

VACCINES

The present invention relates to vaccines, and in particular to a method of identifying microbial polypeptides which are vaccine candidates.

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The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge. The documents listed in the specification are hereby incorporated by reference.

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Microbial infections remain a serious risk to human and animal health, particularly in light of the fact that many pathogenic microorganisms, particularly bacteria, are or may become resistant to anti-microbial agents such as antibiotics.

15 Vaccination provides an alternative approach to combating microbial infections, but it is often difficult to identify suitable immunogens for use in vaccines which are safe and which are effective against a range of different isolates of a pathogenic microorganism, particular a genetically diverse microorganism. Although it is possible to develop vaccines which use as the immunogen
20 substantially intact microorganisms, such as live attenuated bacteria which typically contain one or mutations in a virulence-determining gene, not all microorganisms are amenable to this approach, and it is not always desirable to adopt this approach for a particular microorganism where safety cannot always be guaranteed. Also, some microorganisms express molecules which mimic host
25 proteins, and these are undesirable in a vaccine.

A particular group of microorganisms for which it is important to develop further vaccines is *Neisseria meningitidis* which causes meningococcal disease, a life threatening infection which in the Europe, North America, developing countries
30 and elsewhere remains an important cause of childhood mortality despite the introduction of the conjugate serogroup C polysaccharide vaccine. This is because infections caused by serogroup B strains (*NmB*), which express an α 2-8 linked

polysialic acid capsule, are still prevalent. The term “serogroup” in relation to *N. meningitidis* refers to the polysaccharide capsule expressed on the bacterium. The common serogroup in the UK causing disease is B, while in Africa it is A. Meningococcal septicaemia continues to carry a high case fatality rate; and survivors are often left with major psychological and/or physical disability. After a non-specific prodromal illness, meningococcal septicaemia can present as a fulminant disease that is refractory to appropriate anti-microbial therapy and full supportive measures. Therefore, the best approach to combating the public health menace of meningococcal disease is through prophylactic vaccination.

The non-specific early clinical signs and fulminant course of meningococcal infection mean that therapy is often ineffective. Therefore vaccination is considered the most effective strategy to diminish the global disease burden caused by this pathogen (Feavers (2000) ABC of meningococcal diversity. *Nature* **404**, 451-2). Existing vaccines to prevent serogroup A, C, W135, and Y *N. meningitidis* infections are based on the polysaccharide capsule located on the surface of bacterium (Anderson *et al* (1994) Safety and immunogenicity of meningococcal A and C polysaccharide conjugate vaccine in adults. *Infect Immun.* **62**, 3391-33955; Leach *et al* (1997) Induction of immunologic memory in Gambian children by vaccination in infancy with a group A plus group C meningococcal polysaccharide-protein conjugate vaccine. *J Infect Dis.* **175**, 200-4; Lieberman *et al* (1996). Safety and immunogenicity of a serogroups A/C *Neisseria meningitidis* oligosaccharide-protein conjugate vaccine in young children. A randomized controlled trial. *J. American Med. Assoc.* **275**, 1499-1503). Progress toward a vaccine against serogroup B infections has been more difficult as its capsule, a homopolymer of α 2-8 linked sialic acid, is a relatively poor immunogen in humans. This is because it shares epitopes expressed on a human cell adhesion molecule, N-CAM1 (Finne *et al* (1983) Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet* **2**, 355-357). Indeed, generating immune responses against the serogroup B capsule might actually prove harmful. Thus,

there remains a need for new vaccines to prevent serogroup B *N. meningitidis* infections.

The most validated immunologic correlate of protection against meningococcal
5 disease is the serum bactericidal assay (SBA). The SBA evaluates the ability of
antibodies (usually IgG2a subclass) in serum to mediate complement deposition
on the bacterial cell surface, assembly of the membrane attack complex, and
bacterial lysis. In the SBA, a known number of bacteria are exposed serial
dilutions of the sera with a defined complement source. The number of surviving
10 bacteria is determined, and the SBA is defined as the reciprocal of the highest
dilution of serum that mediates 50% killing. The SBA is predictive of protection
against serogroup C infections, and has been widely used as a surrogate for
immunity against *NmB* infections. Importantly the SBA is a ready marker of
immunity for the pre-clinical assessment of vaccines, and provides a suitable
15 endpoint in clinical trials.

Most efforts at *NmB* vaccine development are directed toward defining effective
protein subunits. There has been a major investment in 'Reverse vaccinology', in
which genome sequences are interrogated for potentially surface expressed
20 proteins which are expressed as heterologous antigens and tested for their ability
to generate meaningful responses in animals. However, this approach is limited by
1) the computer algorithms for predicting surface expressed antigens, 2) failure to
express many of potential immunogens, and 3) the total reliance on murine
immune responses.

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The key to a successful vaccine is to define antigen(s) that elicit protection against
a broad range of disease isolates irrespective of serogroup or clonal group. An
object of the invention is to use a novel genetic screening method (which we have
termed Genetic Screening for Immunogens or GSI) to isolate antigens that are
30 conserved across the genetic diversity of microbial strains and this is exemplified
in relation to meningococcal strains. This may be done by identifying microbial
antigens, such as *N. meningitidis* antigens, by GSI as described in more detail

below; and validated by assessing the function of the immune response elicited by the recombinant antigens and by evaluating the protective efficacy of antigens. Although genetics has been applied in the search for vaccine candidates previously, it has hitherto been difficult to establish high throughput analyses, and
5 has been difficult to differentiate between immunogenic and protective antigens.

A first aspect of the invention provides a method for identifying a polypeptide of a microorganism which polypeptide is associated with an immune response in an animal which has been subjected to the microorganism, the method comprising the
10 steps of

- (1) providing a plurality of different mutants of the microorganism;
- (2) contacting the plurality of mutant microorganisms with antibodies from
15 an animal which has raised an immune response to the microorganism or a part thereof, under conditions whereby if the antibodies bind to the mutant microorganism the mutant microorganism is killed;
- (3) selecting surviving mutant microorganisms from step (2);
- (4) identifying the gene containing the mutation in any surviving mutant
20 microorganism; and
- (5) identifying the polypeptide encoded by the gene.

25 The immune response with which the polypeptide is associated is a functionally important one in the sense that it is one that may be associated with combating infection of the animal by the microorganism.

30 The microorganism may be any microorganism, such as a bacterium or yeast. It is preferred if the microorganism is a pathogenic microorganism, and particularly a pathogenic bacterium such as *Neisseria meningitidis* which causes meningococcal

disease, or *Neisseria gonorrhoeae* which causes gonorrhoea, or *Haemophilus influenzae* which causes at least one type of influenza and middle ear infection.

By “polypeptide associated with an immune response in an animal which has been
5 subjected to the microorganism” we include any such polypeptide. The method of
the invention is able to identify polypeptides of microorganisms which
polypeptides are ones for which antibodies have been raised in an animal when the
immune system of the animal has been in contact with the polypeptide of the
microorganism. Typically, the polypeptide is one which is expressed on the
10 surface of the microorganism. The immune response of the animal is an antibody
response, typically an IgG response.

In one embodiment of the invention, the animal has been subjected to the
microorganism by way of infection with the microorganism, for example a natural
15 infection. Thus, the animal is typically a host for the microorganism. In another
embodiment of the invention, the animal has purposefully been inoculated with
the microorganism (whether live or killed) or part thereof. Either way, the
animal’s immune response has given rise to antibodies directed at the
microorganism, some of which are selective for particular polypeptides of the
20 microorganism and which can be used to identify polypeptides by the method of
the invention.

It will be appreciated that the term “animal” includes human and in a particularly
preferred embodiment of the invention, as discussed in more detail below, the
25 antibodies used in step (2) are ones from a human who is or has been infected with
a microorganism or has been immunised with part of the microorganism.

By using antibodies from the animal which has raised an immune response to the
microorganism or part thereof, immunologically relevant polypeptides may be
30 identified (in the context of immunogenicity and vaccine design, and particularly
polypeptides which are protective).

Thus, it will be appreciated that the polypeptides identified using the method of the invention are antigens or immunogens (these terms being used interchangeably in the specification), typically surface exposed, which are ones that give rise to an immune response in an animal and so are vaccine candidates.

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The plurality (or library) of different mutant microorganisms typically is a sufficient large number to give a high chance (typically >95%) of each gene within the microorganism being mutated. The number of mutants required will depend on the number of genes in the genome of the microorganism. When the mutants are random mutants, the number of mutants required to give a high chance of a mutant of each gene within the genome being represented is around 10 times the number of genes. Typically, therefore, the number of mutant microorganisms provided in step (1) is around 10 to 20 times the number of genes in the microorganism.

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Typically, a bacterium has from 500 to 5000 genes, and so the number of random mutants used is of the order of 5000 to 100,000. In the case of *N. meningitidis* a suitable number of random mutants is around 40, 000.

20 The random mutants may be any type of random mutant such as those induced chemically; preferably, the mutants are insertion mutants, such as transposon mutants, since it is straightforward to identify the position of the insertions (eg using probes or PCR primers which are selective for the inserted element eg transposon), and typically the transposon carries an antibiotic resistance marker which allows selection of the mutants containing the transposon.

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Transposons suitable for integration into the genome of Gram negative bacteria include Tn5, Tn10 and derivatives thereof. Transposons suitable for integration into the genome of Gram positive bacteria include Tn916 and derivatives or analogues thereof. Transposons particularly suited for use with *Staphylococcus aureus* include Tn917 (Cheung *et al* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6462-6466) and Tn918 (Albus *et al* (1991) *Infect. Immun.* **59**, 1008-1014).

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It is particularly preferred if the transposons have the properties of the Tn917 derivatives described by Camilli *et al* (1990) *J. Bacteriol.* **172**, 3738-3744, and are carried by a temperature-sensitive vector such as pE194Ts (Villafane *et al* (1987) *J. Bacteriol.* **169**, 4822-4829).

For *N. meningitidis*, Tn10 is a preferred transposon (see Sun *et al* (2000) *Nature Med.* **6**, 1269-1273), although any transposon and transposase with *in vitro* activity can be used.

It will be appreciated that although transposons are convenient for insertionally inactivating a gene, any other known method, or method developed in the future may be used. A further convenient method of insertionally inactivating a gene, particularly in certain bacteria such as *Streptococcus*, is using insertion-duplication mutagenesis such as that described in Morrison *et al* (1984) *J. Bacteriol.* **159**, 870 with respect to *S. pneumoniae*. The general method may also be applied to other microorganisms, especially bacteria.

For fungi, insertional mutations are created by transformation using DNA fragments or plasmids preferably carrying selectable markers encoding, for example, resistance to hygromycin B or phleomycin (see Smith *et al* (1994) *Infect. Immunol.* **62**, 5247-5254). Random, single integration of DNA fragments encoding hygromycin B resistance into the genome of filamentous fungi, using restriction enzyme mediated integration (REMI; Schiestl & Petes (1991); Lu *et al* (1994) *Proc. Natl. Acad. Sci. USA* **91**, 12649-12653) are known.

A simple insertional mutagenesis technique for a fungus is described in Schiestl & Petes (1994) incorporated herein by reference, and include, for example, the use of Ty elements and ribosomal DNA in yeast.

Random integration of the transposon or other DNA sequence allows isolation of a plurality of independently mutated microorganisms wherein a different gene is insertionally inactivated in each mutant.

5 For some microorganisms, libraries of mutants in which each gene is mutated by a transposon or other insertion element are known. In this case, the plurality of microorganisms may conveniently be produced by pooling one or more representatives of each member of the library. For example, a comprehensive transposon library for *Pseudomonas aeruginosa* is described in Jacobs *et al* (2003)
10 *Proc. Natl. Acad. Sci. USA* **100**, 14339-14344.

In step (2) of the method, the plurality of mutant microorganisms is contacted with antibodies from the animal which has raised as immune response to the microorganism or part thereof. The antibodies may be in any suitable form and
15 from any suitable, convenient source from the animal (including human). Typically, the antibodies are present in serum derived from the animal. However, they may be present in other forms, such as a fraction enriched for IgG. It is preferred if the antibodies are IgG antibodies, but other antibody types may be used, such as IgA and IgM. Although it is preferred if the antibodies are present
20 in or derived from serum, the antibodies may be present in or derived from other body fluids such as saliva.

The antibodies are typically from an animal which is or has been infected with the microorganism. One of the advantages of this embodiment of the method is that it
25 makes use of antibodies from an animal which has raised a relevant immune response in attempting to combat the infection with the microorganism, and such antibodies are likely to bind to polypeptides which are useful in vaccines. Thus, preferred antibodies are ones which are from humans who are or who have been infected with the microorganism, or who have been inoculated with an attenuated
30 (eg vaccine) strain of the microorganism or who have been vaccinated with a vaccine which comprises a part of the microorganism (such as outer membrane component parts). Typically, the antibodies used in step (2) of the method are

from an animal (such as man) which has raised a protective response against the microorganism.

Alternatively, the antibodies are from an animal, such as an experimental animal
5 such as mouse, rabbit, sheep or horse, which has been inoculated with the microorganism and allowed to generate an immune response, preferably a protective immune response. Whether or not a protective response has been raised may be determined by challenging the animal with live bacteria after inoculation. The experimental animals may have been inoculated with a virulent, pathogenic
10 strain of the microorganism, or it may have been inoculated with an avirulent or attenuated strain (whether live or killed).

In a preferred embodiment, the antibodies are raised to a strain of microorganism "heterologous" to the strain used to produce the mutant microorganism. Many
15 pathogenic microorganisms exist in different serogroups or strains, and each serogroup or strain may have polypeptides in common with other serogroups or strains as well as polypeptides which are unique to the serogroup or strains. The advantage of using antibodies raised to one or more heterologous strain(s) is that it increases the chances of identifying a polypeptide which is common to all
20 serogroups of the microorganism (ie conserved, common epitopes). Such polypeptides (or fragments or variants or fusions thereof) are more likely to be effective against the range of serogroups of a particular microorganism. Thus, in a particularly preferred embodiment where the microorganism is *N. meningitidis*, the plurality of mutant microorganisms are derived from a parent serogroup B strain, whereas the antibodies are derived from an animal (such as man) which has
25 raised a response to a serogroup A and/or a serogroup C strain. It will be appreciated that antibodies may be pooled from more than one source. For example, serum from a patient infected with (or convalescing from an infection with) serogroup A strain may be pooled with serum from a patient infected with
30 (or convalescing from an infection with) serogroup C strain. Serum from a patient infected with (or convalescing from an infection with) serogroup B strain may also be pooled. Some microorganisms have, in addition to polypeptide components of

their cell wall or cell membrane, polysaccharide components which often are immunogenic. In a further preferred embodiment, it is convenient to use a strain of the microorganism in which some or all of the polysaccharide components have been eliminated as the strain against which antibodies are produced. Thus, many
5 bacteria have a capsule made predominantly of polysaccharide, but typically strains exist in which the capsule is missing. These "capsule minus" strains may conveniently be used to raise antibodies for use in the second step of the method.

