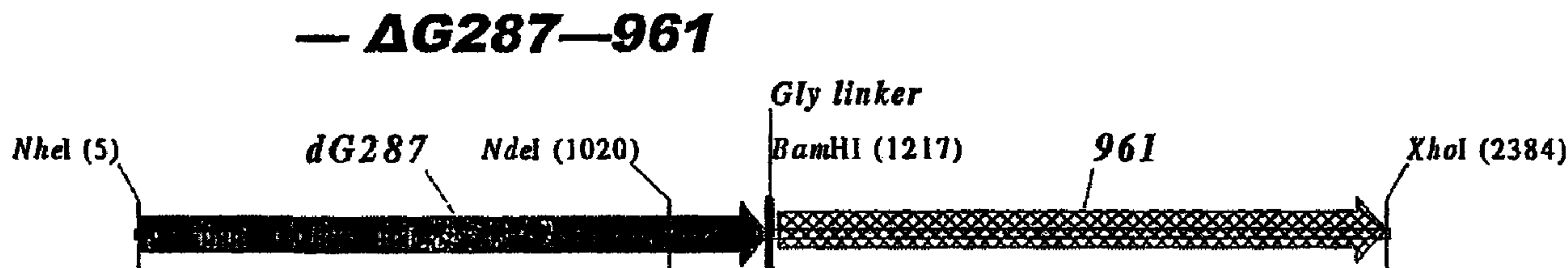




(22) Date de dépôt/Filing Date: 2001/02/28
 (41) Mise à la disp. pub./Open to Public Insp.: 2001/09/07
 (45) Date de délivrance/Issue Date: 2014/05/13
 (62) Demande originale/Original Application: 2 400 562
 (30) Priorités/Priorities: 2000/02/28 (GB0004695.3);
 2000/11/13 (GB0027675.8)

(51) Cl.Int./Int.Cl. *C07K 19/00* (2006.01),
C07K 14/22 (2006.01), *C12N 15/00* (2006.01),
C12N 15/62 (2006.01)
 (72) Inventeurs/Inventors:
 ARICO, MARIA BEATRICE, IT;
 COMANDUCCI, MAURIZIO, IT;
 GALEOTTI, CESIRA, IT;
 MASIGNANI, VEGA, IT;
 GIULIANI, MARZIA MONICA, IT;
 PIZZA, MARIAGRAZIA, IT
 (73) Propriétaire/Owner:
 CHIRON S.R.L., IT
 (74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : EXPRESSIONS HYBRIDES DE PROTEINES DE NEISSERIA
 (54) Title: HYBRID EXPRESSION OF NEISSERIAL PROTEINS



(57) Abrégé/Abstract:

A hybrid protein of formula NH₂-A-B-COOH, wherein A comprises the Neisserial protein ΔG287 and B comprises the Neisserial protein 961, and wherein the amino acid sequence of the hybrid protein is as disclosed in SEQ ID NO: 8 or is a sequence having greater than 70% sequence identity thereto.



ABSTRACT

A hybrid protein of formula $\text{NH}_2\text{-A-B-COOH}$, wherein A comprises the Neisserial protein ΔG287 and B comprises the Neisserial protein 961, and wherein the amino acid sequence of the hybrid protein is as disclosed in SEQ ID NO: 8 or is a sequence having greater than 70% sequence identity thereto.

-1-

HYBRID EXPRESSION OF NEISSERIAL PROTEINS

This application is a divisional application of co-pending application Serial No. 2,400,562, filed February 28, 2001.

TECHNICAL FIELD

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

BACKGROUND ART

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

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

DISCLOSURE OF THE INVENTION

In accordance with the invention, two or more (*e.g.* 3, 4, 5, 6 or more) proteins of the
invention are expressed as a single hybrid protein. It is preferred that no non-Neisserial
fusion partner (*e.g.* GST or poly-His) is used.

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

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

The method will typically involve the steps of: obtaining a first nucleic acid encoding a first
protein of the invention; obtaining a second nucleic acid encoding a second protein of the

-2-

invention; ligating the first and second nucleic acids. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

Where just two proteins are joined, the hybrid protein can be represented simply by the formula $\text{NH}_2\text{-A-B-COOH}$. A and B can each be selected from any Neisserial proteins, and in particular those represented by SEQ#s 1-4326. The method is well suited to the expression of proteins orf1, orf4, orf25, orf40, Orf46/46.1, orf83, 233, 287, 292L, 564, 687, 741, 907, 919, 953, 961 and 983.

The 42 hybrids indicated by 'X' in the following table of form $\text{NH}_2\text{-A-B-COOH}$ are preferred:

\downarrow A : B \rightarrow	ORF46.1	287	741	919	953	961	983
ORF46.1		X	X	X	X	X	X
287	X		X	X	X	X	X
741	X	X		X	X	X	X
919	X	X	X		X	X	X
953	X	X	X	X		X	X
961	X	X	X	X	X		X
983	X	X	X	X	X	X	

10 Preferred proteins to be expressed as hybrids are thus ORF46.1, 287, 741, 919, 953, 961 and 983. These may be used in their essentially full-length form, or poly-glycine deletions (ΔG) forms may be used (*e.g.* $\Delta\text{G-287}$, ΔGTbp2 , ΔG741 , ΔG983 *etc.*), or truncated forms may be used (*e.g.* $\Delta\text{1-287}$, $\Delta\text{2-287}$ *etc.*), or domain-deleted versions may be used (*e.g.* 287B, 287C, 287BC, ORF46₁₋₄₃₃, ORF46₄₃₃₋₆₀₈, ORF46, 961c *etc.*) and so on.

15 Particularly preferred are: (a) a hybrid protein comprising 919 and 287; (b) a hybrid protein comprising 953 and 287; (c) a hybrid protein comprising 287 and ORF46.1; (d) a hybrid protein comprising ORF1 and ORF46.1; (e) a hybrid protein comprising 919 and ORF46.1; (f) a hybrid protein comprising ORF46.1 and 919; (g) a hybrid protein comprising ORF46.1, 287 and 919; (h) a hybrid protein comprising 919 and 519; and (i) a hybrid protein
20 comprising ORF97 and 225.

Further embodiments are shown in the drawings and include $\Delta\text{G287-919}$, $\Delta\text{G287-953}$, $\Delta\text{G287-961}$, $\Delta\text{G983-ORF46.1}$, $\Delta\text{G983-741}$, $\Delta\text{G983-961}$, $\Delta\text{G983-961C}$, $\Delta\text{G741-961}$, $\Delta\text{G741-961C}$, $\Delta\text{G741-983}$, $\Delta\text{G741-ORF46.1}$, ORF46.1-741, ORF46.1-961, ORF46.1-961C,

-3-

961-ORF46.1, 961-741, 961-983, 961C-ORF46.1, 961C-741, 961C-983, 961CL-ORF46.1, 961CL-741, and 961CL-983.

Where 287 is used, it is preferably at the C-terminal end of a hybrid; if it is to be used at the N-terminus, it is preferred to use a ΔG form of 287 is used (*e.g.* as the N-terminus of a hybrid with ORF46.1, 919, 953 or 961).

Where 287 is used, this is preferably from strain 2996 or from strain 394/98.

Where 961 is used, this is preferably at the N-terminus. Domain forms of 961 may be used.

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

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

The fused proteins in the hybrid may be joined directly, or may be joined via a linker peptide *e.g.* via a poly-glycine linker (*i.e.* G_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more) or via a short peptide sequence which facilitates cloning. It is evidently preferred not to join a ΔG protein to the C-terminus of a poly-glycine linker.

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

Host

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

Vectors, hosts etc.

25 As well as the methods described above, the invention provides (a) nucleic acid and vectors useful in these methods (b) host cells containing said vectors (c) proteins expressed or expressible by the methods (d) compositions comprising these proteins, which may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions (e) these compositions for use as medicaments (*e.g.* as vaccines) or as diagnostic reagents (f)

the use of these compositions in the manufacture of (1) a medicament for treating or preventing infection due to Neisserial bacteria (2) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria, and/or (3) a reagent which can raise antibodies against Neisserial bacteria and (g) a method of treating a patient, comprising administering to the patient a therapeutically effective amount of these compositions.

Sequences

The invention also provides a protein or a nucleic acid having any of the sequences set out in the following examples. It also provides proteins and nucleic acid having sequence identity to these. As described above, the degree of 'sequence identity' is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more).

Nomenclature herein

The 2166 protein sequences disclosed in WO99/24578, WO99/36544 and WO99/57280 are referred to herein by the following SEQ# numbers:

Application	Protein sequences	SEQ# herein
WO99/24578	Even SEQ IDs 2-892	SEQ#s 1-446
WO99/36544	Even SEQ IDs 2-90	SEQ#s 447-491
WO99/57280	Even SEQ IDs 2-3020 Even SEQ IDs 3040-3114 SEQ IDs 3115-3241	SEQ#s 492-2001 SEQ#s 2002-2039 SEQ#s 2040-2166

In addition to this SEQ# numbering, the naming conventions used in WO99/24578, WO99/36544 and WO99/57280 are also used (e.g. 'ORF4', 'ORF40', 'ORF40-1' etc. as used in WO99/24578 and WO99/36544; 'm919', 'g919' and 'a919' etc. as used in WO99/57280).

The 2160 proteins NMB0001 to NMB2160 from Tettelin *et al.* [*Science* (2000) 287:1809-1815] are referred to herein as SEQ#s 2167-4326 [see also WO00/66791].

The term 'protein of the invention' as used herein refers to a protein comprising:

- (a) one of sequences SEQ#s 1-4326; or
- (b) a sequence having sequence identity to one of SEQ#s 1-4326; or
- (c) a fragment of one of SEQ#s 1-4326.

-5-

The degree of 'sequence identity' referred to in (b) is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more). This includes mutants and allelic variants [e.g. see WO00/66741]. Identity 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*. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence.

The 'fragment' referred to in (c) should comprise at least *n* consecutive amino acids from one of SEQ#s 1-4326 and, depending on the particular sequence, *n* is 7 or more (eg. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). Preferably the fragment comprises an epitope from one of SEQ#s 1-4326. Preferred fragments are those disclosed in WO00/71574 and WO01/04316.

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

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

It will be appreciated that references to "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) etc.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 to 26 show hybrid proteins according to the invention.

MODES FOR CARRYING OUT THE INVENTION

25 *Example 1 – hybrids of ORF46*

The complete ORF46 protein from *N.meningitidis* (serogroup B, strain 2996) has the following sequence:

1 LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
 51 FGSRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFSDHGHE
 101 VHSPFDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHPAD GYDGPQGGGY

30

-6-

5
10

```

151  PAPKGARDIY  SYDIKGVAQN  IRLNLTDNRS  TGQRLADRFH  NAGSMLTQGV
201  GDGFKRATRY  SPELDRSGNA  AEAFNGTADI  VKNIIGAAGE  IVGAGDAVQG
251  ISEGSNIAVM  HGLGLLSTEN  KMARINDLAD  MAQLKDYAAA  AIRDWAVQNP
301  NAAQGI EAVS  NIFMAALPIK  GIGAVRGKYG  LGGITAHPIK  RSQMGAIALP
351  KGKSAVSDNF  ADAAYAKYPS  PYHSRNIRSN  LEQRYGKENI  TSSTVPPSNG
401  KNVKLADQRH  PKTGVPFDGK  GFPNFEKHVK  YDTKLDIQEL  SGGGIPKAKP
451  VSDAKPRWEV  DRKLNKLTTR  EQVEKNVQEI  RNGNKNSNFS  QHAQLEREIN
501  KLKSADEINF  ADGMGKFTDS  MNDKAFSRLV  KSVKENGFTN  PVVEYVEING
551  KAYIVRGNNR  VFAAEYLGRI  HELKFKKVDF  PVPNTSWKNP  TDVLNESGNV
601  KRPRYRSK*

```

The leader peptide is underlined.

The sequences of ORF46 from other strains can be found in WO00/66741.

15 ORF46 has been fused at its C-terminus and N-terminus with 287, 919, and ORF1. The hybrid proteins were generally insoluble, but gave some good ELISA and bactericidal results (against the homologous 2996 strain):

Protein	ELISA	Bactericidal Ab
Orf1-Orf46.1-His	850	256
919-Orf46.1-His	12900	512
919-287-Orf46-His	n.d.	n.d.
Orf46.1-287His	150	8192
Orf46.1-919His	2800	2048
Orf46.1-287-919His	3200	16384

For comparison, 'triple' hybrids of ORF46.1, 287 (either as a GST fusion, or in Δ G287 form) and 919 were constructed and tested against various strains (including the homologous 2996 strain) *versus* a simple mixture of the three antigens. FCA was used as adjuvant:

	2996	BZ232	MC58	NGH38	F6124	BZ133
Mixture	8192	256	512	1024	>2048	>2048
ORF46.1-287-919his	16384	256	4096	8192	8192	8192
Δ G287-919-ORF46.1his	8192	64	4096	8192	8192	16384
Δ G287-ORF46.1-919his	4096	128	256	8192	512	1024

20 Again, the hybrids show equivalent or superior immunological activity.

Hybrids of two proteins (strain 2996) were compared to the individual proteins against various heterologous strains:

	1000	MC58	F6124 (MenA)
ORF46.1-His	<4	4096	<4
ORF1-His	8	256	128
ORF1-ORF46.1-His	1024	512	1024

Again, the hybrid shows equivalent or superior immunological activity.

-7-

Example 2 – hybrids of Δ G287

The deletion of the (Gly)₆ sequence in 287 was found to have a dramatic effect on protein expression. The protein lacking the N-terminal amino acids up to GGGGGG is called ' Δ G287'. In strain MC58, its basic sequence (leader peptide underlined) is:

```

5      SPDVKS ADTLSKPAAP VVSEKETEAK EDAPQAGSQ QGAPSAQGSQ DMAAVSEENT
      GNGGAVTADN PKNEDEVAQN DMPQNAAGTD SSTPNHTPDP NMLAGNMENQ ATDAGESSQP
      ANQPDMANAA DGMQGDPSA GGQNAAGNTAA QGANQAGNNQ AAGSSDPIPA SNPAPANGGS
10     NFGRVDLANG VLIDGPSQNI TLTHCKGDSC SGNNFLDEEV QLKSEFEKLS DADKISNYKK
      DGKNDKFVGL VADSVQMKGI NQYIIFYKPK PTSFARFRRS ARSRRSLPAE MPLIPVNQAD
      TLIVDGEAVS LTGHSGNIFA PEGNYRYLTY GAEKLPGGSY ALRVQGEPAK GEMLAGAAVY
      NGEVLHFHTE NGRPYPTRGR FAAKVDFGSK SVDGIIDSGD DLHMGTQKFK AAIDGNGFKG
      TWTENSGDV SGKFYGPAGE EVAGKYSYRP TDAERGGFGV FAGKKEQD*

```

15 Δ G287, with or without His-tag (' Δ G287-His' and ' Δ G287K', respectively), are expressed at very good levels in comparison with the '287-His' or '287^{untagged}'.

On the basis of gene variability data, variants of Δ G287-His were expressed in *E. coli* from a number of MenB strains, in particular from strains 2996, MC58, 1000, and BZ232. The results were also good – each of these gave high ELISA titres and also serum bactericidal
20 titres of >8192. Δ G287K, expressed from pET-24b, gave excellent titres in ELISA and the serum bactericidal assay.

Deletion of poly-Gly sequences is also applicable to Tbp2 (NMB0460), 741 (NMB 1870) and 983 (NMB1969). When cloned in pET vector and expressed in *E. coli* without the sequence coding for their leader peptides and without poly-Gly (*i.e.* as " Δ G forms"), the
25 same effect was seen – expression was good in the clones carrying the deletion of the poly-glycine stretch, and poor or absent if the glycines were present in the expressed protein.

Δ G287 was fused directly in-frame upstream of 919, 953, 961 (sequences shown below) and ORF46.1:

```

30      $\Delta$ G287–919
      ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCCGCTCCTGTTGTTGCTGAAAAAGAGACAGAG
      GTAAAAGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGGCAGCCAAGATATGGCGGCA
      GTTTCGGCAGAAAAATACAGGCAATGGCGGTGCGGCAACAACGGACAAACCCAAAAATGAAGACGAGGGACCGCAAAAT
      GATATGCCGCAAAATTCGCGCGAATCCGCAAAATCAAACAGGGAACAACCAACCCCGCGATTCTTCAGATTCCGCCCCC
      GCGTCAAACCCTGCACCTGCGAATGGCGGTAGCAATTTTGGGAAGGGTTGATTTGGCTAATGGCGTTTTTGATTGATGGG
35     CCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGTAATGGTGATAATTTATTGGATGAAGAAGCACCG
      TCAAATCAGAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAATTT
      ACTAATTTGGTTGCGACAGCAGTTCAGCTAATGGAAC TAACAAATATGTCATCATTATAAAGACAAGTCCGCTTCA
      TCTTCATCTGCGCGATTTCAGGCGTTCTGCACGGTCGAGGAGGTCGCTTCCTGCCGAGATGCCGCTAATCCCCGTCAAT
      CAGGCGGATACGCTGATTGTTCGATGGGGAAGCGGTGAGCCTGACGGGGCATTCCGGCAATATCTTCGCGCCCGAAGGG
40     AATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCCCGGCGGATCGTATGCCCTCCGTGTGCAAGGCGAACCGGCA
      AAAGGCGAAATGCTTGCTGGCACGGCCGTGTACAACGGCGAAGTGCTGCATTTTCATACGGAAAACGGCCGTCCGTAC
      CCGACTAGAGGCAGGTTTGCCGCAAAAGTTCGATTTCCGGCAGCAAATCTGTGGACGGCATTATCGACAGCGGCGATGAT
      TTGCATATGGGTACGCAAAATTCAAAGCCCGCATCGATGGAAACGGCTTTAAGGGGACTTGGACGGAAAATGGCGGC
      GGGGATGTTCCGGAAGGTTTTACGGCCCGGCGGCGAGGAAGTGGCGGGAAAATACAGCTATCGCCCCGACAGATGCG

```

GAAAAGGGCGGATTCGGCGTGTTCGCCGGCAAAAAAGAGCAGGATGGATCCGGAGGAGGAGGATGCCAAAGCAAGAGC
 ATCCAAACCTTTCGGCAACCCGACACATCCGTCATCAACGGCCCGGACCGGCCGGTCCGGCATCCCCGACCCCGCCGGA
 ACGACGGTCCGGCGGCGGGGCGGCTCTATACCGTGTACCGCACCTGTCCCTGCCCCACTGGGCGGCGCAGGATTC
 5 GCCAAAAGCCTGCAATCCTTCCGCCTCGGCTGCGCCAATTTGAAAAACCGCCAAGGCTGGCAGGATGTGTGCGCCAA
 GCCTTTCAAACCCCGTCCATTCCTTTCAGGCAAAACAGTTTTTTGAACGCTATTTACGCGCGTGGCAGGTTCAGGC
 AACGGAAGCCTTGCCTGACGGTACCGGCTATTTACGAGCCGGTGTGAAGGGCGACGACAGGCGGACGGCACAAGCC
 CGCTTCCCGATTTACGGTATTCGGCAGGATTTATCTCCGTCCCTGCTGCGGTTTTCGGGAGCGGAAAAGCCCTT
 GTCCGCATCAGGCAGACGGGAAAAACAGCGGCACAATCGACAATACCGGCGGCACACATACCGCCGACCTCTCCCGA
 10 TTCCCATCACCGCGCGCACAACGGCAATCAAAGGCAGGTTTTGAAGGAAGCCGCTTCCCTCCCTACCACACGCGCAAC
 CAAATCAACGGCGGCGCGCTTGACGGCAAAGCCCGATACTCGGTTACGCGAAGACCCCGTCGAACTTTTTTTTATG
 CACATCCAAGGCTCGGGCGGCTGTGAAAACCCCGTCCGGCAAATACATCCGCATCGGCTATGCCGACAAAACGAACAT
 CCTTACGTTTCCATCGGACGCTATATGGCGGACAAAGGCTACCTCAAGCTCGGGCAGACCTCGATGCAGGGCATCAA
 GCCTATATGCGGCAAAATCCGCAACGCTCGCCGAAGTTTTGGGTCAAACCCCGAGCTATATCTTTTCCGCGAGCTT
 15 GCCGGAAGCAGCAATGACGGTCCCGTCCGGCGCACTGGGCACGCCGTTGATGGGGGAATATGCCGGCGCAGTCCGACCG
 CACTACATTACCTTGGGCGCGCCCTTATTTGTGCGCCACCGCCCATCCGGTTACCCGCAAAGCCCTCAACCGCCTGATT
 ATGGCGCAGGATACCGGCAGCGGATTAAGGCGCGGTGCGCGTGGATTATTTTTGGGGATACGGCGACGAAGCCGGC
 GAACTTGCCGCAACAGAAAACACGGGTTACGTCTGGCAGCTCCTACCCAACGGTATGAAGCCCGAATACCGCCCG
 TAACTCGAG

20 1 MASPDVKSAD TLSKPAAPVV AEKETEVEKED APQAGSQGQG APSTQGSQDM
 51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNOQPADSS
 101 DSAPASNPAP ANGGSNFGRV DLANGVLIDG PSQNTLTHC KGDSCNGDNL
 151 LDEEAPSKSE FENLNESERI EKYKKGKSD KFTNLVATAV QANGTNKYVI
 201 IYKDKSASS SARFRRSARS RRSLPAEMPL IPVNQADTLI VDGEAVSLTG
 25 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKEM LAGTAVYNGE
 301 VLHFHTENGR PYPTRGRFAA KVDFGSKSVD GIIDSGDDLH MGTQKFKAAI
 351 DGNGFKGTWT ENGGDVSGR FYGPAGEVA GKYSYRPTDA ERGGFVVFAG
 401 KKEQDGSGGG GCQSKSIQTF PQPDTSVING PDRPVGIPDP AGTTVGGGGA
 451 VYTVVPHLSL PHWAAQDFAK SLQSFRLGCA NLKNRQGWQD VCAQAFQTFV
 30 501 HSFQAKQFFE RYFTPWQVAG NGSLAGTVTG YYEPVLKDD RRTAQARFPI
 551 YGIPDDFISV PLPAGLRSK ALVRIRQTK NSGTIDNTGG THTADLSRFP
 601 ITARTTAIKG RFEGRFLPY HTRNQINGGA LDGKAPILGY AEDPVELFFM
 651 HIQSGRLKT PSGKYIRIGY ADKNEHPYVS IGRYMADKGY LKLGQTSMQG
 701 IKAYMRQNPQ RLAEVLGQNP SYIFFRELQ SSNDGPVQAL GTPLMGEYAG
 35 751 AVDRHYITLG APLFVATAHP VTRKALNRLI MAQDTGSAIK GAVRVDYFWG
 801 YGDEAGELAG KOKTTGYVWQ LLPNGMKPEY RP*

AG287-953

40 ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCCGCTCCTGTTGTTGCTGAAAAAGAGACAGAG
 GTAAAAGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGCGCCATCCACACAAGGCAGCCAAGATATGGCGGCA
 GTTTCGGCAGAAAATACAGGCAATGGCGGTGCGGCAACAACGGACAAACCCAAAAATGAAGACGAGGGACCGCAAAAT
 GATATGCCGCAAAATTCGCGCAATCCGCAAAATCAAACAGGGAACAACCAACCCCGCGATTCCTCAGATTCGCCCCC
 45 GCGTCAAACCCCTGCACCTGCGAATGGCGGTAGCAATTTTGAAGGGTTGATTTGGCTAATGGCGTTTTGATTGATGGG
 CCGTCCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCCTTGTAAATGGTGATAATTTATTGGATGAAGAAGCACC
 TCAAATCAGAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAATTT
 ACTAATTTGGTTGCGACAGCAGTTCAAGCTAATGGAACATAAATATGTCATCATTTATAAAGACAAGTCCGCTTCA
 TCTTCATCTGCGCGATTTCAGGCGTTCTGCACGGTCGAGGAGGTCGCTTCCCTGCCGAGATGCCGCTAATCCCGCTCAAT
 50 CAGGCGGATACGCTGATGTGCGATGGGGAAGCGGTCAGCCTGACGGGGCATTCGGCAATATCTTCGCGCCCCGAAGGG
 AATTACCGGTATCTGACTTACGGGGCGGAAAAATGCCCCGGCGGATCGTATGCCCTCCGTGTGCAAGGCGAACCGGCA
 AAAGGCGAAATGCTTGTGCGCACGGCCGTGTACAACGGCGAAGTGTGCTGATTTTCATACGGAAAACGGCCGTCCGTAC
 CCGACTAGAGGCAGGTTTGGCCGAAAAGTCGATTTCCGGCAGCAAATCTGTGGACGGCATTTATCGACAGCGGCGATGAT
 TTGCATATGGGTACGCAAAAATCAAAGCCGCCATCGATGGAAACGGCTTTAAGGGGACTTGGACGGAAAATGGCGGC
 55 GGGGATGTTTCCGGAAGGTTTTACGGCCCGGCGGCGAGGAAGTGGCGGGAAAATACAGCTATCGCCCGACAGATGCG
 GAAAAGGGCGGATTCGGCGTGTTCGCCGGCAAAAAAGAGCAGGATGGATCCGGAGGAGGAGGCCACCTACAAAGTG
 GACGAATATCACGCCAACGCCCCGTTTCGCCATCGACCATTTCAACACCAGCACCAACGTCGGCGGTTTTTACGGTCTG
 ACCGGTTCCGTGAGTTCGACCAAGCAAACGCGACGGTAAATCGACATCACCATCCCGTTGCCAACCTGCAAAGC
 GGTTCGCAACACTTTACCGACCACCTGAAATCAGCCGACATCTTCGATGCCGCCAATATCCGGACATCCGCTTTGTT
 60 TCCACCAAATTCAACTTCAACGGCAAAAACTGGTTTTCCGTTGACGGCAACCTGACCATGCACGGCAAAAACCGCCCC
 GTCAAACTCAAAGCCGAAAATTCAACTGCTACCAAAGCCCGATGGCGAAAACCGAAGTTTTGCGGGCGGCGACTTCAGC
 ACCACCATCGACCGACCAAATGGGGCGTGGACTACCTCGTTAACGTTGGTATGACCAAAGCGTCCGCATCGACATC
 CAAATCGAGGCAGCCAAACAATAACTCGAG

65 1 MASPDVKSAD TLSKPAAPVV AEKETEVEKED APQAGSQGQG APSTQGSQDM
 51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNOQPADSS
 101 DSAPASNPAP ANGGSNFGRV DLANGVLIDG PSQNTLTHC KGDSCNGDNL
 151 LDEEAPSKSE FENLNESERI EKYKKGKSD KFTNLVATAV QANGTNKYVI

201 IYKDKSASS SARFRRSARS RRS LPAEMPL IPVNQADTLI VDGEAVSLTG
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE
 301 VLHFHTENGR PYPTRGRFAA KVDGFSKSD GIIDSGDDLH MGTQKFKAAL
 351 DGNGFKGTWT ENGGGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVVFAG
 5 401 KKEQDGS GGG GATYKVD EYH ANARFAIDHF NTSTNVGGFY GLTGSVEFDQ
 451 AKRDGKIDIT IPVANLQSGS QHFTDHLKSA DIFDAAQYPD IRFVSTKPNF
 501 NGKKLVSDG NLTMHGKTAP VKLKA EKFN C YQSPMAKTEV CGGDFSTTID
 551 RTKWGV DYL NVGMTKSVRI DIQIEAAKQ*

