asp	Glu Thr 420	Val Asr	l Ile	Asn 425	Ala	Gly	Asn	Asn	Ile 430	Glu	Ile
Thr Arg Asr 435		Asn Ile	Asp 440	Ile	Ala	Thr	Ser	Met 445	Thr	Pro	Gln
Phe Ser Ser 450	Val Ser	Leu Gly 455		Gly	Ala	Asp	Ala 460	Pro	Thr	Leu	Ser
Val Asp Asp 465	) Glu Gly	Ala Leu 470	l Asn	Val	Gly	Ser 475	Lys	Asp	Ala	Asn	L <b>y</b> s 480
Pro Val Arc	JIE Thr 485		. Ala	Pro	Gly 490	Val	Lys	Glu	Gly	Asp 495	Val
Thr Asn Val	Ala Gln 500	Leu Ly:	Gly	Val 505	Ala	Gln	Asn	Leu	Asn 510	Asn	His
Ile Asp Asr 515		Gly Asr	Ala 520	Arg	Ala	Gly	Ile	Ala 525	Gln	Ala	Ile
Ala Thr Ala 530	ı Gly Leu	Val Glr 535		Tyr	Leu	Pro	Gly 540	Lys	Ser	Met	Met
Ala Ile Gly 545	y Gly Gly	Thr Tyr 550	Arg	Gly	Glu	Ala 555	Gly	Tyr	Ala	Ile	Gly 560
Tyr Ser Ser	Ile Ser 565		' Gly	Asn	<b>T</b> rp 570	Ile	Ile	Lys	Gly	Thr 575	Ala
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Val Val Val	. Ser Glu 20	Leu Thr	Arg	Asn 25	His	Thr	Lys	Arg	Ala 30	Ser	Ala
Thr Val Lys 35		Val Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln
Ala Ser Ala 50	ı Asn Asn	Glu Glu 55		Glu	Glu	Asp	Leu 60	Tyr	Leu	Asp	Pro
Val Gln Arc 65	f Thr Val	Ala Val 70	. Leu	Ile	Val	Asn 75	Ser	Asp	Lys	Glu	Gly 80
Thr Gly Glu	ı L <b>y</b> s Glu 85		. Glu	Glu	Asn 90	Ser	Asp	Trp	Ala	Val 95	Tyr

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Ser 145	Phe	Ser	Ala	Asn	Gly 150	Asn	Lys	Val	Asn	Ile 155	Thr	Ser	Asp	Thr	Lys 160
Gly	Leu	Asn	Phe	Ala 165	Lys	Glu	Thr	Ala	Gly 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val	His	Leu	Asn 180	Gly	Ile	Gly	Ser	Thr 185	Leu	Thr	Asp	Thr	Leu 190	Leu	Asn
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Lys	Lys 210	Arg	Ala	Ala	Ser	Val 215	Lys	Asp	Val	Leu	Asn 220	Ala	Gly	Trp	Asn
Ile 225	Lys	Gly	Val	Lys	Pro 230	Gly	Thr	Thr	Ala	Ser 235	Asp	Asn	Val	Asp	Phe 240
Val	Arg	Thr	Tyr	Asp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	L <b>y</b> s 255	Thr
Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys	Asp 265	Asn	Gly	Lys	Lys	Thr 270	Glu	Val
Lys	Ile	Gl <b>y</b> 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	<b>A</b> sp 285	Gly	Lys	Leu
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Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320
Gly	Trp	Arg	Met	L <b>y</b> s 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala
Asp	Lys	Phe	Glu 340	Thr	Val	Thr	Ser	Gly 345	Thr	Asn	Val	Thr	Phe 350	Ala	Ser
Gly	Lys	Gly 355	Thr	Thr	Ala	Thr	Val 360	Ser	Lys	Asp	Asp	Gln 365	Gly	Asn	Ile
	Val 370		-	-		375			-		380				
385	Gln			-	390	-		-		395				-	400
	Gly	-		405		-			410			-	-	415	
	Glu		420					425					430		
	Gly	435			-		440					445			
	Val 450					455					460				
465	Asp				470	-		-	-	475		-			480
Ile	Thr	Asn	Val	Ala 485	Pro	Gly	Val	Lys	Glu 490	Gly	Asp	Val	Thr	Asn 495	Val

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Val Asp	Gly 515	Asn	Ala	Arg	Ala	Gly 520	Ile	Ala	Gln	Ala	Ile 525	Ala	Thr	Ala
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Ile Ser	Asp	Gly	Gly 565	Asn	Trp	Ile	Ile	L <b>y</b> s 570	Gly	Thr	Ala	Ser	Gl <b>y</b> 575	Asn
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Thr Val	Lys	20 Thr	Ala	Val	Leu	Ala	25 Thr	Leu	Leu	Phe	Ala	30 Thr	Val	Gln
	35					40					45			
Ala Ser 50					55					60				
Val Gln 65	Arg	Thr	Val	Ala 70	Val	Leu	Ile	Val	Asn 75	Ser	Asp	Lys	Glu	Gly 80
Thr Gly	Glu	Lys	Glu 85	Lys	Val	Glu	Glu	Asn 90	Ser	Asp	Trp	Ala	Val 95	Tyr
Phe Asn	Glu	L <b>y</b> s 100	Gly	Val	Leu	Thr	Ala 105	Arg	Glu	Ile	Thr	Leu 110	Lys	Ala
Gly Asp	Asn 115	Leu	Lys	Ile	Lys	Gln 120	Asn	Gly	Thr	Asn	Phe 125	Thr	Tyr	Ser
Leu L <b>y</b> s 130	Lys	Asp	Leu	Thr	Asp 135	Leu	Thr	Ser	Val	Gly 140	Thr	Glu	Lys	Leu
Ser Phe 145	Ser	Ala		Gly 150		Lys		Asn			Ser	Asp	Thr	L <b>y</b> s 160
Gly Leu	Asn	Phe	Ala 165	Lys	Glu	Thr	Ala	Gly 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val His	Leu	Asn 180	Gly	Ile	Gly	Ser	<b>T</b> hr 185	Leu	Thr	Asp	Thr	Leu 190	Leu	Asn
Thr Gly	Ala 195		Thr	Asn	Val	Thr 200		Asp	Asn	Val	Thr 205		Asp	Glu
Lys Lys 210		Ala	Ala	Ser	Val 215		Asp	Val	Leu	Asn 220		Gly	Trp	Asn
Ile Lys 225	Gly	Val	Lys	Pro 230		Thr	Thr	Ala	Ser 235	Asp	Asn	Val	Asp	Phe 240
Val Arg	Thr	Tyr			Val	Glu	Phe				Asp	Thr		
Thr Thr	Val		245 Val	Glu	Ser	Lys		250 Asn	Gly	Lys	Lys		255 Glu	Val
		260					265					270		