In relation to *N. meningitidis*, the antibodies may conveniently be present in the
10 following serum sources: from mice immunized by the systemic route using heterologous strains of *N. meningitidis* (ie heterologous to the mutant strain used in the selection); from both acute and convalescent human patients infected with *N. meningitidis*; and from human patients immunized with defined outer membrane vesicles (OMVs) vaccine derived from the serogroup B NmB isolate
15 H44/76. Convalescent sera is preferred since the patient will have raised a significant immune response to the infecting bacteria. In some circumstance, it may be useful to use acute patient serum as a control for the convalescent serum since acute patients may not have raised such a significant immune response. Equivalent sources of antibodies are available with respect to other
20 microorganisms.

Conditions are provided so that when the antibodies bind to the mutant microorganism, the mutant microorganism is killed, whereas when the antibodies do not bind to the mutant microorganism, the mutant microorganism is not killed.

25 In this way, it can be seen that those mutant microorganisms in which a gene encoding a polypeptide which binds to the antibody is mutated so that the antibody no longer binds survive. This provides a very powerful selection for such mutants, and facilitates the identification of the polypeptides which are associated with an immune response in an animal infected with microorganism.
30 As a control, it may be convenient to use wild-type microorganisms which are also killed under the given conditions, or to develop conditions in which all wild type microorganisms are killed.

Conveniently, once the antibodies have been contacted with the mutant microorganism a source of complement is added, such as complement from human, rabbit, mouse, sheep and horse. Conveniently, the complement is from the same source (ie species of animal) as the antibody. Antibodies (generally IgG 2a subclass) mediate complement deposition on the surface of the microorganism, assembly of the membrane attack complex and lysis of the microorganism. Complement-mediated killing is independent of the presence of cells from the blood, but requires the presence of serum. Complement-mediated killing may be inactivated by heating the serum.

Preferably, in this embodiment, the microorganism is a bacterium, either Gram positive or Gram negative. Complement mediated killing is described in Walport (2001) *N. Engl. J. Med.* **344**, 1140-1144, and Walport (2001) *N. Eng J. Med.* **344**, 1058-1066.

The complement deposition approach to killing the microorganisms which retain the ability to bind to the antibodies is particularly suited to use with *N. meningitidis* since the serum bactericidal assay (SBA; see Goldschneider et al (1969) *J. Exp. Med.* **129**, 1307-1326), which is based on the same principle, is used as discussed in the introduction. Of course, in the case of the SBA, the number of surviving bacteria is used to assess the effectiveness of serum in killing bacteria (and using this as a marker of the degree of protection conferred by the strain used to give rise to the antibodies use). As far as the inventors are aware there has never been any suggestion that this method could be adapted to identifying antigens in microorganisms.

As an alternative to using complement to kill the cells to which the antibodies bind, a moiety may be used which binds selectively to the antibodies (which bind the cell) and delivers a cytotoxic agent to the cell. For example, the moiety may be a further antibody which recognizes the antibodies bound to the microorganism and delivers the cytotoxic agent to the cell. Thus, the further antibody may be an

anti-human antibody when the antibody which binds to the mutant microorganism is a human antibody. The cytotoxic agent may be directly cytotoxic or it may be indirectly cytotoxic. By indirectly cytotoxic we include an enzyme that is capable of activating a relatively non-toxic compound to a cytotoxic compound. A similar technique has been used to target tumour cells using tumour-selective antibodies and has been called ADEPT (antibody-directed enzyme prodrug therapy; see WO 88/07378; Bagshawe (1987) *Br. J. Cancer* **56**, 531-532; Bagshawe *et al* (1988) *Br. J. Cancer* **58**, 700-703; and Senter *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4842-4846, all of which are incorporated herein by reference).

Enzyme – prodrug pairs include the following: Alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs, aryl sulphatase useful for converting sulphate-containing prodrugs into free drugs, cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anticancer drug 5-fluorouracil, proteases such as Serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins that are useful for converting peptide-containing prodrugs into free drugs, D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents, carbohydrate-enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs, β -lactamase useful for converting drugs derivatized with β -lactams into free drugs and penicillin amidases useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups into free drugs.

Other enzymes and prodrugs include hydrolases, amidases, sulphatases, lipases, glucuronidases, phosphatases and carboxypeptidases, and prodrugs be prepared from any of the various classes of anti-tumour compounds for example alkylating agents (nitrogen mustards) including cyclophosphamide, bisulphan, chlorambucil and nitrosoureas; intercalating agents including adriamycin and dactinomycin; spindle poisons such as vinca alkaloids; and anti-metabolites including anti-folates, anti-purines, anti-pyrimidines or hydroxyurea.

Also included are cyanogenic prodrugs such as amygdalin which produce cyanide upon action with a carbohydrate cleaving enzyme.

5 Mutant microorganisms which survive the conditions, for example those conditions which kill all wild type (or parent) microorganisms (and indeed the majority of mutants), are selected for further study since such mutant microorganisms are likely to be mutated in a gene which encodes a polypeptide which binds to the antibodies (and therefore is involved in an immune response). In one embodiment, and in order to confirm that the mutations in the surviving
10 mutants are responsible for conferring the ability to withstand killing, the mutation of each mutant may be backcrossed into the parental strain and the ability of the backcrossed mutant to survive the killing conditions confirmed.

The gene containing the mutation is identified using methods well known in the
15 art. For example, when the mutation is an insertion mutation, it is convenient to sequence from the insertion into the flanking DNA of the microorganism. When a transposon has been used to create the mutant microorganisms, it is convenient to identify the gene containing the transposon mutation by digesting genomic DNA from the individual mutant selected in step (3) with a restriction enzyme which
20 cuts outside the transposon, ligating size-fractionated DNA containing the transposon into a plasmid, and selecting plasmid recombinants on the basis of antibiotic resistance conferred by the transposon and not by the plasmid. The microorganism genomic DNA adjacent to the transposon may be sequenced using two primers which anneal to the terminal regions of the transposon, and two
25 primers which anneal close to the polylinker sequences of the plasmid. The sequences may be subjected to DNA database searches to determine if the transposon has interrupted a known gene. Thus, conveniently, sequence obtained by this method is compared against the sequences present in the publicly available databases such as EMBL and GenBank, or a complete genome sequence, if
30 available.

By "gene" we include not only the regions of DNA that code for a polypeptide but also regulatory regions of DNA such as regions of DNA that regulate transcription, translation and, for some microorganisms, splicing of RNA. Thus, the gene includes promoters, transcription terminators, ribosome-binding sequences and for some organisms introns and splice recognition sites.

Typically, sequence information of the identified gene obtained in step 4 is derived. Conveniently, sequences close to the ends of the transposon are used as the hybridisation site of a sequencing primer. The derived sequence or a DNA restriction fragment adjacent to the inactivated gene itself is used to make a hybridisation probe with which to identify, and isolate from a wild-type organism, the corresponding wild type gene.

It is preferred if the hybridisation probing is done under stringent conditions to ensure that the gene, and not a relative, is obtained, at least when identifying the gene.

The gene may be sequenced using standard methods and the polypeptide encoded by the gene identified, for example by translating the coding sequence of the gene, or the sequence may already be available as part of a sequenced microorganism genome.

As described in more detail in the Example, particular genes identified by the method of the invention are the NBM0341 (TspA), NMB0338, NMB1345, NMB0738, NBM0792 (NadC family), NMB0279, NMB2050, NMB1335 (CreA), NMB2035, NMB1351 (Fmu and Fmv), NMB1574 (IlvC), NMB1298 (rsuA), NMB1856 (LysR family), NMB0119, NMB1705 (rfak), NMB2065 (HemK), NMB0339, NMB0401 (putA), NMB1467 (PPX), NMB2056, NMB0808, NMB0774 (upp), NMA0078, NMB0337 (branched-chain amino acid transferase), NMB0191 (ParA family), NMB1710 (glutamate dehydrogenase (gdhA), NMB0062 (rfbA-1) and NMB1583 (hisB) genes of *Neisseria meningitidis*. The genome sequence for *N. meningitidis* is available, for example from The Institute

of Genome Research (TIGR); www.tigr.org. Although these genes form part of the genome that has been sequenced, as far as the inventors are aware, they have not been isolated, the polypeptides they encode have not been produced, and there is no indication that the polypeptides they encode may be useful as a component
5 of a vaccine.

Thus, the invention includes the isolated genes as above and in the Examples and variants and fragments and fusions of such variants and fragments, and includes the polypeptides that the genes encode as described above, along with variants and
10 fragment thereof, and fusions of such fragments and variants. Variants, fragments and fusions are described in more detail below. Preferably, the variants, fragments and fusions of the given genes above are ones which encode a polypeptide which gives rise to neutralizing antibodies against *N. meningitidis*. Similarly, preferably, the variants, fragments and fusions of the polypeptide whose
15 sequence is given above are ones which gives rise to neutralizing antibodies against *N. meningitidis*. The invention also includes isolated polynucleotides encoding the polypeptides whose sequences are given in the Example (preferably the isolated coding region) or encoding the variants, fragments or fusions. The invention also includes expression vectors comprising such polynucleotides and
20 host cells comprising such polynucleotides and vectors (as is described in more detail below). The polypeptides described in the Examples are antigens identified by the method of the invention.

Molecular biological methods for use in the practice of the method of the
25 invention are well known in the art, for example from Sambrook & Russell (2001) Molecular Cloning, a laboratory manual, third edition, Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York, incorporated herein by reference.

30 It will be appreciated that the invention also includes carrying out steps (1) to (4) of the method (but not necessarily step (5)) so that a gene encoding a polypeptide which is associated with an immune response in an animal which has been

subjected to the microorganism is identified. The gene may be cloned and sequenced or may be isolated or synthesised, for example by using the polymerase chain reaction using primers based on its sequence. Variants of the gene may be made, for example by identifying related genes in other microorganisms or in other strains of the microorganism, and cloning, isolating or synthesizing the gene. Typically, variants of the gene are ones which have at least 70% sequence identity, more preferably at least 85% sequence identity, most preferably at least 95% sequence identity with the genes isolated by the method of the invention. Of course, replacements, deletions and insertions may be tolerated. The degree of similarity between one nucleic acid sequence and another can be determined using the GAP program of the University of Wisconsin Computer Group.

Variants of the gene are also ones which hybridise under stringent conditions to the gene. By "stringent" we mean that the gene hybridises to the probe when the gene is immobilised on a membrane and the probe (which, in this case is >200 nucleotides in length) is in solution and the immobilised gene/hybridised probe is washed in 0.1 x SSC at 65°C for 10 min. SSC is 0.15 M NaCl/0.015 M Na citrate.

Fragments of the gene (or the variant gene) may be made which are, for example, 20% or 30% or 40% or 50% or 60% or 70% or 80% or 90% of the total of the gene. Preferred fragments include all or part of the coding sequence. The variant and fragments may be fused to other, unrelated, polynucleotides.

Thus the invention also includes a method for making a polynucleotide the method comprising carrying out steps (1) to (4) of the method of the invention and isolating or synthesising the identified gene or a variant or fragment thereof or a fusion of such gene or variant or fragment. The invention also includes a polynucleotide obtainable or obtained by this method.

Preferably, the polynucleotide encodes a polypeptide which is immunogenic and is reactive with the antibodies from an animal which has been subjected to the microorganism from which the gene was identified.

5 The invention also includes a method of selecting a microorganism mutated in a gene encoding a polypeptide which is associated with an immune response in an animal which has been subjected to the microorganism. This method comprises carrying out steps (1) to (3) of the method of the invention (whether or not steps (4) and (5) are carried out). The invention also included a mutant microorganism
10 obtainable or obtained by this method which is not able to bind to the antibodies.

Although as discussed above the method of the invention is useful in identifying genes and selecting mutant microorganisms, it is particularly preferred if the method is used to identify polypeptides from a microorganism which are
15 associated with an immune response. Once identified, it is desirable to make an antigen based on the polypeptide.

The antigen may be the polypeptide as encoded by the gene identified, and the sequence of the polypeptide may readily be deduced from the gene sequence. In
20 further embodiments, the antigen may be a fragment of the identified polypeptide or may be a variant of the identified polypeptide or may be a fusion of the polypeptide or fragment or variant.

Fragments of the identified polypeptide may be made which are, for example,
25 20% or 30% or 40 % or 50% or 60% or 70% or 80% or 90% of the total of the polypeptide. Typically, fragments are at least 10, 15, 20, 30, 40 , 50, 100 or more amino acids, but less than 500, 400, 300 or 200 amino acids. Variants of the polypeptide may be made. By "variants" we include insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not
30 substantially alter the normal function of the protein. By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu;

Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such variants may be made using the well known methods of protein engineering and site-directed mutagenesis.

5 A particular class of variants are those encoded by variant genes as discussed above, for example from related microorganisms or other strains of the microorganism. Typically the variant polypeptides have at least 70% sequence identity, more preferably at least 85% sequence identity, most preferably at least 95% sequence identity with the polypeptide identified using the method of the invention.

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The percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned
15 optimally.

The alignment may alternatively be carried out using the Clustal W program (Thompson *et al.*, (1994) *Nucleic Acids Res* **22**, 4673-80). The parameters used may be as follows:

20 Fast pairwise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.
Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.
Scoring matrix: BLOSUM.

25 The fusions may be fusions with any suitable polypeptide. Typically, the polypeptide is one which is able to enhance the immune response to the polypeptide it is fused to. The fusion partner may be a polypeptide that facilitates purification, for example by constituting a binding site for a moiety that can be immobilised in, for example, an affinity chromatography column. Thus, the
30 fusion partner may comprise oligo-histidine or other amino acids which bind to cobalt or nickel ions. It may also be an epitope for a monoclonal antibody such as a Myc epitope.

The invention also includes, therefore, a method of making an antigen as described above, and antigens obtainable or obtained by the method.

5 The polynucleotides of the invention may be cloned into vectors, such as expression vectors, as is well known on the art. Such vectors maybe present in host cells, such as bacterial, yeast, mammalian and insect host cells. The antigens of the invention may readily be expressed from polynucleotides in a suitable host cell, and isolated therefrom for use in a vaccine.

10

Typical expression systems include the commercially available pET expression vector series and *E. coli* host cells such as BL21. The polypeptides expressed may be purified by any method known in the art. Conveniently, the antigen is fused to a fusion partner that binds to an affinity column as discussed above, and the fusion
15 is purified using the affinity column (eg such as a nickel or cobalt affinity column).