AG287-961

ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCCGCTCCTGTTGTTGCTGAAAAAGAGACAGAG
 GTAAAAGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGGCAGCCAAGATATGGCGGCA
 GTTTCGGCAGAAAATACAGGCAATGGCGGTGCGGCAACAACGGACAAACCCAAAATGAAGACGAGGGACCGCAAAT
 15 GATATGCCGCAAATTCGCCGAATCCGCAAATCAAACAGGGAACAACCAACCGCCGATTCTTCAGATTCCGCCCCC
 GCGTCAAACCCCTGCACCTGCGAATGGCGGTAGCAATTTTGGAAGGGTTGATTTGGCTAATGGCGTTTTGATTGATGGG
 CCGTCGCAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGTAATGGTGATAATTTATTGGATGAAGAAGCACCG
 TCAAATCAGAAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAAATTT
 ACTAATTTGGTTGCGACAGCAGTTCAAGCTAATGGAAC TAACAAATATGTCATCATTTATAAAGACAAGTCCGCTTCA
 20 TCTTCATCTGCGCGATTTCAGGCGTTCTGCACGGTTCGAGGAGGTCGCTTCCTGCCGAGATGCCGCTAATCCCCGTCAT
 CAGGCGGATACGCTGATTGTGCGATGGGGAAGCGGTTCAGCCTGACGGGGCATTCGGGCAATATCTTCGCGCCCGAAGGG
 AATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCCCGCGGATCGTATGCCCTCCGTGTGCAAGGCGAACCGGCA
 AAAGGCGAAATGCTTGCTGGCACGGCCGTGTACAACGGCGAAGTGCTGCATTTTCATACGGAACGGCCGTCGTTAC
 CCGACTAGAGGCAGGTTTGGCGCAAAGTTCGATTTCCGGCAGCAAATCTGTGGACGGCATTATCGACAGCGCGATGAT
 25 TTGCATATGGGTACGCAAATAATCAAAGCCGCCATCGATGGAAACGGCTTTAAGGGGACTTGGACGGAAAATGGCGGC
 GGGGATGTTTCCGGAAGGTTTACGGCCCGGCCGGCGAGGAAGTGCGGGGAAAATACAGCTATCGCCCGACAGATGCG
 GAAAAGGGCGGATTTCGGCGTGTTTGCCGCAAAAAGAGCAGGATGGATCCGGAGGAGGAGGCCACAAACGACGAC
 GATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCC TACAACAATGGCCAAGAAATCAACGGTTTTCAAAGCTGGA
 GAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGAC
 30 TTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAACGTCGATGCC
 AAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACT
 GATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAAATGGGAGAAAATATAACGACATTTGCTGAAGAGACTAAG
 ACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTGCACAAGCATGCCGAAGCATTCAACGAT
 ATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAATGAAGCCAACAGACGGCC
 35 GAAGAAACCAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCTGCCGCTGGC
 ACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAAC
 AAAGATAATATTTGCTAAAAAGCAAACAGTGGCGACGTGTACACCAGAGAAGAGTCTGACAGCAAATTTGTCAGAATT
 GATGGTCTGAACGCTACTACCGAAAAATGGACACACGCTTTGGCTTCTGCTGAAAAATCCATTGCCGATCAGGATACT
 CGCCTGAACGGTTTTGGATAAAACAGTGTGACACCTGCCCAAAGAAACCCGCCAAGGCCTTGCAGAACAAGCCGCGCTC
 40 TCCGGTCTGTTCCAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCCGGCGGCTACAAATCCGAATCCGGCA
 GTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGGCGTGGCAGTCCGGCACTTCGTCCGGT
 TCTTCCGACGCTACCATGTGCGGCGTCAATTACGAGTGGTAACTCGAG

1 MASPDVKSAD T LSKPAAPVV AEKETEVED APQAGSQGQ APSTQGSQDM
 45 51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNQPADSS
 101 DSAPASNAP ANGGSNFGRV DLANGVLIDG PSQNTLTHC KGDSNGDNL
 151 LDEEAPSKSE FENLNERI EKYKDKGSD KFTNLVATV QANGTNKYVI
 201 IYKDKSASS SARFRRSARS RRS LPAEMPL IPVNQADTLI VDGEAVSLTG
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE
 50 301 VLHFHTENGR PYPTRGRFAA KVDGFSKSD GIIDSGDDLH MGTQKFKAAL
 351 DGNGFKGTWT ENGGGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVVFAG
 401 KKEQDGS GGG GATNDDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE
 451 DGTITKDAT AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDKVKAAE
 501 SEIEKLTKL ADTDAALADT DAALDATNA LNKLGENTT FAEETRTNIV
 55 551 KIDEKLEAVA DTVDKHAFAF NDIADSLDET NTKADEAVKT ANEAKQTAE
 601 TKQNVDAKVK AAETAAGKAE AAAGTANTAA DKAEVAKV TDIKADIATN
 651 KDNIAKKANS ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH
 701 DTRLNGLDKT VSDLRKETRO GLAEQAALSG LFQYVNGRF NVTAAVGGYK
 751 SESAVAIGTG FRFTENFAAK AGVAVGTSSG SSAAYHVGVN YEW*

-10-

	ELISA	Bactericidal
Δ G287-953-His	3834	65536
Δ G287-961-His	108627	65536

The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens (using 287-GST) for 919 and ORF46.1:

	Mixture with 287	Hybrid with Δ G287
919	32000	128000
ORF46.1	128	16000

5 Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained:

Strain	919		ORF46.1	
	Mixture	Hybrid	Mixture	Hybrid
NGH38	1024	32000	-	16384
MC58	512	8192	-	512
BZ232	512	512	-	-
MenA (F6124)	512	32000	-	8192
MenC (C11)	>2048	>2048	-	-
MenC (BZ133)	>4096	64000	-	8192

The hybrid proteins with Δ G287 at the N-terminus are therefore immunologically superior to simple mixtures, with Δ G287-ORF46.1 being particularly effective, even against heterologous strains. Δ G287-ORF46.1K may be expressed in pET-24b.

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

10

 Δ G287NZ-919

15

20

25

ATGGCTAGCCCCGATGTCAAGTCGGCCGACACGCTGTCAAAACCTGCCGCCCTGTTGTTTCTGAAAAGAGACAGAG
 GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGCGCCATCCGCACAAGGCGGTCAAGATATGGCGGCG
 GTTTCGGAAGAAAATACAGGCAATGGCGGTGCGGCAGCAACGGACAAACCCAAAATGAAGACGAGGGGGCGCAAAT
 GATATGCCGCAAATGCCGCCGATACAGATAGTTTGACACCGAATCACACCCCGGCTTCGAATATGCCGGCCGGAAAT
 ATGGAACCAAGCACCGGATGCCGGGAATCGGAGCAGCCGGCAAACCAACCGGATATGGCAAATACGGCCGACGGA
 ATGCAGGGTGACGATCCGTCGGCAGGCGGGGAAAATGCCGGCAATACGGCTGCCCAAGGTACAAATCAAGCCGAAAAC
 AATCAAACCGCCGGTTCTCAAAATCCTGCCCTCTCAACCAATCCTAGCGCCACGAATAGCGGTGGTGATTTTGAAGG
 ACGAACGTGGGCAATTTCTGTTGTGATTGACGGGCCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTTCTTGT
 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGCTAAAATCAGAATTTGAAAATTAAGTGATGCAGACAAAATAAGT
 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTTGTGCGTTTGGTTGCCGATAGTGTGCAGATGAAG
 GGAATCAATCAATATATATCTTTTATAAACCTAAACCCACTTCATTTGCGCGATTAGGCGTTCTGCACGGTCCGAGG
 CGGTCGCTTCCGGCCGAGATGCCGCTGATTCGCCGTCATCAGGCGGATACGCTGATTGTCGATGGGGAAGCGGTCAGC
 CTGACGGGGCATTTCCGGCAATATCTTCGCGCCCGAAGGGAATFACCGGTATCTGACTTACGGGGCGGAAAATTTGCC
 GCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAGGCGAAATGCTCGCGGGCACGGCAGTGTACAACGGC
 GAAGTGCTGCATTTTTCATACGGAAAACGGCCGTCCGTCCCCGTCCAGAGGCAGGTTTGGCCGAAAAGTCGATTTCCGGC
 AGCAAATCTGTGGACGGCATTATCGACAGCGGCGATGGTTTGCATATGGGTACGCAAAAATTCAAAGCCGCCATCGAT
 GGAAACGGCTTTAAGGGGACTTGGACGGAAAATGGCGGCGGGATGTTTCCGGAAAGTTTACGGCCCGCCGGCGAG

GAAGTGGCGGGAAAATACAGCTATCGCCCAACAGATGCGGAAAAGGGCGGATTCGGCGTGTGTTGCCGGCAAAAAGAG
 CAGGATGGATCCGGAGGAGGAGGATGCCAAAGCAAGAGCATCCAAACCTTTCCGCAACCCGACACATCCGTCAAC
 GGCCCGGACCGGCCGGTCCGGCATCCCCGACCCCGCCGGAACGACGGTCCGGCGGGCGGGGCGCTATACCGTTGTA
 5 CCGCACCTGTCCCTGCCCACTGGGCGGGCAGGATTCGCCAAAAGCCTGCAATCCTTCGGCCTCGGCTCGGCCAAT
 TTGAAAACCGCCAAGGCTGGCAGGATGTGTGCGCCCAAGCCTTTCAAACCCCGTCCATTCCTTTCAGGCCAAAACAG
 TTTTGTGAACGCTATTTACGCGGTGGCAGGTTGCAGGCAACGGAAGCCTTGCCGGTACGGTTACCGGCTATTACGAG
 CCGGTGCTGAAGGGCGACGACAGGCGGACGGCACAAGCCCGCTTCCCGATTACGGTATTCGCGACGATTTATCTCC
 GTCCCCCTGCCTGCCGGTTGCGGGAGCGGAAAAGCCCTTGTCCGCATCAGGCAGACGGGAAAAACAGCGGCACAATC
 10 GACAATACCGGCGGCACACATACCGCCGACCTCTCCCGATTCCCCATCACCAGCGCGCACAAACGGCAATCAAAGGCAGG
 TTTGAAGGAAGCCGCTTCCCTCCCTACCACACGCGCAACCAAATCAAACGGCGGGCGCGCTTGACGGCAAAGCCCCGATA
 CTCGGTTACGCCGAAGACCCCGTCAACTTTTTTTTATGCACATCCAAGGCTCGGGCCGTCTGAAAACCCCGTCCGGC
 AAATACATCCGCATCGGCTATGCCGACAAAACGAACATCCCTACGTTTCCATCGGACGCTATATGGCGGACAAAGGC
 TACCTCAAGCTCGGGCAGACCTCGATGCAGGGCATCAAAGCCTATATGCGGCAAAAATCCGCAACGCTCGCCGAAGTT
 15 TTGGGTCAAACCCAGCTATATCTTTTTTCCGCGAGCTTGCCGGAAGCAGCAATGACGGTCCCGTCCGGCGCACTGGGC
 ACGCCGTTGATGGGGGAATATGCCGGCGCAGTCGACCGGCACTACATTACCTTGGGCGCGCCCTTATTTGTCCGCCACC
 GCCCATCCGGTTACCCGCAAAGCCCTCAACCGCCTGATTATGGCGCAGGATACCGGCAGCGCGATTAAAGGCGCGGTG
 CGCGTGGATTATTTTGGGGATACGGCGACGAAGCCGGCGAACTTGCCGGCAAACAGAAAACACGGGTTACGTCTGG
 CAGCTCCTACCCAACGGTATGAAGCCCGAATACCGCCCGTAAAAGCTT

- 20 1 MASPDVKSAD TLSKPAAPVV SEKETEAKED APQAGSQGG APSAQGGQDM
- 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
- 101 PAGNMENQAP DAGESEOPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
- 151 TNQAENQTA GSQNPASSTN PSATNSGGDF GRTNVGNSVV IDGPSQNTL
- 201 THCKGDS CSG NNFLDEEVQL KSEFEKLSDA DKISNYKKG KNDGKNDKRV
- 25 251 GLVADSVQMK GINQYIFYK PKPTSFARFR RSARSRRSLP AEMPLIPVNO
- 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGE
- 351 SKGEMLAGTA VYNGEVLHFH TENGRPSPSR GRFAAKVDFG SKSVDGLIDS
- 401 GDGLHMGTOK FKAIDGNF KGTWTENGGG DVSCKFYGPA GEEVAGKYSY
- 451 RPTDAEKGGF GVFAKKEQD GSGGGCQSK SIQTFPQPT SVINGPDRPV
- 30 501 GIPDPAGTVV GGGGAVYTVV PHLSLPHWAA QDFAKSLQSF RLGCANLKNR
- 551 QGWQDVCAQA FQTPVHSFQA KOFFERYFTP WQVAGNGSLA GTVTGYEYEV
- 601 LKGDDRRTAQ ARFPYIGIPD DFISVPLPAG LRSKALVRI RQTGKNSGTI
- 651 DNTGGTHTAD LSRFPITART TAIKGRFEGS RFLPYHTRNO INGGALDGA
- 701 PILGYAEDPV ELFFMHIQGS GRLKTPSGKY IRIGYADKNE HPYVSIGRYM
- 35 751 ADKGYLKLQ TSMQGIKAYM RQNPORLAEV LGQNPYIFF RELAGSSNDG
- 801 PVGALGTPLM GEYAGAVDRH YITLGAPLFV ATAHVTRKA LNRLIMAQDT
- 851 GSAIKGAVRV DYFWGYGDEA GELAGKQKTT GYVWQLLPNG MKPEYRP*

AG287NZ-953

ATGGCTAGCCCCGATGTCAAGTCGGCGGACACGCTGTCAAACCTGCCGCCCTGTTGTTTCTGAAAAGAGACAGAG
 GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGCGCCATCCGCACAAGGCGGTCAAGATATGGCGGGC
 GTTTCGGAAAGAAAATACAGGCAATGGCGGTGCGGCAGCAACGGACAAACCCAAAATGAAGACGAGGGGGCGCAAAT
 45 GATATGCCGCAAAAATGCCGCCGATACAGATAGTTTGACACCGAATCACACCCCGGCTTCGAATATGCCGGCCGGAAAT
 ATGGAAAACCAAGCACCGGATGCCGGGAATCGGAGCAGCCGGCAAACCAACCGGATATGGCAAATACGGCGGACGGA
 ATGCAGGGTGACGATCCGTCCGCAGGCGGGGAAAATGCCGGCAATACGGCTGCCCAAGGTACAAATCAAGCCGAAAAC
 AATCAAACCGCCGGTTCTCAAATCCTGCCTCTTCAACCAATCCTAGCGCCACGAATAGCGGTGGTGATTTTGGAAAG
 ACGAACGTGGGCAATTTCTGTTGTGATTTGACGGGCGTCCGAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGT
 50 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGCTAAAATCAGAATTTGAAAATTAAGTGATGCAGACAAAATAAGT
 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTTGTCCGTTTGGTTGCCGATAGTGTGCAGATGAAG
 GGAATCAATCAATATATTATCTTTTATAAACCTAAACCCACTTCATTTGCGCGATTTAGGCGTTCTGCACGGTCCGAGG
 CGGTCCGTTCCGGCCGAGATGCCGCTGATTCCCGTCAATCAGGCGGATACGCTGATTGTTCGATGGGGAAGCGGTCCAGC
 CTGACGGGGCATTCCGGCAATATCTTCGCGCCCGAAGGGAATTACCGGTATCTGACTTACGGGGCGGAAAATTTGCC
 55 GCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAGGCGAAATGCTCGCGGGCACGGCAGTGTACAACGGC
 GAAGTGTGCATTTTCATACGGAAAACGGCCGTCCGTCCCGTCCAGAGGCAGGTTTGCCGCAAAAGTGCATTTCCGGC
 AGCAAATCTGTGGACGGCATTATCGACAGCGGGCATGGTTTGCATATGGGTACGCAAAAATTCAAAGCCGCCATCGAT
 GGAAACGGCTTTAAGGGGACTTTGGACGGAAAATGGCGGGCGGGATGTTTCCGGAAAGTTTACGGCCCGCCGGCGAG
 GAAGTGGCGGGAAAATACAGCTATCGCCCAACAGATGCGGAAAAGGGCGGATTCGGCGTGTGTTGCCGGCAAAAAGAG
 CAGGATGGATCCGGAGGAGGAGGACCCTACAAAGTGGACGAATATCACGCCAACGCCGTTTCCGCCATCGACCAT
 60 TTCAACACCAGCACCAACGTCGGCGGTTTTTACGGTCTGACCGGTTCCGTCGAGTTCGACCAAGCAAACCGCGACGGT
 AAAATCGACATCACCATCCCCGTTGCCAACCTGCAAAGCGGTTCCGCAACACTTTACCGACCACCTGAAATCAGCCGAC
 ATCTTCGATGCCGCCAATATCCGGACATCCGCTTTGTTTCCACCAAATTCACCTTCAACGGCAAAAACCTGGTTTCC
 GTTGACGGCAACCTGACCATGCACGGCAAACCGCCCGTCAAACCTCAAAGCCGAAAATTCACCTGCTACCAAAGC
 65 CCGATGGCGAAAACCGAAGTTTGGCGGGCGGACTTCAGCACCACCATCGACCGCACCAAATGGGGCGTGGACTACCTC
 GTTAACGTTGGTATGACCAAAGCGTCCGCATCGACATCAAATCGAGGCAGCCAAACAATAAAAGCTT

1 MASPDVKSAD TLSKPAAPVV SEKETEAKED APQAGSQGG APSAQGGQDM

51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNOTA GSONPASSTN PSATNSGGDF GRTNVGNSVV IDGPSQNIL
 201 THCKGDSCSG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKRV
 5 251 GLVADSVQMK GINQYIIFYK PKPTSFAFR RSARSRRSLP AEMPLIPVNO
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGE
 351 SKGEMLAGTA VYNGEVLHFH TENGRPSPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTOK FKAIDGNF KGTWTFENGGG DVSGKFGPA GEEVAGKYSY
 451 RPTDAEKGGF GVFAKKEQD GSGGGGATYK VDEYHANARF AIDHFNTSTN
 10 501 VGGFYGLTGS VEFDAQKRDG KIDITIPVAN LQSGSQHFTD HLKSADIFDA
 551 AQYPIRIFVS TKFNFNKLL VSVDGNLTMH GKTAPVKLKA EKFNQYQSPM
 601 AKTEVCGGDF STTIDRTKWG VDYLNVNGMT KSVRIDIQIE AAKQ*

AG287NZ-961

15 ATGGCTAGCCCCGATGTCAAGTCGGCGGACACGCTGTCAAACCTGCCGCCCTGTTGTTTCTGAAAAAGACACAGAG
 GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCGCACAAGGCGGTCAAGATATGCGCGCG
 GTTTCGGAAGAAAATACAGGCAATGGCGGTGCGGCAGCAACGGACAAACCCAAAATGAAGACGAGGGGGCGCAAAT
 GATATGCCGCAAATGCCGCCGATACAGATAGTTTGACACCGAATCACACCCCGCTTCGAATATGCCGGCCGAAAT
 ATGGAACCAAGCACCGGATGCCGGGAAATCGGAGCAGCCGGCAAACCAACCGGATATGGCAAATACGGCGGACGGA
 20 ATGCAGGGTGACGATCCGTCGGCAGGCGGGGAAATGCCGGCAATACGGCTGCCAAGGTACAAATCAAGCCGAAAC
 AATCAAACCGCCGTTCTCAAATCCTGCCTCTTCAACCAATCCTAGCGCCACGAATAGCGGTGGTGATTTTGGAAGG
 ACGAACGTGGGCAATCTGTGTGATGACGGGCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATCTTGT
 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGCTAAAATCAGAATTTGAAAAATTAAGTATGCAGACAAAATAAGT
 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTTGTCGGTTTGGTTGCCGATAGTGTGCAGATGAAG
 25 GGAATCAATCAATATATTATCTTTTATAAACCTAAACCCACTTCATTTGCGCGATTTAGGCGTTCTGCACGGTCGAGG
 CGGTCCGTTCCGGCCGAGATGCCGCTGATCCCCTCAATCAGGCGGATACGCTGATGTCGATGGGGAAGCGGTCAGC
 CTGACGGGGCATTCGGCAATATCTTCGCGCCCGAAGGGAATTACCGGTATCTGACTTACGGGGCGGAAAAATGCC
 GCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAGGCGAAATGCTCGCGGGCACGGCAGTGTACAACGGC
 GAAGTGTGATTTTCATACGGAAACGGCCGTCCTCCGTCAGAGGCGAGTTTGCCGCAAAGTCGATTTCCGGC
 30 AGCAAATCTGTGGACGGCATTATCGACAGCGCGATGGTTTGCAATATGGGTACGCAAAAATCAAAGCCGCCATCGAT
 GGAAACGGCTTTAAGGGGACTTTGGACGGAAATGGCGGGGGATGTTTCCGAAAGTTTACGGCCCGCCGGCGAG
 GAAGTGGCGGGAAATACAGCTATCGCCCAACAGATGCGGAAAGGGCGGATTCGGCGTGTGTTGCCGGCAAAGAG
 CAGGATGGATCCGGAGGAGGAGGCCACAAACGACGAGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCC
 TACAACAATGGCCAAGAAATCAACGGTTTCAAAGCTGGAGAGACCATCTACGACATTTGATGAAGACGGCACAATTACC
 35 AAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTG
 ACCAAAACCGTCAATGAAAACAAACAAACGTCGATGCCAAAGTAAAGCTGCAGAATCTGAAATAGAAAAGTTAACA
 ACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAA
 TTGGGAGAAAATATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTAAAAATGATGAAAATTAGAAGCCGTG
 40 GCTGATACCGTCGACAAGCATGCCGAAGCATTCAACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGAC
 GAAGCCGTCAAACCGCCAATGAAGCCAAACAGACGGCCGAAGAAACCAAACAAACGTCGATGCCAAAGTAAAGCT
 GCAGAACTGCAGCAGGCAAAGCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGCCGAAGCTGTGCT
 GCAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATGCTAAAAAGCAAACAGTCCCGACGTG
 TACACCAGAGAAGAGTCTGACAGCAAATTTGTCAGAATGATGGTCTGAACGCTACTACCGAAAAATGGACACACGC
 TTGGCTTCTGCTGAAAAATCCATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCC
 45 AAAGAAACCGCCAAGGCTTGCAGAACAAGCCGCGCTCTCCGGTCTGTTCACCTTACAACGTGGGTCCGTTCAAT
 GTAACGGCTGCAGTCGGCGCTACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACCTT
 GCCGCCAAAGCAGGCGTGGCAGTCGGCACTTCGTCCGGTCTTCCGCGAGCCTACCATGTCCGGCTCAATTACGAGTGG
 TAAAAGCTT

50 1 MASPDVKSAD TSKPAAPVV SEKETEAKED APQAGSQGG APSAQQGQDM
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNOTA GSONPASSTN PSATNSGGDF GRTNVGNSVV IDGPSQNIL
 201 THCKGDSCSG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKRV
 55 251 GLVADSVQMK GINQYIIFYK PKPTSFAFR RSARSRRSLP AEMPLIPVNO
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGE
 351 SKGEMLAGTA VYNGEVLHFH TENGRPSPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTOK FKAIDGNF KGTWTFENGGG DVSGKFGPA GEEVAGKYSY
 451 RPTDAEKGGF GVFAKKEQD GSGGGGATND DDVKKAAATVA IAAAYNNGQE
 60 501 INGFKAGETI YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTLNTRTV
 551 NENKQNVDAK VKAARSEIEK LTTKLADTDA ALADTDAALD ATTNALNKLK
 601 ENITTFAEET KTNIVKIDEK LEAVADTVDK HAEAFNDLAD SLDETNTKAD
 651 EAVKTANEAK QTAETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA
 701 VAAKVTDIKA DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK
 65 751 LDTRLASAER SIADHDTRLN GLDKTVSDLR KETROGLAEQ AALSGLFQPY
 801 NVGRFNVTA VGGYKSESAV AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY
 851 HVGVMYEW*

-13-

Example 3 – hybrids of Δ G983

Protein 983 has the following sequence:

	983		Δ G983
5	1	<u>MRTTPTFPTK TFKPTAMALA VATTLSACLG</u>	GGGGTSAPD FNAGGTGIGS
	51	NSRATTAKSA AVSYAGIKNE MCKDRSMLCA	GRDDVAVTDR DAKINAPPPN
	101	LHTGDFPNPN DAYKNLINLK PAIEAGYTGR	GVEVGIVDTG ESVGSISFPE
	151	LYGRKEHGYN ENYKNYTAYM RKEAPEDGGG	KDIEASFDDE AVIETEAKPT
	201	DIRHVKEIGH IDLVSHIIGG RSV DGRPAGG	IAPDATLHIM NTNDETKNEM
10	251	MVAAIRNAWV KLG ERGVRIV NNSFGTTSRA	GTADLFQIAN SEEQYRQALL
	301	DYSGGDKTDE GIRLMQOSDY GNLSYHIRNK	NMLFIFSTGN DAQAQPNTYA
	351	LLPFYEKDAQ KGIITVAGVD RSGEKFKREM	YGEPGTEPLE YGSNHCGITA
	401	MWCLSAPYEA SVRFTRTNPI QIAGTSFSAP	IVTGTAALLL QKYPWMSNDN
	451	LRTTLLTTAQ DIGAVGVDSK FGWGLLDAGK	AMNGPASFPF GDFTADTKGT
15	501	SDIAYSFRND ISGTGGLIKK GGSQQLHGN	NTYTGKTIIE GGSLVLYGNN
	551	KSDMRVETKG ALIYNGAASG GSLNSDGIVY	LADTDQSGAN ETVHIKGSLO
	601	LDGKGTLYTR LGKLLKVDGT AIIGGKLYMS	ARGKGAGYLN STGRRVPFLS
	651	AAKIGQDYSF FTNIETDGG LSLDSVEKT	AGSEGD TLSY YVRRGNAART
	701	ASAAAHSAPA GLKHAVEQGG SNLENLMVEL	DASESSATPE TVETAADRT
20	751	DMPGIRPYGA TFRAAAVQH ANAADGVRIF	NSLAATVYAD STAAHADMOG
	801	RRLKAVSDGL DHNGTGLRVI AQTQODGGTW	EQGGVEGKMR GSTQTVGLAA
	851	KTGENTTAAA TLGMGRSTWS ENSANAKTDS	ISL FAGIRHD AGDIGYLGKL
	901	FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ	LGALGGVNPV FAATGDLTVE
	951	GGLRYDLLKQ DAF AEKGSAL GWSGNSLTEG	TLVGLAGLKL SQPLSDKAVL
25	1001	FATAGVERDL NGRDYTVTGG FTGATAATGK	TGARNMPHTR LVAGLGADVE
	1051	FGNGWNLAR YSYAGSKQYG NHSGRVGVGY	RF*

 Δ G983 thus has the following basic sequence:

				TSAPD FNAGGTGIGS
30		NSRATTAKSA AVSYAGIKNE MCKDRSMLCA	GRDDVAVTDR DAKINAPPPN	
		LHTGDFPNPN DAYKNLINLK PAIEAGYTGR	GVEVGIVDTG ESVGSISFPE	
		LYGRKEHGYN ENYKNYTAYM RKEAPEDGGG	KDIEASFDDE AVIETEAKPT	
		DIRHVKEIGH IDLVSHIIGG RSV DGRPAGG	IAPDATLHIM NTNDETKNEM	
		MVAAIRNAWV KLG ERGVRIV NNSFGTTSRA	GTADLFQIAN SEEQYRQALL	
35		DYSGGDKTDE GIRLMQOSDY GNLSYHIRNK	NMLFIFSTGN DAQAQPNTYA	
		LLPFYEKDAQ KGIITVAGVD RSGEKFKREM	YGEPGTEPLE YGSNHCGITA	
		MWCLSAPYEA SVRFTRTNPI QIAGTSFSAP	IVTGTAALLL QKYPWMSNDN	
		LRTTLLTTAQ DIGAVGVDSK FGWGLLDAGK	AMNGPASFPF GDFTADTKGT	
		SDIAYSFRND ISGTGGLIKK GGSQQLHGN	NTYTGKTIIE GGSLVLYGNN	
40		KSDMRVETKG ALIYNGAASG GSLNSDGIVY	LADTDQSGAN ETVHIKGSLO	
		LDGKGTLYTR LGKLLKVDGT AIIGGKLYMS	ARGKGAGYLN STGRRVPFLS	
		AAKIGQDYSF FTNIETDGG LSLDSVEKT	AGSEGD TLSY YVRRGNAART	
		ASAAAHSAPA GLKHAVEQGG SNLENLMVEL	DASESSATPE TVETAADRT	
		DMPGIRPYGA TFRAAAVQH ANAADGVRIF	NSLAATVYAD STAAHADMOG	
45		RRLKAVSDGL DHNGTGLRVI AQTQODGGTW	EQGGVEGKMR GSTQTVGLAA	
		KTGENTTAAA TLGMGRSTWS ENSANAKTDS	ISL FAGIRHD AGDIGYLGKL	
		FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ	LGALGGVNPV FAATGDLTVE	
		GGLRYDLLKQ DAF AEKGSAL GWSGNSLTEG	TLVGLAGLKL SQPLSDKAVL	
		FATAGVERDL NGRDYTVTGG FTGATAATGK	TGARNMPHTR LVAGLGADVE	
50		FGNGWNLAR YSYAGSKQYG NHSGRVGVGY	RF*	