Lys       Ile       213       Als       Lys       Th       Ser       201       Ile       Lys       Gu       Lys       Als       Gu       Lys       Als       Gu       Ser       Ser       Ser       Ser       Mat       Als       Gu       Ser       Ser       Ser       Mat       Als       Gu       Mat																
290       295       300         Glu Gly Leu Val Thr Ala Lys Glu Val Ile Asp Ala Zal 315       Asn Lys Ala 320         Gly Trp Arg Met Lys Thr Thr Ala Asn Gly Gln Thr Gly Gln Ala 335       Asp Lys Phe Glu Thr Val Thr Ser Gly Thr Asn Val Thr Phe Ala Ser 355         Gly Lys Gly Thr Thr Ala Thr Yal Ser Lys Asp Asp Gln Gly Asn Ile 355       Asn Sit 20         Glu Asn Ser Gly Thr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gly Ser Gly Lys Val Ile Ser Gly Asn Val Ser Lys Ala Asn Ile Glu Asn Ser Gly Lys Val Ile Ser Gly Asn Val Ser Fro Ser Lys Gly Lys Wet 400         Ser Gly Lys Val Ile Ser Gly Asn Ala Cly Asn Asn Ile Glu Thr Val Asn Ile Asp Ala Cly Asn Asn Ile Glu Thr Val Asn Ile Asp Ala 425         Asn Gly Lys Val Ile Ser Gly Asn Ala Cly Asn Asn Ile Glu Thr Arg 420         Ass Glu Thr Val Asn Ile Asp Ile Ala Thr Ser Met Thr Pato Glu Thr Arg 420         Ass Glu Asp Ala Leu Ash Val Cly Ser Lys Lys Asp Asp Ile Asp Ile Asp Ala 455         Ass Glu Asp Ala Leu Ash Val Gly Ala Asp Ala Pro Thr Leu Ser Val Asp 450         Gly Asp Ala Leu Ash Val Gly Ser Lys Lys Asp Asp Asp Asp Asp Asp Asp Asp Asp As	Lys I	Ile		Ala	Lys	Thr	Ser		Ile	Lys	Glu	Lys	_	Gly	Lys	Leu
305 310 310 315 315 320 Gly Trp Arg Met Lys Thr Thr Thr Ala Aren Gly Gln Thr Gly Gln Ala 335 Aren Lys Phe Glu Thr Val Thr Ser Gly Thr Aren Val Thr Phe Ala Ser 345 Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Aren Aren Val Thr Phe Ala Ser 365 Gly Lys Gly Thr Thr Ala Thr Val Ser Lys Aren Aren Val Aren Gln 355 Thr Val Met Tyr Aren Val Aren Val Gly Aren Ala Leu Aren Val Aren Gln 370 Leu Gln Aren Ser Gly Trp Aren Leu Aren Ser Lys Ala Val Ala Gly Ser 395 Ser Gly Lys Val IIe Ser Gly Aren Val Ser Pro Ser Lys Gly Lys Met 400 Ser Gly Lys Val IIe Ser Gly Aren Val Ser Pro Ser Lys Gly Lys Met 400 Ser Gly Lys Val IIe Ser Gly Aren Val Ser Pro Ser Lys Gly Lys Met 400 Aren Gly Lys Aren Ile Aren Ile Aren Ala Gly Aren Aren Ile Glu Ile Thr Arg 420 Aren Gly Lys Aren Ile Aren Ile Ala Thr Ser Met Thr Pro Gln Phe Ser 430 Aren Gly Lys Aren Ile Aren Ile Ala Thr Ser Met Thr Pro Gln Phe Ser 440 Gly Aren Ala Euu Aren Val Gly Ser Lys Lys Aren Aren Lys Pro Val Aren 440 Her A			Gly	Lys	Asp	Lys		Glu	Asn	Gly	Ser		Thr	Asp	Glu	Gly
As a big by the form of the set		Gly	Leu	Val	Thr		Lys	Glu	Val	Ile		Ala	Val	Asn	Lys	
340345350GlyLysGlyThrThrAlaThrValSerLysAspAspGlnGlyAsnIle370355ThThrAlaThrValSerLysAspAspGlnGlyAsnIle370377SerLysAspAlaAspAspAlaClnSer385SerNanSerSerAspAspGlGlyAsnClnSer395AsnValAspSer395AsnValAspGlyAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAspAsp	Gly 7	Irp	Arg	Met		Thr	Thr	Thr	Ala		Gly	Gln	Thr	Gly		Ala
The Val Met Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gln 370 Thr Val Met Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gln 370 Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala Gly Ser 395 Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met 400 Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met 400 Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile Thr Arg 420 Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Thr Pro Gln Phe Ser 435 Ser Val Ser Leu Gly Ala Gly Ser Lys Lys Asp Asn Lys Pro Val Asp 455 Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 445 Asp Glu Thr Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val 480 Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val 480 Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg Ile Asp Asn 500 Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile Ala Thr Ala 525 Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly 530 Gly Gly Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser 555 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Thr Ala Ser Gly Asn 575 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 <210 > SEQ ID NO 105 <211 > LENCTH: 591 <212 > TYPE PRT $<213 > ORGANISM: Neisseria meningitidis <400 > SEQUENCE: 105Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 15Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 2021 = 20$	Asp I	Lys	Phe		Thr	Val	Thr	Ser		Thr	Asn	Val	Thr		Ala	Ser
370 375 380 Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala Gly Ser 385 390 Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met 405 Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile Thr Arg 420 Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Thr Pro Gln Phe Ser 440 445 445 Ser Val Ser Leu Gly Ala Cly Ala Asp Ala Pro Thr Leu Ser Val Asp 450 Gly Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 455 Gly Asp Ala Leu Asn Val Gly Ser Lys Glu Gly Asp Val Thr Asn Val 465 Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg Ile Asp Asn 500 Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile Ala Thr Ala 510 Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly 530 Gly Gly Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser 545 540 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Thr Ala Ser Gly Asn 555 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 585 580 580 580 580 580 580 580	Gly I	Lys		Thr	Thr	Ala	Thr		Ser	Lys	Asp	Asp		Gly	Asn	Ile
385 390 395 400 Ser Gly Lys Val IIe Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met 405 410 Asn Gly Asn IIe Asn Ala Gly Asn Asn IIe Glu IIe Thr Arg 420 Asn Gly Lys Asn IIe Asn IIe Ala Thr Ser Met Thr Pro Gln Phe Ser 430 Asn Gly Lys Asn IIe Asp IIe Ala Thr Ser Met Thr Pro Gln Phe Ser 435 455 Asn Ala Cly Ala Asp Ala Pro Thr Leu Ser Val Asp 445 445 Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 465 Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val 485 450 Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val 485 450 Asn Ala Arg Ala Gly IIe Ala Gln Ala IIe Ala Thr Ala 500 Fly Asn Ala Arg Ala Gly IIe Ala Gln Ala IIe Ala Thr Ala 510 Ser Val Gly Asn Ala Arg Ala Gly Thr Ala IIe Gly Tyr Ser Sec Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala IIe Gly 530 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 Sec Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 Sec Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 Sec Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580 Sec Arg Gly His Phe Gly Ala Ser Ala Ser Ala Leu Asn Ala Trp 1 2110 LENGTH: 591 2212 TYPE: PRT 2213 ORGANISM: Neisseria meningitidis 2400 SEQUENCE: 105 Met Asn Lys IIe Tyr Arg IIe IIe Trp Asn Ser Ala Leu Asn Ala Trp 1 20 22 25 30 30 30 30 30 30 30 30 30 30 30 30 30			Met	Tyr	Asp	Val		Val	Gly	Asp	Ala		Asn	Val	Asn	Gln
405 410 415 Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile Thr Arg 420 Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Thr Pro Gln Phe Ser 435 440 The Ser Met Thr Pro Gln Phe Ser 445 450 Asp Ala Cly Ala Gly Ala Asp Ala Pro Thr Leu Ser Val Asp 450 450 Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 460 475 Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 460 475 Asp Ala Leu Asn Val Gly Val Lys Glu Gly Asp Val Thr Asn Val 485 480 Asp Ala Cly Val Ala Gly Ala Asp Ala Cly Asp Val Thr Asn Val 485 480 Asp Ala Cly Val Ala Gln Asn Leu Asn Asp Arg Tle Asp Asn 500 Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile Ala Thr Ala 515 515 Asp Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly 530 Cly Gly Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser 540 540 550 550 550 550 550 550 550 550		Gln	Asn	Ser	Gly		Asn	Leu	Asp	Ser		Ala	Val	Ala	Gly	
420425430Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Thr Pro Gln Phe Ser 435Asn Gly Lys Asn Ile Asp Ile Ala Gly Ala Asp Ala Pro Thr Leu Ser Val Asp 455Asp Ala Pro Thr Leu Ser Val Asp 466Asp Ala Pro Thr Leu Ser Val Asp 466Gly Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 465Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val 485Asn Age Ser Val Asp 485Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asp file Asp Asn 500Ser Yal Ser Val Asp Asn Arg Ile Asp Asn 505Asn Arg Ile Asp Asn 510Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Asn Leu Asn Arg Ile Ala Thr Ala 515Ser Met Met Ala Ile Gly 550Thr Ala Ser Ser 560Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly 530Gly Asn Trp Ile Ile Lys Gly Thr Ala Ser Gly Asn 575Ser Arg Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala Ser Gly Asn 570<210> SEQ ID NO 105 <211> LENGTH: S91 <212> TYPE: PRT 213> ORGANISM: Neisseria meningitidisSer Ala Leu Asn Ala Trp 1Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Sr Trp 10The Try Arg Asn His Thr Lys Arg Ala Ser Ala 30	Ser (	Gly	Lys	Val		Ser	Gly	Asn	Val		Pro	Ser	Lys	Gly		Met
435 440 445 Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser Val Asp 450 455 Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 465 Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg 465 Ala Cln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg Tle Asp Asn 500 500 500 500 500 500 500 500 500 500	Asp (	Glu	Thr		Asn	Ile	Asn	Ala		Asn	Asn	Ile	Glu		Thr	Arg
450       455       460         Gly Asp Ala Leu Asn Val Gly Ser Lys $Asp Asp Asp Asp Asp Asp Val Pro Val Arg 480         Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asp Val 485         Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asp Slo Pro Pro Slo Pro Slo Pro Slo Pro Pro Pro Pro Pro Pro Pro Pro Pro Pr$	Asn (	Gly		Asn	Ile	Asp	Ile		Thr	Ser	Met	Thr		Gln	Phe	Ser
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515 $520$ $525$ Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly $530$ Gly Gly Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser $545$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $540$ $575$ $575$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $590$ $59$	Ala (	Gln	Leu		Gly	Val	Ala	Gln		Leu	Asn	Asn	Arg		Asp	Asn
530       535       540         Gly Gly Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser 555       560         Ile Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala Ser Gly Asn 575         Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp 580         <210> SEQ ID NO 105         <211> LENGTH: 591         <212> TYPE: PRT         <213> ORGANISM: Neisseria meningitidis         <400> SEQUENCE: 105         Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 10         Yal Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20	Val A	Asp		Asn	Ala	Arg	Ala		Ile	Ala	Gln	Ala		Ala	Thr	Ala
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<pre>&lt;211&gt; LENGTH: 591 &lt;212&gt; TYPE: PRT &lt;213&gt; ORGANISM: Neisseria meningitidis &lt;400&gt; SEQUENCE: 105 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 5 10 15 Val Val Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala 20 25 30</pre>	Ser 1	Arg	Gly		Phe	Gly	Ala	Ser		Ser	Val	Gly	Tyr		Trp	
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20 25 30		Asn	Lys	Ile		Arg	Ile	Ile	Trp		Ser	Ala	Leu	Asn		Trp
Thr Val Lys Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln	Val V	Val	Val		Glu	Leu	Thr	Arg		His	Thr	Lys	Arg		Ser	Ala
	Thr V	Val	Lys	Thr	Ala	Val	Leu	Ala	Thr	Leu	Leu	Phe	Ala	Thr	Val	Gln