It will be appreciated that the antigen or a polynucleotide encoding the antigen (such as a DNA molecule) is particularly suited for use as in a vaccine. In that
20 case, the antigen is purified from the host cell it is produced in (or if produced by peptide synthesis purified from any contaminants of the synthesis). Typically the antigen contains less than 5% of contaminating material, preferably less than 2%, 1%, 0.5%, 0.1%, 0.01%, before it is formulated for use in a vaccine. The antigen desirably is substantially pyrogen free. Thus, the invention further includes a
25 vaccine comprising the antigen, and method for making a vaccine comprising combining the antigen with a suitable carrier, such as phosphate buffered saline. Whilst it is possible for an antigen of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being
30 compatible with the antigen of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

The vaccine may also conveniently include an adjuvant. Active immunisation of the patient is preferred. In this approach, one or more antigens are prepared in an immunogenic formulation containing suitable adjuvants and carriers and administered to the patient in known ways. Suitable adjuvants include Freund's complete or incomplete adjuvant, muramyl dipeptide, the "Iscoms" of EP 109 942, EP 180 564 and EP 231 039, aluminium hydroxide, saponin, DEAE-dextran, neutral oils (such as miglyol), vegetable oils (such as arachis oil), liposomes, Pluronic polyols or the Ribi adjuvant system (see, for example GB-A-2 189 141). "Pluronic" is a Registered Trade Mark. The patient to be immunised is a patient requiring to be protected from infection with the microorganism.

The aforementioned antigens of the invention (or polynucleotides encoding such antigens) or a formulation thereof may be administered by any conventional method including oral and parenteral (eg subcutaneous or intramuscular) injection. The treatment may consist of a single dose or a plurality of doses over a period of time.

It will be appreciated that the vaccine of the invention, depending on its antigen component (or polynucleotide), may be useful in the fields of human medicine and veterinary medicine.

Diseases caused by microorganisms are known in many animals, such as domestic animals. The vaccines of the invention, when containing an appropriate antigen or polynucleotide encoding an antigen, are useful in man but also in, for example, cows, sheep, pigs, horses, dogs and cats, and in poultry such as chickens, turkeys, ducks and geese.

Thus, the invention also includes a method of vaccinating an individual against a microorganism, the method comprising administering to the individual an antigen (or polynucleotide encoding an antigen) or vaccine as described above. The invention also includes the use of the antigen (or polynucleotide encoding an

antigen) as described above in the manufacture of a vaccine for vaccinating an individual.

The antigen of the invention may be used as the sole antigen in a vaccine or it may
5 be used in combination with other antigens whether directed at the same or different disease microorganisms. In relation to *N. meningitidis*, the antigen obtained which is reactive against NmB may be combined with components used in vaccines for the A and/or C serogroups. It may also conveniently be combined antigenic components which provide protection against *Haemophilus* and/or
10 *Streptococcus pneumoniae*. The additional antigenic components may be polypeptides or they may be other antigenic components such as a polysaccharide. Polysaccharides may also be used to enhance the immune response (see, for example, Makela *et al* (2002) *Expert Rev. Vaccines* **1**, 399-410).

15 It is particularly preferred in the above vaccines and methods of vaccination if the antigen is the polypeptide encoded by any of the genes as described above (and in the Examples), such as the NMB0338 gene, or a variant or fragment or fusion as described above (or a polynucleotide encoding said antigen), and that the disease to be vaccinated against is *Neisseria meningitidis* infection (meningococcal
20 disease).

The invention will now be described in greater detail by reference to the following non-limiting Examples and Figure wherein:

25 Figure 1 is a schematic representation of a preferred embodiment of the method of the invention

Example 1: Genetic screening for immunogens (GSI) in *N. meningitidis*

30 The application of GSI in this example involves screening libraries of insertional mutants of *N. meningitidis* for strains which are less susceptible to killing by bactericidal antibodies.