 Δ G983 was expressed as a hybrid, with ORF46.1, 741, 961 or 961c at its C-terminus:

Δ G983-ORF46.1

55	ATGACTTCTGCGCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
	GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTGCG
	GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTCCAAACCCAAATGACGCA
	TACAAGAATTTGATCAACCTCAAACCTGCAATTGAAGCAGGCATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
	ACAGGCGAATCCGTCCGCAGCATATCCTTTCCCGAAGTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
	AACTATACGGCGTATATGCGGAAGGAAGCGCCTGAAGACGGAGCGGTAAGACATTTGAAGCTTCTTTTCGACGATGAG
60	GCCGTTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAGAAATCGGACACATCGATTTGGTCTCCCAT

ATTATTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACG
AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGCGC
ATCGTCAATAACAGTTTTGGAACAACATCGAGGGCAGGCACCTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAG
5 TACCGCCAAGCGTTGCTCGACTATTCGGCGGTGATAAAACAGACGAGGGTATCCGCTGATGCAACAGAGCGATTAC
GGCAACCTGTCTACCACATCCGTAATAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCC
AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCAGGCGTAGACCAGCAGT
GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTCGCGAAT
ACTGCCATGTGGTGCCTGTCGGCACCCATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTCGCCGA
ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAAC
10 CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCCGGCTGGGGACTGCTG
GATGCGGGTAAGGCCATGAACGGACCCCGCTCCTTTCCGTTCCGGCGACTTACCGCCGATACGAAAGGTACATCCGAT
ATTGCCCTACTCCTTCCGTAACGACATTTTCAGGCACGGGCGGCTGATCAAAAAGGCGGCAGCCAACCTGCAACTGCAC
GGCAACAACACCTATACGGGCAAAACCATTTATCGAAGGCGGTTCCGCTGGTGTGTACGGCAACAACAATCGGATATG
CGCGTCGAAACCAAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTTGTCTAT
15 CTGGCAGATACCGACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACG
CTGTACACACGTTTGGGCAAACCTGCTGAAAGTGGACGGTACGGCGATTATCGGGCGCAAGCTGTACATGTCCGGCACGC
GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCGTGAGTGCCGCCAAAATCGGGCAGGATTAT
TCTTTCTTCAAAACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAA
GGCGACACGCTGTCTATTATGTCCGTCCGGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCGGCGCCCGCC
20 GGTCTGAAACACGCGTAGAACAGGGCGGCAGCAATCTGAAAACCTGATGGTGAAGTGGATGCCTCCGAATCATCC
GCAACACCCGAGACGGTTGAAACTGCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTC
CGCGCAGCGGCAGCGTACAGCATGCGAATGCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTAT
GCCGACAGTACCGCCGCCCATGCCGATATGCAGGGACGCCCTGAAAGCCGATCGGACGGGTGGACCACAACGGC
ACGGGTCTGCGGTCATCGCGCAAACCCAACAGGACGGTGAACGTGGGAACAGGGCGGTGTTGAAGGCAAATGCGC
25 GGCAGTACCCAAACCGTCGGCATTCGCGCAAACCCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGC
AGCACATGGAGCGAAAACAGTGCAAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCGGGC
GATATCGGCTATCTCAAAGGCCTGTTCTCCTACGGACGCTACAAAACAGCATCAGCCGACGACCGGTGCGGACGAA
CATGCGGAAGGCAGCGTCAACGGCAGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGGCGCAACG
GGAGATTTGACGGTGAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAGGCAGTGTCTTTG
30 GGCTGGAGCGGCAACAGCCTCACTGAAGGCAGCTGGTCCGACTCGCGGCTCTGAAGCTGTGCAACCCCTTGAGCGAT
AAAGCCGTCCTGTTTGAACGGCGGGCGTGAACCGGACCTGAACGGACGCGACTACACGGTAACGGCGGGCTTTACC
GGCGGACTGCAGCAACCGGCAAGACGGGGCAGCAATATGCCGCACACCCGCTGTTGGTTCGGGCTGGGCGCGGAT
GTCGAATTCGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGGTTCCAAACAGTACGGCAACCACAGCGGA
CGAGTCGGCGTAGGCTACCGGTTCCCTCGACGGTGGCGGAGGCACTGGATCCTCAGATTTGGCAAACGATTTCTTTTATC
35 CGGCAGGTTCTCGACCGTCAGCATTTGCAACCCGACGGGAAATACCACCTATTCGGCAGCAGGGGGAACTTGCCGAG
CGCAGCGCCATATCGGATTTGGGAAAATACAAAGCCATCAGTTGGGCAACCTGATGATTTCAACAGGCGGCCATTAAA
GGAAATATCGGCTACATTTGCTCCGCTTTTCCGATCACGGGCACGAAGTCCATTTCCCTTCGACAACCATGCCCTACAT
TCCGATTTCTGATGAAGCCGGTAGTCCCGTTGACGGATTTAGCCTTTACCGCATCCATTTGGGACGGATACGAACCCAT
40 CCCGCCGACGGCTATGACGGGCCACAGGGCGGCGGCTATCCCGCTCCCAAAGGCGCGAGGGATATATACAGCTACGAC
ATAAAAGGCGTTGCCAAAATATCCGCCTCAACCTGACCGACAACCGCAGCACCAGGACAACGGCTTGCCGACCGTTTC
CACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGGCAGGATTCAAACCGGCCACCCGATACAGCCCCGAGCTGGAC
AGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAACATCATCGGCGCGGCAGGAGAAATT
GTCGGCGCAGGCGATGCCGTGACGGCATAAGCGAAGGCTCAAACATTTGCTGTATGCACGGCTTGGGTCTGCTTTCC
45 ACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAACTCAAAGACTATGCCGCAGCAGCCATCCGC
GATTTGGGCAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTCAGCAATATCTTTATGGCAGCCATCCCCATC
AAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACGGCACATCTTATCAAGCGGTGCGAGATGGGC
GCGATCGCATTGCCGAAAGGAAATCCGCCGTGACGACAATTTTCCGATGCGGCATACGCCAAAATACCCGTCCCT
TACCATTCCCGAAAATATCCGTTCAAACCTGGAGCAGCGTTACGGCAAAGAAAACATCACCTCCTCAACCGTGCCGCCG
50 TCAAACGGCAAATAATGTCAAACTGGCAGACCAACGCCACCCGAAGACAGGCGTACCCTTTGACGGTAAAGGGTTCCG
AATTTTGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCACCACCCTGA

1 MTSAPDFNAG GTGIGSNSRA TTAKSAVSY AGIKNEMCKD RSMLCAGRDD
51 VAVTDRDAKI NAPPPNLHTG DFPNPNDAYK NLINLKPAIE AGYTGRGVEV
55 101 GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE
151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDLK SHIIGGRSVD GRPAGGLAPD
201 ATLHIMNTND ETKNEMMVAA IRNAWVKLGE RGVRIVNSNF GTTSRAGTAD
251 LFQIANSEEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRKNMLF
301 IFSTGNDAQA QPNTYALLPF YEKDAQKGI TVAGVDRSGE KFKREMYGEP
351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
60 401 TAALLLQKYP WMSNDNLRTT LLTTAQDIGA VGVDSKFGWG LLDAGKAMNG
451 PASFPFGDFT ADTKGTS DIA YSFRNDISGT GGLIKRGGSQ LQLHGNNTYT
501 GKTIEGGSL VLYGNKSDM RVETKGLIY NGAASGGS LN SDGIVYLADT
551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDGTAIIG GKLYMSARGK
601 GAGYLNSTGR RVPFLSAKI GQDYSFFTNI ETDGGLLASL DSVEKTAGSE
65 651 GDTLSYVRR GNAARTASAA AHSAPAGLKH AVEQGSNLE NLMVELDASE
701 SSATPETVET AADRDTMPG IRPYGATFRA AAVQHANA DGVRIFNLSA
751 ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG

5
10
15

801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL
 901 GGVNVPFAAT GDLTVEGGLR YDLLKQDAFA EKGSALGWSG NSLTEGTLVG
 951 LAGLKLSQPL SDKAVLFATA GVERDLNGRD YTVTGGFTGA TAATGKTGAR
 1001 NMPHTRLVAG LGADVEFGNG WNLARYSYA GSKQYGNHSG RVGVGYRFLD
 1051 GGGGTGSSDL ANDSFIRQVL DRQHFEPDGK YHLFGSRGEL AERSGHIGLG
 1101 KIQSHQLGNL MIQQAAIKGN IGYIVRFSDH GHEVHSPFDN HASHSDSDEA
 1151 GSPVDGFSLY RIHWDGYEHH PADGYDGPOG GGYPPAPKAR DIYSYDIKGV
 1201 AQNIRLNLTD NRSTGQRLAD RFHNAGSMLT QGVGDGFKRA TRYSPELDRS
 1251 GNAAEAFNGT ADIVKNIIGA AGEIVGAGDA VQGISEGSNI AVMHGLGLLS
 1301 TENKMARIND LADMAQLKDY AAAAIRDWAY QNPNAOQIE AVSNIFMAAI
 1351 PIKGIGAVRG KYGLGGITAH PIKRSQMGAI ALPKGKSAVS DNFADAAYAK
 1401 YPSPYHSRNI RSNLEQRYGK ENITSSTVPP SNGKNVKLAD QRHPKTGVPF
 1451 DGKGFPNFEK HVKYDTLEHH HHHH*

AG983-741

20
25
30
35
40
45
50
55
60
65

ATGACTTCTGCGCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
 GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTGCCG
 GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTCCAAACCCAAATGACGCA
 TACAAGAATTTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
 ACAGGCGAATCCGTCCGCAGCATATCCTTTCCCGAAGTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
 AACTATACGGCGTATATGCGGAAGGAAGCGCCTGAAGACGGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAG
 GCCGTTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAGAAAGAAATCGGACACATCGATTTGGTCTCCCAT
 ATTATTTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACG
 AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTTGGCGTGGCG
 ATCGTCAATAACAGTTTGGAAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAG
 TACCGCCAAGCGTTGCTCGACTATTTCCGGCGGTGATAAAACAGACGAGGGTATCCGCCCTGATGCAACAGAGCGATTAC
 GGCAACCTGTCTTACCACATCCGTAATAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCC
 AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCAGGCGTAGACCGCAGT
 GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTGCCGAATT
 ACTGCCATGTGGTGCCTGTCCGCACCCATATGAAGCAAGCGTCCGTTTCCACCCGTACAAACCCGATTCAAATTTGCCGGA
 ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAAATACCCGTGGATGAGCAACGACAAC
 CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCCGGCTGGGGACTGCTG
 GATGCCGGTAAGGCCATGAACGGACCCGCGTCTTTCCGTTCCGGCGACTTTTACCGCCGATACGAAAGGTACATCCGAT
 ATTTGCCTACTCCTTCCGTAACGACATTTTCCAGGCACGGCGGCCCTGATCAAAAAGGCGGCAGCCAACCTGCAACTGCAC
 GGCAACAACACCTATACGGGCAAACCAATTATCGAAGGCGGTTCCGCTGGTGTGTACGGCAACAACAATCCGGATATG
 CCGCTCGAAACCAAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTGTCTAT
 CTGGCAGATACCGACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACG
 CTGTACACACGTTTGGGCAAACCTGCTGAAAGTGGACGGTACGGCGATTATCCGGCGCAAGCTGTACATGTCCGCCACGC
 GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCGTGAGTGCCGCCAAAATCCGGCAGGATTAT
 TCTTTCTTCAACAACATCGAAACCGACGGCGGCCCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAA
 GGCGACACGCTGTCTTATGTCCGTCCGGCAATGCCGCACGGACTGCTTCCGCGAGCGGCACATTTCCGCGCCCGCC
 GGTCTGAAACACGCCGTAGAACAGGGCGGCAGCAATCTGGAAAACCTGATGGTCAACTGGATGCCTCCGAATCATCC
 GCAACACCCGAGACGGTTGAAACTGCCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTC
 CGCGCAGCGGCAGCCGTACAGCATGCCAATGCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTAT
 GCCGACAGTACCGCCGCCCATGCCGATATGCAGGGACGCCGCCCTGAAAGCCGTATCGGACGGGTTGGACCACAACGGC
 ACGGGTCTGCGCGTCATCGCGCAAACCCAAACAGGACGGTGGAAACGTGGGAACAGGGCGGTGTTGAAGGCAAATGCCG
 GGCAGTACCCAAACCGTCCGCATTGCCCGCAAACCCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGC
 AGCACATGGAGCGAAAACAGTGCAAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCCGGC
 GATATCCGCTATCTCAAAGCCCTGTTCTCCTACGGACGCTACAAAAACAGCATCAGCCGCAGCACCGGTGCCGGACGAA
 CATGCCGAAGGCAGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGCCTGCAACG
 GGAGATTTGACGGTCAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAGGCAGTGCCTTG
 GGCTGGAGCGGCAACAGCCTCACTGAAGGCACGCTGGTCCGACTCCGGGTCTGAAGCTGTGCAACCCCTTGAGCGAT
 AAAGCCGTCCTGTTGCAACGGCGGGCGTGGAAACCGACCTGAACGGACCGGACTACACGGTAACGGCGGGCTTTACC
 GGCGCGACTGCAGCAACCGGCAAGACGGGGGCAGCAATATGCCGCACACCCGCTGGTGGTGGCGCCGACATCGGTGCCGGGCTTGGC
 GTCGAATTCGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGTTTCCAAACAGTACGGCAACCACAGCGGA
 CGAGTCCGGCTAGGCTACCGGTTCCCTCGAGGGATCCGGAGGGGGTGGTGTGCGCCCGACATCGGTGCCGGGCTTGGC
 GATGCACTAACCGCACCGCTCGACCATAAAGACAAAGTTTTGCAGTCTTTGACGCTGGATCAGTCCGTCAGGAAAAC
 GAGAACTGAAGCTGGCGGCACAAGGTGCCGAAAAAATTTATGAAAACGGTGACAGCCTCAATACGGGCAAATTTGAAG
 AACGACAAGGTACGCCGTTTCGACTTTATCCGCCAAATCGAAGTGGACGGGCAGCTCATTACCTTGGAGAGTGGAGAG
 TTCCAAGTATACAAACAAGCCATTCCGCCCTTAACCGCCTTTTCAGACCGAGCAAATACAAGATTCGGAGCATTCGGG
 AAGATGGTTGCCGAAACGCCAGTTTCAAGATCCGGCAGATAGCGGGCGAACATACATCTTTTGGACAAGCTTCCCGAAGGC
 GGCAGGGCGACATATCGCGGGACGGCGTTCCGTTTCCAGACGATGCCGGCGGAAAACCTGACCTACACCATAGATTTCCGC
 GCCAAGCAGGGAAAACGGCAAATCGAACATTTGAAATCGCCAGAACTCAATGTGACCTGGCCGCCCGGATATCAAG
 CCGGATGGAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTACAACCAAGCCGAGAAAGGCAGTTACTCCCTCGGT

ATCTTTGGCGGAAAAGCCCAGGAAGTTGCCGGCAGCGCGGAAGTGAAAACCGTAAACGGCATAACGCCATATCGGCCTT
GCCGCCAAGCAACTCGAGCACCACCACCACCACCACTGA

5	1	MTSAPDFNAG	GTGIGSNSRA	TTAKSAAVSY	AGIKNEMCKD	RSMLCAGRDD
	51	VAVTDRDAKI	NAPPPNLHTG	DFPNPNDAYK	NLINLKPAIE	AGYTGRGVEV
	101	GIVDTGESVG	SISFPELYGR	KEHGYNENYK	NYTAYMRKEA	PEDGGGKDIE
	151	ASFDEAVIE	TEAKPTDIRH	VKEIGHIDLK	SHIIGGRSVD	GRPAGGIAPD
	201	ATLHIMNTND	ETKNEMMVAA	IRNAWVKLGE	RGVRIVNNNSF	GTTSRAGTAD
	251	LFQIANSEEQ	YRQALLDYSG	GDKTDEGIRL	MQQSDYGNLS	YHIRNKNMLF
10	301	IFSTGNDAQA	QPNTYALLPF	YEKDAQGII	TVAGVDRSGE	KFKREMYGEP
	351	GTEPLEYGSN	HCGITAMWCL	SAPYEASVRF	TRTNPIQIAG	TSFSAPIVTG
	401	TAALLLQKYP	WMSNDNLRTT	LLTTAQDIGA	VGVDKFGWG	LLDAGKAMNG
	451	PASFPFGDFT	ADTKGTS DIA	YSFRNDISGT	GGLIKKGGSQ	LQLHGNNTYT
	501	GKTIIEGSSL	VLYGNKSDM	RVETK GALIY	NGAASGGS LN	SDGIVYLADT
15	551	DQSGANETVH	IKGSLQLDGK	GTLYTRLGKL	LKVDGTAIIG	GKLYMSARGK
	601	GAGYLNSTGR	RVPFLSAAKI	GQDYSFFTNI	ETDGGLLASL	DSVEKTAGSE
	651	GDTLSYVRR	GNAARTASAA	AHSAPAGLKH	AVEQGGSNLE	NLMVELDASE
	701	SSATPETVET	AAADRTDMPG	IRPYGATFRA	AAAVQHANA A	DGVRIFNSLA
	751	ATVYADSTAA	HADMQRRLK	AVSDGLDHNG	TGLRVIAQTQ	QDGGTWEQGG
20	801	VEGKMRGSTQ	TVGIAAKTGE	NTTAAATLGM	GRSTWSENSA	NAKTDSISLF
	851	AGIRHDAGDI	GYLKGLFSYG	RYKNSISRST	GADEHAEGSV	NGTLMQLGAL
	901	GGVNVFPAAT	GDLTVEGGLR	YDLLKQDAFA	EKGSALGWSG	NSLTEGTLVG
	951	LAGLKLQPL	SDKAVLFATA	GVERDLNGRD	YTVTGGFTGA	TAATGKTGAR
	1001	NMPHTRLVAG	LGADVEFGNG	WNLARYSYA	GSKQYGNHSG	RVGVGYRFL E
25	1051	GSGGGVAAD	IGAGLADALT	APLDHKDKGL	QSLTLDQSVR	KNEKLLAAQ
	1101	GAEKTYGNGD	SLNTGKLNKND	KVSRDFIRQ	IEVDGQLITL	ESGEFQVYKQ
	1151	SHSALTAFQT	EQIQDSEHSG	KMVAKRQFRI	GDIAGEHTSF	DKLPEGGRAT
	1201	YRGTAFGSDD	AGGKLTYTID	FAAQGNGKI	EHLKSPELNV	DLAAADIKPD
	1251	GKRHAVISGS	VLYNQAEKGS	YSLGIFGGKA	QEVAGSAEVK	TVNGIRHIGL
30	1301	AAKQLEHHHH	HH*			

AG983-961

ATGACTTCTGCGCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
35 GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGGATGACGTTGCC
GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCA
TACAAGAATTTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
ACAGGCGAATCCGTCGGCAGCATATCCTTTCCCGAAGTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
AACTATACGGCGTATATGCGGAAGGAAGCGCCTGAAGACGGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAG
40 GCCGTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTTGGTCTCCCAT
ATTATTTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTTGCGCCCGATGCGACGCTACACATAATGAATACG
AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGGCG
ATCGTCAATAACAGTTTGGAAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAG
TACCGCCAAGCGTTGCTCGACTATTCGGCGGGTGATAAAACAGACGAGGGTATCCGCCTGATGCAACAGAGCGATTAC
45 GGCAACCTGTCTTACCACATCCGTAATAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCC
AACACATATGCCCTATTGCCATTTTATGAAAAGACGCTCAAAAAGGCATTATCACAGTCGCAGGCGTAGACCGCAGT
GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACC GGGTACAGAACC GCTTGAGTATGGCTCCAACCATTCGGGAAT
ACTGCCATGTGGTGCCGTGTCGGCACCCATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTCGGGA
ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAAC
50 CTGGCTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCCGGCTGGGGACTGCTG
GATGCGGGTAAGGCCATGAACGGACCCGCGTCTTTCCGTTCCGGCGACTTTACCGCCGATACGAAAGGTACATCCGAT
ATTGCCCTACTCCTTCCGTAACGACATTTACAGGCACGGGCGGCTGATCAAAAAGGCGGCAGCCAACCTGCAACTGCAC
GGCAACAACACCTATACGGGCAAACCATTTATCGAAGGCGGTTCCGCTGGTGTGTGTACGGCAACAACAATCGGATATG
CGCGTCGAAACCAAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTGTCTAT
55 CTGGCAGATACCGACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACG
CTGTACACACGTTTGGGCAAACCTGCTGAAAGTGGACGGTACGGCGATTATCGGGCGCAAGCTGTACATGTCGGCACGC
GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCCTGAGTGCCGCCAAAATCGGGCAGGATTAT
TCTTTCTTACAAACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAA
GGCGACACGCTGCTTATTATGTCGGTCGGCAATGCGGCACGGACTGCTTCCGGCAGCGGCACATTCGGCGCCCGCC
60 GGTCTGAAACACGCGGTAGAACAGGGCGGCAGCAATCTGAAAACCTGATGCTTCGAACTGCAATGCTTCGAAATCAATCC

CATGCGGAAGGCAGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGCCGCAACG
 GGAGATTTGACGGTCAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTTCGCCGAAAAAGGCAGTGTCTTG
 GGCTGGAGCGGCAACAGCCTCACTGAAGGCACGCTGGTGGACTCGCGGGTCTGAAGCTGTCGCAACCCTTGAGCGAT
 AAAGCCGTCCTGTTTGAACGGCGGGCGTGAACCGGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTTACC
 5 GCGCGACTGCAGCAACCGGCAAGACGGGGGCACGCAATATGCCGCACACCCGTTCTGGTTGCCGGCCTGGGCGCGGAT
 GTCGAATTCGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGGTTCCAAACAGTACGGCAACCACAGCGGA
 CGAGTCGGCGTAGGCTACCGGTTCTTCGAGGGTGGCGGAGGCACTGGATCCGCCACAAACGACGACGATGTTAAAAA
 GCTGCCACTGTGGCCATTGCTGCTGCCCTACAACAATGGCCAAGAAATCAACGGTTTCAAAGCTGGAGAGACCATCTAC
 GACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGACTTTAAAGGTCTG
 10 GGTCTGAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAAACAAACAAACGTCGATGCCAAAGTAAAAGCT
 GCAGAATCTGAAATAGAAAAGTTAACCAACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACTGATGCCGCTCTG
 GATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTA
 AAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTCAACGATATCGCCGATTCA
 TTGGATGAAACCAACTAAGGCAGACGAAGCCGTCAAACCGCCAATGAAGCCAAACAGACGGCCGAAGAAACCAA
 15 CAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCTGCCGCTGGCACAGCTAATACT
 GCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATT
 GCTAAAAAGCAAACAGTGGCGACGTGTACACCAGAGAAGAGTCTGACAGCAAATTTGTCAGAATTGATGGTCTGAAC
 GCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCACGATACTCGCCTGAACGGT
 TTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGCCCTTGCAGAACAAGCCGCGCTCTCCGGTCTGTTT
 20 CAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCCGCGGCTACAAATCCGAATCGGCAGTCCGCATCGGT
 ACCGGCTTCCGCTTACCGAAAACCTTGGCCGCAAAGCAGGCGTGGCAGTCCGCACTTCGTCCGGTTCTTCCGCAGCC
 TACCATGTCGGCGTCAATTACGAGTGGCTCGAGCACCACCACCACCACCCTGA

1	MTSAPDFNAG	GTGIGSNSRA	TTAKSAVSY	AGIKNEMCKD	RSMLCAGRDD
51	VAVTDRDAKI	NAPPPNLHTG	DFPNPNDAYK	NLNLKPAIE	AGYTGRGVEV
101	GIVDTGESVG	SISFPELYGR	KEHGYNENYK	NYTAYMRKEA	PEDGGGKDIE
151	ASFDDEAVIE	TEAKPTDIRH	VKEIGHIDL	SHIIGGRSVD	GRPAGGIAPD
201	ATLHIMNTND	ETKNEMMVA	IRNAWVKLGE	RGVRIVNSF	GTTSRAGTAD
251	LFQIANSEEQ	YRQALLDYS	GDKTDEGIRL	MQQSDYGNLS	YHIRNKNMLF
301	IFSTGNDAQ	QPNTYALLPF	YEKDAQGII	TVAGVDRSGE	KFKREMYGEP
351	GTEPLEYGSN	HCGITAMWCL	SAPYEASVRF	TRTNPIQIAG	TSFSAPIVTG
401	TAALLLQKYP	WMSNDNLRTP	LLTTAQDIGA	VGVDKFGWG	LLDAGKAMNG
451	PASFPFGDFT	ADTKGTS DIA	YSFRNDISGT	GGLIKKGSQ	LQLHGNNTYT
501	GKTIIEGGS	VLYGNKSDM	RVETKGALIY	NGAASGGSLN	SDGIVYLADT
551	DQSGANETVH	IKGSLQLDGK	GTLYTRLGKL	LKVDGTAIIG	GKLYMSARGK
601	GAGYLNSTGR	RVPFLSAKI	GQDYSFFTNI	ETDGGLLASL	DSVEKTAGSE
651	GDTLSYVRR	GNAARTASAA	AHSAPAGLKH	AVEQGGSNLE	NLMVELDASE
701	SSATPETVET	AAADRTDMPG	IRPYGATFRA	AAAVQHANA	DGVRIFNSLA
751	ATVYADSTAA	HADMQRRLK	AVSDGLDHNG	TGLRVIAQTQ	QDGGTWEQGG
801	VEGKMRGSTQ	TVGLAAKTGE	NTTAAATLGM	GRSTWSENSA	NAKTDSISLF
851	AGIRHDAGDI	GYLKGLFSYG	RYKNSISRST	GADEHAEGSV	NGTLMQLGAL
901	GGVNVFPAAT	GDLTVEGGLR	YDLLKQDAFA	EKGSALGWSG	NSLTEGTLVG
951	LAGLKLQPL	SDKAVLFATA	GVERDLNGRD	YTVTGGFTGA	TAATGKTGAR
1001	NMPHTRLVAG	LGADVEFGNG	WNLARYSYA	GSKQYGNHSG	RVGVGYRFLE
1051	GGGGTGSATN	DDDVKKAATV	AIAAYNNGQ	EINGFKAGET	IYDIDEDGTI
1101	TKKDATAADV	EADDFKGLGL	KKVVTNLTKT	VNENKQNVDA	KVKAASEIE
1151	KLTTKLADTD	AALADTDAAL	DATTNALNKL	GENITTFABE	TKTNIVKIDE
1201	KLEAVADTV	KHAEAFNDIA	DSLDETNTKA	DEAVKTANEA	KQTAEETKON
1251	VDKVKAAET	AAGKAEAAAG	TANTAADKAE	AVAAKVTDIK	ADIATNKDNI
1301	AKKANSADV	TREESDSKVV	RIDGLNATTE	KLDTRLASAE	KSIADHDTRL
1351	NGLDKTVSDL	RKETROGLAE	QAALSGLFQP	YNVGRFNVTA	AVGGYKSESA
1401	VAIGTGRFT	ENFAAKAGVA	VGTSSGSSAA	YHVG VNYEWL	EHHHHHH*