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											_	con	tin	ued	
		35					40					45			
Ala	Ser 50	Ala	Asn	Asn	Glu	Glu 55	Gln	Glu	Glu	Asp	Leu 60	Tyr	Leu	Asp	Pro
Val 65	Gln	Arg	Thr	Val	Ala 70	Val	Leu	Ile	Val	Asn 75	Ser	Asp	Lys	Glu	Gly 80
Thr	Gly	Glu	Lys	Glu 85	Lys	Val	Glu	Glu	Asn 90	Ser	Asp	Trp	Ala	Val 95	Tyr
Phe	Asn	Glu	L <b>y</b> s 100	Gly	Val	Leu	Thr	Ala 105	Arg	Glu	Ile	Thr	Leu 110	Lys	Ala
Gly	Asp	Asn 115	Leu	Lys	Ile	Lys	Gln 120	Asn	Gly	Thr	Asn	Phe 125	Thr	Tyr	Ser
Leu	L <b>y</b> s 130	Lys	Asp	Leu	Thr	Asp 135	Leu	Thr	Ser	Val	Gly 140	Thr	Glu	Lys	Leu
Ser 145	Phe	Ser	Ala	Asn	Gly 150	Asn	Lys	Val	Asn	Ile 155	Thr	Ser	Asp	Thr	L <b>y</b> s 160
Gly	Leu	Asn	Phe	Ala 165	Lys	Glu	Thr	Ala	Gly 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val	His	Leu	<b>A</b> sn 180	Gly	Ile	Gly	Ser	Thr 185	Leu	Thr	Asp	Thr	Leu 190	Leu	Asn
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Val	Arg	Thr	Tyr	Asp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	L <b>y</b> s 255	Thr
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Lys	Ile	Gl <b>y</b> 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	<b>As</b> p 285	Gly	Lys	Leu
Val	Thr 290	Gly	Lys	Asp	Lys	Gly 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly
Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320
Gly	Trp	Arg	Met	L <b>y</b> s 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala
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Thr	Val 370	Met	Tyr	Asp	Val	Asn 375		Gly	Asp	Ala	Leu 380	Asn	Val	Asn	Gln
Leu 385	Gln	Asn	Ser	Gly	Trp 390		Leu	Asp	Ser	L <b>y</b> s 395		Val	Ala	Gly	Ser 400
Ser	Gly	Lys	Val	Ile 405	Ser	Gly	Asn	Val	Ser 410	Pro	Ser	Lys	Gly	L <b>y</b> s 415	Met
Asp	Glu	Thr	Val 420	Asn	Ile	Asn	Ala	Gl <b>y</b> 425		Asn	Ile	Glu	Ile 430	Thr	Arg

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Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser Val Asp Gly Asp Ala Leu Asn Val Gly Ser Lys Lys Asp Asn Lys Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val Ala Gln Leu Lys Gly Val Ala Gln Asn Leu Asn Asn Arg Ile Asp Asn Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile Ala Thr Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly Gly Gly Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser Ile Ser Asp Gly Gly Asn Trp Ile Ile Lys Gly Thr Ala Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp <210> SEQ ID NO 106 <211> LENGTH: 592 <212> TYPE: PRT <213> ORGANISM: Neisseria meningitidis <400> SEQUENCE: 106 Met Asn Lys Ile Tyr Arg Ile Ile Trp Asn Ser Ala Leu Asn Ala Trp 1 5 10 15 Val Ala Val Ser Glu Leu Thr Arg Asn His Thr Lys Arg Ala Ser Ala Thr Val Lys Thr Ala Val Leu Ala Thr Leu Leu Phe Ala Thr Val Gln Ala Asn Ala Thr Asp Glu Asp Glu Glu Glu Glu Leu Glu Ser Val Gln Arg Ser Val Val Gly Ser Ile Gln Ala Ser Met Glu Gly Ser Gly Glu65707580 Leu Glu Thr Ile Ser Leu Ser Met Thr Asn Asp Ser Lys Glu Phe Val Asp Pro Tyr Ile Val Val Thr Leu Lys Ala Gly Asp Asn Leu Lys Ile Lys Gln Asn Thr Asn Glu Asn Thr Asn Ala Ser Ser Phe Thr Tyr Ser Leu Lys Lys Asp Leu Thr Gly Leu Ile Asn Val Glu Thr Glu Lys Leu Ser Phe Gly Ala Asn Gly Lys Lys Val Asn Ile Ile Ser Asp Thr Lys145150155160 Gly Leu Asn Phe Ala Lys Glu Thr Ala Gly Thr Asn Gly Asp Thr Thr 165 170 175 Val His Leu Asn Gly Ile Gly Ser Thr Leu Thr Asp Thr Leu Ala Gly Ser Ser Ala Ser His Val Asp Ala Gly Asn Gln Ser Thr His Tyr Thr Arg Ala Ala Ser Ile Lys Asp Val Leu Asn Ala Gly Trp Asn Ile Lys

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Val	Arg	Thr	Tyr	Asp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	L <b>y</b> s 255	Thr					
Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys	<b>A</b> sp 265	Asn	Gly	Lys	Arg	Thr 270	Glu	Val					
Lys	Ile	Gly 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	<b>A</b> sp 285	Gly	Lys	Leu					
Val	Thr 290	Gly	Lys	Gly	Lys	Gly 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly					
Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320					
Gly	Trp	Arg	Met	L <b>y</b> s 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala					
Asp	Lys	Phe	Glu 340	Thr	Val	Thr	Ser	Gly 345	Thr	Asn	Val	Thr	Phe 350	Ala	Ser					
Gly	Lys	Gly 355	Thr	Thr	Ala	Thr	Val 360	Ser	Lys	Asp	Asp	Gln 365	Gly	Asn	Ile					
Thr	Val 370	Met	Tyr	Asp	Val	Asn 375	Val	Gly	Asp	Ala	Leu 380	Asn	Val	Asn	Gln					
Leu 385	Gln	Asn	Ser	Gly	Trp 390	Asn	Leu	Asp	Ser	L <b>y</b> s 395	Ala	Val	Ala	Gly	Ser 400					
Ser	Gly	Lys	Val	Ile 405	Ser	Gly	Asn	Val	Ser 410	Pro	Ser	Lys	Gly	Lys 415	Met					
Asp	Glu	Thr	Val 420	Asn	Ile	Asn	Ala	Gl <b>y</b> 425	Asn	Asn	Ile	Glu	Ile 430	Ser	Arg					
Asn	Gly	L <b>y</b> s 435	Asn	Ile	Asp	Ile	Ala 440	Thr	Ser	Met	Ala	Pro 445	Gln	Phe	Ser					
Ser	Val 450	Ser	Leu	Gly	Ala	Gl <b>y</b> 455	Ala	Asp	Ala	Pro	Thr 460	Leu	Ser	Val	Asp					
Asp 465	Glu	Gly	Ala	Leu	Asn 470	Val	Gly	Ser	Lys	Asp 475	Ala	Asn	Lys	Pro	Val 480					
Arg	Ile	Thr	Asn	Val 485	Ala	Pro	Gly	Val	L <b>y</b> s 490	Glu	Gly	Asp	Val	Thr 495	Asn					
Val	Ala	Gln	Leu 500	Lys	Gly	Val	Ala	Gln 505	Asn	Leu	Asn	Asn	Arg 510	Ile	Asp					
Asn	Val	Asp 515	Gly	Asn	Ala	Arg	Ala 520	Gly	Ile	Ala	Gln	Ala 525	Ile	Ala	Thr					
Ala	Gly 530	Leu	Val	Gln	Ala	<b>Ty</b> r 535	Leu	Pro	Gly	Lys	Ser 540	Met	Met	Ala	Ile					
Gly 545	Gly	Gly	Thr	Tyr	Arg 550	Gly	Glu	Ala	Gly	<b>Ty</b> r 555	Ala	Ile	Gly	Tyr	Ser 560					
Ser	Ile	Ser	Asp	Gly 565	Gly	Asn	Trp	Ile	Ile 570	Lys	Gly	Thr	Ala	Ser 575	Gly					
Asn	Ser	Arg	Gly 580	His	Phe	Gly	Ala	Ser 585	Ala	Ser	Val	Gly	<b>Ty</b> r 590	Gln	Trp					