We have demonstrated the effectiveness of GSI by screening a library of mutants of the sequenced *NmB* isolate, MC58, with sera raised in mice against a capsule minus of the same strain. A total of 40,000 mutants was analysed with sera raised in mice by intraperitoneal immunisation with the homologous strain; the SBA of this sera is around 2,000 against the wild-type strain. Surviving mutants were detected when the library was exposed to serum at a 1:560 dilution (which kills all wild-type bacteria). To establish whether the transposon insertion in the surviving mutants was responsible for the ability to withstand killing, the mutations were backcrossed into the parental strain, and the backcrossed mutants were confirmed as being more resistant to killing than the wild-type in the SBA. The sequence of the gene affected by the transposon was examined by isolating the transposon insertion site by marker rescue. We found that two of the genes affected were TspA and NMB0338. TspA is a surface antigen which elicits strong CD4⁺ T cell responses and is recognized by sera from patients (Kizil *et al* (1999) *Infect Immun.* 67, 3533-41). NMB0338 is a gene of previously unknown function which encodes a polypeptide that is predicted to contain two transmembrane domains, and is located at the cell surface. The amino acid sequence encoded by NMB0338 is:

```

20 MERNGVFGKIVGNRILRMSSEHAAASYPKPKCSFKLAQSWFRVRSCLGGVFTIYGA
    NMKLIYTVIKIIILLFLLLAVINTDAVTFSYLPGQKFDLPLIVVLFGAFFVVGII
    FGMFALFGRLLSLRGENGLRAEVKKNARLTGKELTAPPAQNAPESTKQP

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There are several practical advantages of using *NmB* for GSI aside from the public health imperative: a) the bacterium is genetically tractable; b) killing of the bacterium by effector immune mechanism is straightforward to assay; c) the genome sequences are available for three isolates of different serogroups and clonal lineages (IV-A, ET-5, and ET-37 for serogroups A, B, and C, respectively); and d) well-characterised clinical resources are available for this work.

30

GSI has two potential limitations. First, targets of bactericidal antibodies may be essential. This is unlikely as all known targets of bactericidal antibodies in *NmB*

are non-essential, and no currently licensed bacterial vaccine targets an essential gene product. Second, sera will contain antibodies to multiple antigens, and, loss of a single antigen may not affect the survival of mutants. We have already shown that even during selection with sera raised against the homologous strain, relevant
5 antigens were still identified using appropriate dilutions of sera.

The major advantages of GSI are that 1) the high throughput steps do not involve technically demanding or costly procedures (such as protein expression/purification and immunisation), and 2) human samples can be used in
10 the assay rather than relying solely on animal data. GSI will rapidly pinpoint the subset of surface proteins that elicit bactericidal activity, allowing more detailed analysis of a smaller number of candidates.

1. *Identification of targets of bactericidal antibodies using GSI*

15 Murine sera raised against heterologous strains, and human sera, are used to identify cross-reactive antigens. The sera are obtained from:

- i) mice immunised by the systemic route with heterologous strains: the strains will be selected and/or constructed to avoid isolates with the same immunotype and sub-serotype.
- 20 ii) acute and convalescent sera from patients infected with known isolates of *N. meningitidis* (provided by Dr R. Wall, Northwick Park)
- iii) pre- and post-immunisation samples (provided by the Meningococcal Reference Laboratory) from volunteers receiving defined outer membrane vesicle (OMVs) vaccines derived from the NmB isolate,
25 H44/76.

Each of these sources of sera has specific advantages and disadvantages.

Serum source	Advantages	Disadvantages
Murine	1) Defined antigenic exposure. 2) Use of genetically modified strains to generate immune response. 3) Naïve samples available 4) Examine individuals responses	1) Animal source of material
Patient sera	1) Human material 2) Known strain exposure 3) Acute and convalescent sera available	1) Background immunity 2) Limited material
Sera following immunisation with H4476 OMVs	1) Human material 2) Defined antigenic exposure 3) Pre and post immunisation sera available 4) Examine individuals responses	1) Background immunity 2) Limited material

a) Sera from animals immunised with heterologous strains (ie the sequenced serogroup A or C strains) are used in GSI to select the library of MC58 mutants. We have shown that immunisation with live, attenuated *NmB* elicits cross-reactive bactericidal antibody responses against serogroup A and C strains. The antigen absent in mutants with enhanced survival in the face of human sera are identified by marker rescue of the disrupted gene.

b) Mutations are identified that confer resistance against killing by heterologous sera, and it is determined whether the gene product is also a target for killing of the sequenced, serogroup A and C strains, Z2491 and FAM18 respectively. The genome databases are inspected for homologues of the genes. If a homologue is present, the transposon insertion is amplified from the MC58 mutant and introduced into the serogroup A and C strains by transformation. The relative survival of the mutant and wild-type strain of each serogroup are

compared. Thus, GSI can quickly give information whether the targets of bactericidal activity are conserved and accessible in diverse strains of *N. meningitidis*, irrespective of serogroup, immunotype and subserotype.

- 5 c) Mutants with enhanced survival against sera raised in mice are tested using human sera from either convalescent patients or vaccinees receiving heterologous OMV vaccines (derived from H44/76). This addresses the important question of whether the targets are capable of eliciting bactericidal antibodies in human. With other vaccine approaches, this information is only gained at the late, expensive
10 stage of clinical trials that requires GMP manufacture of vaccine candidates.

The advantages are that GSI is a high-throughput analysis performed using simple, available techniques. Antigens which elicit bactericidal antibodies in humans and which mediate killing of multiple strains can be identified rapidly as GSI is
15 flexible with respect to the bacterial strain and sera used. Mutants selected using human sera are analysed in the same way as those selected by murine sera.

2. *Assessment of the antibody response of recombinant GSI antigens*

- 20 Proteins which are targets of bactericidal antibodies that are recognised by sera from convalescent patients and vaccines are expressed in *E. coli* using commercially available vectors. The corresponding open reading frames are amplified by PCR from MC58, and ligated into vectors such as pCR Topo CT or pBAD/His, to allow protein expression under the control of a T7 or arabinose-
25 inducible promoter, respectively. Purification of the recombinant proteins from total cellular protein is performed via the His Tag fused to the C terminus of the protein on a Nickel or Cobalt column.

- Adult New Zealand White rabbits are immunized on two occasions separated by
30 four weeks by subcutaneous injection with 25 µg of purified protein with Freund's incomplete adjuvant. Sera from animals will be checked prior to immunisation for pre-existing anti-*Nm* antibodies by whole cell ELISA. Animals which have an

initial serum titre of <1:2 are used for immunisation experiments. Post-immunisation serum are obtained two weeks after the second immunisation. To confirm that specific antibodies have been raised, pre- and post-immunisation serum is tested by i) Western analysis against the purified protein and ii) ELISA
5 using cells from the wild-type and the corresponding mutant (generated by GSI).

SBAs will be performed against MC58 (the homologous strain), and the sequenced serogroup A and C strains with the rabbit immune serum. The assay will be performed in triplicate on at least two occasions. SBAs of >8 will be
10 considered significant. The results provide evidence of whether the protein candidates can elicit bactericidal antibodies as recombinant proteins.

3. *Establishing the protective efficacy of GSI antigens*

15 All the candidates are tested for their ability to protect animals against live bacterial challenge as this allows any aspect of immunity (cellular or humoral) to be assessed in a single assay. We have established a model of active immunisation and protection against live bacterial infection. In this model, adult mice are immunised on days 0 and 21, and on day 28 receive live bacterial challenge of 10^6
20 or 10^7 CFU of MC58 intraperitoneally in iron dextran (as the supplemental iron source). The model is similar to that described for evaluation of the protective efficacy of immunisation with Tbps Danve *et al* (1993) *Vaccine* **11**, 1214-1220. Non-immunised animals develop bacteraemia within 4 hours of infection, and show signs of systemic illness by 24 hours. We have already been able to
25 demonstrate the protective efficacy of both attenuated *Nm* strains and a protein antigen against live meningococcal challenge; PorA is an outer membrane protein that elicits bactericidal antibodies, but which is not a lead vaccine candidate because of extensive antigenic variation(Bart *et al* (1999) *Infect Immun.* **67**, 3832-3846.

30

Six week old, BALB/c mice (group size, 35 animals) receive 25 µg of recombinant protein with Freund's incomplete adjuvant subcutaneously on days