AG983-961c

ATGACTTCTGCGCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
 GCAGTATCTTACGCCGATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTGCG
 GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCA
 TACAAGAATTTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
 60 ACAGGCGAATCCGTCCGCAGCATATCTTTCCCGAAGTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
 AACTATACGGCGTATATGCGGAAGGAAGCGCCTGAAGACGGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAG
 GCCGTTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAGAAATCGGACACATCGATTTGGTCTCCCAT
 ATTATTTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATGCGCCCGATGCGACGCTACACATAATGAATACG
 AATGATGAAACCAAGAACGAAATGATGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGGCG
 65 ATCGTCAATAACAGTTTGGAAACAACATCGAGGGCAGGCACTGCCGACCTTTTCAAATAGCCAATTCGGAGGAGCAG
 TACCGCAAAGCGTTGCTCGACTATTCCGGCGGTGATAAAACAGACGAGGGTATCCGCCTGATGCAACAGAGCGATTAC
 GGCAACCTGTCTTACCACATCCGTAATAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCC

AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCAGGCGTAGACCGCAGT
 GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTCGGGAATT
 ACTGCCATGTGGTGCCTGTTCGGCACCCCTATGAAGCAAGCGTCCGTTTACCCCGTACAAACCCGATTCAAATTCGCCGA
 5 ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAAC
 CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCGGCTGGGGACTGCTG
 GATGCGGGTAAGGCCATGAACGGACCCGCGTCCTTTCCGTTCCGGCGACTTTACCGCCGATACGAAAGGTACATCCGAT
 ATTGCCTACTCCTTCCGTAACGACATTTACGGCACGGGCGGCTGATCAAAAAGGCGGCAGCCAACCTGCAACTGCAC
 GGCAACAACACCTATACGGGCAAAACCATTTATCGAAGGCGGTTTCGCTGGTGTGTGTACGGCAACAACAATCGGATATG
 10 CGCGTCGAAACCAAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTTGTCTAT
 CTGGCAGATACCGACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACG
 CTGTACACACGTTTGGGCAAACTGCTGAAAGTGGACGGTACGGCGATTATCGGGCGGCAAGCTGTACATGTCCGGCACGC
 GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCCTGAGTGCCGCCAAAATCGGGCAGGATTAT
 TCTTTCTTCAACAACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAA
 15 GCGACACGCTGTCTATTATGTCCGTCGCGGCAATGCGGCACGGACTGCTTCCGGCAGCGGCACATTCGCGCCCGCC
 GGTCTGAAACACGCGGTAGAACAGGGCGGCAGCAATCTGGAAAACCTGATGGTTCGAACTGGATGCCTCCGAATCATCC
 GCAACACCCGAGACGGTTGAAACTGCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTC
 CGCGCAGCGGCAGCCGTACAGCATGCGAATGCCGCGCAGCGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTAT
 GCCGACAGTACCGCCGCCCATGCCGATATGCAGGGACGCCGCTGAAAGCCGTATCGGACGGGTTGGACCACAACGGC
 ACCGGTCTGCGCGTCATCGCGCAAACCCAAACAGGACGGTGGAACTGGGAACAGGGCGGTGTTGAAGGCAAAATGCGC
 20 GGCAGTACCCAAACCGTCCGCATTGCCGCGAAAACCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGC
 AGCACATGGAGCGAAAACAGTGCAAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCGGGC
 GATATCGGCTATCTCAAAGCCCTGTTCTCCTACGGACGCTACAAAACAGCATCAGCCGACGACCCGGTGCAGGACGAA
 CATGCGGAAGGCAGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGCCGCAACG
 25 GGAGATTTGACGGTCAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAGGCAGTGCCTTG
 GGCTGGAGCGGCAACAGCCTCACTGAAGGCACGCTGGTTCGGACTCGCGGGTCTGAAGCTGTGCAACCCCTTGAGCGAT
 AAAGCCGTCCTGTTTGCAACGGCGGGCGTGGAAACGGACCTGAACGGACCGGACTACACGGTAAACGGCGGCTTTACC
 GCGCGACTGCAGCAACCGGCAAGACGGGGCACGCAATATGCCGACACCCGCTGGTTCGGCGCTGGGCGCGGAT
 GTCGAATTCGGCAACGGCTGGAACGGCTTGGCAGCTTACAGCTACGCCGGTTCCAAACAGTACGGCAACCACAGCGGA
 30 CGAGTCGGCGTAGGCTACCGGTTCCCTCGAGGGTGGCGGAGGCACTGGATCCGCCACAAACGACGACGATGTTAAAAA
 GCTGCCACTGTGGCCATTGCTGCTGCCCTACAACAATGGCCAAAGAAATCAACGGTTTCAAAGCTGGAGAGACCATCTAC
 GACATTTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGACTTTAAAGGTCTG
 GGTCTGAAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAACGTCGATGCCAAAGTAAAAGCT
 GCAGAACTCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACTGATGCCGCTCTG
 35 GATGCAACCACCAACGCCTTGAATAAAATGGGAGAAAATATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTA
 AAAATGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTCAACGATATCGCCGATTCA
 TTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAATGAAGCCAAACAGACGGCCGAAGAAACCAA
 CAAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCTGCCGCTGGCACAGCTAATACT
 GCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATT
 40 GCTAAAAAGCAAACAGTGCAGGACGCTGACACCAGAGAAGAGTCTGACAGCAAATTTGTGAGAAATGATGGTCTGAAC
 GCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTCGGGATCACGATACTCGCCTGAACGGT
 TTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCTTGCAGAACAAAGCCGCTCTCCGGTCTGTTTC
 CAACCTTACAACGTGGGTCTCGAGCACCACCACCACCCTGA

45 1 MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSMLCAGRDD
 51 VAVTDRDAKI NAPPNLTHTG DFPNPNDAYK NLINLKPAIE AGYTGRGVEV
 101 GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE
 151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDL V SHIIGGRSVD GRPAGGIAPD
 201 ATLHIMNTND ETKNEMVAA IRNAWVKLGE RGVRIVNSNF GTTSRAGTAD
 251 LFQIANSEEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRNKNMLF
 50 301 IFSTGNDQA QPNTYALLPF YEKDAQGII TVAGVDRSGE KFKREMYGEP
 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
 401 TAALLLQKYP WMSNDNLRTT LLFTAQDIGA VGVDSKFGWG LLDAGKAMNG
 451 PASFPFGDFT ADTKGTS DIA YSFRNDISGT GGLIKKGSQ LQLHGNNTYT
 501 GKTIIEGSL VLYGNKSDM RVETKGALIY NGAASGSLN SDGIVYLADT
 55 551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDGTALIG GKLYMSARGK
 601 GAGYLNSTGR RVPFLSAKI GQDYSFFTNI ETDGGLLASL DSVEKTAGSE
 651 GDTLSSYYVRR GNAARTASAA AHSAPAGLKH AVEQGGSNLE NLMVELDASE
 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANA DGVRIFNLSLA
 751 ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG
 60 801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL
 901 GGVNVPPFAAT GDLTVEGGLR YDLLKQDAFA EKGSALGWSG NSLTEGTLVG
 951 LAGLKLQPL SDKAVLFATA GVERDLNGRD YTVTGGFTGA TAATGKTGAR
 1001 NMPHTRLVAG LGADVEFGNG WNLARYSYA GSKQYGNHSG RVGVGYRFL
 65 1051 GGGGTGSATN DDDVKAATV AIAAYNNGQ EINGFKAGET IYDIDEDGTI
 1101 TKKDATAADV EADDFKGLGL KKVVTNLTKT VNENKQNVDA KVKAASESIE
 1151 KLTKLADTD AALADTDAAL DATFNALNKL GENITTFABE TRTNIVKIDE

-19-

1201 KLEAVADTVD KHAEAFNDIA DSLDETNTKA DEAVKTANEA KQTAEETKQN
 1251 VDAKVKAAET AAGKAEAAAG TANTAADKAE AVAAKVTDIK ADIATNKDNI
 1301 AKKANSADV TREESDSKVV RIDGLNATTE KLDTRLASAE KSIADHDTRL
 1351 NGLDKTVSDL RKETROGLAE QAALSGLFQP YNVGLEHHHH HH*

5

Example 4 – hybrids of ΔG741

Protein 741 has the following sequence:

1 VNRTAFCCLS LTTALILTAC SSSGGGVAAD IGAGLADALT APLDHKDKGL
 51 QSLTLDQSVR KNEKCLKLAAQ GAEKTYGNGD SLNTGKCLKND KVSRLFDFIRQ
 101 IEVDGQLITL ESGEFQVYKQ SHSALTAFQT EQIQDSEHSG KMVAKRQFRI
 151 GDIAGEHTSF DKLPEGGRAT YRGTAFGSDD AGGKLYTYTID FAAKQNGKI
 201 EHLKSPELNV DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA
 251 QEVAGSAEVK TVNGIRHIGL AAKQ*

10

15

ΔG741 thus has the following basic sequence:

VAAD IGAGLADALT APLDHKDKGL
 QSLTLDQSVR KNEKCLKLAAQ GAEKTYGNGD SLNTGKCLKND KVSRLFDFIRQ
 IEVDGQLITL ESGEFQVYKQ SHSALTAFQT EQIQDSEHSG KMVAKRQFRI
 GDIAGEHTSF DKLPEGGRAT YRGTAFGSDD AGGKLYTYTID FAAKQNGKI
 EHLKSPELNV DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA
 QEVAGSAEVK TVNGIRHIGL AAKQ*

20

ΔG741 was fused directly in-frame upstream of proteins 961, 961c, 983 and ORF46.1:

ΔG741-961

ATGGTCCGCCGACATCGGTGCGGGGCTTGCCGATGCACCTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGCAG
 TCTTTGACGCTGGATCAGTCCGTGAGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGA
 AACGGTGACAGCCTCAATACGGGCAAATGAAGAACGACAAGGTCAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
 GACGGGCAGCTCATTTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCGCGCTTAACCGCCTTTCAG
 ACCGAGCAAATACAAGATTCGGAGCATTCGGGAAGATGGTTGCGAAACGCCAGTTCAGAATCGGCGACATAGCGGGC
 GAACATACATCTTTGACAAGCTTCCCGAAGGCGGCGAGGCGACATATCGCGGGACGGCGTTCCGGTTCAGACGATGCC
 GCGGAAAACTGACCTACACCATAGATTTCCGCCCAAGCAGGGAAACGGCAAATCGAACATTTGAAATCGCCAGAA
 CTCAATGTCGACCTGGCCGCGCCGATATCAAGCCGGATGGAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTG
 AAAACCGTAAACGGCATAACCCATATCGGCCTTGCCGCAAGCAACTCGAGGGTGGCGGAGGCACTGGATCCGCCACA
 AACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTC
 AAAGCTGGAGAGACCATCTACGACATGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGATGTTGAA
 GCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAAAC
 GTCGATGCCAAAGTAAAGCTGCAGAACTCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTA
 GCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAA
 GAGACTAAGACAAATATCGTAAAAATGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCA
 TTCAACGATATCGCCGATTCATTTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAATGAAGCCAAA
 CAGACGGCCGAAGAAACCAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCT
 GCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAAGCTGATATC
 GCTACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGACAGCAAATTT
 GTCAGAAATGATGGTCTGAACGCTACTACCGAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTTCCGAT
 CACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCTTGCAGAACAA
 GCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCGGCGGCTACAAATCC
 GAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACCTTTGCCGCCAAAGCAGGCGTGGCAGTCGGCACT
 TCGTCCGGTCTTCCGCAGCCTACCATGTCGGCGTCAATTACGAGTGGCTCGAGCACCACCACCACCACCCTGA

25

30

35

40

45

50

1 MVAADIGAGL ADALTAFLDH KDKGLQSLTL DQSVRKNEKL KLAAQGAEKT
 51 YNGDSLNTG KLKNDKVSFR DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQIQD SEHSKQMVAK RQFRIGDIAG EHTSFDKLP EGRATYRGTA
 151 FGSDDAGGKL TYTIDFAAQ GNGKIEHLKS PELNVDLAAA DIKPDGKRHA
 201 VISGSVLYNQ AEKGSYSLGI FGGKAQEVAG SAEVKTVNGI RHIGLAAKQL
 251 EGGGGTGSAT NDDDVKKAAT VALAAAYNNG QEINGFKAGE TIYDIDEDGT
 301 ITRKDATAAD VEADDFKGLG LKKVVTNLTK TVNENKQNV DAKVKAASEI
 351 EKLTKLADT DAALADTDA LDATTNALNK LGENITTFAE ETKTNIVKID
 60 401 EKLEAVADTV DKHAEAFNDI ADSLDETNTK ADEAVKTANE AKQTAEETKQ

55

60

451 NVDKVKAAE TAAGKAEAAA GTANTAADKA EAVAAKVTDI KADIATNKDN
 501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR
 551 LNGLDKTVSD LRKETROGLA EQAALSGLFQ PYNVGRFNVV AAVGGYKSES
 601 AVAIGTGFRF TENFAAKAGV AVGTSSGSSA AYHVGVNYEW LEHHHHHH*

5

AG741-961c

ATGGTCGCCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACCAGCACCGCTCGACCATAAAGACAAAGGTTTGCAG
 TCTTTGACGCTGGATCAGTCCGTGAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGA
 AACGGTGACAGCCTCAATACGGGCAAATGGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
 GACGGGCAGCTCATTTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCGCGCTTAACCGCCTTTCAG
 ACCGAGCAAATACAAGATTCCGAGCATTCGCGGAAGATGGTTGCGAAACGCCAGTTCAGAATCGGCGACATAGCGGGC
 GAACATACATCTTTTGACAAGCTTCCCAGAGGCGGCAGGGCGACATATCGCGGGACGGCGTTCCGTTTCAGACGATGCC
 GGCGGAAAACTGACCTACACCATAGATTTCCGCGCCAAGCAGGGAAACGGCAAATCGAACATTTGAAATCGCCAGAA
 CTCAATGTCGACCTGGCCCGCCGATATCAAGCCGGATGGAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTG
 AAAACCGTAAACGGCATAACCCATATCGGCCTTGCCGCCAAGCAACTCGAGGGTGGCGGAGGCACTGGATCCGCCACA
 AACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTC
 AAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGATGTTGAA
 GCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAAAC
 GTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTA
 GCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAA
 GAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCA
 TTCAACGATATCGCCGATTTCATTTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAATGAAGCCAAA
 CAGACGGCCGAAGAAACCAAAACAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCT
 GCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATC
 GCTACGAACAAAGATAATATTTGCTAAAAAGCAAACAGTGCAGCAGTGTACACCAGAGAAGAGTCTGACAGCAAATTT
 GTCAGAAATGATGGTCTGAACGCTACTACCGAAAATTTGGACACACGCTTGGCTTCTGCTGAAAATCCATTGCCGAT
 CACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACAGCTGCGCAAAGAAACCCGCCAAGGCCCTTGCAGAACAA
 GCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTCTCGAGCACCACCACCACCACCCTGA

10

15

20

25

30

1 MVAADIGAGL ADALTAPLDH KDKGLQSLTL DQSVRKNEKL KLAAQGAEKT
 51 YNGDSLNTG KLKNDKVSFR DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQIQD SEHSGKMAK RQFRIGDIAG EHTSFDKLEPE GGRATYRGT
 151 FGSDDAGGKL TYTIDFAAKQ GNGKIEHLKS PELNVDLAAA DIKPDGKRHA
 201 VISGSVLYNQ AERGSYSLGI FGGKAQEVAG SAEVKTVNGI RHIGLAAKQL
 251 EGGGGTGSAT NDDDVKKAAT VAIAAYNNG QEINGFKAGE TIYDIDEDGT
 301 ITTKDATAAD VEADDFKGLG LKKVVTNLTK TVNENKQNV DAKVKAASEI
 351 EKLITTKLADT DAALADTDA LDATTNALNK LGENITTFAE ETKTNIVKID
 401 EKLEAVADTV DKHAEAFNDI ADSLDEFNTK ADEAVKTANE AKQTAETKQ
 451 NVDKVKAAE TAAGKAEAAA GTANTAADKA EAVAAKVTDI KADIATNKDN
 501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR
 551 LNGLDKTVSD LRKETROGLA EQAALSGLFQ PYNVGLEHHH HHH*

35

40

45

AG741-983

ATGGTCGCCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACCAGCACCGCTCGACCATAAAGACAAAGGTTTGCAG
 TCTTTGACGCTGGATCAGTCCGTGAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGA
 AACGGTGACAGCCTCAATACGGGCAAATGGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
 GACGGGCAGCTCATTTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCGCGCTTAACCGCCTTTCAG
 ACCGAGCAAATACAAGATTCCGAGCATTCGCGGAAGATGGTTGCGAAACGCCAGTTCAGAATCGGCGACATAGCGGGC
 GAACATACATCTTTTGACAAGCTTCCCAGAGGCGGCAGGGCGACATATCGCGGGACGGCGTTCCGTTTCAGACGATGCC
 GGCGGAAAACTGACCTACACCATAGATTTCCGCGCCAAGCAGGGAAACGGCAAATCGAACATTTGAAATCGCCAGAA
 CTCAATGTCGACCTGGCCCGCCGATATCAAGCCGGATGGAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTG
 AAAACCGTAAACGGCATAACCCATATCGGCCTTGCCGCCAAGCAACTCGAGGGATCCGGCGGAGGCGGCACCTTCTGCG
 CCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCAGCAGTATCTTAC
 GCCGGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCGGGTGGGATGACGTTGCGGTTACAGACAGG
 GATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCATACAAGAATTTG
 ATCAACCTCAAACCTGCAATTTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGACACAGGCGAATCC
 GTCGGCAGCATATCCTTTCCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTAACAAAATATACGGCG
 TATATGCGGAAGGAAGCGCCTGAAGACGGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAGGCCGTTATAGAG
 ACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTTGGTCTCCCATATTTATGGCGGG
 CGTTCCGTTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACGAATGATGAAACC
 AAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGTCAAGCTGGGCGAACGTGGCGTGCAGCATCGTCAATAAC
 AGTTTGGAAACAACATCGAGGGCAGGCACTGCCGACCTTTCCAAATAGCCAATTCGGAGGAGCAGTACCGCCAAGCG
 TTGCTCGACTATTCGGCGGTGATAAAACAGACGAGGGTATCCGCTGATGCAACAGAGCGATTACGGCAACCTGTCC

50

55

60

65

5
10
15
20
25
30

TACCACATCCGTAATAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACATATGCC
 CTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCAGGCGTAGACCGCAGTGGAGAAAAGTTC
 AAACGGGAAATGTATGGAGAACCGGTACAGAACCGCTTGAGTATGGCTCCAACCAATTGCGGAATTACTGCCATGTGG
 TGCCGTGTCGGCACCCCTATGAAGCAAGCGTCCGTTTCACCCGTACAAACCCGATTCAAATGCCGGAACATCCTTTTCC
 GCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGTACCACG
 TTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCGGCTGGGGACTGCTGGATGCGGGTAAG
 GCCATGAACGGACCCGCGTCCCTTCCGTTCCGGCGACTTTACCGCCGATACGAAAGGTACATCCGATATTGCCTACTCC
 TTCCGTAACGACATTTTCAGGCACGGGCGGCTGATCAAAAAGGCGGCAGCCAACTGCAACTGCACGGCAACAACACC
 TATACGGGCAAACCAATTATCGAAGGCGGTTGCTGGTGTGTACGGCAACAACAATCGGATATGCGCGTCAAAACC
 AAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCTGAACAGCGACGGCATTGCTCTATCTGGCAGATACC
 GACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACGCTGTACACACGT
 TTGGGCAAACCTGCTGAAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTACATGTCGGCAGCGGGCAAGGGGCA
 GGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCCTGAGTGCCGCCAAAATCGGGCAGGATATTCTTTCTTCA
 AACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGCGACACGCTG
 TCCTATTATGTCCGTCGCGGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCGCGCCCCGCGGTCTGAAACAC
 GCCGTAGAACAGGGCGGCAGCAATCTGGAAAACCTGATGGTCAACTGGATGCCTCCGAATCATCCGCAACACCCGAG
 ACGGTTGAAACTGCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTCGCGCAGCGGCA
 GCCGTACAGCATGCGAATGCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTATGCCGACAGTACC
 GCCGCCCATGCCGATATGCAGGGACGCCGCTGAAAGCCGTATCGGACGGGTTGGACCACAACGGCAGCGGTCTGCGC
 GTCATCGCGCAAACCCAAACAGGACGGTGGAAACGTGGGAACAGGGCGGTGTTGAAGGCAAATGCGCGGCAGTACCCAA
 ACCGTCGGCATTGCCGCGAAAACCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGCAGCACATGGAGC
 GAAAACAGTGCAAATGCAAAAACCGACAGCATTAGTCTGTTTCAGGCATACGGCAGCATGCGGGCGATATCGGCTAT
 CTCAAAGGCCTGTTCTCCTACGGACGCTACAAAAACAGCATCAGCCGCAGCACCGGTGCGGACGAACATGCGGAAGGC
 AGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGGCCGCAACGGGAGATTTGACG
 GTCGAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAGGCAGTGTCTTGGGCTGGAGCGGC
 AACAGCCTCACTGAAGGCACGCTGGTCCGACTCGCGGTCTGAAGCTGTGCAACCCCTTGAGCGATAAAGCCGCTCTG
 TTTGCAACGGCGGGCGTGGAACGCGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTACCGGCGCGACTGCA
 GCAACCGGCAAGACGGGGGCACGCAATATGCCGCACACCCGCTGCTGTTGCCGGCCTGGGCGCGGATGTGCAATTCGGC
 AACGGCTGGAACGGCTTGGCAGTTACAGCTACGCCGTTCCAAACAGTACGGCAACCACAGCGGACGAGTCCGGCGTA
 GGCTACCGGTTCCCTCGAGCACCACCACCACCACCTGA

35
40
45
50
55
60

1	MVAADIGAGL	ADAL/TAPLDH	KDKGLQSLTL	DQSVRKNEKL	KLAAQGAEKT
51	YNGDSLNTG	KLKNDKVSFR	DFIRQIEVDG	QLITLESGEF	QVYKQSHSAL
101	TAFQTEQIQD	SEHSGKMVAK	RQFRIGDIAG	EHTSFDKLE	GGRATYRGTA
151	FGSDDAGGKL	TYTIDFAAKQ	GNGKIEHLKS	PELNVDLAAA	DIKPDGKRHA
201	VISGSVLYNQ	AEKGSYSLGI	FGGKAQEVAG	SAEVKTVNGI	RHIGLAAKQL
251	EGSGGGGTS	PDFNAGGTGI	GSNSRATTAK	SAVSYAGIK	NEMCKDRSML
301	CAGRDDVAVT	DRDAKINAPP	PNLHTGDFPN	PNDAYKNLIN	LKPAIEAGYT
351	GRGVEVGIVD	TGESVGSISF	PELYGRKEHG	YNENYKNYTA	YMRKEAPEDG
401	GGKDIEASFD	DEAVIETEAK	PTDIRHVKEI	GHIDLVSIII	GGRSVDGRPA
451	GGIAPDATHL	IMNPNDETKN	EMMVAAIRNA	WVKLGERGVR	IVNNSFGTTS
501	RAGTADLFQI	ANSEEQYRQA	LLDYSGGDKT	DEGIRLMQOS	DYGNLSYHIR
551	NKNMLFIFST	GNDAAQAQNT	YALLPFYEK	AQKGIITVAG	VDRSGEKFKR
601	EMYGEPGTEP	LEYGSNHCIG	TAMWCLSAPY	EASVRFTRTN	PIQLAGTSFS
651	APIVTGTAAL	LLQKYPWMSN	DNLRTPLLT	AQDIGAVGVD	SKFGWGLLDA
701	GKAMNGPAS	PFGDFTADTK	GTSDIAYSFR	NDISGTGGLI	KKGGSQLQLH
751	GNNTYTGKTI	IEGGLVLYG	NNKSDMRVET	KGALIYNGAA	SGGSLNSDGI
801	VYLADTDQSG	ANETVHIKGS	LQLDGRGTLY	TRLGKLLKVD	GTAIIGGKLY
851	MSARGKGAGY	LNSTGRRVFP	LSAAKIGQDY	SFFTNIETDG	GLLASLDSVE
901	KTAGSEGDTL	SYVRRGNAA	RTASAAHSA	PAGLKHAVEQ	GGSNLENLMV
951	ELDASESSAT	PETVETAAD	RTDMPGIRPY	GATFRAAAV	QHANAADGVR
1001	IFNSLAATVY	ADSTAAHAD	QRRRLKAVSD	GLDHNGTGLR	VIAQTQDGG
1051	TWEQGGVEGK	MARGSTQVGI	AAKTGENTTA	AATLGMGRST	WSENSANAKT
1101	DSISLFAGIR	HDAGDIGYLK	GLFSYGRYKN	SISRSTGADE	HAEGSVNGTL
1151	MQLGALGGVN	VPFAATGDLT	VEGGLRYDLL	KQDAFAEKGS	ALGWSGNSLT
1201	EGTLVGLAGL	KLSQPLSDKA	VLFATAGVER	DLNGRDYTVT	GGFTGATAAT
1251	GKTGARNMPH	TRLVAGLGAD	VEFGNGWNGL	ARYSYAGSKQ	YGNHSGRVGV
1301	GYRFLEHHHH	HH*			

AG741-ORF46.1

65

ATGGTCGCCCGGACATCGGTGCGGGGCTTGCCGATGCACATAACCGCACCGCTCGACCATAAAGACAAAGGTTTGCAG
 TCTTTGACGCTGGATCAGTCCGTCAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGA
 AACGGTGACAGCCTCAATACGGGCAAATGGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
 GACGGGCGAGCTCATTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAGCCATTCGCGCTTAACCGCCTTTCAG
 ACCGAGCAAATACAAGATTCGGAGCATTCGGGAAGATGGTTGCGAAACGCCAGTTCAGAATCGGCGACATAGCGGGC
 GAACATACATCTTTGACAAGCTTCCCGAAGGCGGCAGGGCGACATATCGCGGGACGGCGTTCCGTTTCAGACGATGCC

-22-

GCGGAAACTGACCTACACCATAGATTTCCGCCCAAGCAGGGAAACGGCAAATCGAACATTTGAAATCGCCAGAA
 CTCAATGTCGACCTCGCCGCCCGCCGATATCAAGCCGGATGGAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCCAGGAAGTTGCCGGCAGCCGGAAGTG
 5 AAAACCGTAAACGGCATAACCCATATCGGCCTTGCCGCCAAGCAACTCGACGGTGGCCGGAGGCACTGGATCCTCAGAT
 TTGGCAAACGATTTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTTCGAACCCGACGGGAAATACCACCTATTCCGC
 AGCAGGGGGGAACTTGCCGAGCGCAGCGGCCATATCGGATTGGGAAAATAACAAAGCCATCAGTTGGGCAACCTGATG
 ATTCAACAGGCGGCCATTAAAGGAAATATCGGCTACATTTGTCCGCTTTTCCGATCACGGGCACGAAGTCCATTTCCCC
 TTCGACAACCATGCCTCACATTCGGATTTCTGATGAAGCCGGTAGTCCCGTTGACGGATTTAGCCTTTACCGCATCCAT
 10 TGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGCGGCTATCCCGCTCCCAAAGGCGCG
 AGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCCTCAACCTGACCGACAACCGCAGCACCCGGA
 CAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAACCGCGCCACC
 CGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAACATC
 ATCGGCGCGGCAGGAGAAATTTGTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTGCTGTATG
 CACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAACTCAAAGAC
 15 TATGCCGACAGCCATCCCGGATTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTGAGCAATATC
 TTTATGGCAGCCATCCCATCAAAGGGATTGGAGCTGTTCGGGGAAAATACGGCTTGGGCGGCATCACGGCACATCCT
 ATCAAGCGGTGCGAGATGGGCGCGATCGCATTGCCGAAAGGGAAAATCCGCCGTGACGACAATTTTGGCGATGCGGCA
 TACGCCAAATACCCGTCCCTTACCATTCCCGAAATATCCGTTCAAACCTGGAGCAGCGTTACGGCAAAGAAAACATC
 ACCTCCTCAACCGTGCCGCCGTCAAACGGCAAATAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTACCG
 20 TTTGACGGTAAAGGGTTTCCGAATTTGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCACCACCACCTGA

	1	MVAADIGAGL	ADALTAPLDH	KDKGLQSLTL	DQSVRKNEKL	KLAAQGAEKT
	51	YNGDSLNTG	KLKNDKVSFR	DFIRQIEVDG	QLITLESGEF	QVYKQSHSAL
25	101	TAFQTEQIQD	SEHSGKMAK	RQFRIGDIAG	EHTSFDKLE	GGRATYRGTA
	151	FGSDDAGGKL	TYTIDFAAKQ	GNGKIEHLKS	PELNVDLAAA	DIKPDGKRHA
	201	VISGSVLYNQ	AEKGSYSLGI	FGKKAQEVAG	SAEVKTVNGI	RHIGLAAKQL
	251	DGGGGTGSSD	LANDSFIRQV	LDRQHFEPDG	KYHLFGSRGE	LAERSGHIGL
	301	GKIQSHQLGN	LMIQQAARKG	NIGYIVRFS	HGHEVHSPFD	NHASHSDSDE
	351	AGSPVDGFSL	YRIHWDGYEH	HPADGYDGPQ	GGYPAPKGA	RDIYSYDIKG
30	401	VAQNIRLNL	DNRSTGQRLA	DRFHNAGSML	TQVGDGFKR	ATRYSPELDR
	451	SGNAAEAFNG	TADIVKNIIG	AAGEIVGAGD	AVQGISSEGSN	IAVMHGLGLL
	501	STENKMARIN	DLADMAQLKD	YAAAIRDWA	VQNPNAAQGI	EAVSNIFMAA
	551	IPIKGIGAVR	GKYGLGGITA	HPIKRSQMG	IALPKGKSAV	SDNFADAAYA
	601	KYPSPYHSRN	IRSNLEQRYG	KENITSSTVP	PSNGKNVKLA	DQRHPKTGVP
35	651	FDGKGFNFE	KHVKYDTLEH	HHHHH*		

Example 5 – hybrids of 287

Expression of 287 as full-length with a C-terminal His-tag, or without its leader peptide but
 with a C-terminal His-tag, gives fairly low expression levels. Better expression is achieved
 40 using a N-terminal GST-fusion. As an alternative to using GST as an N-terminal fusion
 partner, 287 was placed at the C-terminus of protein 919 ('919-287'), of protein 953 ('953-
 287'), and of proteins ORF46.1 ('ORF46.1-287'). In both cases, the leader peptides were
 deleted, and the hybrids were direct in-frame fusions.