<211> LENGTH: 592 <212> TYPE: PRT <213> ORGANISM: Neisseria meningitidis

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Thr	Val	Lys 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln
Ala	Asn 50	Ala	Thr	Asp	Glu	Asp 55	Glu	Glu	Glu	Glu	Leu 60	Glu	Ser	Val	Gln
Arg 65	Ser	Val	Val	Gly	Ser 70	Ile	Gln	Ala	Ser	Met 75	Glu	Gly	Ser	Gly	Glu 80
Leu	Glu	Thr	Ile	Ser 85	Leu	Ser	Met	Thr	Asn 90	Asp	Ser	Lys	Glu	Phe 95	Val
Asp	Pro	Tyr	Ile 100	Val	Val	Thr	Leu	Lys 105	Ala	Gly	Asp	Asn	Leu 110	Lys	Ile
Lys	Gln	Asn 115	Thr	Asn	Glu	Asn	Thr 120	Asn	Ala	Ser	Ser	Phe 125	Thr	Tyr	Ser
Leu	Lys 130	Lys	Asp	Leu	Thr	Gly 135	Leu	Ile	Asn	Val	Glu 140	Thr	Glu	Lys	Leu
Ser 145	Phe	Gly	Ala	Asn	Gly 150	Lys	Lys	Val	Asn	Ile 155	Ile	Ser	Asp	Thr	Lys 160
Gly	Leu	Asn	Phe	Ala 165	Lys	Glu	Thr	Ala	Gl <b>y</b> 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val	His	Leu	Asn 180	Gly	Ile	Gly	Ser	Thr 185	Leu	Thr	Asp	Thr	Leu 190	Ala	Gly
Ser	Ser	Ala 195	Ser	His	Val	Asp	Ala 200	Gly	Asn	Gln	Ser	Thr 205	His	Tyr	Thr
Arg	Ala 210	Ala	Ser	Ile	Lys	Asp 215	Val	Leu	Asn	Ala	Gly 220	Trp	Asn	Ile	Lys
Gly 225	Val	Lys	Thr	Gly	Ser 230	Thr	Thr	Gly	Gln	Ser 235	Glu	Asn	Val	Asp	Phe 240
Val	Arg	Thr	Tyr	Asp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	L <b>y</b> s 255	Thr
Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys	Asp 265	Asn	Gly	Lys	Arg	Thr 270	Glu	Val
Lys	Ile	Gl <b>y</b> 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	Asp 285	Gly	Lys	Leu
Val	Thr 290	Gly	Lys	Gly	Lys	Gl <b>y</b> 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly
Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320
Gly	Trp	Arg	Met	L <b>ys</b> 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala
Asp	Lys	Phe	Glu 340	Thr	Val	Thr	Ser	Gly 345	Thr	Asn	Val	Thr	Phe 350	Ala	Ser
Gly	Lys	Gly 355	Thr	Thr	Ala	Thr	Val 360	Ser	Lys	Asp	Asp	Gln 365	Gly	Asn	Ile
Thr	Val 370	Met	Tyr	Asp	Val	Asn 375	Val	Gly	Asp	Ala	Leu 380	Asn	Val	Asn	Gln
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385					390					395					400
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Asp	Glu	Thr	Val 420	Asn	Ile	Asn	Ala	Gly 425	Asn	Asn	Ile	Glu	Ile 430	Ser	Arg
Asn	Gly	L <b>y</b> s 435	Asn	Ile	Asp	Ile	Ala 440	Thr	Ser	Met	Ala	Pro 445	Gln	Phe	Ser
Ser	Val 450	Ser	Leu	Gly	Ala	Gly 455	Ala	Asp	Ala	Pro	Thr 460	Leu	Ser	Val	Asp
Asp 465		Gly	Ala	Leu	Asn 470	Val	Gly	Ser	Lys	Авр 475		Asn	Lys	Pro	Val 480
	Ile	Thr	Asn			Pro	Gly	Val	-		Gly	Asp	Val		
Val	Ala	Gln	Leu	485 Lys	Gly	Val	Ala	Gln	490 Asn	Leu	Asn	Asn	Arg	495 Ile	Asp
Asn	Val	Asp	500 Glv	Asn	Ala	Arq	Ala	505 Glv	Tle	Ala	Gln	Ala	510 Tle	Ala	- Thr
		515	-			-	520	-				525			
Ala	G1y 530	Leu	Val	Gln	Ala	<b>Ty</b> r 535	Leu	Pro	GIY	Lys	Ser 540	Met	Met	Ala	Ile
Gly 545	Gly	Gly	Thr	Tyr	Arg 550	Gly	Glu	Ala	Gly	<b>Ty</b> r 555	Ala	Ile	Gly	Tyr	Ser 560
Ser	Ile	Ser	Asp	Gly 565		Asn	Trp	Ile	Ile 570	Lys	Gly	Thr	Ala	Ser 575	Gly
Asn	Ser	Arg	Gly 580	His	Phe	Gly	Ala	Ser 585	Ala	Ser	Val	Gly	<b>Ty</b> r 590	Gln	Trp
-210		II QI		100											
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		Lvs			Ara	Ile	Tle	Tro	Asn	Ser	Ala	Leu	Asn	Ala	Trp
1		-		5	-			-	10					15	-
Val	Val	Val	Ser 20	Glu	Leu	Thr	Arg	Asn 25	His	Thr	Lys	Arg	A1a 30	Ser	Ala
Thr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Ser	Ala 45	Thr	Val	Gln
Ala	Asn 50	Ala	Thr	Asp	Thr	Asp 55		Asp	Glu	Glu	Leu 60	Glu	Ser	Val	Val
Arg 65	Ser	Ala	Leu	Val	Leu 70	Gln	Phe	Met	Ile	Asp 75	Lys	Glu	Gly	Asn	Gly 80
Glu	Ile	Glu	Ser	Thr 85		Asp	Ile	Gly	Trp 90		Ile	Tyr	Tyr	Asp 95	Asp
His	Asn	Thr	Leu 100		Gly	Ala	Thr	Val 105	Thr	Leu	Lys	Ala	Gly 110	Asp	Asn
Leu	Lys	Ile 115	Lys		Ser	Gly	L <b>y</b> s 120	Asp	Phe	Thr	Tyr	Ser 125		Lys	Lys
Glu		Lys		Leu	Thr	Ser	Val		Thr				Ser	Phe	Gly
Ala	130 Asn		Asn	Lys	Val	135 Asn		Thr	Ser		140 Thr	Lys	Gly	Leu	Asn
145		-1		1-	150					155		1.5	-1		160

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Asn	Gly	Ile	Gly 180		Thr	Leu	Thr	<b>A</b> sp 185	Thr	Leu	Ala	Gly	Ser 190	Ser	Ala					
Ser	His	Val 195	Asp	Ala	Gly	Asn	Gln 200	Ser	Thr	His	Tyr	Thr 205	Arg	Ala	Ala					
Ser	Ile 210	Lys	Asp	Val	Leu	Asn 215	Ala	Gly	Trp	Asn	Ile 220	Lys	Gly	Val	Lys					
Thr 225	Gly	Ser	Thr	Thr	Gly 230	Gln	Ser	Glu	Asn	Val 235	Asp	Phe	Val	Arg	Thr 240					
Tyr	Asp	Thr	Val	Glu 245	Phe	Leu	Ser	Ala	<b>A</b> sp 250	Thr	Lys	Thr	Thr	Thr 255	Val					
Asn	Val	Glu	Ser 260	Lys	Asp	Asn	Gly	Lys 265	Arg	Thr	Glu	Val	L <b>y</b> s 270	Ile	Gly					
Ala	Lys	Thr 275	Ser	Val	Ile	Lys	Glu 280	Lys	Asp	Gly	Lys	Leu 285	Val	Thr	Gly					
Lys	Gly 290		Gly	Glu	Asn	Gly 295	Ser	Ser	Thr	Asp	Glu 300	Gly	Glu	Gly	Leu					
Val 305	Thr	Ala	Lys	Glu	Val 310	Ile	Asp	Ala	Val	Asn 315	Lys	Ala	Gly	Trp	Arg 320					
Met	Lys	Thr	Thr	Thr 325	Ala	Asn	Gly	Gln	Thr 330	Gly	Gln	Ala	Asp	Lys 335	Phe					
Glu	Thr	Val	Thr 340	Ser	Gly	Thr	Lys	Val 345	Thr	Phe	Ala	Ser	Gly 350	Asn	Gly					
Thr	Thr	Ala 355	Thr	Val	Ser	Lys	Asp 360	Asp	Gln	Gly	Asn	Ile 365	Thr	Val	Lys					
Tyr	Asp 370		Asn	Val	Gly	Asp 375	Ala	Leu	Asn	Val	Asn 380	Gln	Leu	Gln	Asn					
Ser 385	Gly	Trp	Asn	Leu	Asp 390	Ser	Lys	Ala	Val	Ala 395	Gly	Ser	Ser	Gly	L <b>y</b> s 400					
Val	Ile	Ser	Gly	Asn 405	Val	Ser	Pro	Ser	Lys 410	Gly	Lys	Met	Asp	Glu 415	Thr					
Val	Asn	Ile	Asn 420	Ala	Gly	Asn	Asn	Ile 425	Glu	Ile	Thr	Arg	Asn 430	Gly	Lys					
Asn	Ile	Asp 435	Ile	Ala	Thr	Ser	Met 440	Thr	Pro	Gln	Phe	Ser 445	Ser	Val	Ser					
Leu	Gly 450	Ala	Gly	Ala	Asp	Ala 455	Pro	Thr	Leu	Ser	Val 460	Asp	Asp	Glu	Gly					
Ala 465	Leu	Asn	Val	Gly	Ser 470	Lys	Asp	Ala	Asn	L <b>y</b> s 475	Pro	Val	Arg	Ile	Thr 480					
Asn	Val	Ala	Pro	Gly 485	Val	Lys	Glu	Gly	Asp 490	Val	Thr	Asn	Val	Ala 495	Gln					
Leu	Lys	Gly	Val 500	Ala	Gln	Asn	Leu	Asn 505	Asn	Arg	Ile	Asp	Asn 510	Val	Asp					
Gly	Asn	Ala 515	Arg	Ala	Gly	Ile	Ala 520	Gln	Ala	Ile	Ala	Thr 525	Ala	Gly	Leu					
Ala	Gln 530	Ala	Tyr	Leu	Pro	Gly 535	Lys	Ser	Met	Met	Ala 540	Ile	Gly	Gly	Gly					
Thr 545	Tyr	Arg	Gly	Glu	Ala 550	Gly	Tyr	Ala	Ile	Gly 555	Tyr	Ser	Ser	Ile	Ser 560					
Asp	Thr	Gly	Asn	Trp	Val	Ile	Lys	Gly	Thr	Ala	Ser	Gly	Asn	Ser	Arg					