day 0 and 21, then are challenged with 10^6 (15 animals) or 10^7 (15 animals) CFU of MC58 intraperitoneally on day 28. Two challenge doses are used to examine the vaccine efficacy at a high and low challenge dose; sera are obtained on day 28 from the remaining five animals in each group, and from five animals before the first immunisation and stored at -70°C for further immunological assays. Animals in control groups receive either i) adjuvant alone, ii) recombinant refolded PorA, and iii) a live, attenuated *Nm* strain. To reduce the overall number of animals in control groups, sets of five candidates will be tested at one time (number of groups = 5 candidates + 3 controls). Survival of animals in the groups is compared by Mann Whitney U Test. With group sizes of 15 mice/dose, the experiments are powered to show a 25% difference in survival between groups.

For vaccines which show significant protection against challenge, a repeat experiment is performed to confirm the finding. Furthermore, to establish that vaccination with a candidate also elicits protection against bacteraemia, levels of bacteraemia are determined during the second experiment; blood is sampled at 22 hr post-infection in immunised and un-immunised animals (bacteraemia is maximal at this time). The results are analysed using a two-tailed Student-T test to determine if there is a significant reduction in bacteraemia in vaccinated animals.

Further materials and methods used

Mutagenesis of Neisseria meningitidis

5 For work with *Neisseria meningitidis*, mutants were constructed by *in vitro* mutagenesis. Genomic DNA from *N. meningitidis* was subjected to mutagenesis with a Tn5 derivative containing a marker encoding resistance to kanamycin, and an origin of replication which is functional in *E. coli*. These elements are bound by composite Tn5 ends. Transposition reactions were carried out with a
10 hyperactive variant of Tn5 and the DNA repaired with T4 DNA polymerase and ligase in the presence of ATP and nucleotides. The repaired DNA was used to transform *N. meningitidis* to kanamycin resistance. Southern analysis confirmed that each mutant contained a single insertion of the transposon only.

15 *Serum bactericidal assays (SBAs)*

Bacteria were grown overnight on solid media (brain heart infusion media with Levanthals supplement) and then re-streaked to solid media for four hours on the morning of experiments. After this time, bacteria were harvested into phosphate
20 buffered saline and enumerated. SBAs were performed in a 1 ml volume, containing a complement source (baby rabbit or human) and approximately 10^5 colony forming units. The bacteria were collected at the end of the incubation and plated to solid media to recover surviving bacteria.

25 *Isolating the transposon insertion sites*

Genomic DNA will be recovered from mutants of interest by standard methods and digested with *PvuII*, *EcoRV*, and *DraI* for three hours, then purified by phenol extraction. The DNA will then be self-ligated in a 100 microlitre volume
30 overnight at 16°C in the presence of T4 DNA ligase, precipitated, then used to transform *E. coli* to kanamycin resistance by electroporation.

Example 2: Further screening and results thereof

GSI has been used to screen a library of approximately 40,000 insertional mutants of MC58. The library was constructed by *in vitro* Tn5 mutagenesis, using a transposon harbouring the origin of replication from pACYC184.

MC58 was chosen as it is a serogroup B isolate of *N. meningitidis*, and the complete genome sequence of this strain is known.

The library is always screened in parallel with the wild-type strain as a control, and the number of colonies recovered from the library and the wild-type is shown.

Selection with murine sera

Initially the library was analysed using sera from animals immunised with the attenuated strain YH102. Adult mice (Balb/C) received 10⁸ colony forming units intra-peritoneally on three occasions, and sera was collected 10 days after the final immunisation,

The screen identified several mutants with enhanced resistance to serum killing: This was confirmed by isolating individual mutants, reconstructing the mutation in the original genetic background, and re-testing the individual mutants for their susceptibility to complement mediated lysis against the wild-type. The transposon insertions are in the following gene:

25

NMB0341 (TspA) DNA sequence

ATGCCCCGCGCCGACTGCCCCGCGATGCCCCGATGATGACGAAATTTACAGACTGTACG
 CGGTCAAACCGTATTTCAGCCGCCAACCACAGGGGATACATCTTGAAAAACAACAGACAA
 ATCAAACCTGATTGCCGCTCCGTGCGAGTTGCCGCATCCTTTTCAGGCACATGCTGGACTG
 30 GGCGGACTGAATATCCAGTCCAACCTTGACGAACCCTTTTCGGGCAGCATTACCGTAACC
 GGCGAAGAAGCCAAAGCCCTGCTAGGCGGCGGCAGCGTTACCGTTTCCGAAAAAGGCCTG
 ACCGCCAAAGTCCACAAAGTTGGGCGACAAAGCCGTCAATTGCCGTTTCTTCCGAACAGGCA
 GTCCGCGATCCCGTCTGGTGTTCGCGATCGGCGCAGGCGCACAGGTACGCGAATACACC
 GCCATCCTCGATCCTGTCTGGCTACTCGCCCAAACCAAATCTGCACTTTCAGACGGCAAG
 35 ACACACCGCAAAACCGCTCCGACAGCAGAGTCCCAAGAAAATCAAACGCCAAAGCCCTC
 CGCAAAACCGATAAAAAAGACAGCGCAACGCAGCCGTCAAACCGGCATACAACGGCAAA
 ACCCATACCGTCCGCAAAAGGCGAAACGGTCAAACAGATTGCCGCCGCCATCCGCCGAAA
 CACCTGACGCTCGAACAGGTTGCCGATGCGTGTGAAGGCAAACCCAAATGTTTCCGCA

CACGGCAGACTGCGTGCGGGCAGCGTGCTTCACATTCCGAATCTGAACAGGATCAAAGCG
 GAACAACCCAAACCGCAAACGGCGAAACCCAAAGCCGAAACCGCATCCATGCCGTCGGAA
 CCGTCCAAACAGGCAACGGTAGAGAAACCGGTTGAAAAACCTGAAGCAAAAGTTGCCGCG
 CCCGAAGCAAAAGCGGAAAAACCGGCCGTTTCGACCCGAACCTGTACCCGCTGCAAATACT
 5 GCCGCATCGGAAACCGCTGCCGAATCCGCCCCCAAGAAGCCGCCGCTTCTGCCATCGAC
 ACGCCGACCGACGAAACCGGTAAACGCCGTTTCCGAACCTGTCGAACAGGTTTCTGCCGAA
 GAAGAAACCGAAAGCGGACTGTTTGACGGTCTGTTTCGGCGGTTTCGTACACCTTGCTGCTT
 GCCGGCGGAGGCGCGGCATTAATCGCCCTGCTGCTGCTTTTGCGCCTTGCCCAATCCAAA
 CGCGCGCGCCGTACCGAAGAATCCGTCCCTGAGGAAGAGCCTGACCTTGACGACGCGGCA
 10 GACGACGGCATAGAAATCACCTTTGCCGAAGTCGAAACTCCGGCAACGCCCGAACCCGCT
 CCGAAAAACGATGTAACGACACACTTGCCCTAGATGGGGAATCTGAAGAAGAGTTATCG
 GCAAAACAAACGTTTCGATGTGCGAAACCGATACGCCCTTCCAACCGCATCGACTTGGATTTT
 GACAGCCTGGCAGCCGCGCAAAACGGCATTTTATCCGGCGCACTTACGCAGGATGAAGAA
 ACCCAAAAACGCGCGGATGCCGATTGGAACGCCATCGAATCCACAGACAGCGTGTACGAG
 15 CCCGAGACCTTCAACCCGTACAACCCGTGTCGAAATCGTCATCGACACGCCCGAACCGGAA
 TCTGTGCGCCAAACTGCCGAAAAACAAACCGGAAACCGTCGATACCGATTTCTCCGACAAC
 CTGCCCTCAAACAACCATATCGGCACAGAAGAAACAGCTTCCGCAAAACCTGCCTCACCC
 TCCGGACTGGCAGGCTTCTGAAGGCTTCTCGCCCGAAACCATCTTGAAAAAACAGTT
 GCCGAAGTCCAAACACCGGAAGAGTTGCACGATTTCCTGAAAGTGTACGAAACCGATGCC
 20 GTCGCGGAAACTGCGCCTGAAACGCCCGATTTCAACGCCCGCCGAGACGATTTGTCCGCA
 TTGCTTCAACCTGCCGAAGCACCCTCCGTTGAGGAAAAATATAACGGAAACCGTTGCCGAA
 ACACCCGACTTCAACGCCACCGCAGACGATTTGTCCGCATTACTTCAACCTTCTAAAGTA
 CCTGCCGTTGAGGAAAAATGCAGCGGAAACCGTTGCCGATGATTTGTCCGCACTGTTGCAA
 CCTGCTGAAGCACCGGCCGTTGAGGAAAAATGTAACGGAAACCGTTGCCGAAACACCCGAT
 25 TTCAACGCCACCGCAGACGATTTGTCCGCATTACTTCAACCTTCTGAAGCACCTGCCGTT
 GAGGAAAAATGCAGCGGAAACCGTTGCCGATGATTTGTCCGCACTGTTGCAACCTGCTGAA
 GCACCGGCCGTTGAGGAAAAATGCAGCGGAAATCACTTTGGAAACGCCTGATTCCAACACC
 TCTGAGGCAGACGCTTTGCCCGACTTCTGAAAGACGGCGAGGAGGAAACGGTAGATTGG
 AGCATCTACCTCTCGGAAGAAAAATATCCCAAATAATGCAGATACAGTTTCCCTTCGGAA
 30 TCTGTAGGTTCTGACGCGCCTTCCGGAAGCAATACGACCTTGCCGAAATGTATCTCGAA
 ATCGGCGACCGCGATGCCGCTGCCGAGACAGTGCAGAAATTGCTGGAAGAAGCGGAAGGC
 GACGTACTCAAACGTGCCCAAGCATTTGGCGCAGGAATTGGGTATTTGA

NBM0341 Protein sequence

35 MPAGRLPRRCFMMTKFTDCTRSNRIQPPTHRGIYILKNNRQIKLIAASVAVAASFQAHAGL
 GGLNIQSNLDEPFSGSITVTGEEAKALLGGGSVTVSEKGLTAKVHKLGDKAVIAVSSEQA
 VRDPVLVFRIGAGAQVREYTAILDPVGYSPTKLSALSDGKTHRKTAPTAESENQNAKAL
 RKTDKDSANAAVKPAYNGKTHTVRKGETVKQIAAAIRPKHLTLEQVADALLKANPNVSA
 HGRRLRAGSVLHIPNLNRIKAEQPKPQTAKPKAETASMPSEPSKQATVEKPVEKPEAKVAA
 40 PEAKAEKPAVRPEPVPAAANTAASETAESAPEAAASAITPTDETGNVSEPVEQVSAE
 EETESGLFDGLFGGSYTLTLAGGGAALIALLLLRLAQSKRARRTEESVPEEEDLDDAA
 DDGIEITFAEVETPATPEPAPKNDVNDTLALDGESEEEELSAKQTFDVETDTPSNRIDLDF
 DSLAAAQNGILSGALTQDEETQKRADADWNAIESTDSVYEPETFPNPNPVEIVIDTPEPE
 SVAQTAENKPEVTVDTFSDNLPNNHIGTEETASAKPASPSGLAGFLKASSPETILEKTV
 45 AEVQTPEELHDFLKVYETDAVAETAPETPDFNAAADDLSALLQPAEAPSVEENITETVAE
 TPDFNATADDLSALLQPSKVPVEENAAETVADDLSALLQPAEAPAVEENVETETVAETPD
 FNATADDLSALLQPEAPAVEENAAETVADDLSALLQPAEAPAVEENAAEITLETTPDSNT
 SEADALPDFLKDGEETVDWSIYLSEENIPNNADTSFPSESVGSDAPSEAKYDLAEMYLE
 50 IGDRDAAAETVQKLLEEAEGDVLKRAQALAEELGI

NMB0338 DNA sequence

ATGGAAAGGAACGGTGTAATTTGGTAAAATTGTCGGCAATCGCATACTCCGTATGTCGTCC
 GAACACGCTGCCGCATCCTATCCGAAACCGTGCAAATCGTTTAACTAGCGCAATCTTGG
 TTCAGAGTGCGAAGCTGTCTGGGCGGCGTTTTTATTTACGGAGCAAACATGAACTTATC
 55 TATACCGTCATCAAAATCATTATCCTGCTGCTCTTCTGCTGCTTGCCGTCATTAATACG
 GATGCCGTTACCTTTTCTACCTGCCGGGGCAAAAATTCGATTTGCCGCTGATTGTGCTGA
 TTGTTCCGGCGCATTTGTAGTCGGTATTATTTTTGGAATGTTTGCCTTGTTCCGACGGTTG
 TTGTCGTTACGTGGCGAGAACGGCAGGTTGCGTGCCGAAGTAAAGAAAAATGCGCGTTTG

ACGGGGAAGGAGCTGACCGCACCACCGGCGCAAAATGCGCCCGAATCTACCAAACAGCCT
TAA

NMB0338 Protein sequence

5 MERNGVFGKIVGNRIIRMSSEHAAASYPKPKCSFKLAQSWFRVRSCLGGVFIYGANMKLI
YTVIKIIILLLLFLLAVINTDAVTFSYLPGQKFDLPLIVVLFGAFVVGIIIFGMFALFGRL
LSLRGENGRRLRAEVKKNARLTGKELTAPPAQNAPESTKQP

Analysis of the polypeptide indicates that it is predicted to have two membrane
10 spanning domains, from residues 54 to 70 and 88 to 107. Thus, fragments from
the regions 1 to 53, and 108 to the end (C-terminal) may be particularly useful as
immunogens.

NMB1345 DNA sequence

15 ATGAAAAAACCTTTGATTTGCGTTGCGGCAGCATTGCTCGGCGTTGCTTTGGGCACGCCT
TATTATTTGGGTGTCAAAGCCGAAGAAAGCTTGACGCAGCAGCAAAAAATATTGCAGGAA
ACGGGCTTCTTGACCGTCGAATCGCACCAATATGAGCGCGGCTGGTTTACCTCTATGGAA
ACGACGGTCATCCGTCTGAAACCCGAGTTGCTGAATAATGCCCGAAAATACCTGCCGGAT
AACCTGAAAACAGTGTTGGAACAGCCGGTTACGCTGGTTAACCATATCACGCACGGCCCT
20 TTCGCCGGCGGATTTCGGCACGCAGGCGTACATTGAAACCGAGTTCAAATACGCGCCTGAA
ACGGAAAAAGTTCTGGAACGCTTTTTTGGAAAACAAGTCCCGGCTTCCCTTGCCAATACC
GTTTATTTTAACGGCAGCGGTAAAATGGAAGTCAGTGTTCCCGCCTTCGATTATGAAGAG
CTGTCGGGCATCAGGCTGCACTGGGAAGGCCGTGACGGGAGAAAACGGTTTATCAAAAAGGT
TTCAAAAAGCTACCGGAACGGCTATGATGCCCCCTTGTTTAAATCAAGCTGGCAGACAAA
25 GGCGATGCCGCGTTTGAAAAAGTGCAATTCGATTTCGGAAACTTCAGACGGCATCAATCCG
CTTGCTTTGGGCAGCAGCAATCTGACCTTGGAAAAATTCTCCCTAGAATGGAAAGAGGGT
GTCGATTACAACGTCAAGTTAAACGAACTGGTCAATCTTGTTACCGATTTGCAGATTGGC
GCGTTTATCAATCCCAACGGCAGCATCGCACCTTCCAAAATCGAAGTCGGCAAACCTGGCT