To generate the 953-287 hybrid, the leader peptides of the two proteins were omitted by
 45 designing the forward primer downstream from the leader of each sequence; the stop codon
 sequence was omitted in the 953 reverse primer but included in the 287 reverse primer. For
 the 953 gene, the 5' and the 3' primers used for amplification included a *NdeI* and a *BamHI*
 restriction sites respectively, whereas for the amplification of the 287 gene the 5' and the 3'
 primers included a *BamHI* and a *XhoI* restriction sites respectively. In this way a sequential
 50 directional cloning of the two genes in pET21b+, using *NdeI-BamHI* (to clone the first gene)
 and subsequently *BamHI-XhoI* (to clone the second gene) could be achieved.

-23-

The 919-287 hybrid was obtained by cloning the sequence coding for the mature portion of 287 into the *XhoI* site at the 3'-end of the 919-His clone in pET21b+. The primers used for amplification of the 287 gene were designed for introducing a *SalI* restriction site at the 5'- and a *XhoI* site at the 3'- of the PCR fragment. Since the cohesive ends produced by the *SalI* and *XhoI* restriction enzymes are compatible, the 287 PCR product digested with *SalI-XhoI* could be inserted in the pET21b-919 clone cleaved with *XhoI*.

The ORF46.1-287 hybrid was obtained similarly.

The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens:

	Mixture with 287	Hybrid with 287
919	32000	16000
953	8192	8192
ORF46.1	128	8192

10 Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained for 919-287 and 953-287:

<i>Strain</i>	919		953		ORF46.1	
	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>
MC58	512	1024	512	1024	-	1024
NGH38	1024	2048	2048	4096	-	4096
BZ232	512	128	1024	16	-	-
MenA (F6124)	512	2048	2048	32	-	1024
MenC (C11)	>2048	n.d.	>2048	n.d.	-	n.d.
MenC (BZ133)	>4096	>8192	>4096	<16	-	2048

Hybrids of ORF46.1 and 919 were also constructed. Best results (four-fold higher titre) were achieved with 919 at the N-terminus.

15 Hybrids 919-519His, ORF97-225His and 225-ORF97His were also tested. These gave moderate ELISA titres and bactericidal antibody responses.

As hybrids of two proteins A & B may be either NH₂-A-B-COOH or NH₂-B-A-COOH, the "reverse" hybrids with 287 at the N-terminus were also made, but using ΔG287. A panel of strains was used, including homologous strain 2996. FCA was used as adjuvant:

-24-

Strain	287 & 919		287 & 953		287 & ORF46.1	
	$\Delta G287-919$	919-287	$\Delta G287-953$	953-287	$\Delta G287-46.1$	46.1-287
2996	128000	16000	65536	8192	16384	8192
BZ232	256	128	128	<4	<4	<4
1000	2048	<4	<4	<4	<4	<4
MC58	8192	1024	16384	1024	512	128
NGH38	32000	2048	>2048	4096	16384	4096
394/98	4096	32	256	128	128	16
MenA (F6124)	32000	2048	>2048	32	8192	1024
MenC (BZ133)	64000	>8192	>8192	<16	8192	2048

Better bactericidal titres are generally seen with 287 at the N-terminus.

When fused to protein 961 [$\text{NH}_2\text{-}\Delta\text{G287-961-COOH}$ – sequence shown above], the resulting protein is insoluble and must be denatured and renatured for purification. Following renaturation, around 50% of the protein was found to remain insoluble. The soluble and insoluble proteins were compared, and much better bactericidal titres were obtained with the soluble protein (FCA as adjuvant):

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

Titres with the insoluble form were, however, improved by using alum adjuvant instead:

Insoluble	32768	128	4096	>2048	>2048	2048
------------------	-------	-----	------	-------	-------	------

961c was also used in hybrid proteins (see above). As 961 and its domain variants direct efficient expression, they are ideally suited as the N-terminal portion of a hybrid protein.

Example 23 – further hybrids

Further hybrid proteins of the invention are shown in the drawings and have the sequences set out below. These are advantageous when compared to the individual proteins:

15

ORF46.1-741

20

ATGTCAGATTGGCAAACGATTC'TTTTATCCGGCAGGTTCTCGACCGTCAGCATTTCGAACCCGACGGGAAATACCAC
CTATTCGGCAGCAGGGGGGAAC'TTGCCGAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGC
AACCTGATGATTCAACAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTC
CATTCCCCTTCGACAACCATGCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTTAGCCTTTAC
CGCATCCATTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGGCTATCCCGCTCCC
AAAGGCGCGAGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCCTCAACCTGACCGACAACCGC
AGCACCGGACAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAA
CGCGCCACCCGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTT
AAAAACATCATCGGCGCGCAGGAGAAATTGTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATT

5 GCTGTCATGCACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGCAGCAGCCATCCCGCATTTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTC
 AGCAATATCTTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACG
 GCACATCCTATCAAGCGGTGCGCAGATGGGCGCGATCGCATTTGCCGAAAGGGAAATCCGCCGTGAGCGACAATTTTGCC
 10 GATGCGGCATACGCCAAATACCCGTCCCTTACCATTCCCGAAATATCCGTTCAAAC TTGGAGCAGCGTTACGGCAA
 GAAAACATCACCTCCTCAACCGTGCCGCCGTCAAACGGCAAATAATGTCAAAC TTGGCAGACCAACGCCACCCGAAGACA
 GCGGTACCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGGGATCCGGAGGGGGTGGT
 GTCGCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGCAGTCT
 TTGACGCTGGATCAGTCCGTGAGAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAA ACTTATGGAAAC
 15 GGTGACAGCCTCAATACGGGCAAATTGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAAATCGAAGTGGAC
 GGGCAGCTCATTACCTTGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTCAGACC
 GAGCAAATACAAGATTCGGAGCATTCGGGGAAGATGGTTGCGAAACGCCAGTTTCAAGATCGGGACATAGCGGGCGAA
 CATACTCTTTTGACAAGCTTCCCGAAGCGCGCAGGGCGACATATCGCGGGACGGCGTTCCGGTTTTCAGACGATGCCGGC
 GGAAAAC TGACCTACACCATAGATTTCCGCCCAAGCAGGGAAACGCCAAAATCGAACATTTGAAATCGCCAGAACTC
 20 AATGTCGACCTGGCCGCCCGATATCAAGCCGGATGGAAAACGCCATGCCGTATCAGCGGTTCCGTCCTTTACAAC
 CAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTGAAA
 ACCGTAACCGGCATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGCACCACCACCACCACCCTGA

20 1 MSDLANDSFI RQVLDROHFE PDGKYHLFGS RGELAERSGH IGLGKIQSHQ
 51 LGNLMIQQA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 101 FSLYRIHWDG YEHPADGYD GPQGGGYPAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRA TRYSPE LDRSGNAAEA
 201 FNGTADIVKN IIGAAGEIVG AGDAVQGI SE GSNIAVMHGL GLLSTENKMA
 25 251 RINDLADMAQ LKDYAAAIR DWAVQNPNA QGIEAVSNIF MAAPIKIGI
 301 AVRGKYGLGG ITAHPKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNLEQ RYKENTSS TVPPSNGKNV KLADQRHPKT GVPFDGKGF
 401 NFEKHVKYDT GSGGGVAAD IGAGLADALT APLDHKDKGL QSLTLDQSVR
 451 KNEKLLAAQ GAEKTYGNGD SLNTGKLNKND KVS RFD FIRQ IEVDGQLITL
 501 ESGEFQVYKQ SHSALTAFTQ EQIQDSEHSG KMVAKRQFRI GDIAGEHTSF
 30 551 DKLPBGG RAT YRGTAFGSDD AGGKLYTID FAAKQNGKI EHLKSPELNV
 601 DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA QEVAGSAEVK
 651 TVNGIRHIGL AAKQLEHHH HH*

35 ORF46.1-961

ATGTCAGATTTGGCAAACGATTTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTCGAACCCGACGGGAAATACCAC
 CTATTCGGCAGCAGGGGGGAAC TTGCCGAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGC
 AACCTGATGATTCAACAGGCGGCCATTAAGGAAATATCGGCTACATTTGTCGCTTTTCCGATCACGGGCACGAAGTC
 40 CATTCCCTTCGACAACCATGCCTCACATTCGGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTTAGCCTTTAC
 CGCATCCATTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGGCTATCCCGCTCCC
 AAAGGCGCGAGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCTCAACCTGACCGACAACCGC
 AGCACCGGACAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAA
 CGCGCCACCCGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTT
 AAAACATCATCGGCGCGCAGGAGAAATTTGTCGGCGCAGGCGATGCCGTGACGGGCATAAGCGAAGGCTCAAACATT
 45 GCTGTCATGCACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGCAGCAGCCATCCCGCATTTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTC
 AGCAATATCTTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACG
 GCACATCCTATCAAGCGGTGCGCAGATGGGCGCGATCGCATTTGCCGAAAGGGAAATCCGCCGTGAGCGACAATTTTGCC
 GATGCCGCATACGCCAAATACCCGTCCCTTACCATTCCCGAAATATCCGTTCAAAC TTGGAGCAGCGTTACGGCAA
 50 GAAAACATCACCTCCTCAACCGTGCCGCCGTCAAACGGCAAATAATGTCAAAC TTGGCAGACCAACGCCACCCGAAGACA
 GCGGTACCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGGGATCCGGAGGAGGAGGA
 GCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAAC
 GGTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTAACAAAAAGACGCAACTGCAGCCGAT
 55 GTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAACCGTCAATGAAAAACAA
 CAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCC
 GCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACATTT
 GCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCC
 GAAGCATTCAACGATATCGCCGATTCATTGGATGAAACCAACTAAGGCAGACGAAGCCGTCAAACCGCCAATGAA
 60 GCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAAGCC
 GAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAAGCT
 GATATCGCTACGAACAAAGATAATTTGCTAAAAAAGCAAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGACAGC
 AAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATT
 65 GCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCCTTGCA
 GAACAAGCCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCCGGCGCTAC
 AAATCCGAATCGGCAGTCCCATCGGTACCGGCTTCCGCTTTACCGAAAAC TTGCCGCCAAGCAGGCGTGGCAGTC
 GGCACCTCGTCCGGTTCTTCCGACGCTACCATGTCCGGCTCAATTACGAGTGGCTCGAGCACCACCACCACCAC
 TGA

1 MSDLANDSFI RQVLDRQHFE PDGKYHLFGS RGELAERSGH IGLGKIQSHQ
 51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 5 101 FSLYRIHWDG YEHPADGYD GPQGGYPAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRATRYSP E LDRSGNAAEA
 201 FNGTADIVKN IIGAAGEIVG AGDAVQGISE GSNIAVMHGL GLLSTENKMA
 251 RINDLADMAQ LKDYAAAAIR DWAVQNPNA QGIEAVSNIF MAAIPIKGIG
 301 AVRGKYGLGG ITAHPKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNLEQ RYGKENITSS TVPPSNGKNV KLADQRHPKT GVPFDGKGF
 10 401 NFEKHVKYDT GSGGGGATND DDVKKAAATVA IAAAYNNGQE INGFKAGETI
 451 YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV NENKQNVDAK
 501 VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKLK ENITTFAEET
 551 KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD EAVKTANEAK
 601 QTAEETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA VAAKVTDIKA
 15 651 DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK LDTRLASA EK
 701 SIADHDTRLN GLDKTVSDLR KETROGLAEQ AALSGLFQPY NVGRFNVTA A
 751 VGGYKSESAV AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY HVG VNYEWLE
 801 HHHHHH*

ORF46.1-961c

ATGTCAGATTTGGCAAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTTCGAACCCGACGGGAAATACCAC
 CTATTCGGCAGCAGGGGGAACTTGCCGAGCGCAGCGGCCATATCGGATTTGGGAAAAATACAAAGCCATCAGTTGGGC
 AACCTGATGATTCAACAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTC
 25 CATTCCTCCCTTCGACAACCATGCCTCACATTCCGATTTCTGATGAAGCCGGTAGTCCCGTTGACGGATTTAGCCTTTAC
 CGCATCCATTTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGCGGCTATCCCGCTCCC
 AAAGGCGCGAGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCCCTCAACCTGACCGACAACCGC
 AGCACCGGACAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAA
 CGCGCCACCCGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTT
 30 AAAAAATCATCGGCGCGCAGGAGAAATTTGTCGGCGCAGGCGATGCCGTGACGGGCATAAGCGAAGGCTCAAACATTT
 GCTGTTCATGCACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGAGCAGCCATCCGCGATTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTC
 AGCAATATCTTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAAATACGGCTTGGGCGGCATCAG
 GCACATCCTATCAAGCGGTGCGCAGATGGGCGCGATCGCATTGCCGAAAGGGAAATCCGCCGTCAGCGACAATTTTGCC
 35 GATGCGGCATACGCCAAATACCCGCTCCCTTACCATTCCCGAAATATCCGTTCAAACCTTGGAGCAGCGTTACGGCAA
 GAAAACATCACCTCTCAACCGTGCCGCGCTCAAACGGCAAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACA
 GCGGTACCGTTTGGCGTAAAGGGTTTCCGAATTTTGGAGAAGCAGTGAATATGATACGGGATCCGGAGGAGGAGGA
 GCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAAC
 40 GGTTTCAAAGCTGGAGAGACCATCTACGACATGATGAAGACGGCACAATFACCAAAAAGACGCAACTGCAGCCGAT
 GTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAAAACCGTCAATGAAAACAAA
 CAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACTGATGCC
 GCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTTGGGAGAAAATATAACGACATTT
 GCTGAAGAGACTAAGACAAATATCGTAAAAATGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCC
 GAAGCATTCACGATATCGCCGATTCAATTGGATGAAACCAACTAAGGCAGACGAAGCCGTCAAACCCGCAATGAA
 45 GCCAAACAGACGGCCGAAGAAACCAAACAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAAGCC
 GAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAAGTTACCGACATCAAAGCT
 GATATCGCTACGAACAAAGATAATATGCTAAAAAGCAAACAGTCCGACGCTGTACACCAGAGAAGAGTCTGACAGC
 AAATTTGTCAGAAATGATGGTCTGAACGCTACTACCGAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATT
 50 GCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCAAGGCCTTGCA
 GAACAAGCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTCTCGAGCACCACCACCACCCTGA

1 MSDLANDSFI RQVLDRQHFE PDGKYHLFGS RGELAERSGH IGLGKIQSHQ
 51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 55 101 FSLYRIHWDG YEHPADGYD GPQGGYPAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRATRYSP E LDRSGNAAEA
 201 FNGTADIVKN IIGAAGEIVG AGDAVQGISE GSNIAVMHGL GLLSTENKMA
 251 RINDLADMAQ LKDYAAAAIR DWAVQNPNA QGIEAVSNIF MAAIPIKGIG
 301 AVRGKYGLGG ITAHPKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNLEQ RYGKENITSS TVPPSNGKNV KLADQRHPKT GVPFDGKGF
 60 401 NFEKHVKYDT GSGGGGATND DDVKKAAATVA IAAAYNNGQE INGFKAGETI
 451 YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV NENKQNVDAK
 501 VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKLK ENITTFAEET
 551 KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD EAVKTANEAK
 601 QTAEETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA VAAKVTDIKA
 65 651 DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK LDTRLASA EK
 701 SIADHDTRLN GLDKTVSDLR KETROGLAEQ AALSGLFQPY NVGLEHHHHH
 751 H*

961-ORF46.1

5 ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCC
GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAC
AAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATGGGAGAAAATATAACGACA
10 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
GCCGAAGCATTCAACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAAT
GAAGCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAA
GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAA
GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAAACAGTGCCGACGTTACACCAGAGAAGAGTCTGAC
15 AGCAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCTT
GCAGAACAAGCCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGGTTCATGTAACGGCTGCAGTCGGCGGC
TACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACCTTTGCCGCCAAAGCAGGCGTGGCA
GTCGGCACTTCGTCGGTCTTCCGCAGCCTACCATGTCGGCGTCAATTACGAGTGGGGATCCGGAGGAGGAGGATCA
GATTTGGCAAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTCGAACCCGACGGGAAATACCACCTATTC
20 GGCAGCAGGGGGAACTTGCCGAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTG
ATGATTCAACAGGCGGCCATTAAGGAAATATCGGCTACATTGTCGCTTTTCCGATCACGGGCACGAAGTCCATTCC
CCCTTCGACAACCATGCCTCACATTCGGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCTTTACCGCATC
CATTTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGCGCTATCCCGCTCCCAAAGGC
GCGAGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCCTCAACCTGACCGACAACCGCAGCACC
25 GGACAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGGAAGGAGTAGGCGACGGATTCAAACGCGCC
ACCCGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAAC
ATCATCGGCGCGGAGGAGAAATTGTCGGCGCAGGCGATGCCGTGACGGGCATAAGCGAAGGCTCAAACATTGCTGTC
ATGCACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTGGCAGATATGGCGCAACTCAA
GACTATGCCGCAGCAGCCATCCGCGATTGGGCAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTGAGCAAT
30 ATCTTTATGGCAGCCATCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACGGCACAT
CCTATCAAGCGGTCGAGATGGGCGGATCGCATTTGCCGAAAGGAAATCCGCCGTGACGGACAATTTGGCCGATGCG
GCATACGCCAAATACCGTCCCTTACCATTCCCGAAATATCCGTTCAAACCTGGAGCAGCGTTACGGCAAAGAAAAC
ATCACCTCTCAACCGTCCCGCCGTCAAACGGCAAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTA
CCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCACCACCAC
35 TGA

1 MATNDDVKK AATVAIAAY NNGQEBNGFK AGETIYDIDE DGTITKDDAT
51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDAKVKAEE SEIEKLTTKL
101 ADTDAALADT DAALDATNA LNKLGENTTT FAEETKTNIV KIDEKLEAVA
40 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAEE TRQNVDAKVK
201 AAETAAGKAE AAAGTANTAA DKAEVAHAVK TDIKADIATN KDNIAKKANS
251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
301 VSDLRKETRO GLAEQAALSG LFQYVNVGRF NVTAAVGGYK SESAVAIGTG
45 351 FRFTENFAAK AGVAVGTSSG SSAAYHVGVN YEWGSGGGGS DLANDSFIRQ
401 VLDRQHFEFD GKYHLFGSRG ELAERSGHIG LGKIQSHQLG NLMIQQAIAK
451 GNIGYIVRFS DHGHEVHSPF DNHASHSDSD EAGSPVDGFS LYRIHWGDE
501 HHPADGYDGP QGGGYPAPKG ARDIYSYDIK GVAQNIRLNL TDNRSTGQRL
551 ADRFHNAGSM LTQGVGDGFK RATRYSPELD RSGNAEAFN GTADIVKNI I
601 GAAGEIVGAG DAVQGI SEGS NIAVMHGLGL LSTENKMARI NDLADMAQLK
50 651 DYAAAIRDW AVQNPNAAG IEAVSNIFMA APIKIGIGAV RGKYGLGGIT
701 AHPIKRSQMG AIALPKGKSA VSDNFADAAY AKYSPYHSR NIRSNEQRY
751 GKENITSSTV PPSNGKNVKL ADQRHPKTGV PFDGKGFPMF EKHVKYDTLE
801 HHHHHH*

961-741

55 ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCC
GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAC
60 AAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATGGGAGAAAATATAACGACA
TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
GCCGAAGCATTCAACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAAT
GAAGCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAA
GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAA
65 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAAACAGTGCCGACGTTACACCAGAGAAGAGTCTGAC
AGCAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCTT

GCAGAACAAGCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTCCGGTTCATGTAACGGCTGCAGTCGGCGGC
TACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACCTTTGCCGCCAAAGCAGGCGTGGCA
GTCGGCACTTCGTCCGGTCTTCCGCAGCCTACCATGTCCGGCGTCAATTACGAGTGGGGATCCGGAGGGGGTGGTGT
5 GCGGCCGACATCGGTGCGGGGCTTGCAGTGCCTAACCACCGCTCGACCATAAAGACAAAGGTTTGCAGTCTTTG
ACGCTGGATCAGTCCGTTCAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGAAACGGT
GACAGCCTCAATACGGGCAAAATTGAAGAACGACAAGGTCAGCCGTTTCGACTTTATCCGCCAAATCGAAGTGGACGGG
CAGCTCATTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTTCAGACCGAG
CAAATACAAGATTCCGGAGCATTCGGGGAAGATGGTTGCGAAACGCCAGTTCAGAAATCGGGCAGATAGCGGGCGAACAT
10 ACATCTTTTGACAAGCTTCCCGAAGGCGGCAGGGCGACATATCGCGGGACGGCGTTCGGTTCAGACGATGCCGGCGGA
AAACTGACCTACACCATAGATTTCCCGGCCAAGCAGGGAAACGGCAAAATCGAACATTTGAAATCGCCAGAACTCAAT
GTCGACCTGGCCCGCCGATATCAAGCCGGATGGAAAACGCCATGCCGTATCAGCGGTTCCGTCTTTACAACCAA
GCCGAGAAAGGAGTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTGAACACC
GTAAACGGCATAACGCATATCGGCCTTGCAGCAAGCAACTCGAGCACCACCACCACCACCCTGA

15 1 MATNDDVKK AATVAIAAAY NNGQINGFK AGETIYDIDE DGTITKRDAT
51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDKVKAAE SEIEKLTTKL
101 ADTDAALADT DAALDATTNA LNKLGENTTT FAEETKTNIV KIDEKLEAVA
151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
201 AAETAAGKAE AAAGTANTAA DKAEVAKAV TDIKADIATN KDNIKKANS
20 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
301 VSDLRKETRO GLAEQAALSG LFQPYNVGRF NVTAAVGGYK SESAVAIGTG
351 FRFTENFAAK AGVAVGTSSG SSAAYHVGUN YEWGSGGGV AADIGAGLAD
401 ALTAPLDHKD KGLQSLTLDQ SVRKNEKLLK AAQGAEKTYG NGDSLNTGKL
451 KNDKVSRLFDF IRQIEVDGQL ITLESGEFQV YKQSHSALTA FQTEQIQDSE
25 501 HSGKMAKRO FRIGDLAGEH TSFDKLEPEG RATYRGTAFG SDDAGGKLT
551 TIDFAAKQGN GKIEHLKSPE LNVDLAAADI KPDGKRHAVI SGSVLYNQAE
601 KGSYSLGIFG GKAQEVAGSA EVKTVNGIRH IGLAAKQLEH HHHHH*

961-983

ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCC
35 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAC
AAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTTGAATAAATTGGGAGAAAAATATAACGACA
TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
GCCGAAGCATTCAACGATATCGCCGATTCAATGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAAT
GAAGCCAAACAGACGGCCGAAGAAACCAAAACAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAA
40 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAAGTTACCGACATCAA
GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAAACAGTGGCCGACGTGTACACCAGAGAAGAGTCTGAC
AGCAAATTTGTCAGAAATGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCCT
GCAGAACAAGCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTCCGGTTCATGTAACGGCTGCAGTCGGCGGC
45 TACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACCTTTGCCGCCAAAGCAGGCGTGGCA
GTCGGCACTTCGTCCGGTCTTCCGCAGCCTACCATGTCCGGCGTCAATTACGAGTGGGGATCCGGCGGAGGCGGCACT
TCTGCGCCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCAGCAGTA
TCTTACGCCGGTATCAAGAACGAAATGTGCAAGACAGAAGCATGCTCTGTGCCGGTCCGGGATGACGTTGCCGTTACA
GACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCATACAAG
AATTTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATAACAGGACGCGGGGTAGAGGTAGGTATCGTCGACACAGGC
50 GAATCCGTCCGCAGCATATCCTTTCCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAAACCTAT
ACGGCGTATATGCGGAAGGAAGCGCTGAAGACGGAGGCGGTAAAGACATTTGAAGCTTCTTTGACGATGAGGCCGTT
ATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTTGGTCTCCCATATTTAT
GGCGGGCGTTCGGTGGACGGCAGACCTGCAGGCGGTATTTGCCCGGATGCGACGCTACACATAATGAATACGAATGAT
55 GAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTTGGCGTGCATCGTC
AATAACAGTTTTTGAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAGTACCGC
CAAGCGTTGCTCGACTATTCCGGCGGTGATAAAACAGACGAGGGTATCCGCCTGATGCAACAGAGCGATTACGGCAAC
CTGTCCTACCACATCCGTAATAAAAAACATGCTTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACA
TATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTTATCACAGTCCGAGGCGTAGACCGCAGTGGAGAA
AAGTTCAAACGGGAAATGTATGGAGAACC GGTTACAGAACCCTTGAGTATGGCTCCAACCATTCGCGGAATTACTGCC
60 ATGTGGTGCCTGTCCGCACCCATGAAGCAAGCGTCCGTTTCACCCGTACAAACCCGATTCAAATTCGCGGAACATCC
TTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGT
ACCAGTTGCTGACGACGGCTCAGGACATCGGTGCGTGGACAGCAAGTTCCGGTGGGGACTGCTGGATGCG
GGTAAGGCCATGAACGGACCCGCGTCTTTCCGTTCCGGCGACTTTACCGCCGATACGAAAGGTACATCCGATATTGCC
TACTCCTTCCGTAACGACATTTTCAGGCACGGCGGCTGATCAAAAAGGCGGCAGCCAACTGCAACTGCACGGCAAC
65 AACACCTATACGGGCAAAACCATTTATCGAAGCGGTTCCGCTGGTGTGTACGGCAACAACAATCGGATATGCGCGTC
GAAACCAAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTTGCTATCTGGCA
GATACCGACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACGCTGTAC

ACACGTTTGGGCAAACCTGCTGAAAGTGGACGGTACGGCGATTATCGGGCGGCAAGCTGTACATGTCGGCACGCGGCAAG
 GGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCTGAGTGCCGCCAAAATCGGGCAGGATTATTCCTTTC
 TTCACAAACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGCGAC
 ACGCTGTCTTATTATGTCGGTCCGGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCGCGCCCCGCGGTCTG
 5 AAACACGCCGTAGAACAGGGCGGCAGCAATCTGGAAAACCTGATGGTCGAACTGGATGCCCTCCGAATCATCCGCAACA
 CCCGAGACGGTTGAAACTGCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTCGCGCA
 GCGGCAGCCGTACAGCATGCGAATGCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTATGCCGAC
 AGTACCGCCGCCATGCCGATATGCAGGGACGCCGCTGAAAGCCGTATCGGACGGGTGGACCACAACGGCAGCGGT
 CTGCGCGTCATCGCGCAAACCCAAACAGGACGGTGGAAACGTGGGAACAGGGCGGTGTTGAAGGCAAAATGCGCGGCAGT
 10 ACCCAAACCGTCGGCATTTGCCGCGAAAACCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGCAGCACA
 TGGAGCGAAAACAGTGCAAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCGGGCGATATC
 GGCTATCTCAAAGGCCTGTTCTCTACGGACGCTACAAAACAGCATCAGCCGACAGACCGGTGCGGACGAACATGCG
 GAAGGCAGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTTCGCGCAACGGGAGAT
 TTGACGGTTCGAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCCGCCGAAAAGGCAGTGCCTTTGGGCTGG
 15 AGCGGCAACAGCCTCACTGAAGGCACGCTGGTCCGACTCGCGGGTCTGAAGCTGTGCGCAACCCTTGAGCGATAAAGCC
 GTCCTGTTTGCACGGCGGGCGTGGAAACGCGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTTACCGGCGCG
 ACTGCAGCAACCGGCAAGACGGGGGACGCAATATGCCGCACACCCGCTCTGGTTGCCGGCCTGGGCGCGGATGTCGAA
 TTCGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACCGCGGTTCAAACAGTACGGCAACCACAGCGGACGAGTC
 GCGGTAGGCTACCGGTTCTCGAGCACCACCACCACCACCTGA

20
25
30
35
40
45
50

1	MATNDDVKK	AATVAIAAAY	NNGQEINGFK	AGETIYDIDE	DGTITKDAT
51	AADVEADDFK	GLGLKVVVN	LTKFVNENKQ	NVDAKVKAAE	SEIEKLTTKL
101	ADTDAALADT	DAALDATTNA	LNKLGENTIT	FAEETKTNIV	KIDKLEAVA
151	DTVDKHAEBF	NDIADSLDET	NTKADEAVKT	ANEAKQTAEE	TKQNVDAKVK
201	AAETAAGKAE	AAAGTANTAA	DKAEVAKV	TDIKADIATN	KDNIARKANS
251	ADVYTREESD	SKFVRIDGLN	ATTEKLDTRL	ASAEKSIADH	DTRLNGLDKT
301	VSDLRKETRQ	GLAEQAALSG	LFQPYNVGRF	NVTAAVGGYK	SESAVAIGTG
351	FRFTENFAAK	AGVAVGTSSG	SSAAYHVGVN	YEWSSGGGT	SAPDFNAGGT
401	GIGSNSRATT	AKSAAVSYAG	IKNEMCKDRS	MLCAGRDDVA	VTDRDAKINA
451	PPPNLHTGDF	PNPNDAYKNL	INLKPAIRAG	YTGRGVEVGI	VDTGESVGS
501	SFPPELYGRKE	HGYNENYKNY	TAYMRKEAPE	DGGGKDIEAS	FDDEAVIETE
551	AKPTDIRHVK	EIGHIDLVS	IIGGRSVDGR	PAGGLAPDAT	LHIMNTNDET
601	KNEMVAAIR	NAWVKLGERG	VRIVNNSFGT	TSRAGTADLF	QIANSEEQYR
651	QALLDYSGGD	KTDEGIRLMQ	QSDYGNLSYH	IRKNMLFIF	STGNDAQAQP
701	NTYALLPFYE	KDAQGIIITV	AGVDRSGEKF	KREMYGEPGT	EPLEYGSNHC
751	GITAMWCLSA	PYEASVRFTR	TNPIQIAGTS	FSAPIVTGTA	ALLLOKYPWM
801	SNDNLRITLL	TTAQDIGAVG	VDSKFGWGLL	DAGKAMNGPA	SFPFGDFTAD
851	TKGTSDIAYS	FRNDISGTGG	LIKKGGSQLO	LHGNNITYTGK	TIIEGGSVL
901	YGNNKSDMRV	ETKALYNG	AASGSLNSD	GIVYLADTDQ	SGANETVHIK
951	GSLQLDGKGT	LYTRLGKLLK	VDGTAIIGK	LYMSARGKGA	GYLNSTGRRV
1001	PFLSAAKIGQ	DYSFFTNIET	DGGLLASLDS	VEKTAGSEGD	TLSYVRRGN
1051	AARTASAAAH	SAPAGLKHAV	EQGGSNLENL	MVELDASESS	ATPETVETAA
1101	ADRTDMPGIR	PYGATFRAAA	AVQHANAADG	VRIFNSLAAT	VYADSTAHA
1151	DMQGRRLKAV	SDGLDHNGTG	LRVIAQTQOD	GGTWEQGGVE	GKMRGSTQTV
1201	GIAAKTGENT	TAAATLGMGR	STWSSENSANA	KTDSISLIFAG	IRHDAGDIGY
1251	LKGLFSYGRY	KNSISRSTGA	DEHAEGSVNG	TLMQLGALGG	VNVPPAATGD
1301	LTVEGGLRYD	LLKQDAFAEK	GSALGWSGNS	LTEGTLVGLA	GLKLSQPLSD
1351	KAVLFATAGV	ERDLNDRDYT	VTGGFTGATA	ATGKTGARNM	PHTRLVAGLG
1401	ADVEFGNGWN	GLARYSYAGS	KQYGNHSGRV	GVGYRFLEHH	HHHH*

961c-ORF46.1

ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCC
 55 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAC
 AAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 GCCGAAGCATTCAACGATATCGCCGATTGATGGAATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAAT
 60 GAAGCCAAACAGACGGCCGAAGAAACCAAAACAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAA
 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCGCTGCAAAAGTTACCGACATCAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAAACAGTGCCGACGTTGTACACCAGAGAAGAGTCTGAC
 AGCAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATCAGGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCTT
 65 GCAGAACAAAGCCGCTCTCCGGTCTGTTCCAACCTTACAACGTTGGGTGGATCCGGAGGAGGAGGATCAGATTTGGCA
 AACGATTTCTTTTATCCGGCAGGTTCTCGACCGTACGATTTTCGAACCCGACGGGAAATACCACCTATTCGGCAGCAGG
 GGGAACTTGCCGAGCGCAGCGGCCATATCGGATTTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTGATGATCAA

CAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTCCATTCCCCCTTCGAC
 AACCATGCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCCTTGACGGATTTAGCCTTTACCGCATCCATTGGGAC
 GGATACGAACACCATCCC GCCGACGGCTATGACGGGCCACAGGGCGGGCTATCCCCTCCCAAAGGCGGAGGGAT
 ATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCTCAACCTGACCGACAACCGCAGCACCGGACAACGG
 CTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAACGCGCCACCCGATAC
 AGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAACATCATCGGC
 GCGGCAGGAGAAATTTGTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTGCTGTATGCACGGC
 TTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAACTCAAAGACTATGCC
 GCAGCAGCCATCCGCGATTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTGAGCAATATCTTTATG
 GCAGCCATCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACGGCACATCTTATCAAG
 CGGTGCGAGATGGGCGGATCGCATTTGCCGAAAGGAAAATCCGCCGTCAGCGACAATTTGCCGATGCGGCATACGCC
 AAATACCCGTTCCCTTACCATTCCCGAAAATATCCGTTCAAACCTTGAGCAGCGTTACGGCAAAGAAAACATCACCTCC
 TCAACCGTGC CGCCGTCAAAACGGCAAATAATGTCAAACCTGGCAGACCAACGCCACCCGAAAGACAGGCGTACCGTTTGAC
 GGTAAAGGGTTTCCGAATTTTGGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCACCACCACCCTGA

5

10

15

1 MATNDDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKDAT
 51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDAKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATINA LNKLGENTIT FAEBTKTNIV KIDEKLEAVA
 151 DTVDKHAFAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAABV TDIKADIATN KDNIACKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 301 VSDLRKETRQ GLAEQAALSG LFQPVNNGS GGGGSDLAND SFIRQVLDRO
 351 HFEPDGKYLH FGSRGELAER SHHIGLGIQ SHQLGNLMIQ QAAIKGNIGY
 401 IVRFSDHGHE VHSFPDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHPAD
 451 GYDGPQGGY PPKGARDIY SYDIKVAQN IRLNLTNRS TGQRLADRFH
 501 NAGSMLTQGV GDGFKRATRY SPELDRSNA ABAFNGTADI VKNLIGAAGE
 551 IVGAGDAVQG ISEGSNIAMV HGLGLLSTEN KMARINDLAD MAQLKDYAAA
 601 AIRDWAVQNP NAAQIEAVS NIFMAAIPK GIGAVRGKYG LGGITAHPIK
 651 RSQMGAIALP KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI
 701 TSSTVPPSNG KNVKLADQRH PKTGVPFDGK GFFNFEKHVK YDTLEHHHHH
 751 H*

20

25

30

961c-741

ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCCTGACCAAAACCGTCAATGAAAAC
 AAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTTGAATAAATTTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTTAGAAGCCGTGGCTGATACCGTGCACAAGCAT
 GCCGAAGCATTCAACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAA
 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTCCGACGTGTACACCAGAGAAGAGTCTGAC
 AGCAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCTT
 GCAGAACAAGCCGCGCTCTCCGGTCTGTTCCAACTTACAACGTGGGTGGATCCGGAGGGGGTGGTGTGCGCCGCCGAC
 ATCGGTGCGGGCTTGCCGATGCACTAACCACCGCTCGACCATAAAGACAAAGGTTTGCAGTCTTTGACGCTGGAT
 CAGTCCGTGAGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAATTTATGGAACGGTGCAGCCTC
 AATACGGGCAAATTTGAAGAACGACAAGGTGAGCCGTTTCGACTTTTATCCGCCAAATCGAAGTGGACGGGCAGCTCAT
 ACCTTGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCGCCCTTAACCGCCTTTCAGACCGAGCAAATACAA
 GATTCGGAGCATTCGGGAAGATGGTTGCGAAACGCCAGTTTCAAGATCGGCGACATAGCGGGCGAACATACATCTTTT
 GACAAGCTTCCCGAAGGCGGACGGGCGACATATCGCGGGACGGCGTTCCGTTTTCAGACGATGCGGGCGGAAAACCTGACC
 TACACCATAGATTTCCGCCCAAGCAGGGAAACGGCAAATCGAACATTTGAAATCGCCAGAACTCAATGTGACCTG
 GCCGCCCGGATATCAAGCCGGATGGAACCGCCATGCCGTCATCAGCGGTTCCGTCTTTTACAACCAAGCCGAGAAA
 GGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTGAACCGGTAACGGC
 ATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGCACCACCACCACCACCCTGA

35

40

45

50

55

60

65

1 MATNDDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKDAT
 51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDAKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATINA LNKLGENTIT FAEBTKTNIV KIDEKLEAVA
 151 DTVDKHAFAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAABV TDIKADIATN KDNIACKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 301 VSDLRKETRQ GLAEQAALSG LFQPVNNGS GGGGVAADIG AGLADALTAP
 351 LDHKDKGLQS LTLDQSVRKN EKLKLAQGA EKTYGNGDSL NTGKLNKDKV
 401 SRFDLIRQIE VDGQLITLES GEFQVYKQSH SALTAFQTEQ IQDSEHSGKM

451 VAKRQFRIGD IAGEHTSFDK LPEGGRATYR GTAFGSDDAG GKLYTIDFA
 501 AKQGNKIEH LKSPELNVDL AAADIKPDGK RHAVISGSVL YNQAEGSYS
 551 LGIFGGKAQE VAGSAEVKTV NGIRHIGLAA KQLEHHHHHH *

5
 10
 15
 20
 25
 30
 35
 40
 45
 50
 55
 60

961c-983

ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAAC
 AAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 GCCGAAGCATTCAACGATATCGCCGATTCATTTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAACAAAACCGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCCAAA
 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAAGTTACCGACATCAAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAAACAGTGGCCGACGTGTACACCAGAGAAGAGTCTGAC
 AGCAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCCTT
 GCAGAACAAGCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGGATCCGGCCGAGGCCGCACTTCTGCGCCC
 GACTTCAATGCAGGCCGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCAGCAGTATCTTACGCC
 GGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTGCGGTTACAGACAGGGAT
 GCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTCCAAACCCAAATGACGCATACAAGAATTTGATC
 AACCTCAAACCTGCAATTGAAGCAGGCTATAACAGGACCGGGGTAGAGGTAGGTATCGTCGACACAGGCCAATCCGTC
 GGCAGCATATCCTTTCCCGAAGCTGTATGGCAGAAAAGAACACGGCTATAACGAAAAATTACAAAAACTATACGGCGTAT
 ATCGCGAAGGAAGCGCCTGAAGACGGAGCGGTAAGACATTGAAGCTTCTTTTCGACGATGAGGCCGTTATAGAGACT
 GAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTTGGTCTCCCATATTATTGGCGGGCGT
 TCCGTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACGAATGATGAAACCAAG
 AACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTTGGCGTGCATCGTCAATAACAGT
 TTTGGAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAAATTCGGAGGAGCAGTACCGCCAAGCGTTG
 CTCGACTATTCCGGCGGTGATAAAAACAGACGAGGGTATCCGCCTGATGCAACAGAGCGATTACGGCAACCTGTCCTAC
 CACATCCGTAATAAAAACATGCTTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACATATGCCCTA
 TTGCCATTTTATGAAAAGACGCTCAAAAAGGCATTATCACAGTGCAGGCCGTAGACCGCAGTGGAGAAAAGTTCAA
 CGGGAAATGTATGGAGAACCAGGTTACAGAACCCTTGAGTATGGCTCCAACCATTGCGGAATTACTGCCATGTGGTGC
 CTGTGCGCACCCATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATGCGCGAACATCCTTTTCCGCA
 CCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGTACCACGTTG
 CTGACGACGGCTCAGGACATCGGTGCAGTCCGGCTGGACAGCAAGTTCCGGCTGGGGACTGCTGGATGCGGGTAAGGCC
 ATGAACGGACCCGCGTCTTTCCGTTCCGGCGACTTTACCGCCGATACGAAAGGTACATCCGATATTGCCTACTCCTTC
 CGTAACGACATTTTCAGGCACGGCGGCTGATCAAAAAGGCGGCAGCCAACTGCAACTGCACGGCAACAACACCTAT
 ACGGGCAAACCATTTATCGAAGGCGGTTCCGCTGGTGTGTACGGCAACAACAATCGGATATGCGCGTCGAAACCAA
 GGTGCGCTGATTTATAACGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTTGCTATCTGGCAGATACCGAC
 CAATCCGGCGCAAACGAAACCGTACACATCAAAGGCGAGTCTGCAGCTGGACGGCAAAGGTACGCTGTACACAGCTTG
 GGCAAACCTGCTGAAAAGTGGACGGTACGGCGATTATCGGGCGCAAGCTGTACATGTGCGCACGGCGCAAGGGGGCAGGC
 TATCTCAACAGTACCGGACGACGTGTCCCTTCTGAGTGCAGCCAAATCGGGCAGGATTTATCTTTCTTCACAAAC
 ATCGAAACCGACGGCGGCTGCTGGCTTCCCTGACAGCGTCAAAAAACAGCGGGCAGTGAAGGGCAGACGCTGTCC
 TATTATGTCCGTGCGCGCAATGCGGCACGGACTGCTTCCGGCAGCGGCACATTCGCGCCCGCCGGTCTGAAACACGCC
 GTAGAACAGGGCGGCAGCAATCTGGAACCTGATGGTCAACTGGATGCCCTCCGAATCATCCGCAACACCCGAGACG
 GTTGAACCTGCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTCCGCGCAGCGGCAGCC
 GTACAGCATGCGAATGCCCGGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTATGCCGACAGTACCGCC
 GCCCATGCCGATATGCAGGGACGCCGCTGAAAGCCGATCGGACGGGTTGGACCACAACGGCACGGGTCTGCGCGTC
 ATCGCGCAAACCCAACAGGACGGTGGAAACGTGGGAACAGGGCGGTGTTGAAGGCAAATGCGCGGCAGTACCCAAACC
 GTCGGCATTGCCGCGAAAACCGGCGAAAATACGACAGCAGCCGACACTGGGCATGGGACGCAGCACATGGAGCGAA
 AACAGTCAAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCGGGCGATATCGGCTATCTC
 AAAGGCTGTCTCTACGGACGCTACAAAACAGCATCAGCCGACGACCCGGTGGCGACGAACATGCGGAAGGCAGC
 GTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGGCCGCAACGGGAGATTTGACGGTC
 GAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAAGGCAGTGTCTTGGGCTGGAGCGGCAAC
 AGCCTCACTGAAGGCACGCTGGTCCGACTCGCGGGTCTGAAGCTGTGCAACCCTTGAGCGATAAAGCCGTCCTGTTT
 GCAACGGCGGGCGTGGAAACGCGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTTACCGGCGGACTGCAGCA
 ACCGGCAAGACGGGGGACGCAATATGCCGCACACCCGCTGTTGGTGGCCGGCTGGGGCGGGATGTGCAATTCGGCAAC
 GGCTGGAACGGCTTGGCACGTTACAGCTACGCCGGTTCCAAACAGTACGGCAACCACAGCGGACGAGTCCGGCTAGGC
 TACCGGTTCTCGAGCACCACCACCACCACCCTGA

1 MATNDDVKK AATVAIAAY NNGQEINGFK AGETIYDIDE DGTITKKDAT
 51 AADVEADDFK GLGLKRVVN LTKTVNENKQ NVDKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATNA LNKLGENTT FAEETKTNIV KIDEKLEAVA
 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAARKV TDIKADIATN KDNIKAKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT

5
10
15
20

301	VSDLRKETRO	GLAEQAALSG	LFQPYNVGGS	GGGGTSAPDF	NAGGTGIGSN
351	SRATTAKSAA	VSYAGIKNEM	CKDRSMLCAG	RDDVAVTDRD	AKINAPPPNL
401	HTGDFPNPND	AYKNLNLKLP	AIEAGYTGRG	VEVGIVDTGE	SVGSISFPEL
451	YGRKEHGYNE	NYKNYTAYMR	KEAPEDGGGK	DIEASFDDEA	VIETEAKPTD
501	IRHVKEIGHI	DLVSHIIGGR	SVDGRPAGGI	APDATLHIMN	TNDETKNEMM
551	VAAIRNAWVK	LGERGVRIVN	NSFGTTSRAG	TADLFQIANS	EEQYRQALLD
601	YSGGDKTDEG	IRLMQQSDYG	NLSYHIRNKN	MLFIFSTGND	AQAQPNTYAL
651	LPFYEKDAQK	GIITVAGVDR	SGEKFKREMY	GEPGTEPLEY	GSNHCGITAM
701	WCLSAPYEAS	VRFTRTNPIQ	IAGTSFSAPI	VTGTAALLLQ	KYPWMSNDNL
751	RTTLLTTAQD	IGAVGVDSKF	GWGLLDAGKA	MNGPASFPFG	DF'TADTKGTS
801	DIAYSFRNDI	SGTGGLIKKG	GSOLQLHGNN	TYTGKTIIEG	GSLVLYGNK
851	SDMRVETKGA	LIYNGAASGG	SLNSDGIVYL	ADTDQSGANE	TVHIKGSLOL
901	DGKGTLYTRL	GKLLKVDGTA	IIGGKLYMSA	RGKGAGYLNS	TGRRVPFLSA
951	AKIGQDYSFF	TNIETDGGLL	ASLDSVEKTA	GSEGDLSY	VRRGNAARTA
1001	SAAAHSAFAG	LKHAVEQGGG	NLENLMVELD	ASESSATPET	VETAAADRTD
1051	MFGIRPYGAT	FRAAAVQHA	NAADGVRIFN	SLAATVYADS	TAAHADMOGR
1101	RLKAVSDGLD	HNGTGLRVIA	QTQODGGTWE	QGGVEGKMRG	STQTVGIAAK
1151	TGENTTAAAT	LGMGRSTWSE	NSANAKTDSI	SLFAGIRHDA	GDIGYLKGLF
1201	SYGRYKNSIS	RSTGADEHAE	GSVNGTLMQL	GALGGVNVPF	AATGDLTVEG
1251	GLRYDLLKQD	AFAEKGSALG	WSGNSLTEGT	LVGLAGLKLS	QPLSDKAVLF
1301	ATAGVERDLN	GRDYTVTGGF	TGATAATGKT	GARNMPHTRL	VAGLGADVEF
1351	GNGWNLARY	SYAGSKQYGN	HSGRVGVGYR	FLEHHHHH*	

25

961cL-ORF46.1

30
35
40
45
50
55

ATGAAACACTTCCATCCAAAGTACTGACCACAGCCATCCTTGCCACTTTCGTAGCGGCGCACTGGCAGCCACAAAC
GACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTCAA
GCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGATGTTGAAGCC
GACGACTTTAAAGGCTCTGGGTCGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAACGTC
GATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCA
GATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAAGAG
ACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTC
AACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAATGAAGCCAAACAG
ACGGCCGAAGAAACCAACAAACAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAAGCCGAAGCTGCC
GCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCT
ACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGCAGCAGAAATTTGTC
AGAATTGATGGTCTGAACGCTACTACCAGAAAATTGGACACACGCTTTGGCTTCTGCTGAAAATCCATTGCCGATCAC
GATACTCGCCTGAACGGTTTGGATAAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCTTGCAGAACAGCC
GCGCTCTCCGGTCTGTCCAACCTTACAACGTGGGTGGATCCGGAGGAGGAGGATCAGATTTGGCAAACGATTCCTTTT
ATCCGGCAGGTTCTCGACCGTCAGCATTTGCAACCCGACGGGAAATACCACCTATTCGGCAGCAGGGGGGAACTTGCC
GAGCGCAGCGGCCATATCGGATTTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTGATGATTC AACAGGCGGCCATT
AAAGGAAATATCGGCTACATTTGCTCCGCTTTTCCGATCACGGGCACGAAGTCCATTCCTCCCTTCGACAACCATGCCTCA
CATTCGGATTCGTGATGAAGCCGGTAGTCCCCTTGACGGATTTAGCCTTTACCGCATCCATTGGGACGGATACGAACAC
CATCCCGCCGACGGCTATGACGGGCCACAGGGCGGGCTATCCCGCTCCCAAAGGCGGAGGGATATATACAGCTAC
GACATAAAAGGCGTTGCCAAAATATCCGCCTCAACCTGACCGACAACCGCAGCACCAGGACAACGGCTTGCCGACCGT
TTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGGCAGGATTCAAACCGGCCACCCGATACAGCCCCGAGCTG
GACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAACATCATCGGCGCGGCAGGAGAA
ATTGTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTTGCTGTGATGCACGGCTTGGGCTGTGCTT
TCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAACTCAAAGACTATGCCGACGAGCCATC
CGCGATTGGGCAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTGAGCAATATCTTTATGGCAGCCATCCCC
ATCAAAGGGATTGGAGCTGTTCGGGGAAAATACGGCTTGGGCGGCATCACGGCACATCCTATCAAGCGGTGCGAGATG
GGCGGATCGCATTGCCGAAAGGGAAAATCCGCCGTGAGCGACAATTTTGCCGATGCCGCATACGCCAAATACCCGTCC
CCTTACCATTCCCGAAATATCCGTTCAAACCTTGGAGCAGCGTTACGGCAAAGAAAACATCACCTCCTCAACCGTCCCG
CCGTCAAACGGCAAATAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTACCGTTTGACGGTAAAGGGTTT
CCGAATTTTGAGAAGCACGTGAAATATGATACGTAACCTCGAG

60

65

1	MKHFPSKVL	TAILATFCSG	ALAATNDDDV	KKAATVAIAA	AYNNGQEING
51	FKAGETIYDI	DEDGTITKGD	ATAADVEADD	FKGLGLKKVV	TNLTKTVNEN
101	KQNVDAKVA	AESEIEKLT	KLADTDAALA	DTDAALDATT	NALNKLGENI
151	TTFAEETKTN	IVKIDEKLEA	VADTVDKHAE	AFNDIADSLD	ETNTRADEAV
201	KTANEAKQTA	EETKQNVDAK	VKAAETAAGK	AEAAAGTANT	AADKAEAVAA
251	KVTDIKADIA	TNKDNIAKKA	NSADVYTREE	SDSKFVRIDG	LNATTEKLDT
301	RLASAEKSIA	DHDTRLNGLD	KTVSDLRKET	RQGLAEQAAL	SGLFQPYNVG
351	GSGGGGSDLA	NDSFIRQVLD	RQHFEPDGKY	HLFGSRGELA	ERSGHIGLGLK
401	IQSHQLGNLM	IQQAIAKONI	GYIVRFSDHG	HEVHSPFDNH	ASHSDSDEAG
451	SPVDGFSLYR	IHWGDEYHHP	ADGYDGPQGG	GYPAPKGARD	IYSYDIKQVA
501	QNIRLNLTDN	RSTGQRLADR	FHNAGSMLTQ	GVGDGFKRAT	RYSPELDRSG

551 NAAEAFNGTA DIVKNIIGAA GEIVGAGDAV QGISEGSNIA VMHGLGLLST
 601 ENKMARINDL ADMAQLKDYA AAAIRDWAVQ NPNAAQGIEA VSNIFMAAIP
 651 IKGIGAVRGK YGLGGITAHF IKRSQMGAlA LPKGKSAVSD NFADAAYAKY
 701 PSPYHSRNIR SNLEORYGKE NITSSTVPPS NGKNVKLADQ RHPKTGVPPD
 751 GKGFPNFEKH VKYDT*

961cL-741

10 ATGAAACACTTTCCATCCAAAGTACTGACCACAGCCATCCTTGCCACTTTCTGTAGCGGCGCACTGGCAGCCACAAAC
 GACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTCAA
 GCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGATGTTGAAGCC
 GACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAAACGTC
 GATGCCAAAGTAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCA
 15 GATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAAGAG
 ACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTC
 AACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCAATGAAGCCAAACAG
 ACGGCCGAAGAAACCAAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAAGCCGAAGCTGCC
 GCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAAGTTACCGACATCAAAGCTGATATCGCT
 20 ACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCAGGACGTGTACACCAGAGAAGAGTCTGACAGCAAATTTGTC
 AGAATTTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTTGGCTTCTGCTGAAAAATCCATTGCCGATCAC
 GATACTCGCCTGAACGGTTTGGATAAAAACAGTGTGAGACCTGCGCAAAGAAACCCGCCAAGGCCCTTGCAGAACAGCC
 GCGCTCTCCGGTCTGTTCACCTTACAACGTTGGTGGATCCGGAGGGGGTGGTGTGCGCCGCCGACATCGGTGCCGGG
 CTTGCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGCAGTCTTTGACGCTGGATCAGTCCGTCAGG
 25 AAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAAGTTATGGAAACGGTGACAGCCTCAATACGGGCAA
 TTGAAGAACGACAAGGTCAGCCGTTTCGACTTTATCCGCCAAATCGAAGTGGACGGGCAGCTCATTACCTTGGAGAGT
 GGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTCAGACCGAGCAAATACAAGATTCCGGAGCAT
 TCCGGGAAGATGGTTGCGAAACGCCAGTTCAGAAATCGGGCAGATAGCGGGCGAACATACATCTTTTGACAAGCTTCCC
 GAAGGCCGGCAGGGCGACATATCGCGGGACGGCGTTCGGTTTCAGACGATGCCGGCGGAAAACCTGACCTACACCATAGAT
 30 TTCGCCGCCAAGCAGGGAAACGGCAAATCGAACATTTGAAATCGCCAGAACTCAATGTGACCTGGCCGCCCGCCGAT
 ATCAAGCCGGATGGAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTACAACCAAGCCGAGAAAGGCAGTTACTCC
 CTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTGAACCCGTAACGGCATAACCCATATC
 GGCCTTGCCGCCAAGCAACTCGAGCACCACCACCACCACCCTGA