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Val	Val	Val	Ser 20	Glu	Leu	Thr	Arg	Asn 25	His	Thr	Lys	Arg	Ala 30	Ser	Ala
Thr	Val	Ala 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Ser	Ala 45	Thr	Val	Gln
Ala	Asn 50	Ala	Thr	Asp	Thr	Asp 55	Glu	Asp	Glu	Glu	Leu 60	Glu	Ser	Val	Val
Arg 65	Ser	Ala	Leu	Val	Leu 70	Gln	Phe	Met	Ile	Asp 75	Lys	Glu	Gly	Asn	Gly 80
Glu	Ile	Glu	Ser	Thr 85	Gly	Asp	Ile	Gly	Trp 90	Ser	Ile	Tyr	Tyr	Asp 95	Asp
His	Asn	Thr	Leu 100	His	Gly	Ala	Thr	Val 105	Thr	Leu	Lys	Ala	Gly 110	Asp	Asn
Leu	Lys	Ile 115	Lys	Gln	Ser	Gly	L <b>y</b> s 120	Asp	Phe	Thr	Tyr	Ser 125	Leu	Lys	Lys
Glu	Leu 130	Lys	Asp	Leu	Thr	Ser 135	Val	Glu	Thr	Glu	L <b>y</b> s 140	Leu	Ser	Phe	Gly
Ala 145	Asn	Gly	Asn	Lys	Val 150	Asn	Ile	Thr	Ser	Asp 155	Thr	Lys	Gly	Leu	Asn 160
Phe	Ala	Lys	Glu	Thr 165	Ala	Gly	Thr	Asn	Gly 170	Asp	Pro	Thr	Val	His 175	Leu
Asn	Gly	Ile	Gly 180	Ser	Thr	Leu	Thr	Asp 185	Thr	Leu	Ala	Gly	Ser 190	Ser	Ala
Ser	His	Val 195	Asp	Ala	Gly	Asn	Gln 200	Ser	Thr	His	Tyr	Thr 205	Arg	Ala	Ala
	Ile 210	-	-			215		-	-		220	-	-		-
225	Gly				230					235					240
-	Asp			245					250		-			255	
	Val		260					265					270		
	Lys	275				-	280	-	-	-	-	285			-
-	Gly 290	-	-			295				-	300	-		-	
Val 305	Thr	Ala	Lys	Glu	Val 310	Ile	Asp	Ala	Val	Asn 315	Lys	Ala	Gly	Trp	Arg 320
Met	Lys	Thr	Thr	Thr 325	Ala	Asn	Gly	Gln	Thr 330	Gly	Gln	Ala	Asp	Lys 335	Phe

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											-	con	tin	ued						
Glu	Thr	Val	Thr 340	Ser	Gly	Thr	Lys	Val 345	Thr	Phe	Ala	Ser	Gly 350	Asn	Gly					
Thr	Thr	Ala 355	Thr	Val	Ser	Lys	Asp 360	Asp	Gln	Gly	Asn	Ile 365	Thr	Val	Lys					
Tyr	<b>A</b> sp 370	Val	Asn	Val	Gly	<b>A</b> sp 375	Ala	Leu	Asn	Val	Asn 380	Gln	Leu	Gln	Asn					
Ser 385	Gly	Trp	Asn	Leu	Asp 390	Ser	Lys	Ala	Val	Ala 395	Gly	Ser	Ser	Gly	Lys 400					
Val	Ile	Ser	Gly	Asn 405	Val	Ser	Pro	Ser	Lys 410	Gly	Lys	Met	Asp	Glu 415	Thr					
Val	Asn	Ile	Asn 420	Ala	Gly	Asn	Asn	Ile 425	Glu	Ile	Thr	Arg	Asn 430	Gly	Lys					
Asn	Ile	Asp 435		Ala	Thr	Ser	Met 440		Pro	Gln	Phe	Ser 445		Val	Ser					
Leu	Gly 450		Gly	Ala	Asp	Ala 455		Thr	Leu	Ser	Val 460		Asp	Glu	Gly					
Ala 465		Asn	Val	Gly	Ser 470		Asp	Ala	Asn	Lys 475		Val	Arg	Ile	Thr 480					
	Val	Ala	Pro	Gly 485		Lys	Glu	Gly	_		Thr	Asn	Val							
Leu	Lys	Gly		485 Ala	Gln	Asn	Leu		490 Asn	Arg	Ile	Asp		495 Val	Asp					
Gly	Asn		500 Arg	Ala	Gly	Ile		505 Gln	Ala	Ile	Ala		510 Ala	Gly	Leu					
Ala		515 Ala	Tyr	Leu	Pro		520 Lys	Ser	Met	Met	Ala	525 Ile	Gly	Gly	Gly					
Thr	530 <b>Ty</b> r	Arg	Gly	Glu	Ala	535 Gly	Tyr	Ala	Ile	Gly	540 Tyr	Ser	Ser	Ile	Ser					
545 Asp	Thr	Gly	Asn	Trp	550 Val	Ile	Lys	Gly	Thr	555 Ala	Ser	Gly	Asn	Ser	560 Arg					
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1			580					585	1	-1-										
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				Nei	sseri	ia me	eninç	gitic	lis											
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Val	Ala	Val	Ser 20	Glu	Leu	Thr	Arg	Asn 25	His	Thr	Lys	Arg	Ala 30	Ser	Ala					
Thr	Val	Lys 35	Thr	Ala	Val	Leu	Ala 40	Thr	Leu	Leu	Phe	Ala 45	Thr	Val	Gln					
Ala	Asn 50	Ala	Thr	Asp	Glu	Asp 55	Glu	Glu	Glu	Glu	Leu 60	Glu	Ser	Val	Gln					
Arg 65	Ser	Val	Val	Gly	Ser 70	Ile	Gln	Ala	Ser	Met 75	Glu	Gly	Ser	Gly	Glu 80					
Leu	Glu	Thr	Ile	Ser 85	Leu	Ser	Met	Thr	Asn 90	Asp	Ser	Lys	Glu	Phe 95	Val					
Asp	Pro	Tyr	Ile 100	Val	Val	Thr	Leu	L <b>y</b> s 105	Ala	Gly	Asp	Asn	Leu 110	Lys	Ile					