TTTTCACCAAGACCGGGGAATCAGGCGCGTTTATCAACAGTGAAGGGCAGTTCCGTTTC
30 GATACTAGGTGTACGGCGATGAAAAATACGGCCCGCTGGACATCCATATCGCTGCCGAA
CACCTCGATGCTTCTGCCTTAACCGTATTGAAACGCAAGTTTGACAAAATTTCCGCCAAA
AAAATGACCGAGGAACAAAATCCGCAATGATTTGATTGCCGCCGTCAAAGGAGAGGCTTCC
GGACTGTTACCAACAATCCCGTATTGGACATTAAACTTTCCGATTACGCTGCCATCG
GGAAAAATCGATGTGGGCGGAAAAATCATGTTTAAAGACATGAAGAAGGAAGATTTGAAT
35 CAATTGGGTTTGATGCTGAAGAAAACCGAAGCCGACATCAGAATGAGTATTCCTCAAAAA
ATGCTGGAAGACTTGGCGGTGAGTCAAGCAGGCAATATTTTCAGCGTCAATGCCGAAGAT
GAGGCGGAAGGCAGGGCAAGTCTTGACGACATCAACGAGACCTTGCGCCTGATGGTGGAC
AGTACGGTTCAGAGTATGGCAAGGGAAAAATATCTGACTTTGAACGGCGACCAGATTGAT
ACTGCCATTTCTCTGAAAAACAATCAGTTGAAATGAACGGTAAAACGTTGCAAAACGAA
40 CCGGAGCCGATTTTGATGAAGGCGGTATGGTTTCAGAGCCGCAGCAGTAA

NMB1345 Protein sequence

45 MKKPLISVAAALLGVALGTPYYLGVKAEESLTQQQKILQETGFLTVESHQYERGWFTSME
TTVIRLKPPELLNNARKYLPDNLKTVLEQPVTLVNHITHGPFAGGFGTQAYIETEFKYAPE
TEKVLERFFGKQVPASLAN TVYFNGSGKMEVSVPAFDYEELSGIRLHWEGLTGETVYQKG
FKSYRNGYDAPLFKIKLADKGDAAFEKVHFDSETSDGINPLALGSSNLTLEKFSLEWKEG
VDYNVKNELVNLVTDLQIGAFINPNSIAPSKIEVGKLAFTSTKTGESGAFINSEGQFRF
DVLVYGDEKYGPLDIHIAAEHLDAALVGLKRFQISAKKMTTEEQIRNDLIAAVKGEAS
GLFTNNPVLDIKTFRFTLP SGKIDVGGKIMFKDMKKEDLNQLGLMLKKTEADIRMSI PQK
50 MLEDLAVSQAGNIFSVNAEDEAEGRASLDDINETLRMLVDSTVQSMAREKYLTINGDQID
TAISLKNQKLKLNKTLQNEPEPDFDEGGMVSEPPQQ

Selection with vaccinees sera

Sera from the Meningococcal Reference Laboratory in Manchester has been made available to us. This sera has come from a clinical trial of OMV immunisation of
 5 volunteers.

Mutants selected by vaccinee C1 sera (screened once)

The following sequences were isolated

10

NMB0338 (as above)

NMB0738 DNA sequence

15 ATGAAGATCGTCCTGATTAGCGGCCTGTCCGGTTCGGGCAAGTCCGTCGCACTGCGCCAA
 ATGGAAGATTCGGGTATTCTGCGTGGACAATTTGCCTTTGGAAATGTTGCCCCGCGCTG
 GTGTCGTATCATATCGAACGTGCGGACGAAACCGAATTGGCGGTGAGCGTCGATGTGCGT
 TCCGGCATTGACATCGGACAGGCGCGGGAACAGATTGCCTCTCTGCGCAGACTGGGGCAC
 AGGGTTGAAGTTTGTGTCGAGGCGGAAGAAAGCGTGTGGTCCGCCGGTTTCCGAA
 ACCAGGCGAGGACATCCTCTGAGCAATCAGGATATGACCTTGTGGAAAGCTTAAAGAAA
 20 GAACGGGAATGGCTGTCCCGCTTAAAGAAATCGCCTATTGTATCGACACTTCCAAGATG
 AATGCCCAACAGCTCCGCCATGCAGTCCGGCAGTGGCTGAAGGTCGAACGTACCGGGCTG
 CTGGTGATTTTGGAGTCCTTCGGGTTCAAATACGGTGTGCCGAACAACGCGGATTTTATG
 TTGATATGCGCAGCCTGCCAACCCTATTACGATCCCGAGTTGAGGCCTTACACCGGT
 ATGGACAAGCCGTTTGGGATTATTTGGACGGACAGCCGCTTGTGCAGGAAATGGTTGAC
 25 GACATCGAAAGGTTTGTACGCATTGGTTACCGCGTTTGGAGGATGAAAGCAGGAGCTAC
 GTTACCGTCGCCATCGGTTGCACGGGAGGACAGCACCCTTCGGTCTATATTGTGCAAAAA
 CTCGCCCCAAGGTTGAAAGGGCGTTATGAATTGCTGATACGGCACAGACAGGCGCAAAAC
 CTGTCAGACCGCTAA

30 NMB0738 Protein sequence

MKIVLISGLSGSGKSVALRQMEDSGYFCVDNLPLEMLPALVSYHIERADETELAVSVDVR
 SGIDIGQAREQIASLRRLGHRVEVLVFEAEESVLVRRFSETRRGHPLSNQDMTLLESLKK
 EREWLFPLKEIAYCIDTSKMNAQQLRHAVRQWLKVERTGLLVILESFGFKYGVPPNADFM
 FDMRSLPNPYDPELRPYTGMDKPVWDYLDGQPLVQEMVDDIERFVTHWLPRLLEDERSY
 35 VTVAIGCTGGQHRSVYIVEKLARRLKGRYELLIRHRQAQNLSDR

NMB0792 NadC family (transporter) DNA sequence

ATGAACCTGCATGCAAAGGACAAAACCCAGCATCCCGAAAACGTCGAGCTGCTCAGTGCG
 CAGAAGCCGATTACCGACTTTAAGGGCCTGCTGACCACCATTATTTCCGCCGTCGTCTGT
 40 TTCGGCATTACACATCCTGCCTTACAGCCCCGATGCCAATAAAGGTATCGCGCTGCTG
 ATTTTCGTTGCCGCACTTTGGTTTACCGAGGCCGTCCACATTACCGTAACCGCACTGATG
 GTGCCGATTCTCGCCGTCGTACTCGGTTTCCCCGACATGGACATCAAAAAGGCGATGGCT
 GATTTTTCCAACCGATTATCTACATTTTTTTCGGCGGCTTCGCGCTTGCCACCGCCCTG
 CATATGCAGCGGCTGGACCGTAAAATCGCCGTGAGCCTGTTGCGCCTGTGCGCGGCAAT
 45 ATGAAAGTGGCGGTTTTGATGTTGTTCTCTGTTACCGCCTTTCTGTCCATGTGGATCAGC
 AACACCGCCACCGCCGCGATGATGCTGCCTCTAGCAATGGGTATGCTGAGCCACCTCGAC
 CAGGAAAAAGAACAAAAACCTACGTCTTCCTCCTGCTCGGCATCGCCTATTGCGCCAGC
 ATCGGCGGCTTGGGCACGCTCGTCGGCTCGCCGCCAACCTGATTGCCGCCAAAGCCCTA

AATCTGGACTTCGTCGGCTGGATGAAGCTCGGCCTGCCGATGATGCTGTTGATTCTGCCC
TTGATGCTGCTCTCCCTGTACGTCATCCTCAAACCTAATTTGAACGAACGCGTGGAAATC
AAAGCCGAATCCATCCCTTGGACGCTGCACCGCGTGATCGCGCTGTTGATTTTCTTGCC
ACAGCCGCCGCGTGGATATTAGCTCCAAAATCAAACCGCCTTCGGCATTTCCAATCCC
5 GACACCGTTATCGCCCTGAGTGCCGCCGTGCGCGTCTGCTCTTCGGCGTGGCGCAATGG
AAGGAAGTCGCCCCGAATACCGACTGGGGCGTGTTGATGCTCTTCGGCGGCGGCATCAGC
CTGAGCACGCTGTTGAAAACATCCGGCGCGTCCGAAGCCTTGGGACAGCAGGTTGCCGCC
ACCTTTTCCGGCGCGCCCGCATTTTTTGGTGATACTCATCGTCGCCCGCTTCATTATTTTT
CTGACCGAGTTCACCAGCAACACCGCCTCCGCCGATTGCTTGTACCGATTTTCTCCGGC
10 ATCGCTATGCAGATGGGGCTGCCGAACAAGTCTTGGTATTTCGTATCGGCATCGGCACA
TCTTGTGCTTCATGCTGCCGGTTGCCACACCGCCTAACGCGATTGTGTTCCGGCACGGGC
TTAATCAAGCAACGCGAAATGATGAATGTCCGCATACTGCTGAACATCCTCTGCGTAGTA
TTGGTTGCTCTGTGGGCTTATGCTGTACTGATGTAA

15 NMB0792 Protein sequence

MNLHAKDKTQHPENVELLSAQKPITDFKGLLTIIISAVVCFGIYHILPYSPPDANKGIAL
IFVAALWFTEAVHITVTALMVPILAVVLGFPDMDIKKAMADFSNPPIIYIFFGGFALATAL
HMQRDLDRKIAVSLRLSRGNMKVAVLMLFLVTAFLSMWISNTATAAMMLPLAMGMLSHLD
QEKEHKTYVFLLLGIAYCASIGGLGLTVGSPNLI AAKALNLDVFGWMKLG LPMMLLILP
20 LMLLSLYVILKPNLNERVEIKAESIPWTLHRVIALLI FLATAAAWIFSSKIKTAFGISNP
DTVIALSAAVAVVFGVAQWKEVARNTDWGVLMFLGGGISLSTLLKTS GASEALGQQVAA
TFSGAPFLVILIVAAFIIFLTEFTSNTASAALLVPIFSGIAMQMGLPEQVLV FVIGIGA
SCAFMLPVATPPNAIVFGTGLIKQREMMNVGILLNLCVVLVALWAYAVLM

25 NMB0279 DNA sequence

ATGCAACGACAAATCAAACCTGAAAAATTGGCTTCAGACCGTTTATCCCGAACGGGACTTC
GATCTGACTTTTGGCGGCGCGGATGCTGATTTCCGCCGCTATTTCCGTGCAACGTTTCA
GACGGCAGCAGTGTCTGTCATGGATGCACCGCCGACAAGATGAGTGTGCGACCTTAT
TTGAAAGTGCAGAACTGTTTGACATGGTCAATGTGCCGAGGTATTGCACGCGGACACG
30 GATCTGGGGTTTGTGGTATTGAACGACTTGGGCAATACGACGTTTTTGACCGCAATGCTT
CAGGAACAGGGCGAAACGGCGCACAAAGCCCTGCTTTTGGAGGCAATCGGCGAGTTGGTC
GAATTGCAGAAGGCGAGCCGTGAAGGGGTTTTGCCCGAATATGACCGTGAAACGATGTTG
CGCGAAATCAACCTGTTCCCGGAATGGTTTGTGCGAAAAGAATTGGGGCGCGAATTAACA
TTCAAACAACGCCAACTTTGGCAGCAAACCGTCGATACGCTGCTGCCGCCCTGTTGGCG
35 CAGCCCAAAGTCTATGTGCACCGCGACTTTATCGTCCGCAACCTGATGCTGACGCGCGGC
AGGCCGGGCGTTTTAGACTTCCAAGACGCGCTTTACGGCCCGATTTCCTACGATTTGGTG
TCGCTGTTGCGCGATGCCTTTATCGAATGGGAAGAAGAATTGTCTTGACTTGGTTATC
CGCTACTGGGAAAAGGCGCGGGCTGCCGCTTCCCGTCCCGAAGCGTTTGACGAGTTT
TACCGCTGGTTTCAATGGATGGGCGTGCAGCGGCACCTGAAGGTTGCAGGCATCTCGCA
40 CGCCTGTACTACCGCGACGGCAAAGACAAATACCGTCCGGAAATCCCGCGTTTCTTAAAC
TATCTGCGCCGCTATCGCGCGTTTATGCCGAACTCGCCCCGCTCTACGCGCTCTTGGTC
GAACTGGTCGGCGATGAAGAACTGGAACGGGCTTTACGTTTTAA

NMB0279 Protein sequence

45 MQRQIKLKNWLQTVYPERDFDLTFAAADADFRRYFRATFS DGSSVVCMDAPPDKMSVAPY
LKVQKLFDMVNPQVLHADTDLGFVVLNDLGNNTTFLTAMLQEQGETAHKALLLEAIGELV
ELQKASREGVLPEYDRETMREINLFPWFVAKELGRELT FKRQLWQQTVDTLPLPLLA
QPKVYVHRDFIVRNLMLTRGRPGVLD FQDALYGPISYDLVSLLRDAFIEWEEEFVLDLVI
RYWEKARAAGLPVPEAFDEFYRWF EWMGVQRHLKVAGIFARLYYRDGDKYRPEI PRFLN
50 YLRRVSRRYAELAPLYALLVELVGDEELETGFTF

NMB2050 DNA sequence

ATGGAAGTGAAGGCGGTTTGGCGGCGTTGGTGTCGGGCGTGTTGTTTACA
TGTTTGCTGATGAAGGGCCGTTT CAGGGCGAGTTTGCCGTTTGAACGCGCACCTGGCG
55 GAAAAGGCGGCAAGATGTGATTTTGTGCAACAGGCACACGGCAAACCGTGT CGGAATTG
GCGGTGTTGGACGGGAAATACCGGCATTTGCAGGACGAAAATTATGCTTTGGGCAACCGT
TTTTCCGCAGCCGAAAAGCAGATTGCCCATTTGCAGGAAAAGAGGCGGAGTCGGCGCGG
CTGAAGCAGTCGTATATCGAGTTGCAGGAAAAGGCACAGGGTTTGGCGGTTGAAAACGAA

CGTTTGGCAACGCAGCTCGGACAGGAACGGAAGGCGTTTGCCGACCAATATGCCTTGGAA
 CGCCAAATCCGCCAAAGAATCGAAACCGATTTGGAAGAAAGCCGCCAAACTGTCCGCGAC
 GTGCAAAACGACCTTTCCGATGTCGGCAACCGTTTGGCGCAGCCGAAAAACAGATTGCC
 CATTTGCAGGAAAAAGAGGCGGAAGCGGAGCGGTTGAGGCAGTCGCATACCGAGTTGCAG
 5 GAAAAGGCACAGGGTTTGGCGGTTGAAAACGAACGTTTGGCAACGCAAATCGAACAGGAA
 CGCCTTGCTTCTGAAGAGAAGCTGTCCTTGCTGGGCGAGGCGCGCAAAAGTTTGAGCGAT
 CAGTTTCAAAATCTTGCCAACACGATTTTGAAGAAAAAAGCCGCCGTTTACCGAGCAG
 AACCGCGAGCAGCTCCATCAGGTTTTGAACCGCTAAACGAACGCATCCACGGTTTCGGC
 GAGTTGGTCAAGCAAACCTATGATAAAGAATCGCGCGAGCGGCTGACGTTGGAAAAACGAA
 10 TTGAAACGGCTTCAGGGGTTGAACGCGCAGCTGCACAGCGAGGCAAAGGCCCTGACCAAC
 GCGCTGACCGGTACGCAGAATAAGGTTTCAGGGCAATTGGGGCGAGATGATTCTGAAACG
 GTTTTGGAAAAATTCGCGCCTTCAGAAAGGGCGGGAATATGTGGTTTCAGGCGGCATCCGTC
 CGAAAAAGAGGAAGACGCGGCGACGCGCCGCTCCAGCCCGACGTTTTTGGTCAACCTGCCC
 GACAACAAGCAGATTGTGATTGATTCCAAGGTCTCGCTGACAGCTTATGTGCGCTACACG
 15 CAGGCGGCGGATGCGGATACGCGGCGACGCGAAGTGGCGGCACACGTTGCCAGCATCCGT
 GCACACATGAAAGGCTTGTGCTGAAGGATTACACCGATTTGGAAGGTGTGAACACATTG
 GATTTCTGCTTTATGTTTATCCCTGTGCAACCGGCTTACCTGTTGGCGTTGCAGAATGAC
 GCGGGCTTGTCCAAGAGTGTTCGACAAACGATTATGCTGGTTCGGCCCCAGTACGCTG
 CTGGCGACTTTGAGGACGGTGGCGAATATTTGGCGCAACGAACAGCAAAATCAGAACGCA
 20 CTGGCGATTGCGGACGAAGGCGGCAAGCTGTACGACAAGTTTGTTCGGCTTCGTACAGACG
 CTCGAAAGCGTCGGCAAAGGCATCGATCAGGCGCAAAGCAGTTTTTCAGACGGCATTCAAG
 CAACTTGCCGAAGGGCGCGGGAATCTGGTTCGGACGCGCCGAGAAACTGCGTCTGTTGGGC
 GTGAAGGCAGGCAAAACACTTCAACGGGATTTGGTTCGAGCGTTCCAATGAAACAACGGCG
 TTGTCGGAATCTTTGGAATACGCGGCAGAAGATGAAGCAGTCTGA

25

NMB2050 Protein sequence

MEIMTVLLPLAALVSGVLF TWLLMKGRFQGEFAGLNAHLAEKAARCDFVEQAHGKTVSEL
 AVLDBGKYRHLQDENYALGNRFSAAEKQIAHLQKEKEAESARLKQSYIELQEKAOGLAVENE
 RLATQLGQERKAFADQYALERQIRQRIETDLEESRQTVRDVQNDLSDVGNRFAAAEKQIA
 30 HLQKEKEAEERLRQSHTELQEKAOGLAVENERLATQIEQERLASEEKLSSLGEARKSLSD
 QFQNLANTILEEKSRRFTEQNREQLHQVLNPLNERIHGFELVKQTYDKESRERLTLENE
 LKRLQGLNAQLHSEAKALTNALTGTONKVQGNWEMILETVLENSGLQKGREYVVQAASV
 RKEEDGGTRRLQPDVLVNLDPNKQIVIDSKVSLTAYVRYTQAADADTAARELAHVASIR
 AHMKGLSLKDYTDLEGVNLTDFVFMFIPVEPAYLLALQNDAGLFQECFDRIMLVGPSTL
 35 LATLRVTANIWRNEQQNQNALAIADDEGGKLYDKFVGFVQTLESVKGIDQAQSSFQATAFK
 QLAEGRGNLVGRAEKLRLLVGVKAGKQLQORDLVERSNETTALSESLEYAAEDEAV

NMB1335 CreA protein DNA sequence

ATGAACAGACTGCTACTGCTGTCTGCCCGCTCCTGCTGACTGCCTGCGGCAGCGGCGAA
 40 ACCGATAAAATCGGACGGGCAAGTACCGTTTTCAACATACTGGGCAAAACGACCGTATC
 GAAGTGAAGGATTTCGACGATCCCGACGTTCAAGGGGTTGCCTGTTATATTTCTGATGCA
 AAAAAAGGCGGCTTGAAGGAAATGGTCAATTTGGAAGAGGACGCGTCCGACGCATCGGTT
 TCGTGCCTTCAGACGGCATCTTCGATTTCTTTTACGAAACCGCCGTGCGCAAACCGAAA
 GAAGTTTTCAAACACGGTGCAGCTTCGCGTTCAAGAGCCGGCAGATTGTCCGTTATTAC
 45 GACCCCAAACGCAAAACCTTCGCCTATTTGGTGTACAGCGATAAAATCATCCAAGGCTCG
 CCGAAAAATTCCTTAAGCGCGGTTTCCTGTTTCGGCGGCGGCATACCGCAAACCGATGGG
 GTGCAAGCCGATACTTCGGCAACCTGCTTGCCGCGCGCCTGCATGATTTCCAACCCGATA
 GAAATCTCGACAAACGCTGA

NMB1335 Protein sequence

MNRLLLLSAAVLLTACSGETDKIGRASTVFNILLKNDRIEVEGFDDPDVQGVACYISYA
 KKGGLKEMVNLEEDASDASVSCVQTASSISFDETAVRKPKEVFKHGASFAFKSRQIVRY
 DPKRKTFAYLVSYSKIIQGSFKNLSAVSCFGGGIPQTDGVQADTSNLLAGACMISNPI
 55 ENLDKR

NMB2035 DNA sequence

ATGACCGCCTTTGTCCACACCCTTTTCAGACGGCATGGAACTGACCGTCGAAATCAAGCGC
 CGTGCCAAGAAAAACCTGATTATCCGCCCCGCCGGCACACATACCGTCCGCATCAGCGTC

CCACCTGCTTCTCCGTCTCCGCTCTAAACCGCTGGCTGTATGAAAACGAAGCCGCTCCTG