35 1 MKHFPSKVL TAILATFCSG ALAATNDDDV KKAATVAIAA AYNNGQEING
 51 FKAGETIYDI DEDGTITKRD ATAADVEADD FKGLGLKVV TNLTKTVNEN
 101 KQNVDAKVA AESEIEKLT KLADTDAALA DTDALDATT NALNKLGENI
 151 TTFABETKTN IVKIDKLEA VADTVDKHAE AFNDIADSLD ETNTKADEAV
 201 KTANEAKQTA BETKQNVDAK VKAAETAAGK AEAAGTANT AADKAEAVAA
 251 KVTDIKADIA TNKDNIAKKA NSADVYTRIE SDSKFVRIDG LNATTEKLDT
 40 301 RLSAEKSIA DHDTRLNGLD KTVSDLRKET RQGLAEQAAL SGLFQFYNVG
 351 GSGGGGVAAD IGAGLADALT APLDHDKGL QSLTLDQSVR KNEKLLKLAQ
 401 GAERTYNGD SLNTGKLNKND KVSRFDFIRQ IEVDGQLITL ESGEFQVYKQ
 451 SHSALTAFQT EQIQDSEHSG KMKVAKRQFRI GDIAGEHTSF DKLPEGGRAT
 501 YRGTAFGSD AGGKLTYYTID FAAKQNGKI EHLKSPELNV DLAAADIKPD
 45 551 GKRHAVISGS VLYNQAEKGS YSLGIFGGKA QEVAGSAEVK TVNGIRHIGL
 601 AAKQLEHHHH HH*

961cL-983

50 ATGAAACACTTTCCATCCAAAGTACTGACCACAGCCATCCTTGCCACTTTCTGTAGCGGCGCACTGGCAGCCACAAAC
 GACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTCAA
 GCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGATGTTGAAGCC
 GACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAAACGTC
 GATGCCAAAGTAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCA
 55 GATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAAGAG
 ACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTC
 AACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCAATGAAGCCAAACAG
 ACGGCCGAAGAAACCAAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAAACTGCAGCAGGCAAAGCCGAAGCTGCC
 GCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAAGTTACCGACATCAAAGCTGATATCGCT
 60 ACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCAGGACGTGTACACCAGAGAAGAGTCTGACAGCAAATTTGTC
 AGAATTTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTTGGCTTCTGCTGAAAAATCCATTGCCGATCAC
 GATACTCGCCTGAACGGTTTGGATAAAAACAGTGTGAGACCTGCGCAAAGAAACCCGCCAAGGCCCTTGCAGAACAGCC
 GCGCTCTCCGGTCTGTTCACCTTACAACGTTGGTGGATCCGGCGGAGGGCGGCCTTCTGCGCCCGACTTCAATGCA
 GCGGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCAGCAGTATCTTACCGCGGTATCAAGAAC
 65 GAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTGCGGTTACAGACAGGGATGCCAAAATCAAT
 GCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAACCCCAATGACGCATACAAGAATTTGATCAACCTCAAACCT
 GCAATTTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGACACAGGCGAATCCGTCGGCAGCATATCC

5 TTTCCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAAACTATACGGCGTATATGCGGAAGGAA
 GCGCCTGAAGACGGAGGCGGTAAAGACATGAAGCTTCTTTTCGACGATGAGGCCGTTATAGAGACTGAAGCAAAGCCG
 ACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTTGGTCTCCCATATATTTGGCGGGCGTTCCGTTGGACGGC
 10 AGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACGAATGATGAAACCAAGAACGAAATGATG
 GTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGCGCATCGTCAATAACAGTTTTGGAACAACA
 TCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAGTACCGCCAAGCGTTGCTCGACTATTC
 GCGGTTGATAAAAACAGACGAGGGTATCCGCCCTGATGCAACAGAGCGATTACGGCAACCTGTCTACCACATCCGTAAT
 AAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACATATGCCCTATTGCCATTTTAT
 15 GAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCAGGCGTAGACCGCAGTGGAGAAAAGTTCAAACGGGAAATGTAT
 GGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCAATTGCGGAATTACTGCCATGTGGTGCCTGTCCGCACCC
 TATGAAGCAAGCGTCCGTTTCACCCGTACAAACCCGATTCAAATTGCCGGAACATCCTTTTCCGCACCCATCGTAACC
 GGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGTACCACGTTGCTGACGACGGCT
 CAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCGGCTGGGGACTGCTGGATGCGGGTAAGGCCATGAACGGACCC
 GCGTCTTTCCGTTCCGGCGACTTTACC GCCGATACGAAAGGTACATCCGATATTGCCTACTCCTTCCGTAACGACATT
 20 TCAGGCACGGGCGGCCTGATCAAAAAGGCGGCAGCCAACTGCAACTGCACGGCAACAACACCTATACGGGCAAAACC
 ATTATCGAAGGCGGTTCCGTTGGTGTGTGACGGCAACAACAATCGGATATGCGCGTCGAAACCAAGGTGCGCTGATT
 TATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTGTCCTATCTGGCAGATACCGACCAATCCGGCGCA
 AACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAAGGTACGCTGTACACACGTTTGGGCAAACTGCTG
 AAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTACATGTCGGCAGCGGCAAGGGGGCAGGCTATCTCAACAGT
 25 ACCGGACGACGTGTTCCCTTCCCTGAGTCCCGCAAATCGGGCAGGATTATTTCTTTCACAAACATCGAAACCGAC
 GCGGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGCGACACGCTGTCCTATTATGTCCGT
 CGCGGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCGCGCCCGCCGCTCTGAAACACGCCGTAGAACAGGGC
 GGCAGCAATCTGGAAAACCTGATGGTTCGAACTGGATGCCTCCGAATCATCCGCAACACCCGAGACGGTTGAAACTGCG
 GCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTCCGCGCAGCGGCAGCCGTACAGCATGCG
 30 AATGCCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTATGCCGACAGTACCGCCGCCATGCCGAT
 ATGCAGGGACGCCGCTGAAAGCCGTATCGGACGGGTTGGACCACAACGGCACGGGTCTGCGCGTCATCGCGCAAACC
 CAACAGGACGGTGGAAACGTGGGAACAGGGCGGTTGAAAGGCAAAATGCGCGGCAGTACCCAAACCGTCCGCATTGCC
 CGGAAAACCGCGGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGCAGCACATGGAGCGAAAACAGTGCAAAT
 GCAAAAACCGACAGCATTAGTCTGTGTTGTCAGGCATACGGCAGATGCGGGCGATATCGGCTATCTCAAAGGCCTGTT
 35 TCCTACGGACGCTACAAAACAGCATCAGCCGACACCGGTGCGGACGAACATGCGGAAGGCAGCGTCAACGGCAGC
 CTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTCCGCAACGGGAGATTGACGGTTCGAAGGCGGTCTG
 CGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAGGCAGTGCCTTTGGGCTGGAGCGGCAACAGCCTCACTGAA
 GGCACGCTGGTCCGACTCGCGGGTCTGAAGCTGTGCGCAACCCCTTGAAGCGATAAAGCCGTCCTGTTGCAACGGCGGGC
 GTGGAACCGGACCTGAACGGACCGACTACACGGTAACGGGCGGCTTTACCGGCGCGACTGCAGCAACCGGCAAGACG
 GGGGCACGCAATATGCCGCACACCCGCTCTGGTTGCCGGCCTGGGCGCGGATGTGCAATTCGGCAACGGCTGGAACGGC
 TTGGCACGTTACAGCTACGCCGGTTCCAAACAGTACGGCAACCACAGCGGACGAGTCCGGCTAGGCTACCGGTTCTGA
 CTCGAG

40 1 MKHFPSKVL TAILATFCSG ALAATNDDDV KKAATVAIAA AYNNGQEING
 51 FKAGETIYDI DEDGTITK KD ATAADVEADD FKGLGLKVV TNLTKTVNEN
 101 KQNVDAKVA AESEIEKLT KLADTDAALA DTDALDATT NALNKLGENI
 151 TTFAEETKTN IVKIDEKLEA VADTVDKHAE AFNDIADSLD ETNTKADEAV
 201 KTANEAKQTA EETKQNVDAK VKAAETAAGK AEAAAGTANT AADKAEAVAA
 251 KVTDIKADIA TNKDNIAKKA NSADVYTREE SDSKFVRIDG LNATTEKLDI
 45 301 RLASAEKSIA DHDTRLNGLD KTVSDLRKET RQGLAEQAAL SGLFQPYNVG
 351 GSGGGGTSAP DFNAGGTGIG SNSRATTAKS AAVSYAGIKN EMCKDRSMLC
 401 AGRDDVAVTD RDAKINAPP NLHTGDFPNP NDAYKNLINL KPAIEAGYTG
 451 RGVEVGIVDT GESVGSISFP ELYGRKEHGY NENYKNYTAY MRKEAPEDGG
 501 GKDIEASFDD EAVIETEAKP TDIRHVKEIG HIDLVSHIIG GRSVDGRPAG
 50 551 GIAPDATHI MNTNDETKNE MMVAAIRNAW VKLGERGVRI VNNSFGTTSR
 601 AGTADLFQIA NSEEQYRQAL LDYSGGDKTD EGIRLMQOSD YGNLSYHIRN
 651 KNMLFIFSTG NDAQAQPNTY ALLPFYEKDA QKGIITVAGV DRSGEKFRE
 701 MYGEPGTEPL EYGSNHCGIT AMWCLSAPYE ASVRFTRTNP IQIAGTSFSA
 751 PIVTGTALL LQKYPWMSND NLRTLLTTA QDIGAVGVDS KFGWLLDAG
 55 801 KAMNGPASEP FGDEFTADTKG TSDIAYSFRN DLSGTGLIK KGGSQLQLHG
 851 NNTYTGKTI EGGSLVLYGN NKSDMRVETK GALIYNGAAS GGSLNSDGIV
 901 YLADTDQSGA NETVHIKGS LLDGKGTLYT RLGKLLKVDG TAIIGGKLYM
 951 SARGKGAGYL NSTGRRVPFL SAAKIGQDYS FFTNIETDGG LLASLDSVEK
 1001 TAGSEGDILS YVRRGNAAR TASAAHSAP AGLKHAVEQG GSNLENLMVE
 60 1051 LDASESATP ETVETAAADR TDMPGIRPYG ATFRAAAVQ HANAADGVRI
 1101 FNSLAATVYA DSTAAHADMQ GRRLKAVSDG LDHNGTGLRV IAQTQDGGT
 1151 WEQGGVEGKM RGSTQTVGIA AKTGENTTAA ATLGMRSTW SENSANAKTD
 1201 SISLFIAGIRH DAGDIGYLKG LFSYGRYKNS ISRSTGADEH AEGSVNGTLM
 1251 QLGALGGVNV PFAATGDLTV EGGLRYDLLK QDAFAEKGS LGWSGNSLTE
 65 1301 GTLVGLAGLK LSQPLSDKAV LFATAGVERD LNDRDYTVT GFTGATAATG
 1351 KTGARNMPHT RLVAGLGADV EFGNGWNGLA RYSYAGSKQY GNHSGRVGVG
 1401 YRF*

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention. For instance, the use of proteins from other strains is envisaged [e.g. see WO00/66741 for polymorphic sequences for ORF4, ORF40, ORF46, 225, 235, 287, 519, 726, 919 and 953].

5

EXPERIMENTAL DETAILS

Cloning strategy and oligonucleotide design

Genes coding for antigens of interest were amplified by PCR, using oligonucleotides designed on the basis of the genomic sequence of *N. meningitidis* B MC58. Genomic DNA from strain 2996 was always used as a template in PCR reactions, unless otherwise specified, and the amplified fragments were cloned in the expression vector pET21b+ (Novagen) to express the protein as C-terminal His-tagged product, or in pET-24b+(Novagen) to express the protein in 'untagged' form (e.g. ΔG 287K).

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

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

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

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

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

The melting temperatures of the selected oligonucleotides were usually 65-70°C for the whole oligo and 50-60°C for the hybridising region alone.

Oligonucleotides were synthesised using a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2.0ml 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 centrifuged and the pellets resuspended in water.

		Sequences	Restriction site
fu (961)-	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCC	BamHI

741(MC58)-His	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAGGC	XhoI
fu (961)-983-His	Fwd	CGCGGATCC - GGCGGAGGCGGCACTT	BamHI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
fu (961)- Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTTGGCAAACGATTC	BamHI
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu (961 c-L)- 741(MC58)	Fwd	CGCGGATCC -GGAGGGGGTGGTGTTCG	BamHI
	Rev	CCCGCTCGAG-TTATTGCTTGGCGGCAAG	XhoI
fu (961c-L)-983	Fwd	CGCGGATCC - GGCGGAGGCGGCACTT	BamHI
	Rev	CCCGCTCGAG-TCAGAACCGGTAGCCTAC	XhoI
fu (961c-L)- Orf46.1	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTTGGCAAACGATTC	BamHI
	Rev	CCCGCTCGAG-TTACGTATCATATTTACGTGC	XhoI
fu-(ΔG287)-919- His	Fwd	CGCGGATCCGGTGGTGGTGGT- CAAAGCAAGAGCATCCAAACC	BamHI
	Rev	CCCAAGCTT-TTCGGGCGGTATTCGGGCTTC	HindIII
fu-(ΔG287)-953- His	Fwd	CGCGGATCCGGTGGTGGTGGT- GCCACCTACAAAGTGGAC	BamHI
	Rev	GCCCAAGCTT-TTGTITGGCTGCCTCGAT	HindIII
fu-(ΔG287)-961- His	Fwd	CGCGGATCCGGTGGTGGTGGT-ACAAGCGACGACG	BamHI
	Rev	GCCCAAGCTT-CCACTCGTAATTGACGCC	HindIII
fu-(ΔG287)- Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTTGGCAAACGATTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu-(ΔG287-919)- Orf46.1-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT- TCAGATTTGGCAAACGATTC	HindIII
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu-(ΔG287- Orf46.1)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT- CAAAGCAAGAGCATCCAAACC	HindIII
	Rev	CCCGCTCGAG-CGGGCGGTATTCGGGCTT	XhoI
fu ΔG287(394.98)- ...	Fwd	CGCGGATCCGCTAGC-CCCGATGTTAAATCGGC	NheI
	Rev	CGGGGATCC-ATCCTGCTCTTTTTGCCGG	BamHI
fu Orf1-(Orf46.1)- His	Fwd	CGCGGATCCGCTAGC-GGACACACTTATTTCCGCATC	NheI
	Rev	CGCGGATCC-CCAGCGGTAGCCTAATTTGAT	
fu (Orf1)-Orf46.1- His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTTGGCAAACGATTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu (919)-Orf46.1- His	Fwd1	GCGGCGTCGACGGTGGCGGAGGCACTGGATCCTCAG	SalI
	Fwd2	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTC	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
Fu (orf46)-287-His	Fwd	CGGGGATCCGGGGGGCGGCGGTGGCG	BamHI
	Rev	CCCAAGCTTATCCTGCTCTTTTTGCCGGC	HindIII
Fu (orf46)-919-His	Fwd	CGCGGATCCGGTGGTGGTGGTCAAAGCAAGAGCATCCA AACC	BamHI
	Rev	CCCAAGCTTTCGGGCGGTATTCGGGCTTC	HindIII

-37-

Fu (orf46-919)-287-His	Fwd	CCCCAAGCTTGGGGGCGGCGGTGGCG	HindIII
	Rev	CCCGCTCGAGATCCTGCTCTTTTTTGCCGGC	XhoI
Fu (orf46-287)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGTCAAAGCAAGAGCAT CCAAACC	HindIII
	Rev	CCCGCTCGAGCGGGCGGTATTCGGGCTT	XhoI
(Δ G741)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(Δ G741)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(Δ G741)-983-His	Fwd	GCGGCCTCGAG- GGATCCGGCGGAGGGCGGCACTTCTGCG	XhoI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
(Δ G741)-orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTC	Sall
	Fwd2	GCGGCCTCGAGCGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACAGTGC	XhoI
(Δ G983)-741(MC58)-His	Fwd	GCGGCCTCGAG-GGATCCGGAGGGGGTGGTGTGCGCC	XhoI
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAG	XhoI
(Δ G983)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(Δ G983)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(Δ G983)-Orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTC	Sall
	Fwd2	GCGGCCTCGAGCGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACAGTGC	XhoI

* This primer was used as a Reverse primer for all the C terminal fusions of 287 to the His-tag.

⁵ Forward primers used in combination with the 287-His Reverse primer.

NB – All PCR reactions use strain 2996 unless otherwise specified (e.g. strain MC58)

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

Preparation of chromosomal DNA templates

N.meningitidis strains 2996, MC58, 394.98, 1000 and BZ232 (and others) were grown to exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in 5ml buffer (20% w/v sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10ml of lysis solution (50mM NaCl, 1% Na-Sarkosyl, 50 μ g/ml Proteinase K), and the suspension incubated at 37°C for 2 hours. Two

-38-

phenol extractions (equilibrated to pH 8) and one CHCl_3 /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes of ethanol, and collected by centrifugation. The pellet was washed once with 70%(v/v) ethanol and redissolved in 4.0ml TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The
5 DNA concentration was measured by reading OD_{260} .

PCR Amplification

The standard PCR protocol was as follows: 200ng of genomic DNA from 2996, MC581000, or BZ232 strains or 10ng of plasmid DNA preparation of recombinant clones were used as template in the presence of 40 μM of each oligonucleotide primer, 400-800 μM dNTPs
10 solution, 1x PCR buffer (including 1.5mM MgCl_2), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, Boehringer Mannheim ExpandTM Long Template).

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

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

Digestion of PCR fragments and of the cloning vectors

The purified DNA corresponding to the amplified fragment was digested with the appropriate restriction enzymes for cloning into pET-21b+, pET22b+ or pET-24b+. Digested
25 fragments were purified using the QIAquick PCR purification kit (following the manufacturer's instructions) and eluted with either H_2O or 10mM Tris, pH 8.5. Plasmid vectors were digested with the appropriate restriction enzymes, loaded onto a 1.0% agarose gel and the band corresponding to the digested vector purified using the Qiagen QIAquick Gel Extraction Kit.

Cloning

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

- 5 Recombinant plasmid was transformed into competent *E.coli* DH5 or HB101 by incubating the ligase reaction solution and bacteria for 40 minutes on ice, then at 37°C for 3 minutes. This was followed by the addition of 800µl LB broth and incubation at 37°C for 20 minutes. The cells were centrifuged at maximum speed in an Eppendorf microfuge, resuspended in approximately 200µl of the supernatant and plated onto LB ampicillin (100mg/ml) agar.
- 10 Screening for recombinant clones was performed by growing randomly selected colonies overnight at 37°C in 4.0ml of LB broth + 100µg/ml ampicillin. Cells were pelleted and plasmid DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions. Approximately 1µg of each individual miniprep was digested with the appropriate restriction enzymes and the digest loaded onto a 1-1.5% agarose gel
- 15 (depending on the expected insert size), in parallel with the molecular weight marker (1kb DNA Ladder, GIBCO). Positive clones were selected on the basis of the size of insert.

Expression

- After cloning each gene into the expression vector, recombinant plasmids were transformed into *E.coli* strains suitable for expression of the recombinant protein. 1µl of each construct
- 20 was used to transform *E.coli* BL21-DE3 as described above. Single recombinant colonies were inoculated into 2ml LB+Amp (100µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100µg/ml) in 100ml flasks, to give an OD₆₀₀ between 0.1 and 0.2. The flasks were incubated at 30°C or at 37°C in a gyratory water bath shaker until OD₆₀₀ indicated exponential growth suitable for induction of expression (0.4-0.8 OD). Protein
 - 25 expression was induced by addition of 1.0mM IPTG. After 3 hours incubation at 30°C or 37°C the OD₆₀₀ was measured and expression examined. 1.0ml of each sample was centrifuged in a microfuge, the pellet resuspended in PBS and analysed by SDS-PAGE and Coomassie Blue staining.

Purification of His-tagged proteins

- 30 Various forms of 287 were cloned from strains 2996 and MC58. They were constructed with a C-terminus His-tagged fusion and included a mature form (aa 18-427), constructs with

deletions ($\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$) and clones composed of either B or C domains. For each clone purified as a His-fusion, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 µg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 µg/ml) liquid medium and grown overnight at 37°C with shaking.

5 The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 µg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD₅₅₀ reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000g for 15 min at 4°C. The bacterial pellet was resuspended in 7.5 ml of

10 either (i) cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0) for soluble proteins or (ii) buffer B (10mM Tris-HCl, 100 mM phosphate buffer, pH 8.8 and, optionally, 8M urea) for insoluble proteins. Proteins purified in a soluble form included 287-His, $\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$ 287-His, $\Delta 4$ 287MC58-His, 287c-His and 287cMC58-His. Protein 287bMC58-His was insoluble and purified accordingly. Cells were disrupted by

15 sonication on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13000xg for 30 min at 4°C. For insoluble proteins, pellets were resuspended in 2.0 ml buffer C (6 M guanidine hydrochloride, 100 mM phosphate buffer, 10 mM Tris- HCl, pH 7.5 and treated with 10 passes of a Dounce^{*} homogenizer. The homogenate was centrifuged at 13000g for 30 min and the supernatant retained. Supernatants for both soluble and insoluble

20 preparations were mixed with 150µl Ni²⁺-resin (previously equilibrated with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose^{*} Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700g for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml

25 buffer A or B for 10 min, resuspended in 1.0 ml buffer A or B and loaded onto a disposable column. The resin continued to be washed with either (i) buffer A at 4°C or (ii) buffer B at room temperature, until the OD₂₈₀ of the flow-through reached 0.02-0.01. The resin was further washed with either (i) cold buffer C (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8.0) or (ii) buffer D (10mM Tris-HCl, 100mM phosphate buffer, pH 6.3 and,

30 optionally, 8M urea) until OD₂₈₀ of the flow-through reached 0.02-0.01. The His-fusion protein was eluted by addition of 700µl of either (i) cold elution buffer A (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) or (ii) elution buffer B (10 mM Tris-HCl, 100 mM phosphate buffer, pH 4.5 and, optionally, 8M urea) and fractions

*Trade-mark

collected until the OD₂₈₀ indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analysed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

Renaturation of denatured His-fusion proteins.

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

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

Immunization

- 15 Balb/C mice were immunized with antigens on days 0, 21 and 35 and sera analyzed at day 49.

Sera analysis – ELISA

- The acapsulated MenB M7 and the capsulated strains were plated on chocolate agar plates and incubated overnight at 37°C with 5% CO₂. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into Mueller-Hinton Broth (Difco)
20 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 0.4–0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and bacteria were washed twice with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 1 hour at 37°C and then overnight at 4°C with stirring. 100µl bacterial cells
25 were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20* in PBS). 200µl of saturation buffer (2.7% polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to
30 each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 100µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells

*Trade-mark

-42-

were washed three times with PBT buffer. 100µl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10µl of H₂O₂) were added to each well and the plates were left at room temperature for 20 minutes. 100µl 12.5% H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA titers were calculated arbitrarily as the dilution of sera which gave an OD₄₉₀ value of 0.4 above the level of preimmune sera. The ELISA was considered positive when the dilution of sera with OD₄₉₀ of 0.4 was higher than 1:400.

Sera analysis – FACS Scan bacteria binding assay

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C with 5% CO₂. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following 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 4000rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA in PBS, 0.4% NaN₃) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.05. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:100, 1:200, 1:400) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan (Laser Power 15mW) setting were: FL2 on; FSC-H threshold:92; FSC PMT Voltage: E 01; SSC PMT: 474; Amp. Gains 6.1; FL-2 PMT: 586; compensation values: 0.

Sera analysis – bactericidal assay

N. meningitidis strain 2996 was grown overnight at 37°C on chocolate agar plates (starting from a frozen stock) with 5% CO₂. Colonies were collected and used to inoculate 7ml Mueller-Hinton broth, containing 0.25% glucose to reach an OD₆₂₀ of 0.05-0.08. The culture was incubated for approximately 1.5 hours at 37 degrees with shaking until the OD₆₂₀ reached the value of 0.23-0.24. Bacteria were diluted in 50mM Phosphate buffer pH 7.2 containing 10mM MgCl₂, 10mM CaCl₂ and 0.5% (w/v) BSA (assay buffer) at the working dilution of 10⁵ CFU/ml. The total volume of the final reaction mixture was 50 µl with 25 µl

of serial two fold dilution of test serum, 12.5 µl of bacteria at the working dilution, 12.5 µl of baby rabbit complement (final concentration 25%).

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

Sera analysis – western blots

Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded onto a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA
15 at 4°C, using transfer buffer (0.3% Tris base, 1.44% glycine, 20% (v/v) 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
20 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.

The OMVs were prepared as follows: *N. meningitidis* strain 2996 was grown overnight at 37 degrees with 5% CO₂ on 5 GC plates, harvested with a loop and resuspended in 10 ml of
25 20mM Tris-HCl pH 7.5, 2 mM EDTA. Heat inactivation was performed at 56°C for 45 minutes and the bacteria disrupted by sonication for 5 minutes on ice (50% duty cycle, 50% output , Branson sonifier 3 mm microtip). Unbroken cells were removed by centrifugation at 5000g for 10 minutes, the supernatant containing the total cell envelope fraction recovered and further centrifuged overnight at 50000g at the temperature of 4°C . The pellet containing
30 the membranes was resuspended in 2% sarkosyl, 20mM Tris-HCl pH 7.5, 2 mM EDTA and incubated at room temperature for 20 minutes to solubilise the inner membranes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, the supernatant was further centrifuged at 50000g for 3 hours. The pellet, containing the outer membranes

*Trade-mark

-44-

was washed in PBS and resuspended in the same buffer. Protein concentration was measured by the D.C. Bio-Rad Protein assay (Modified Lowry method), using BSA as a standard.

Total cell extracts were prepared as follows: *N. meningitidis* strain 2996 was grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl.

5 Heat inactivation was performed at 56°C for 30 minutes.

CLAIMS:

1. A hybrid protein of formula $\text{NH}_2\text{-A-B-COOH}$, wherein A comprises the Neisserial protein ΔG287 and B comprises the Neisserial protein 961, and wherein the amino acid sequence of the hybrid protein is as disclosed in SEQ ID NO: 8 or is a sequence having greater than 70% sequence identity thereto.
2. The protein of claim 1, wherein ΔG287 is from strain 2996 or 394/98.
3. The protein of claim 1, wherein 961 is from strain 2996 or 394/98.
4. The protein of claim 1, wherein A and B are from the same strain.
5. The hybrid protein of claim 1, comprising the amino acid sequence recited in SEQ ID NO: 8.