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Lys	Gln	Asn 115	Thr	Asn	Glu	Asn	Thr 120	Asn	Ala	Ser	Ser	Phe 125	Thr	Tyr	Ser
Leu	Lys 130	Lys	Asp	Leu	Thr	Gly 135	Leu	Ile	Asn	Val	Glu 140	Thr	Glu	Lys	Leu
Ser 145	Phe	Gly	Ala	Asn	Gly 150	Lys	Lys	Val	Asn	Ile 155	Ile	Ser	Asp	Thr	Lys 160
Gly	Leu	Asn	Phe	Ala 165	Lys	Glu	Thr	Ala	Gly 170	Thr	Asn	Gly	Asp	Thr 175	Thr
Val	His	Leu	Asn 180	Gly	Ile	Gly	Ser	Thr 185	Leu	Thr	Asp	Met	Leu 190	Leu	Asn
Thr	Gly	Ala 195	Thr	Thr	Asn	Val	Thr 200	Asn	Asp	Asn	Val	Thr 205	Asp	Asp	Glu
Lys	Lys 210	Arg	Ala	Ala	Ser	Val 215	Lys	Asp	Val	Leu	Asn 220	Ala	Gly	Trp	Asn
Ile 225	Lys	Gly	Val	Lys	Pro 230	Gly	Thr	Thr	Ala	Ser 235	Asp	Asn	Val	Asp	Phe 240
Val	Arg	Thr	Tyr	<b>A</b> sp 245	Thr	Val	Glu	Phe	Leu 250	Ser	Ala	Asp	Thr	Lys 255	Thr
Thr	Thr	Val	Asn 260	Val	Glu	Ser	Lys	Asp 265	Asn	Gly	Lys	Lys	Thr 270	Glu	Val
Lys	Ile	Gly 275	Ala	Lys	Thr	Ser	Val 280	Ile	Lys	Glu	Lys	Asp 285	Gly	Lys	Leu
Val	Thr 290	Gly	Lys	Gly	Lys	Gl <b>y</b> 295	Glu	Asn	Gly	Ser	Ser 300	Thr	Asp	Glu	Gly
Glu 305	Gly	Leu	Val	Thr	Ala 310	Lys	Glu	Val	Ile	Asp 315	Ala	Val	Asn	Lys	Ala 320
Gly	Trp	Arg	Met	L <b>y</b> s 325	Thr	Thr	Thr	Ala	Asn 330	Gly	Gln	Thr	Gly	Gln 335	Ala
Asp	Lys	Phe	Glu 340	Thr	Val	Thr	Ser	Gl <b>y</b> 345	Thr	Asn	Val	Thr	Phe 350	Ala	Ser
Gly	Lys	Gly 355	Thr	Thr	Ala	Thr	Val 360	Ser	Lys	Asp	Asp	Gln 365	Gly	Asn	Ile
Thr	Val 370	Met	Tyr	Asp	Val	Asn 375	Val	Gly	Asp	Ala	Leu 380	Asn	Val	Asn	Gln
Leu 385	Gln	Asn	Ser	Gly	Trp 390	Asn	Leu	Asp	Ser	L <b>y</b> s 395	Ala	Val	Ala	Gly	Ser 400
Ser	Gly	Lys	Val	Ile 405	Ser	Gly	Asn	Val	Ser 410	Pro	Ser	Lys	Gly	Lys 415	Met
Asp	Glu	Thr	Val 420	Asn	Ile	Asn	Ala	Gl <b>y</b> 425	Asn	Asn	Ile	Glu	Ile 430	Thr	Arg
Asn	Gly	L <b>y</b> s 435	Asn	Ile	Asp	Ile	Ala 440	Thr	Ser	Met	Thr	Pro 445	Gln	Phe	Ser
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Asp 465	Lys	Gly	Ala	Leu	Asn 470	Val	Gly	Ser	Lys	Asp 475	Ala	Asn	Lys	Pro	Val 480
Arg	Ile	Thr	Asn	Val 485	Ala	Pro	Gly	Val	Lys 490	Glu	Gly	Asp	Val	Thr 495	Asn
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Ile Lys Glu Lys Asp Gly Lys Leu Val

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Glu

Thr	Gly	L <b>y</b> s 300	Gly	Lys	Gly
Gly	Leu	Val	Thr	Ala	Lys

Asn Gly Ser Ser Thr Asp Glu Gly Glu G Glu Val Ile Asp Ala Val Asn Lys Ala Gly Trp Arg Met Lys Thr Thr Thr Ala Asn Gly Gln Thr Gly Gln Ala Asp Lys Phe Glu Thr Val Thr Ser Gly Thr Lys Val Thr Phe Ala Ser Gly Asn Gly Thr Thr Ala Thr Val Ser Lys Asp Asp Gln Gly Asn Ile Thr Val Lys Tyr Asp Val Asn Val Gly Asp Ala Leu Asn Val Asn Gln Leu Gln Asn Ser Gly Trp Asn Leu Asp Ser Lys Ala Val Ala Gly Ser Ser Gly Lys Val Ile Ser Gly Asn Val Ser Pro Ser Lys Gly Lys Met Asp Glu Thr Val Asn Ile Asn Ala Gly Asn Asn Ile Glu Ile Thr Arg Asn Gly Lys Asn Ile Asp Ile Ala Thr Ser Met Thr Pro Gln Phe Ser Ser Val Ser Leu Gly Ala Gly Ala Asp Ala Pro Thr Leu Ser Val Asp Asp Glu Gly Ala Leu Asn Val Gly Ser Lys Asp Ala Asn Lys Pro Val Arg Ile Thr Asn Val Ala Pro Gly Val Lys Glu Gly Asp Val Thr Asn Val Ala Gln Leu Lys Gly Val Ala 500 505 510 Gln Asn Leu Asn Asn Arg Ile Asp Asn Val Asp Gly Asn Ala Arg Ala Gly Ile Ala Gln Ala Ile Ala Thr Ala Gly Leu Val Gln Ala Tyr Leu Pro Gly Lys Ser Met Met Ala Ile Gly Gly Gly Thr Tyr Arg Gly Glu Ala Gly Tyr Ala Ile Gly Tyr Ser Ser Ile Ser Asp Gly Gly Asn Trp 565 570 575 Ile Ile Lys Gly Thr Ala Ser Gly Asn Ser Arg Gly His Phe Gly Ala Ser Ala Ser Val Gly Tyr Gln Trp <210> SEQ ID NO 112 <211> LENGTH: 32 <212> TYPE: DNA <213> ORGANISM: Neisseria meningitidis <400> SEQUENCE: 112 cgcggatccc atatgtcgcc gcaaaattcc ga <210> SEQ ID NO 113

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- Ile I 145		Ser	Asp	Thr	L <b>y</b> s 150		Leu	Asn	Phe	Ala 155		Glu	Thr	Ala	Gly 160
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Leu T	ſhr	Ser			Thr	Glu	Lys			Phe	Gly	Ala			Asp
Lys V	/al	Asp	100 Ile	Thr	Ser	Asp	Ala	105 Asn	Gly	Leu	Lys	Leu	110 Ala	Lys	Thr
Gly A	Asn	115 Glv	Asn	Val	Hi∝	Len	120 Asn	Glv	Leu	Asn	Ser	125 Thr	Leu	Pro	Asn
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Thr Xaa Xaa Xaa 35	Leu Xaa Xaa	a Xaa Xaa Xaa 40	Xaa Xaa Xaa Val 45	Xaa Xaa
Xaa Xaa Leu Pro 50	Xaa Xaa Xaa 5		Lys Xaa Xaa Xaa 60	Xaa Xaa
Gly Xaa Xaa Xaa 65	Xaa Xaa Asj 70	o Xaa Glu Xaa	Xaa Asn Ala Xaa 75	Lys Pro 80
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Pro Lys Asn Pro 20		l Val Ile Leu 25	Asp Leu Gly Ile 30	Leu Asp
Thr Phe Asp Ala 35	Leu Lys Le	1 Asn Asp Lys 40	Val Ala Gly Val 45	Pro Ala
Lys Asn Leu Pro 50	Lys Tyr Len 5!		L <b>y</b> s Asn L <b>y</b> s Pro 60	Ser Val
Gly Gly Val Glr 65	Gln Val Asj 70	p Phe Glu Ala	Ile Asn Ala Leu 75	L <b>y</b> s Pro 80
Asp Leu Ile Ile	lle Ser Gl 85	y Arg Gln Ser 90	Lys Phe Tyr Asp	L <b>y</b> s Leu 95
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Glu Thr Phe Tyr 50	Gly Lys Glu 5		Val Leu Gly Thr 60	Gly Val
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Glu 385	Ile	Val	Gly	Glu	Ala 390	Leu	Thr	Asn	Gly	L <b>y</b> s 395	Asn	Pro	Asp	Thr	Leu 400
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Ala	Ala	Glu 435	Val	Ala	Val	Lys	Asn 440	Asn	Gln	Leu	Ser	Asp 445	Xaa	Glu	Gly
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Gln 465	Leu	Сув	Arg	Lys	Asn 470	Thr	Val	Lys	Lys	<b>Ty</b> r 475	Gln	Asn	Val	Ala	Asp 480
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Glu	Lys	Ala	Arg	Ala 85	Asp	Ala	Lys	Ile	Ile 90	Leu	Lys	Gly	Ile	Val 95	Asn
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Lys	Gln	Ala 115	Gly	Ser	Gly	Ser	Thr 120	Val	Glu	Thr	Leu	L <b>y</b> s 125		Pro	Ser
Phe			Pro	Ala	Leu	Pro 135		Leu	Thr	Ala	Pro 140		Gly	Tyr	Ile
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Val	Asn	Trp	Asn	165 Gln	Val	Gln	Leu	Ala	170 Tyr	Asp	Lys	Trp	Asp	175 Tyr	Lys
Gln	Glu	Gly	180 Leu	Thr	Gly	Ala	Gly	185 Ala	Ala	Ile	Ile	Ala	190 Leu	Ala	Val
		195			Gly		200					205			
	210				_	215	_		-		220		_		
225					Ala 230		_			235					240
GIn	Ala	Ser	Val	Ser 245	Phe	lle	Asn	Asn	L <b>y</b> s 250	GIY	Asn	lle	GIY	Asn 255	Thr
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Val	Ala	Thr 275	Ala	Gly	Val	Ala	<b>A</b> sp 280	Lys	Ile	Gly	Ala	Ser 285	Ala	Leu	Asn
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Xaa	Xaa	Xaa 35	Xaa	Xaa	Xaa	Xaa	Xaa 40	Val	Val	Arg	Gln	Pro 45	Ile	Lys	Arg
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Ile 385	Glu	Ser	Lys	Leu	Thr 390	Val	Gly	Gln	Leu	Ile 395	Ala	Phe	Asn	Met	Leu 400