 CGGCAAACTGCGGAAAACACCGCCGCCGCAAAC TGCCGAAAACCGGCTGCCCCGAATCC
 ATCCTCTTCCACGGCAGACAGCTTGCCCTCACCGCCCATCAAGACACGCAAATCCTGCTG
 ATGCCGTCTGAAATCCGTGTTCCCGAAGGCGCACCCGAAAAACAGCTTGCGCTGCTGCGG
 5 GACTTTTTGGAACGGCAGGCGCACAGTTACCTGATTCCTCGCTCGAACGCCACGCCCGC
 ACCACACAACTGTTCCTCGCTCCTCCTCGCTGACCTCTGCCAAAACCTTCTGGGGCGTG
 TGCCGCAAAACACAGGCATACGCTTCAACTGGCGGCTGGTCGGCGCACCGGAATACGTT
 GCCGACTATGTCTGCATACAGAACTCTGCCACCTCGCCCATCCCGACCACAGCCCCGCC
 TTTTGGGAACTGACCCGCCGCTTCGCCCCCTACACGCCCAAAGCGAAACAGTGGCTCAA
 10 ATCCACGGCAGGGAAC TTTTCGCCTTAGGCTGA

NMB2035 Protein sequence

MTAFVHTLSDGMELTVEIKRRAKKNLIIRPAGHTVIRISVPPCFSVSALNRWLYENEAVL
 RQTLAKTPPPQTAENRLPESILFHGRQLALTAHQDTQILLMPSEIRVPEGAPKQLALLR
 15 DFLERQAHSYLIPLERHARTTQLFPASSSLTSAKTFWGVCRKTTGIRFNWRLVGAPEYV
 ADYVCIHCHLAHPDHSPAFWELTRRFAPYTPKAKQWLKIHGRELFALG

NMB1351 Fmu and Fmv protein DNA sequence

ATGAACGCCGACAACTCGACCATACCGCCAAAGTTTTGGCTGAAATGCTGACTTTCAAA
 20 CAGCTGCCGATGCCGTCTCTCCGCCTATTTCCGCGAACACAAAAGCTCGGCAGTCAA
 GATCGCCACGAAATCGCCGAAACCGCCTTTGCCGCGCTGCGCCACTATCAAAAAATCAGT
 ACCGCCCTACGCCGTCCGCACGCGCAGCCGCGCAAAGCCGCTCTCGCCGCACTGGTTCTC
 GGCAGAAACACCAACATCAGCCAAATCAAAGACCTGCTTGATGAAGAAGAAACAGCGTTC
 CTCGGCAATTTGAAAGCCCGTAAAACCGAGTTTTTCAGACAGCCTGAATACCGCCGAGAA
 25 TTGCCGCAATGGCTGGTGAACAACCTGAAACAGCATTGGCGCGAAGAAGAAATCCTCGCT
 TTCGGCCGCACTCAACCAGCCTGCCCCGCTCGACATCCGCGTCAACACTTTGAAAGGC
 AAACGCGATAAAGTGCTGCCGCTGTTGCAAGCCGAAAGTGCCGATGCAGAGGCAACGCCT
 TATTGCCTTTGGGGCATCCGCTGAAAAACAAAATCGCGCTTAACAAACACGAACTGTTT
 TTAGACGGCAGCTGGAAGTCCAAGACGAAGGCAGCCAGCTGCTTGCTTATTGGTGGGC
 30 GCAAAACGAGGCGAAATCATTGTGATTTCTGTGCCGCTGCCGGCGGTAACCTTTGGCT
 GTCCGTGCGCAATGGCGAACAAGGCAGAAATCTACGCCTTCGATATCGCCGAAAAACGC
 CTTGCCAACCTCAAAACCGGTATGACCCGCGCCGACTGACCAATATCCACCCCGAACGC
 ATCGGCAGCGAACACGATGCCCGTATCGCCCGACTGGCAGGCAAAGCCGACCGTGTGTTG
 GTGGACGCGCCCTGCTCCGGTTTGGGCACTTTACGCCGCAATCCCGACCTCAAATACCGC
 35 CAATCCGCCGAAACCGTTCGCCAACCTTTTGGAACAGCAACACAGCATCCTCGATGCCGCC
 TCCAAACTGGTAAACCGCAAGGACGTTTGGTGACGCCACTTGCAGCATCCTGCCCGAA
 GAAACGAGCTGCAAGTCGAACGTTTCTGTCCGAACATCCCGAATTTGAACCGCTCAAC
 TGCGCCGAACTGCTTGCCGGTTTGAAAATCGATTGGATACCGGCAAATACCTGCGCCTC
 AACTCCGCCCGACACCAACCGACGGCTTCTTCGCCGCCGTATTGCAACGCAATAA
 40

NMB1351 Protein sequence

MNAQLDHTAKVLAEMLTFKQPADAVLSAYFREHKKLGSQDRHEIAETAFAALRHYQKIS
 TALRRPHAQPRKAALALVLRSTNISQIKDLLDEETAFLGNLKARKTEFSDSLNTAAE
 LPQWLVEQLKQHWREEEILAFGRSINQPAPLDIRVNTLKGRDKVLPLLQAESADAEATP
 45 YSPWGIRLKNKIALNKHELFLDGTLEVQDEGSQLLALLVGAKRGEIIVDFCAGAGGKTLA
 VQAQMANKGRIYAFDIAEKRLANLKPRMTRAGLTNIHPERIGSEHDARIARLAGKADRVL
 VDAPCSGLGLRRNPDLKYRQSAETVANLLEQQHSILDAASKLVKPGRLVYATCSILPE
 ENELQVERFLSEHPEFEPVNCAELLAGLKIDLDTGKYLRLNSARHQTDGFFAAVLQRK

NMB1574 IlvC DNA sequence

ATGCAAGTCTATTACGATAAAGATGCCGATCTGTCCCTAATCAAAGGCAAAACCGTTGCC
 ATCATCGGTTACGGTTCGCAAGGTCATGCCCATGCCGCCAACCTGAAAGATTCGGGTGTA
 AACGTGGTGATTGGTCTGCGCCAAGGTTCTTCTTGAAAAAAGCCGAAGCAGCCGGTCAT
 GTCGTCAAAACCGTTGCTGAAGCGACCAAGAAGCCGATGTCGTTATGCTGCTGCTGCCT
 55 GACGAAACCATGCCTGCCGTCTATCACGCCGAAGTTACAGCCAATTTGAAAGAAGGCGCA
 ACGCTGGCATTTGCACACGGCTTCAACGTGCACTACAACCAAATCGTTCGCGCTGCCGAC
 TTGGACGTGATTATGGTTGCCCCCAAAGTCCGGGCCATACCGTACGCAGTGAATACAAA
 CGCGGCGGGCGGCTGCCTTCTCTGATTGCCGTTTACCAAGACAATTCGGGCAAAGCCAA

GACATCGCCCTGTCTTATGCGGCTGCCAACGGCGGCACCAAAGGCGGTGTGATTGAAACC
 ACTTTCCGCGAAGAAACCGAAACCGATCTGTTTCGGCGAACAAGCCGTATTGTGCGGCGGC
 GTGGTCGAGTTGATCAAGGCGGGTTTTGAAACCCTGACCGAAGCCGGTTACGCGCCTGAA
 ATGGCTTACTTCGAATGTCTGCACGAAATGAACTGATCGTTGACCTGATTTTTCGAAGGC
 5 GGTATTGCCAATATGAACTACTCCATTTCCAACAATGCGGAGTACGGCGAATACGTTACC
 GGCCCTGAAGTGGTCAATGCTTCCAGCAAAGAAGCCATGCGCAATGCCCTGAAACGCATT
 CAAACCGGCGAATACGCAAAAATGTTTATCCAAGAGGGTAATGTCAACTATGCGTCTATG
 ACTGCCCCGCCCGCTCTGAATGCCGACCACCAAGTTGAAAAAGTCGGCGCACAACTGCGT
 GCCATGATGCCTTGATTACTGCCAACAAATTGTTGACCAAGACAAAACTGA

10

NMB1574 Protein sequence

MQVYYDKDADLSLIKGTVAIIIGYGSQGHAAHANLKDSGVNVVIGLRQSSWKKAEEAAGH
 VVKTVAEATKEADVVMILLPDETMFAVYHAEVTANLKEGATLAFAHGFNVHYNQIVPRAD
 LDVIMVAPKGPGHVTRSEYKRGGVPSLIAVYQDNSGKAKDIALSYAAANGGKGGVIET
 15 TFREETETDLFGEQAVLCGGVVELIKAGFETLLEAGYAPEMAYFECLHEMKLIVDLIFEG
 GIANMNYSSISNNAEYGEYVTGPEVVNASSKEAMRNALKRIQTGEYAKMFIQEGNVNYASM
 TARRRLNADHQVEKVGQALRAMMPWITANKLVDQDKN

NMB1298 rsuA DNA sequence

20 ATGAAACTTATCAAATACCTGCAATATCAAGGCATAGGAAGCCGCAAGCAGTGCCAATGG
 CTGATTGCCGGCGGTTATGTTTTTCATCAACGGAACTGCATGGACGACACCGATGCAGAC
 ATCGATTCTCATCCGTCGAAACGTTGGATATTGACGGGGAAGCAGTAACCGTCGTTCCC
 GAACCCTATTTCTACATCATGCTCAACAAGCCTGAAGATTACGAACTTCGCACAAACCC
 AAGCACTACCGCAGCGTATTGAGCCTGTTCCCCGACAATATGCGGAACATCGATATGCAG
 25 GCGGTGCGCAGGCTGGATGCAGATACGACCGGCGTATTGCTGATTACCAACGACGGCAAA
 CTGAACCACAGCCTGACTTCGCCGAGCAGAAAAATTTCCCAAGCTGTACGAAGTAACGCTC
 AAACACCCACAGGAGAAACGCTCTGCGAAACCTTGAAAAACGGCGTGCTGCTCCACGAC
 GAAAACGAAACCGTTTGTGCCGCCGATGCCGTTTTGAAAAACCGACCACCCTGCTGCTG
 ACCATTACCGAAGGAAAATACCAACAGTCAAACGCATGATCGCCGCCGCCGGAACCGC
 30 GTGCAACACCTTCATCGCCGCGGATTTCGCACATCTGGAAACAGAAAACCTCAAACCCGGG
 GAATGGAAATTTATCGAATGTCCAAAATTCTGA

NMB1298 Protein sequence

35 MKLIKYLQYQIGSRKQCQWLIAGGYVFINGT CMDDTDADIDSSSVETLDIDGEAVTVVP
 EPYFYIMLNKPEDYETSHKPKHYRSVFSLFDPNMRNIDMQAVGRLDADTTGVLLITNDGK
 LNHSLTSPSRKIPKLYEVTLKHPTGETLCE TLKNGVLLHDENETVCAADAVLKNPTLLLL
 TITEGKYHQVKRMIAAAGNRVQHLHRRRFAHLETENLKPGEWKFI ECPKF

NMB1856 Lys R family (transcription regulator) DNA sequence

40 ATGAAAACCAATTGAGAAGAACTGACCGTATTTGTTCAAGTGGTGGAAAGCGGCAGCTTC
 AGCCGTGCGGCGGAGCAGTTGGCGATGGCAAATTCTGCCGTAAGCCGCATCGTCAAACGG
 CTGGAGGAAAAGTTGGGTGTGAACCTGCTCAACCGCACACGCGGCAACTCAGTCTGACG
 GAAGAAGGCGCGCAATATTTCCGCCGCGCGCAGAGAAATCCTGCAAGAAATGGCAGCGGCG
 GAAACCGAAATGCTGGCAGTGCACGAAATACCGCAAGGCGTGTGAGCGTGATTCCGCG
 45 ATGCCGATGGTGTGCTGCTGCTGGCGCCGCTGGCAGCAAAATTCACGAACGCTATCCG
 CATATCCGACTTTTCGCTCGTTTCTTCCGAAGGCTATATCAATCTGATTGAACGCAAAGTC
 GATATTGCCTTACGGGCCGAGAGAAATGGACGATTCCGGGCTGCGTGCACGCCATCTGTTT
 GACAGCCGCTTCCGCGTAATCGCCAGTCTGAAATACCTGGCAAAACACGGCACGCCGCAA
 TCTACAGAAGAGCTTGCCGGCCACCAATGTTTAGGCTTCACCGAACCCGGTTCTCTAAAT
 50 ACATGGGCGGTTTTAGATGCGCAGGGAAATCCCTATAAGATTTACCGCACTTTACCGCC
 AGCAGCGGTGAAATCTTACGCTCGTTGTGCCTTTCAGGTTGCGGTATTGTTTGCTTATCA
 GATTTTTTGGTTGACAACGACATCGCTGAAGGAAAGTTAATCCCCTGCTCGCCGAACAA
 ACCTCCGATAAAACACACCCCTTTAATGCTGTTTATTACAGCGATAAAGCCGTCAATCTC
 CGCTTACGCGTATTTTTGGATTTTTTAGTGGAGGAACTGGGAAACAATCTCTGTGGATAA

55

NMB1856 Protein sequence

MKTNSEELTVFVQVVEGSGFSRAAEQLAMANSVSRIVKRLEEKLGVNLLNRTTRQLSLT

EEGAQYFRRAQRILQEMAAAETEMLA VHEI PQGVLSVDSAMPVHLHLLAPLA AKFNERYP
HIRLSLVSSEGYINLIERKVDIALRAGELDDSGLRARHLFDSRFRVIASPEYLAKHGTPQ
STEELAGHQCLGFTEPGSLNTWAVLDAQGNPYKISPHFTASSGEILRSICLSGCGIVCLS
DFLVDNDIAEGKLIPLLAEQTS DKTHPFAVYYS DKA VNLRLRVFLDFLVEELGNNLCG

5

NMB0119 DNA sequence

ATGATGAAGGATTTGAATTTGAGCAACAGCCTGTTCAAAGGCTACAACGACAAACATGGC
TTAATGATTTGTGGCTATGAATGGGGTTGGAGTAAAGCCGATGAGGCTGCTTATGTAGCA
GGTGAATACAACTCCCTGAAAACAAAATCGACCATA CATTGCAAACAAATCCCTCTAT
10 TTCGGAGAGCAGGCAAAAAAGTGGCGTTACGACAATACGATAAAAAATTGGTTTGAAATG
TGGGGACACCCCTTAGACGAAAATGGATTGGGCGGTGCATTTGAAAAATCCCTGGTTCAA
ACCAACTGGGCTGCTACACAGGGCAACACTATCGACAATCCCGACAAGTTCACACAACCC
GAGCACATCGATAATTTTCTCTACCACATCGAAAACTGCGTCCGAAAGTCATCCTCTTC
ATGGGCAGCAGGTTGGCGGATTTTCTGAACAACCAAAATGTACTGCCACGCTTCGAGCAG
15 TTGGTCGGTAAGCAGACCAACCGCTGGAGACGGTGCAAAAAGAATTTGACGGTACACGT
TTCAATGTCAAATTCCAATCGTTTGAAGATTGCGAAGTCGTCTGCTTTCCCATCCAGT
GCCAGTCGCGGTCTATCTTACGATTACATCGCCTTGTTTGCGCCTGAAATGAACCGGATT
TTATCGGACTTTAAACAACACGCGGATTCAAATAA

20 NMB0119 Protein sequence

MMKDLNLSNSLFGKYNDKHGLMICGYEWGWSKADEAAYVAGEYKLPENKIDHTFANKSLY
FGEQAKKWRYDNTIKNWFEMWGHPLDENG LGGA FEKSLVQTNWAATQGN TIDNPKFTQP
EHIDNFLYHIEKLRPKVILFMGSRLADFLNNQNVLP RFEQLVGKQTKPLETVQKEFDGTR
FNVKFQSFEDCEVVCFFPHPSASRGLSYDYIALFAPEMNRILSDFKTTTRGFK

25

NMB1705 rfaK DNA sequence

ATGGAAAAAGAATTCAGGATATTAAATATCGTATCGGCCAAGATTTGGGGTGGAGGCGAA
CAATATGTCTATGATGTTTCAAAGCATTGGGGCTTCGGGGCTGCACAATGTTTACCGCC
GTCAATAAAAAATAATGAATTGATGCACAGGCGATTTTCCGAAGTTTCTTCCGTTTTTACA
30 ACGCGCCTTCACACGCTCAACGGGCTGTTTTCGCTCTACGCACTTACCCGCTTTATCCGG
AAAAACCGCATTTCCACCTGATGATACACACCGGCAAAATTGCCGCCTTATCCATACTT
TTGAAAAAACTGACCGGGGTGCGCCTGATATTTGTCAAACATAATGTCGTCGCCAACAAA
ACCGATTTTTTACCACCGCCTGATACAGAAAAACACAGACCGCTTTATTTGCGTTTCCCGT
CTGGTTTACGATGTGCAAACCGCCGACAATCCCTTTAAAGAAAAATACCGGATTGTTTCAT
35 AACGGTATCGATACCGGCCGTTTCCCTCCCTCTCAAGAAAAACCCGACAGCCGTTTTTTTT
ACCGTCGCCTACGCCGGCAGGATCAGTCCAGAAAAAGGATTGGAAAACCTGATTGAAGCC
TGTGTGATACTGCATCGGAAATATCCTCAAATCAGGCTCAAATTGGCAGGGGACGGACAT
CCGGATTATATGTGCCGCCTGAAGCGGGACGTATCTGCTTCAGGAGCAGAACCATTGTGTT
TCTTTTGAAGGGTTTACCGAAAACTTGCTTCGTTTTACCGCCAAAGCGATGTGCTGGTT
40 TTGCCCAGCCTCGTCCCGGAGGCATTGCTTTGTCATTATGCGAGGCGATGTACTGCCGA
ACGGCGGTGATTTCCAATACTTTGGGGGCGCAAAAGGAAATTGTCGAACATCATCAATCG
GGGATTCTGCTGGACAGGCTGACACCTGAATCTTTGGCGGACGAAATCGAACGCCTCGTC
TTGAACCTTGAACGAAAAACGCACCTGGCAACGGCAGCTCATCAATGCGTCGCCGCCCGT
TTTACCATCAACCATAACCGCCGACAAATTATTGGATGCAATATAA

45

NMB1705 Protein sequence

MEKEFRILNIVSAKIWGGGEQYVYDVSKALGLRGCTMFTAVNKNNELMHRRFSEVSSVFT
TRLHTLNLGLSLYALTRFIRKNRISHLMIHTGKIAALSILLKKLTGVRLIFVKHNVVANK
TDFYHRLIQKNTDRFICVSRVLVDVQTADNPFKEKYRIVHNGIDTGRFPPSQEKPD SRFF
50 TVAYAGRISPEKGLENLIEACVILHRKYPQIRLKL AGDGH PDYMCRLKRDVSASGAEPFV
SFEFGTEKLASFYRQSDVVVLP SLVPEAFGLSLCEAMYCRTAVISNTLGAQKEIVEHHQS
GILLDR LTPESLADEIERLVLPETKNALATAAHQCVAARFTINHTADKLLDAI

55 NMB2065 Hemk protein DNA sequence

ATGCAGGAACAGAATCGGAAACCAAGTTTCCCATAGTGATGTTGCTGGTGTGCGTTGCC
CTGTGGATAGCGTCTTTATCCAATGTTGCATTTTATTTGGGCAATCATGGAAGCATGGAG
GGTTTGACCGTTTGGATTTGGGGTCGATATTTGCTTCTTTGGATATCAGGTATTGTGCG

GTCTATGCGAATTATGTTTGGTTGGCGGCCATTGTTTTGCTGGCGTTGCGGAAGAAGGTC
 GTGCCTGTCCATGCGGCACCTTTGGGGCTTGGCGTTGGTGGCTTTCAGTGTGAAAGCCGTA
 TACGTCGATGAAGCAGGGAATACATCGGATATTGTGCGCTACGGTGCCAGGATTTTATTG
 TGGTATGCCGCATTTGCGGTTGCCACCATCGGTACGTTTGCCGGAAGAATAAGGAAAGA
 5 AAAGCCGCATCAGCGGCAGACGGGATAAAAATGACGTTTGATAAATGGTTGGGCTTGTCA
 AAAGTGCCTAAAAATGAAGCAAGAATGCTGCTACAATATGTTTCGGAATATACGCGCGTG
 CAGTTGTTGACGCGGGGCGGGGAAGAAATGCCGGACGAAGTCCGACAGCGGGCGGACAGG
 CTGGCGCAACGCCGTCTGAACGGCGAGCCGTTGCCTATATTTTAGGTGTGCGCGAATTT
 10 TATGGCAGACGCTTTACAGTCAATCCGAGCGTGCTGATTCCGCGCCCCGAAACCGAACAT
 TTGGTCAAGCCGTATTGGCGCGCCTGCCGAAAACGGGCGCGTGTGGGATTTGGGGACG
 GGCAGCGGCGCGGTTGCCGTAACCGTCGCGCTCGAACGCCCCGATGCGTTTGTGCGCGCA
 TCCGACATCAGCCCCCGCCCTTGAAACGGCGCGGAAAATGCGGCGGATTTGGGCGCG