FIGURE 1 — ΔG287—919

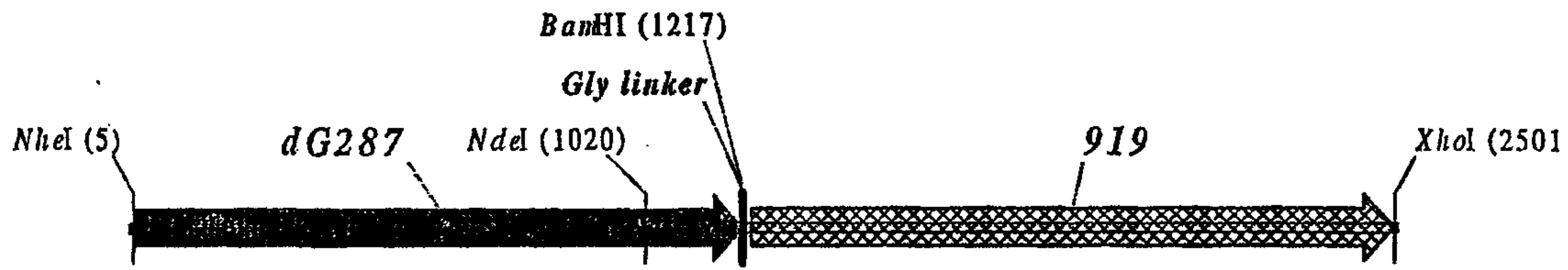


FIGURE 2 — ΔG287—953

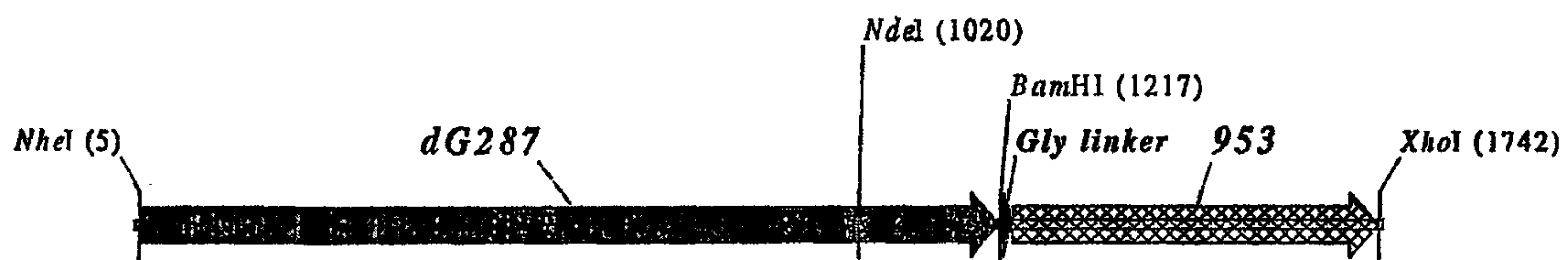


FIGURE 3 — ΔG287—961

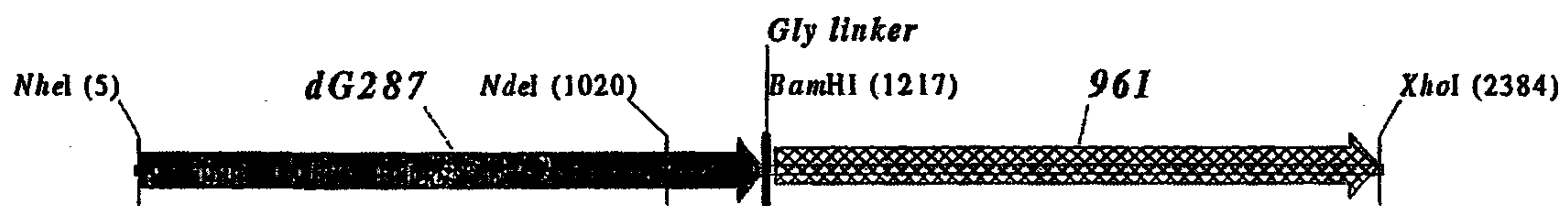


FIGURE 4 — ΔG287NZ—919

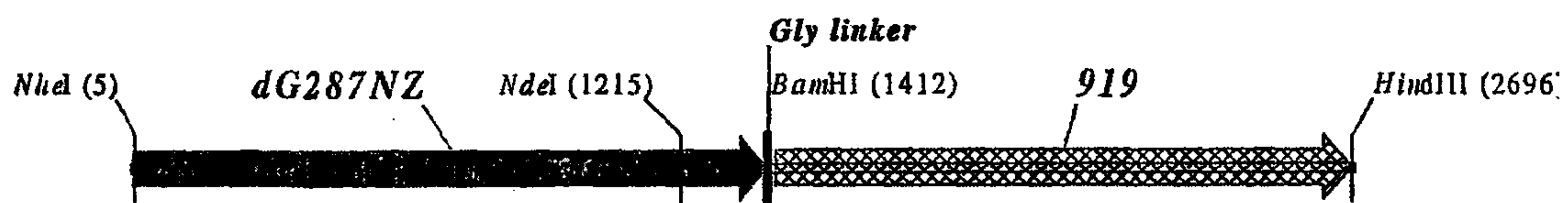


FIGURE 5 — ΔG287NZ—953

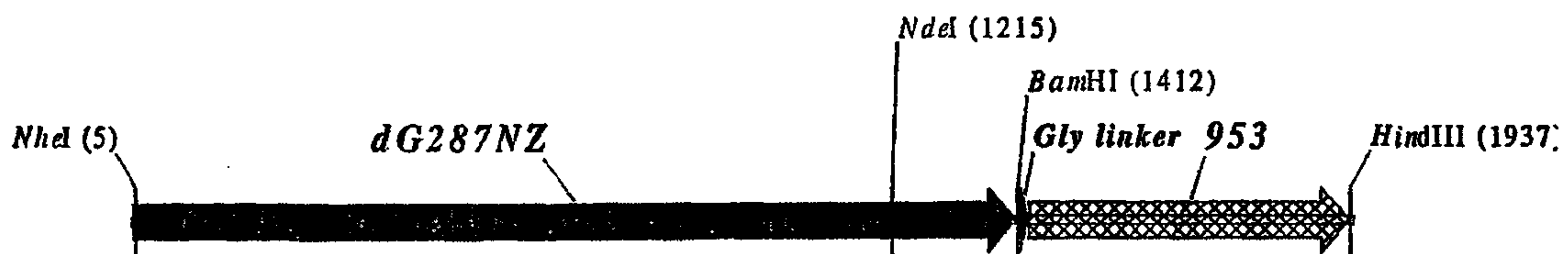


FIGURE 6 — ΔG287NZ—961

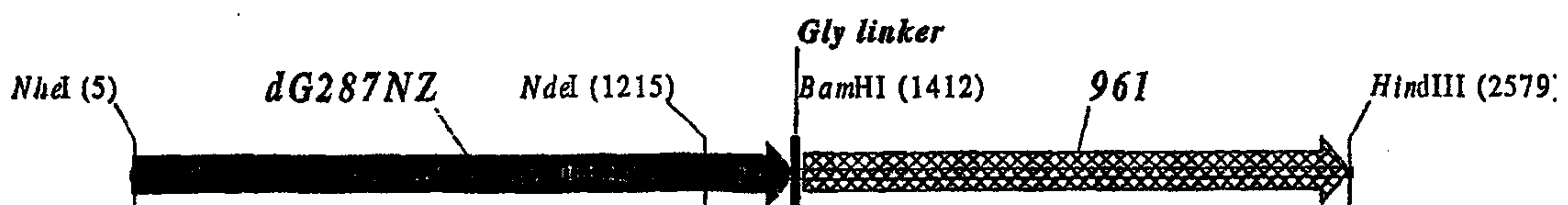


FIGURE 7 — ΔG983-ORF46.1



FIGURE 8 — ΔG983-741



FIGURE 9 — ΔG983-961



FIGURE 10 — ΔG983-961c

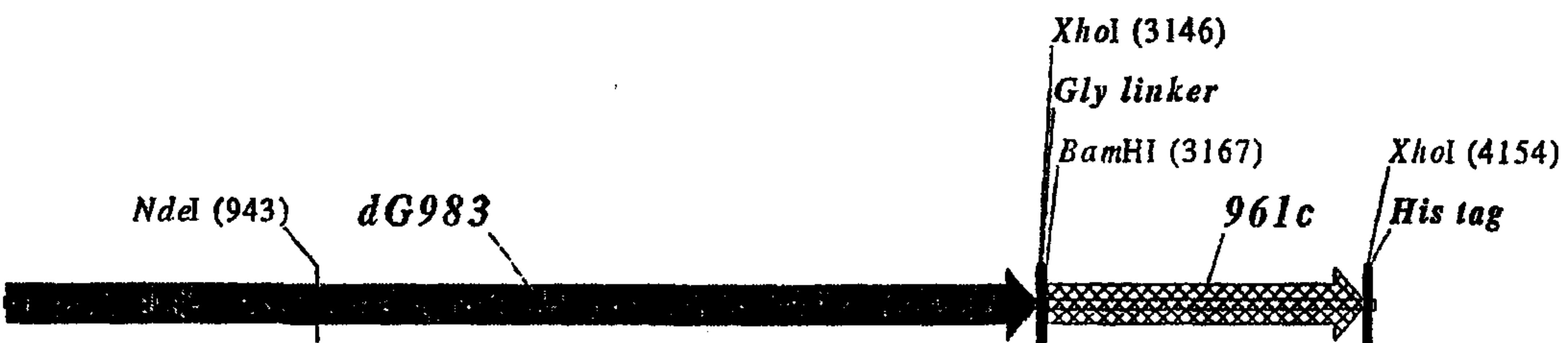


FIGURE 11 — ΔG741-961

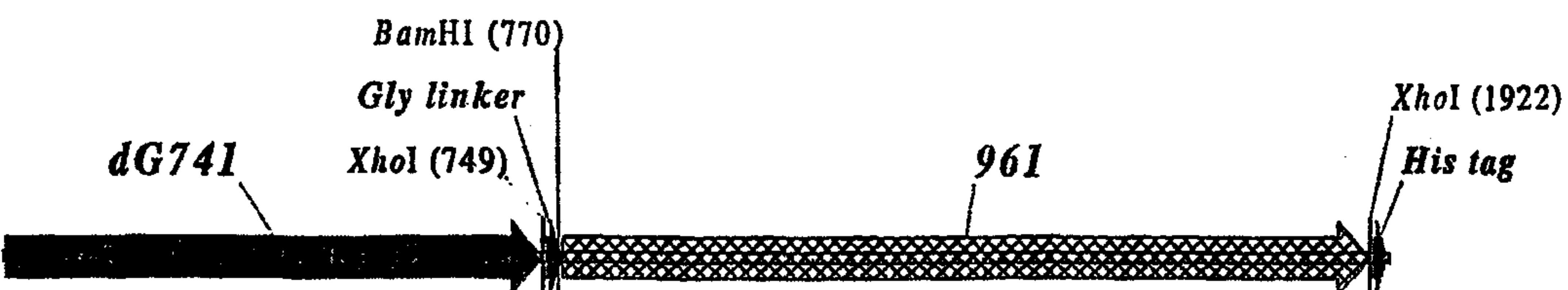


FIGURE 12 — ΔG741-961c

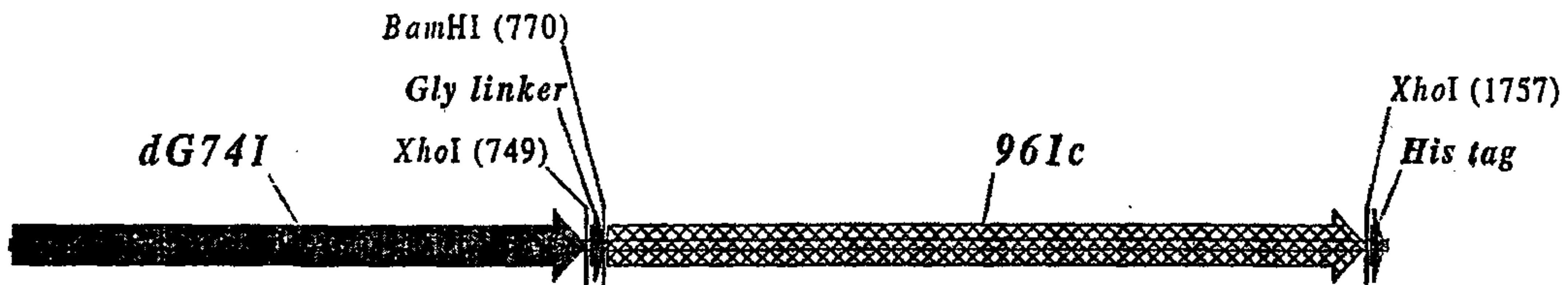


FIGURE 13 — ΔG741-983

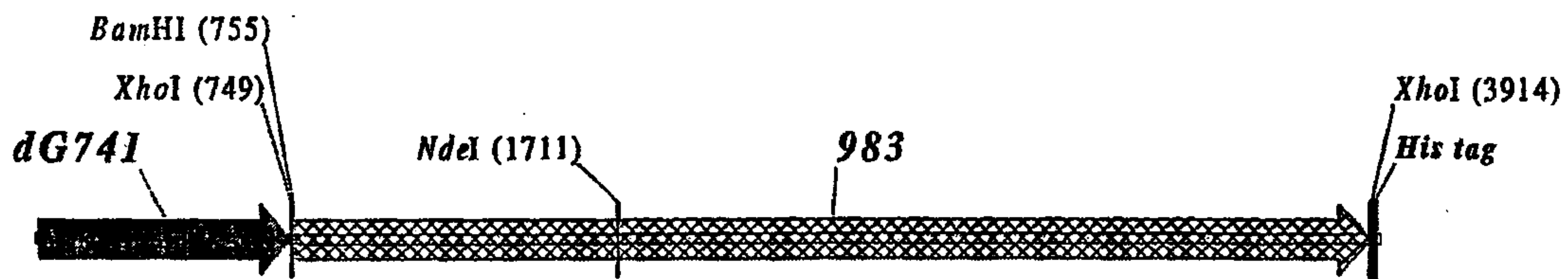


FIGURE 14 — ΔG741-ORF46.1

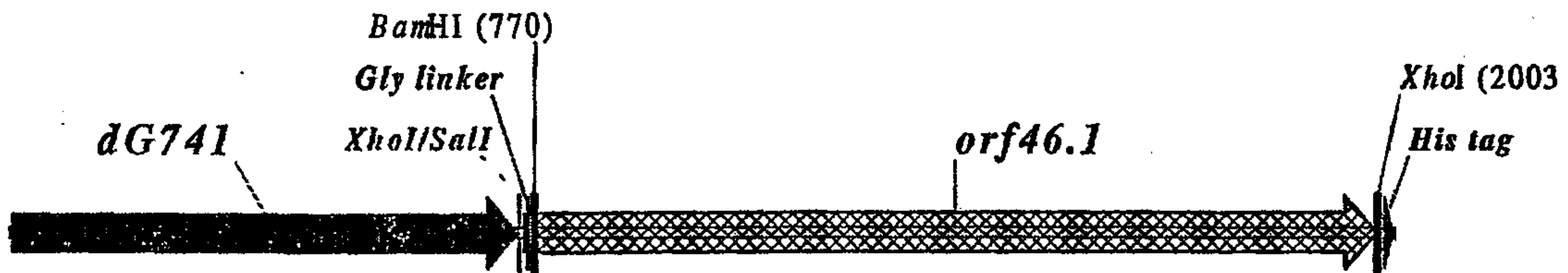


FIGURE 15 — ORF46.1-741

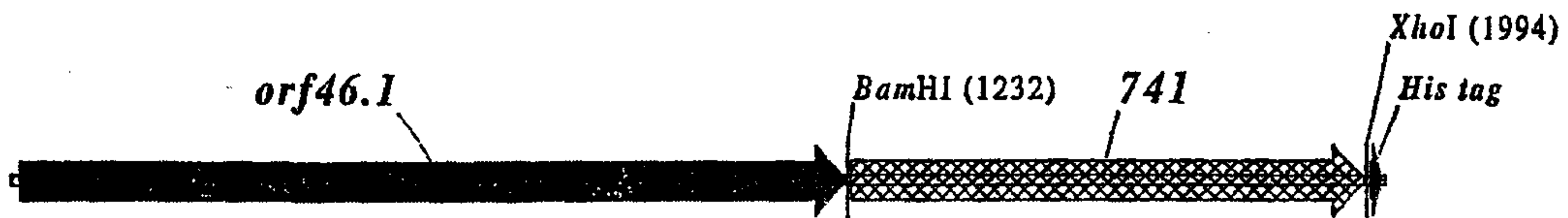


FIGURE 16 — ORF46.1-961

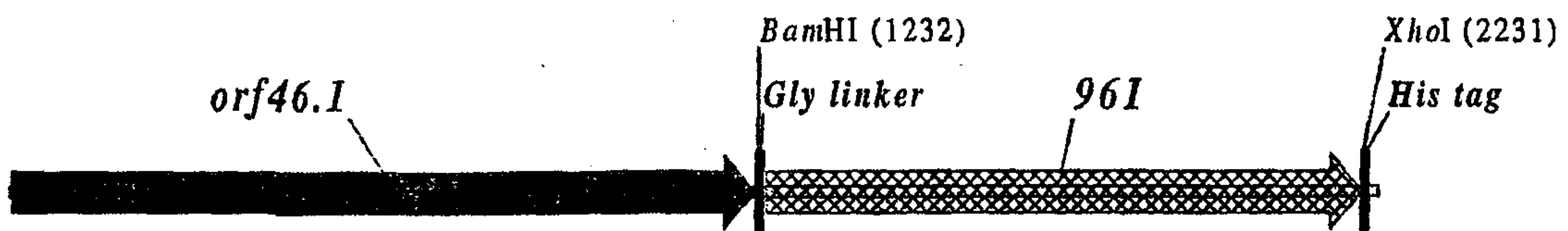


FIGURE 17 — ORF46.1—961c

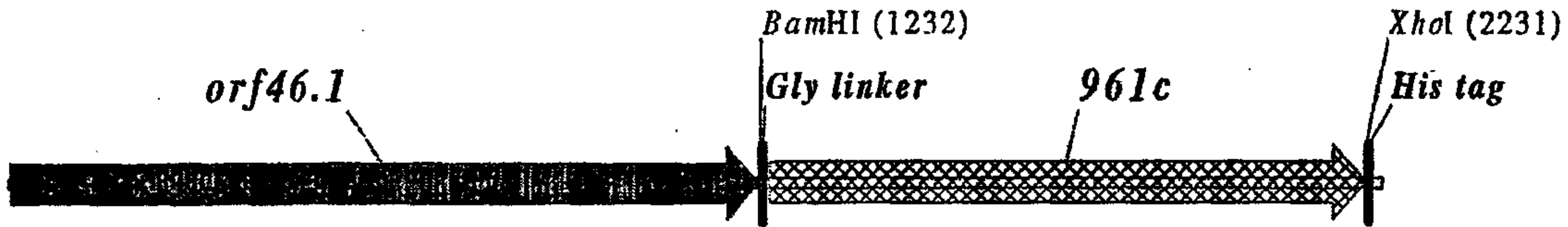


FIGURE 18 — 961-ORF46.1

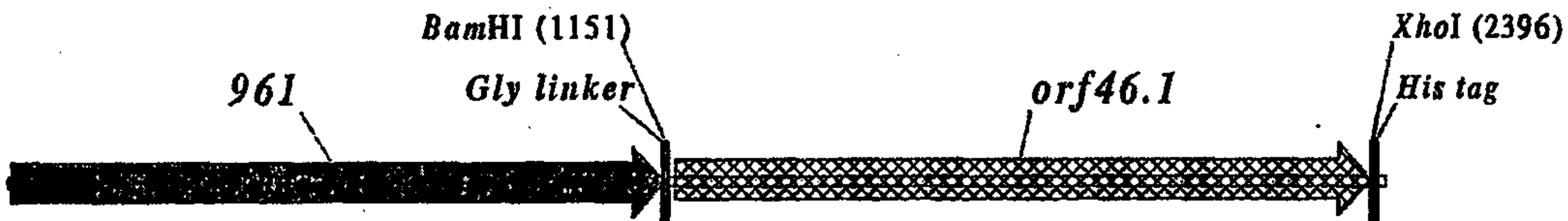


FIGURE 19 — 961-741

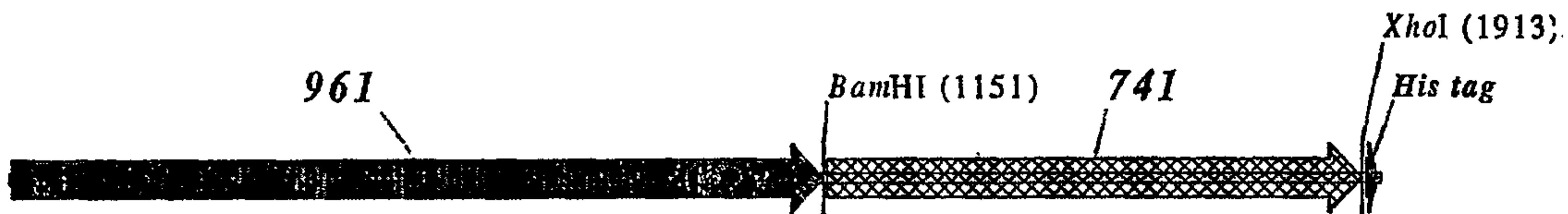


FIGURE 20 — 961-983

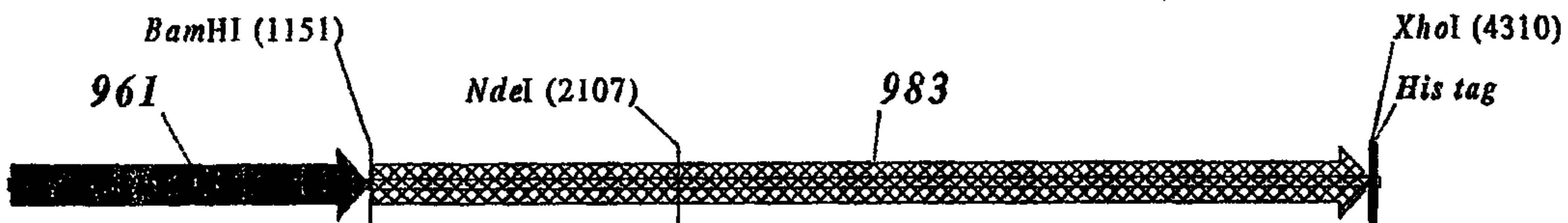


FIGURE 21 — 961c-ORF46.1

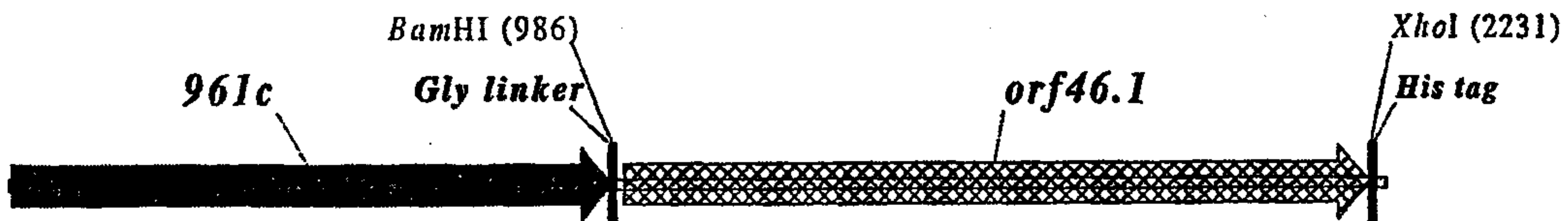


FIGURE 22 — 961c-741



FIGURE 23 — 961c-983

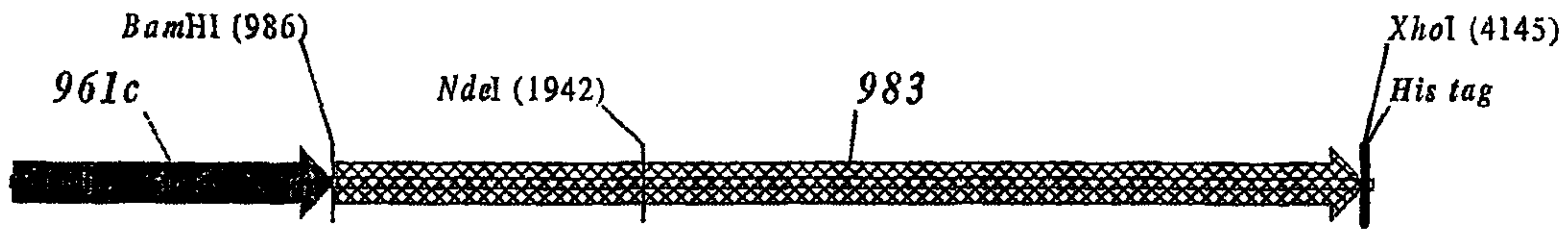


FIGURE 24 — 961cL-ORF46.1

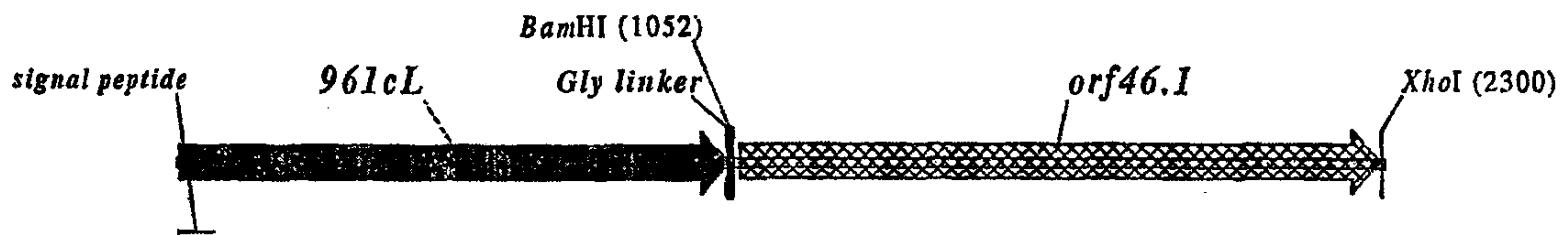


FIGURE 25 — 961cL-741

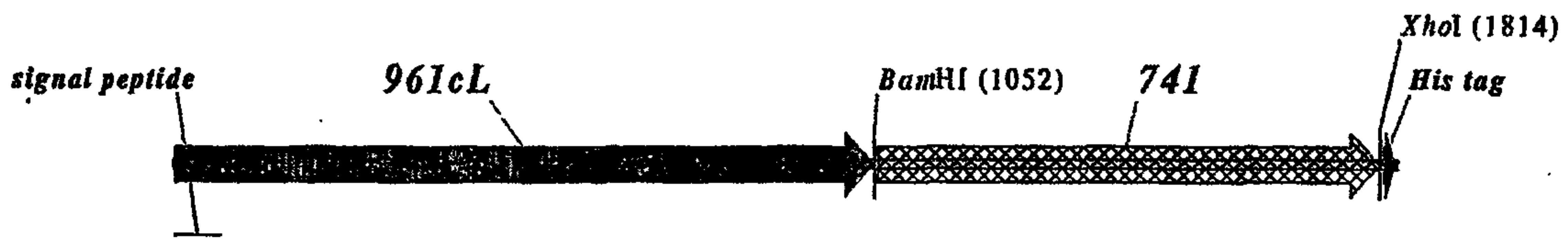
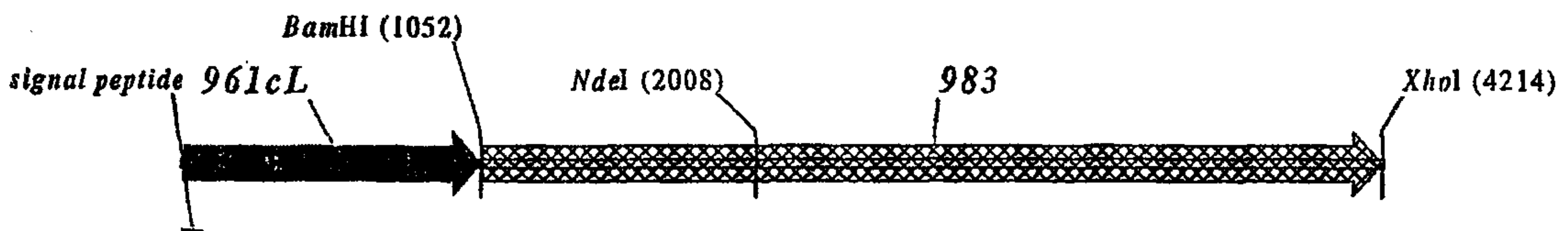


FIGURE 26 — 961cL-983



— ΔG287—961