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Glu	Ala	Ala	Lys	Leu 565	Ala	Gly	Ala	His	Glu 570	Phe	Ile	Met	Glu	Leu 575	
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Glu 625	Arg	Ala	Ile	Met	Gln 630	Asn	Met	Gln	Ala	Ile 635	Cys	Ala	Asn	Arg	Thr 640
Val	Leu	Ile	Ile	Ala 645	His	Arg	Leu	Ser	<b>T</b> hr 650	Val	Lys	Thr	Ala	His 655	Arg
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Ile 385	Xaa	Xaa	Xaa	Leu	Xaa 390	Xaa	Gly	Gln	Leu	Ile 395	Ala	Phe	Asn	Met	Leu 400
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Xaa	Gly 450	Xaa	Ile	Thr	Phe	Xaa 455	Xaa	Xaa	Xaa	Phe	Arg 460	Tyr	Lys	Xaa	Asp
Xaa 465	Xaa	Xaa	Ile	Leu	Xaa 470	Asp	Xaa	Asn	Leu	Xaa 475	Ile	Xaa	Xaa	Gly	Glu 480
Val	Xaa	Gly	Ile	Val 485	Gly	Arg	Ser	Gly	Ser 490	Gly	Lys	Ser	Thr	Leu 495	Thr
Lys	Leu	Xaa	Gln 500	Arg	Xaa	Tyr	Xaa	Pro 505	Xaa	Xaa	Gly	Xaa	Val 510	Leu	Xaa
Asp	Gly	Xaa 515	Asp	Leu	Ala	Leu	Ala 520	Xaa	Pro	Xaa	Trp	Leu 525	Arg	Arg	Gln
Val	Gl <b>y</b> 530	Val	Val	Leu	Gln	Xaa 535	Asn	Val	Leu	Leu	Asn 540	Arg	Ser	Ile	Arg
<b>A</b> sp 545	Asn	Ile	Ala	Leu	Xaa 550	Asp	Xaa	Gly	Met	Pro 555	Xaa	Glu	Xaa	Ile	Xaa 560
Xaa	Ala	Ala	Lys	Leu 565	Ala	Gly	Ala	His	Glu 570	Phe	Ile	Xaa	Glu	Leu 575	Xaa
Glu	Gly	Tyr	Xaa 580	Thr	Xaa	Val	Gly	Glu 585	Gln	Gly	Ala	Gly	Leu 590	Ser	Gly
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Glu 625	Xaa	Xaa	Ile	Met	Xaa 630	Asn	Met	Xaa	Xaa	Ile 635	Суз	Xaa	Xaa	Arg	Thr 640
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Glu	Leu	Leu 675	Ala	Xaa	Pro	Asn	Gl <b>y</b> 680	Xaa	Tyr	Xaa	Tyr	Leu 685	Xaa	Xaa	Leu
Gln	Xaa 690														
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				170											
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Ser	Leu	Glu 35	Leu	Lys	Ala	Lys	Gln 40	Val	Lys	Lys	Ala	Ile 45	Asp	Arg	Leu
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Ser	Leu	Tyr	Gln 100	Gly	Lys	Leu	Ile	Leu 105	Val	Ala	Ser	Arg	Ala 110	Ser	Ile
Val	Gly	L <b>y</b> s 115	Leu	Ala	Lys	Phe	<b>A</b> sp 120	Phe	Thr	Trp	Phe	Ile 125	Pro	Ala	Val
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Leu 145	Gln	Ile	Phe	Ala	Leu 150	Ile	Thr	Pro	Leu	Phe 155	Phe	Gln	Val	Val	Met 160
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Gln	Ile	Arg	Asn	Phe 245	Leu	Thr	Gly	Gln	<b>A</b> la 250	Leu	Thr	Ser	Val	Leu 255	Asp
Leu	Met	Phe	Ser 260	Phe	Ile	Phe	Phe	Ala 265	Val	Met	Trp	Tyr	<b>Ty</b> r 270	Ser	Pro
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Lys	Leu	Ala	Gly	Ala 565	His	Glu	Phe	Ile	Ser 570	Glu	Leu	Arg	Glu	Gly 575	Tyr
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Arg	His	Leu	Leu 100	Ala	Leu	Pro	Ile	Ser 105	Tyr	Phe	Glu	Ala	Arg 110	Arg	Val
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		QUEN				рта	100-11	Iorue							
Met 1	Asn	Leu	Ile	Ser 5	Arg	Tyr	Ile	Ile	Arg 10	Gln	Met	Ala	Val	Met 15	Ala
Val	Tyr	Ala	Leu 20	Leu	Ala	Phe	Leu	Ala 25	Leu	Tyr	Ser	Phe	Phe 30	Glu	Ile
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		Leu													

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Lys Lys Leu	Leu Leu 100	Ile Leu	Ser Gln 105	Phe Gly	7 Phe Ile	Phe Ala 110	Ile
Ala Thr Val 115	Ala Leu (	Gly Glu	Trp Val 120	Ala Pro	o Thr Leu 125	Ser Gln	Lys
Ala Glu Asn 130	Ile Lys J	Ala Ala 135	Ala Ile	Asn Gly	v Lys Ile 140	Ser Thr	Gly
Asn Thr Gly 145		Leu L <b>y</b> s 150	Glu Lys	Asn Ser 155		Asn Val	Arg 160
Glu Met Leu	Pro Asp 1 165	His Thr	Leu Leu	Gly Ile 170	e Lys Ile	Trp Ala 175	Arg
Asn Asp Lys	Asn Glu 1 180	Leu Ala	Glu Ala 185	Val Glu	ı Ala Asp	Ser Ala 190	Val
Leu Asn Ser 195	Asp Gly :	Ser Trp	Gln Leu 200	Lys Asr	l Ile Arg 205	Arg Ser	Thr
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Gln	Lys	Ala	Ala	Glu 965	Leu	Asn	Gln	Lys	Ser 970	Lys	Glu	Leu	Glu	Gln 975	Gln
Ile	Ala	Gln	Leu 980	Lys	Lys	Ser	Ser	Pro 985	Lys	Ser	Lys	Leu	Ile 990	Pro	Thr
Leu	Gln	Glu 995	Glu	Arg	Asp		Leu 1000	Ala	Phe	Tyr		Gln 1005	Ala	Ile	Asn
	Glu 1010	Val	Lys	Gly		L <b>y</b> s 1015	Pro	Lys	Gly		Glu 1020	Tyr	Leu	Gln	Ala
L <b>y</b> s 1025	Leu 5	Ser	Ala		Asn 1030	Ile	Asp	Leu		Ser L035	Ala	Gln	Gly		Glu 1040
Ile	Ser	Gly		Asp L045	Ile	Thr	Ala		L <b>y</b> s 1050	Lys	Leu	Asn		His 1055	Ala
Ala	Gly		Leu 1060	Pro	Lys	Ala		Asp 1065	Ser	Glu	Ala		Ala 1070	Ile	Leu
Ile	Asp 1	Gl <b>y</b> 1075	Ile	Thr	Asp		<b>Ty</b> r 1080	Glu	Ile	Gly		Pro 1085	Thr	Tyr	Lys
	His 1090	Tyr	Asp	Lys		Ala 1095	Leu	Asn	Lys		Ser 1100	Arg	Leu	Thr	Gly
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Ile	Ile	Ile		Ala L125	Ser	Glu	Ile		Ala 130	Pro	Ser	Gly		Ile 1135	Asp
Ile	Lys		His L140	Ser	Asp	Ile		Leu 1145	Glu	Ala	Gly		Asn 150	Asp	Ala
Tyr	Thr	Phe 1155	Leu	Lys	Thr		Gly 1160	Lys	Ser	Gly		Ile 1165	Ile	Arg	Lys
	Lys 1170	Phe	Thr	Ser		Arg 1175	Asp	His	Leu		Met 1180	Pro	Ala	Pro	Val
Glu 1185	Leu 5	Thr	Ala		Gly 1190	Ile	Thr	Leu		Ala 195	Gly	Gly	Asn		Glu 1200
Ala	Asn	Thr		Arg L205	Phe	Asn	Ala		Ala 1210	Gly	Lys	Val		Leu 1215	Val
Ala	Gly		Glu 1220	Leu	Gln	Leu		Ala 1225	Glu	Glu	Gly		His 230	Lys	His
Glu	Leu	Asp 1235	Val	Gln	Lys		Arg 1240	Arg	Phe	Ile		Ile 1245	Lys	Val	Gly
	Ser 1250	Asn	Tyr	Ser		Asn 1255	Glu	Leu	Asn		Thr 1260	Lys	Leu	Pro	Val
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Thr Val Trp Gln Lys Gln Ala Gly Arg Gly Ser Thr Ile Glu Thr Leu 1330 1335 1340
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Gly Gly Tyr Ile Val Asp Ile Pro Lys Gly Asn Leu Lys Thr Glu Ile 1365 1370 1375
Glu Lys Leu Ala Lys Gln Pro Glu Tyr Ala Tyr Leu Lys Gln Leu Gln 1380 1385 1390
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Thr Ser Asp Thr Val Lys Gln Ile Val Thr Ser Ala Leu Thr Ala Gly 1505 1510 1515 1520
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Arg Thr Glu Leu Phe Ser Ser Thr Gly Asn Gln Thr Ile Ala Asn Leu 1540 1545 1550
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Leu	Gly 50	Val	Ala	Ala	Phe	Ser 55	Pro	Ala	Pro	Ala	Ser 60	Gly	Ile	Ile	Ala
Asp 65	Lys	Ser	Ala	Pro	L <b>y</b> s 70	Asn	Gln	Gln	Ala	Val 75	Ile	Leu	Gln	Thr	Ala 80
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Ser	Val	Asn	Arg 100	Phe	Lys	Gln	Phe	<b>A</b> sp 105	Val	Asp	Glu	Lys	Gly 110	Val	Ile
Leu	Asn	Asn 115	Ser	Arg	Ser	Asn	Thr 120	Gln	Thr	Gln	Leu	Gly 125	Gly	Trp	Ile
Gln	Gly 130	Asn	Pro	His	Leu	Ala 135	Arg	Gly	Glu	Ala	Arg 140	Val	Ile	Val	Asn
Gln 145	Ile	Asp	Ser	Ser	Asn 150	Pro	Ser	Leu	Leu	Asn 155	Gly	Tyr	Ile	Glu	Val 160
Gly	Gly	Lys	Arg	Ala 165	Glu	Val	Val	Val	Ala 170	Asn	Pro	Ser	Gly	Ile 175	Arg
Val	Asn	Gly	Gly 180	Gly	Leu	Ile	Asn	<b>Ala</b> 185	Ala	Ser	Val	Thr	Leu 190	Thr	Ser
Gly	Val	Pro 195	Val	Leu	Asn	Asn	Gly 200	Asn	Leu	Thr	Gly	Phe 205	Asp	Val	Ser
Ser	Gly 210	Lys	Val	Val	Ile	Gl <b>y</b> 215	Gly	Lys	Gly	Leu	Asp 220	Thr	Ser	Asp	Ala
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Asp	Gly	Ser	Leu 260	Ala	Lys	Thr	Ala	Ser 265	Ala	Pro	Ser	Ser	Ser 270	Asp	Ser
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 225
 230
 235
 240

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Ile ?	<b>f</b> hr	Ile 355	Ser	Ala	Gln	Thr	Val 360	Asp	Asn	Arg	Gln	Gly 365	Phe	Ile	Arg
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Arg	Gln	Ser	L <b>y</b> s 420	Val	Lys	Ala	Asp	His 425	Ala	Ser	Val	Thr	Gly 430	Gln	Ser	
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Leu 145	Ile	Asn	Thr	Ala	Val 150	Asn	Gly	Gly	Ser	Leu 155	Lys	Asp	Xaa	Leu	Glu 160
Ala	Asn	Ile	Leu	Ala 165	Ala	Leu	Val	Asn	Thr 170	Ala	His	Gly	Glu	Ala 175	Ala
Ser	Lys	Ile	L <b>y</b> s 180	Gln	Leu	Asp	Gln	His 185	Tyr	Ile	Val	His	L <b>y</b> s 190	Ile	Ala
His	Ala	Ile 195	Ala	Gly	Cys	Ala	Ala 200	Ala	Ala	Ala	Asn	Lys 205	Gly	Lys	Сув
Gln	Asp 210	Gly	Ala	Ile	Gly	Ala 215	Ala	Val	Gly	Glu	Ile 220	Val	Gly	Glu	Ala
Leu 225		Asn	Gly	Lys	Asn 230		Asp	Thr	Leu	Thr 235	Ala	Lys	Glu	Arg	Glu 240
	Ile	Leu	Ala	<b>Ty</b> r 245		Lys	Leu	Val	Ala 250		Thr	Val	Ser	Gly 255	
Val	Gly	Gly	Asp 260	Val	Asn	Ala	Ala	Ala 265		Ala	Ala	Glu	Val 270		Val
Lys	Asn	Asn 275		Leu	Ser	Asp	Xaa 280		Gly	Arg	Glu	Phe 285		Asn	Glu
Met	Thr 290		Cys	Ala	Lys	Gln 295		Xaa	Pro	Gln	Leu 300		Arg	Lys	Asn
Thr 305		Lys	Lys	Tyr	Gln 310		Val	Ala	Asp	Lys 315		Leu	Ala	Ala	Ser 320
_	Ala	Ile	Суз	Thr 325		Ile	Ser	Arg	Ser 330	515					
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		UHER EQUEN			LION	: Des	scriț	ptior	1 OI	Art	Incla	al Se	equer	nce:	DRF51a
Tyr 1	Lys	Leu	Leu	Ala 5	Ile	Gly	Ser	Val	Val 10	Gly	Ser	Ile	Leu	Gly 15	Val
Lys	Leu	Leu	Leu 20	Ile	Leu	Pro	Val	Ser 25	Trp	Leu	Leu	Leu	Leu 30	Met	Ala
Ile	Ile	Thr 35	Leu	Tyr	Tyr	Ser	Val 40	Asn	Gly	Ile	Leu	Asn 45	Val	Сув	Ala
Lys	Ala 50	Lys	Asn	Ile	Gln	Val 55	Val	Ala	Asn	Asn	Lys 60	Asn	Met	Val	Leu
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	Ile	Leu	Leu	Ile 85		Leu	Leu	Ser	Glu 90		Glu	Asn	Lys	Asn 95	
Ile	Ala	Lys	Ser 100	Ser	Asn	Leu	Cys	<b>Ty</b> r 105		Leu	Ala	Lys	Ile 110		Gln
Ile	Tyr	Met 115		Arg	Asp	Gln	<b>Ty</b> r 120		Leu	Leu	Asn	L <b>y</b> s 125		Glu	Tyr
		113					120					123			

## -continued

Gly Leu Ile Phe Leu Leu Ser Val Leu Ser Val Ile Gly Leu Tyr Val 130 135 140 Gly Ile Arg Leu Arg Thr Lys Ile Ser Pro Asn Phe Phe Lys Met Leu 150 145 155 160 Ile Phe Ile Val Leu Leu Val Leu Ala Leu Lys Ile Gly Tyr Ser Gly 165 170 Leu Ile Lys Leu 180 <210> SEQ ID NO 195 <211> LENGTH: 180 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: ORF82a <400> SEQUENCE: 195 Met Arg His Met Lys Asn Lys Asn Tyr Leu Leu Val Phe Ile Val Leu 1 5 10 15 His Ile Thr Leu Ile Val Ile Asn Ile Val Phe Gly Tyr Phe Val Phe 25 Leu Phe Asp Phe Phe Ala Phe Leu Phe Phe Ala Asn Val Phe Leu Ala 35 40 45 Val Asn Leu Leu Phe Leu Glu Lys Asn Ile Lys Asn Lys Leu Leu Phe 55 Leu Leu Pro Ile Ser Ile Ile Ile Trp Met Val Ile His Ile Ser Met 70 65 75 80 Ile Asn Ile Lys Phe Tyr Lys Phe Glu His Gln Ile Lys Glu Gln Asn 85 90 Ile Ser Ser Ile Thr Gly Val Ile Lys Pro His Asp Ser Tyr Asn Tyr 100 105 110 Val Tyr Asp Ser Asn Gly Tyr Ala Lys Leu Lys Asp Asn His Arg Tyr 115 120 Gly Arg Val Ile Arg Glu Thr Pro Tyr Ile Asp Val Val Ala Ser Asp 135 130 140 Val Lys Asn Lys Ser Ile Arg Leu Ser Leu Val Cys Gly Ile His Ser 150 155 145 160 Tyr Ala Pro Cys Ala Asn Phe Ile Lys Phe Ala Lys Lys Pro Val Lys 165 170 Ile Tyr Phe Tyr 180

1-17. (canceled)

**18**. An isolated polypeptide comprising a member selected from the group consisting of

(a) the amino acid sequence of SEQ ID NO: 4; and

(b) an immunogenic fragment of at least 15 contiguous amino acids of SEQ ID NO: 4, wherein the immunogenic fragment, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell meditated immune response that recognizes the isolated polypeptide SEQ ID NO: 4.

**19**. The isolated polypeptide of claim 18, wherein the polypeptide is according to (a).

**20**. The isolated polypeptide of claim 18, wherein the polypeptide is according to (b).

**21**. The isolated polypeptide of claim 18, wherein the immunogenic fragment of (b) comprises at least 20 contiguous amino acids of SEQ ID NO:4; wherein the immunogenic fragment, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell meditated immune response that recognizes the polypeptide SEQ ID NO: 4.

**22**. The isolated polypeptide of claim 18, wherein the isolated polypeptide consists of SEQ ID NO: 4.

**23**. A fusion protein comprising the isolated polypeptide of claim 18.

**24**. An immunogenic composition comprising the polypeptide of claim 18, and a pharmaceutically acceptable carrier.

**25**. The isolated polypeptide of claim 18, wherein the isolated polypeptide is a recombinant polypeptide.

26. The isolated polypeptide of claim 19, wherein the isolated polypeptide is a recombinant polypeptide.

27. The isolated polypeptide of claim 20, wherein the isolated polypeptide is a recombinant polypeptide.

**28**. An immunogenic composition comprising the isolated polypeptide of claim 19.

**29**. An immunogenic composition comprising the isolated polypeptide of claim 20.

**30**. A fusion protein comprising the isolated polypeptide of claim 19.

**31**. A fusion protein comprising the isolated polypeptide of claim 20.

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