 CGGGTCGAATTTGCACACCGGTTTCGTGGTTTCGACACCGATATGCCGTCTGAAGGGAAATGG
 GACATCATCGTGTCCAACCCGCCCTATATCGAAAACGGCGATAAACATTTGTTGCAAGGC
 15 GATTTGCGGTTTGAGCCGCAATCGCGCTGACCGACTTTTCAGACGGCCTAAGCTGCATC
 CGCACCTTGGCGCAAGGCGCGCCCGACCGTTTGGCGGAAGGCGGTTTTTTATTGCTGGAA
 CACGTTTTCGATCAGGGCGCGGCGGTGCGCGGCGTGTGGCGGAGAATGGTTTTTCAGGA
 GTGGAACCCCTGCCGATTGCGCGGTTTGGACAGGGTTACGCTGGGGAAGTATATGAAG
 CATTTGAAATAA

20

NMB2065 Protein sequence

MQEQNRKPSFPIVMLLVSVLWIASLSNVAFYLGNGHSMGLTVLILGSI FASLDIRYCA
 VYANYVWLAAIVLLALRKKVVPVHAALWGLALVAFSVKAVYVDEAGNTSDIVRYGAGFYI
 WYAAFAVATIGTFAGKNKERKAASAADGIKMTFDKWLGLSKLPKNEARMLLQYVSEYTRV
 25 QLLTRGGEEMPDEVRQADRLAQRRNLNGEPVAYILGVREFYGRFTVNPSVLIIPRPEH
 LVEAVLARLPENGRVWDLGTGSGAVAVTVALERPDAFVRASDISPPALETARKNAADLGA
 RVEFAHGSWFDTDMPSEGKWDIIVSNPPYIENGDKHLLQGDRLRFEPQIALTDFSDGLSCI
 RTLAQGAPDRLAEGGFLLLEHGFDOGAAVRGVLAENGFSGVETLPDLAGLDRVTLGKYMK
 HLK

30

Mutants selected by vacinee's 17 D sera (Screened once only)

NMB0339 DNA sequence

ATGGACAACGAATTGTGGATTATCCTGCTGCCGATTATCCTTTTGCCCGTCTTCTTCGCG
 35 ATGGGCTGGTTTGCCGCCCCGCTGGATATGAAAACCGTATTGAAGCAGGCAAAAAGCATC
 CCTTCGGGATTTTATAAAAGCTTGGACGCTTTGGTCGACCGCAACAGCGGGCGCGCGGCA
 AGGGAGTTGGCGGAAGTCGTCGACGGCCGGCCGAATCGTATGATTTGAACCTCACCCCTC
 GGCAAACCTTTACCGCCAGCGTGGCGAAAACGACAAAGCCATCAACATACACCGGACAATG
 CTCGATTCTCCCGATACGGTCGGCGAAAAGCGCGCGCGCTCCTGTTTGAATTGGCGCAA
 40 AACTACCAAAGTGCGGGGTTGGTCGATCGTGCCGAACAGATTTTTTTGGGGCTGCAAGAC
 GGTAAAATGGCGCGTGAAGCCAGACAGCACCTGCTCAATATCTACCAACAGGACAGGGAT
 TGGGAAAAGCGGTTGAAACCGCCCGGCTGCTCAGCCATGACGATCAGACCTATCAGTTT
 GAAATCGCCAGTTTTATTGCGAAGTTGCCAAGCCGCGCTGTTCAAGTCCAATTTTCGAT
 GTCGCGCGTTTCAATGTCGGCAAGGCACTCGAAGCCAACAAAAAATGCACCCGCGCCAAC
 45 ATGATTTTGGGCGACATCGAACACCGACAAGGCAATTTCCCTGCCGCGCTCGAAGCCTAT
 GCCGCCATCGAGCAGCAAAACCATGCATACTTGAGCATGGTCGGCGAGAAGCTTTACGAA
 GCCTATGCCGCGCAGGGAAAACCTGAAGAAGGCTTGAACCGTCTGACAGGATATATGCAG
 ACGTTTCCCGAAGTTGACCTGATCAATGTCGTGTACGAGAAATCCCTGCTGCTTAAGTGC
 GAGAAAGAAGCGCGCAAAACCGCCGTCGAGCTTGTCCGCCGAAGCCCGACCTTAACGGC
 50 GTGTACCGCTGCTCGGTTTGAACCTCAGCGATATGAATCCGGCTTGGAAAGCCGATGCC
 GACATGATGCGTTTCGGTTATCGGACGGCAGCTACAGCGCAGCGTGATGTACCGTTGCCGC
 AACTGCCACTTCAAATCCCAAGTCTTTTTCTGGCACTGCCCCGCTGCAACAAATGGCAG
 ACGTTTACCCCGAATAAAAATCGAAGTTTAA

55

NMB0339 Protein sequence

MDNELWIILLPIILLPVFFAMGWFAARVDMKTVLKQAKSIPSGFYKSLDALVDRNSGRAA
 RELAEVVDGRPQSYDLNLTGLKLYRQRGENDKAINIHRTMLDSPDTVGEKRARVLFELAQ

NYQSAGLVDRAEQIFLGLQDGKMAREARQHLLNIYQQDRDWEKAVETARLLSHDDQTYQF
 EIAQFYCELAQAALFKSNFDVARFNVGKALEANKKCTRANMILGDIHRQGNFPAAVEAY
 AAIEQQNHAYLSMVGEKLYEAYAAQKGPEEGLNRLTGVMQTFPELDLINVVYEKSLLLKC
 EKEAAQTAVELVRRKPDNLNGVYRLLGLKLSDMNPAWKADADMMSVIGRQLQRSVMYRCR
 5 NCHFKSQVFFWHCPACNKWQTFTPNKIEV

Selection with patient's sera

We have a collection of acute and convalescent sera available to us for screening.

10 This is from individuals infected with different serogroup of *N. meningitidis*.

Screens have been performed with acute (A) or convalescent (C) sera. The period between the acute infection and collection of sera was from 2 weeks to 3 months.

NMB0401 putA DNA sequence

15 ATGTTTCATTTTGCATTTCCGGCACAACTGCCCTGCGCCAAGCGATAACCGATGCCTAC
 CGCCGTAATGAAATCGAAGCCGTACAGGATATGTTGCAACGTGCACAGATGAGCGACGAA
 GAGCGCAACGCCGCCCTCCGAGCTTGCCCGCCGTTTGGTTACCCAAGTCCGCGCCGGCCGC
 ACCAAAGCCGGCGGCGTGATGCGCTGATGCACGAGTTTTCACTCTCCAGCGAAGAAGGC
 ATCGCGCTGATGTGTCTGGCAGAAGCCCTGCTGCGTATCCCCGACAACGCCACGCGCGAC
 20 CGCCTGATTGCCGACAAGATTTAGACGGCAACTGGAAGGCCATTTGAACAACAGCCCT
 TCCCTCTTCGTCAATGCTGCCGCCCTGGGGCCTGCTGATTACCGGCAAACGACCGCCACA
 AACGACAAACAAATGAGTTCGCGACTCAGCCGCCGTGATCAGCAAAGGCGGCGCACCGCTC
 ATCCGCCAAGGCGTAAATTACGCCATGCGGCTTCTGGGCAAACAGTTCGTAAACGGACAG
 ACCATTGAAGAAGCCCTGCAAAACGGCAAAGAACGCGAAAAAATGGGCTACCGCTTCTCC
 25 TTCGATATGTTGGGCGAAGCCGCCCTACACCAAGCCGATGCCGACCGCTACTACCGGAC
 TATGTCGAAGCCATCCAGCCATCGGCAAAGATGCGGCGAGGACAAGGCGTTTACGAAGGT
 AACGGTATTTCCGTCAAACCTTTCCGCCATCCATCCGCGCTACTCGCGCACCCAACACGGC
 CGCGTGATGGGCGAAGCTGTTGCCGCGCCTGAAAGAGCTGTTCTTTTGGGTAAAAAATAC
 GATATCGGTATCAACATCGATGCCGAAGAAGCCAACCGTCTGGAGCTGTCTTTGGATTG
 30 ATGGAGGCTTTGGTTTCAGACCCTGACTTGGCTGGCTACAAAGGTATCGGTTTCGTGTC
 CAAGCCTACCAAAAACGTTGTCCGTTTCGTTATCGACTACCTGATCGACCTTGCCCGCCGC
 AACAAACCAAACTAATGATCCGCCCTCGTCAAAGGCGCGTATTGGGACAGCGAAATCAAA
 TGGGCGCAAGTGGACGGCTTGAACGGCTATCCGACCTACACCCGCAAAGTCCACACCGAC
 ATCTCCTACCTCGCCTGCGCGCGCAAACCTGCTTTCGCGCAAGACGCGGTATTCCCGCAA
 35 TTTGCCACCCACAACGCCTACACTTTGGGCGCAATCTACCAAATGGGTAAAGGCAAAGAT
 TTTGAACACCAATGCCTGCACGGTATGGGCGAAACCCTGTACGACCAAGTCGTGGGCCG
 CAAAACCTTAGGCCGCCGCGTGCGCGTGACGCCCCAGTCGGCACACACGAAACCCCTGCTC
 GCCTACTTGGTGCGCCGCTGTTGGAAAACGGCGCGAAGTTCGTCTTTTCGTCAACCAAAATC
 GTCGATGAAAACATCAGCATCGACACGCTCATCCGCAGCCCGTTTCGACACCATCGCCGAA
 40 CAAGGCATCCACCTGCACAACGCCCTGCCGCTGCCGCGCGATTGTTGACGGCAAATGCCGT
 CTGAACTCGCAAGGCGTGGACTTGAGCAACGAAAACGTATTGCAGCAGCTTCAAGAACAG
 ATGAACAAAGCCGCCGCGCAAGACTTCCACGCCGCATCCATCGTCAACGGCAAAGCCCGC
 GATGTCGGCGAAGCGCAACCGATTAAAAACCCCTGCCGACCACGACGACATCGTCGGCACA
 GTCAGCTTTGCCGATGCCGCGCTTGCCCAAGAAGCGGTTGGCGCAGCCGTTGCCGCGTTC
 45 CCCGAATGGAGTGCAGACACTGCCGCCGAACGCGCCGCTGCCTGCGCCGTTTTGCCGAT
 TTGCTGGAGCAGCACACCCAGCACTGATGCTTGCCGTGCGCGAAGCAGGCAAAACG
 CTGAACAACGCCATTGCCGAAGTGCGCGAAGCCGTCGATTTCTGCCGCTACTACGCAAAAC
 GAAGCCGAACATACCCCTGCCTCAAGACGCAAAAGCCGTCGGCGCGATTGTCGCCATCAGC
 CCGTGGAACCTTCCCGCTCGCCATCTTTACCGGCGAAGTCGTTTCCGCATTGGCGGCAGGC
 50 AACACCGTCATCGCCAAACCCGCCGAACAAACAGCCTGATTGCCGTTTATGCCGTTTCC
 CTCATGCACGAAGCCGGCATCCCGACTTCCGCCCTGCAACTCGTCTCTCGGCGCAGGCGAC
 GTGGGTGCGGCATTGACCAACGATGCCCGCATCGGCGGCGTGATTTTACCGGCTCGACC

GAAGTGGCGCGCCTGATCAACAAAGCCCTTGCCAAACGCGGCGACAATCCCGTCCTGATT
 GCCGAAACGGCGGACAAAACGCCATGATTGTCGATTCCACCGCACTTGCCGAGCAAGTC
 TGGCGCGACGTATTGAACTCCGCCCTTCGACAGCGCGGGACAACGCTGCTCCGCCCTGCGC
 ATTTTGTGCGTCCAAGAAGACGTTGCCGACCGTATGCTCGACATGATCAAAGGCGCTATG
 5 GACGAACTCGTCGTCGGCAAACCGATTACGCTCACTACCGATGTCGGCCCCGTCATCGAT
 GCCGAAGCACAGCAAAAACCTGTTGAACCACATCAACAAAATGAAAGGTGTTGCCAAGTCC
 TACCACGAAGTCAAAAACCGCCGCCGATGTCGATTCCAAAAAATCCACGTTCTGTTCCGCCCC
 ATCCTGTTTGAATTGAACAACCTCAACGAACTGCAACGCGAAGTCTTCGGTCCCGTCTCTG
 CACGTCGTCCGCTACCGCGCCGACGAACTCGACAACGTCAACGACCAAATCAACAGCAAA
 10 GGCTACGCCCTGACCCACGGCGTACACAGCCGCATCGAAGGCACGGTACGCCACATCCGC
 AGCCGCATCGAAGCCGGCAACGTTTACGTCAACCGCAACATCGTCGGCGCAGTCGTGCGC
 GTACAGCCCTTCGGCGGACACGGTCTGTCCGGCACAGGCCCAAAGCAGGCGGTTTCTGTTT
 TACCTGCAAAAACTGACCCGCGCCGCGCAATGGGTTGCCCGACCTGAGCCAAATCGGA
 CAGGCGGACGAAGCCGCACTCAAACGCCCTCGAAGCACTGGTTCAAAACTACCGTTCAAC
 15 GCCGAAGAGAAAAAAGCCGCAGCGGCCGCTTTGGGACACGCCCGCATCCGCACCCTGCGC
 CGTGCCGAAACCGTCCTTACCGGACCGACCGGCGAGCGCAACAGCATCTCATGGCACGCG
 CCCAAACGCGTTTGGATACACGGCGGCAGCACGGTTCAAGCCTTTGCCGCACTGACCGAA
 CTTGCCGCCCTCCGGCATAACAGGCAGTGGTCAACCCGACAGCCCCCTTGGCTTCTTACACT
 GCCGACTTGAAGGTCTGCTGCTGGTCAACGGCAAACCCGAAACCGCCGGCATCAGCCAC
 20 GTTGCCGCCCTGTCGCTTTGGACAGCGCGCGCAAACAGGAAGTTGCCGCCACGACGGC
 GCACTCATCCGCATCCTCCCTTCGGAAAACGGAAGTCTGCAAGTGTGTTGAAGAA
 ATCTCTTGACGCGTCAACACCACAGCCGCCGCGGCAACGCCAGCCTGATGGCGGTGCGC
 GACTGA

25 NMB0401 Protein sequence

MFHFAFPAQTALRQAITDAYRRNEIEAVQDMLQRAQMSDEERNAASELARRLVTQVRAGR
 TKAGGVDAIMHEFSLSSSEEGIALMCLAEALLRIPDNATRDRLIADKISDGNWKSHLNNSP
 SLFVNAAAWGLLITGKLITATNDKQMSSALSRLISKGGAPLIROGVNYAMRLLGKQFVTGQ
 TIEEALQNGKEREKMGYRFSFDMLEAAAYTQADADRYRDRDYVEAIHAIGKDAAGQGVYEG
 30 NGISVKLSAIHPYRSRTQHGRVMGELLPRLEKFLGKKYDIGINIDAEENRLESLDL
 MEALVSDPDLAGYKIGFVVQAYQKRCPPFVIDYLDLARRNNQKLMIRLVKGAYWDSEIK
 WAQVDGLNGYPTTYTRKVHTDISYLACARKLLSAQDAVFPQFATHNAYTLGAIYQMGKGKD
 FEHQCLHGMGETLYDQVVGFPQNLGRVRVYAPVGTHTLLAYLVRRLLENGANSFVNQI
 VDENISIDTLIRSPFDITAEQGIHLHNAIPLPRDLYGKRLNSQGVDSLSENVLQQLQEQ
 35 MNKAAAQDFHAASIVNGKARDVGEAQPIKNPADHDDIVGTVSFADAALAEAVGAVAFAF
 PEWSATPAAERAACLRRFADLLEQHTPALMMLAVREAGKTLNNAIAEVREAVDFCRYAN
 EAEHTLPQDAKAVGAIVAISPWNPPLAIFTGEVVSALAAGNTVIAKPAEQTSIAGYAVS
 LMHEAGIPTSAQLVLGAGDVGAALTNDARIGGVI FTGSTEVARLINKALAKRGDNPVLI
 AETGGQNAMIVDSTALAEQVCADVLNSAFDSAGQRCALRILCVQEDVADRMLDMIKGAM
 40 DELVVGKPIQLTTDVGPVIDAEAQONLLNHINKMKGVAKS YHEVKTAAVDVSKKSTFVRP
 ILFELNNLNEQREVFGPVLHVVRVRADELNDVIDQINSKGYALTHGVHSRIEGTVRHIR
 SRIEAGNVYVNRNIVGAVVGVPFGGHGLSGTGPKAGGSFYQLKLTRAGEWVAPTLSQIG
 QADEAALKRLEALVHKLPFNAEEKKAAAAALGHARIRTLRRAETVLTGPTGERNSISWHA
 PKRVWIHGGSTVQAFALTELAASGIQAVVEPDSPLASYTADLEGLLLVNGKPETAGISH
 45 VAALSPLDSARKQELAHDGALIRILPSENGLDILQVFEEISCSVNTTAAGGNASLMAVA
 D

NMB1335 CreA

50 DNA and Protein sequences given above

NMB1467 PPX DNA sequence

ATGACCACCACCCCCGAAACGTCCTCGCCTCCGTCGATTTGGGTTCCAACAGTTTCCGC
 CTCCAGATTTGCGAAAACAACAACGGACAATTAAGTCAATCGATTCTGTTCAAACAGATG
 55 GTGCGCTTCGCCGCCGGACTGGACGAACAGAAAAATCTGAGTGCCGCTTCCCAAGAACAG

GCTTTGGACTGTCTGGCAAAATTCGGCGAACGCCTGCGCGGCTTCCGCCCTGAACAGGTA
CGCGCCGTGGCAACCAACACATTCCGCGTTGCCAAAACATCGCAGATTTCTTCCCAAA
GCCGAAGCGGCATTGGGTTTCCCCATCGAAATCATCGCCGGGCGCGAAGAGGCGCGGCTG
ATTTATACCGCGTGATCCACACCCTCCCCCGGGCGGCGGCAAAATGCTGGTTATCGAC
5 ATCGGCGGCGGTTTCGACAGAATTTGTCATCGGCTCGACGCTGAATCCCGACATTACCGAA
AGCCTGCCCTTGGGCTGCGTAACCTACAGCCTGCGCTTCTTCCAAAACAAAATCACCGCC
AAAGACTTCCAATCTGCCATTTCCGCCGCCCGCAACGAAATCCAGCGTATCAGCAAAAAT
ATGAGGCGCGAAGGTTGGGATTTCCGCCGTCGGCACATCGGGTTCGGCAAAATCCATCCGC
GACGTGCTTGCCGCCGAAATGCCCCAAGAGGCGGACATTACCTACAAAGGCATGCGCGCC
10 CTCGCCGAACGCATCATCGAAGCCGGTTCGGTCAAAAAAGCCAAATTTGAAAACCTGAAA
CCGGAACGCATCGAAGTTTTCGCCGCGGACTTGCCGTGATGATGGCGGCGTTTGAGGAA
ATGAAACTCGACAGGATGACCGTAACCGAAGCCGCCCTGCGCGACGGCGTGTTTTACGAT
TTGATCGGGCGCGGTTTAAACGAAGATATGCGCGGACAAACGGTTGCCGAGTTCCAACAC
CGCTACCACGTCAGCCTCAATCAGGCGAAACGCCGCCGAGACCGCGCAAACCTTTATG
15 GACAGCCTCTGCCACGCTAAAAACGTTACAGTTCAAGAGCTTGCTTGTGGCAACAGTAT
CTCGGACGCGCCGCCGCGCTGCACGAAATCGGTTTGGACATCGCCACACCGGCTATCAC
AAGCATTCGCCTACATCCTCGAAAACGCCGATATGCCGGGTTTCTCACGCAAAGAACAG
ACCATACTTGCCCAACTGGTCATCGGTCATCGCGGCGATATGAAAAAATGAGCGGCATC
ATCGGCACCAACGAAATGTTGTGGTATGCCGTTTTGTCCCTGCGCCTTGCCGCACTGTTT
20 TGCCGTTTCGCGCAAGACCTGTCTTTCGCAAAAATATGCAGTTGCGCACGGATACGGAA
AGCTGCGGCTTCATCCTGCGTATTGACAGGGAATGGCTGGAACGCCATCCCCTGATTGCC
GACGCATTGGAATATGAAAAGCGTCCAATGGCAAAAATCAATATGCCGTTCAAAGTCGAG
GCCGTCTGA

25 NMB1467 Protein sequence

MTTTPANVLASVDLGSNSFRLQICENNNQGLKVIDSFKQMVRFAAGLDEQKNLSAASQEQ
ALDCLAKFGERLRGRPEQVRVATNTFRVAKNIADFLPKAEALGFPIEIIAGREEARL
IYTGVIHTLPPGGGKMLVIDIGGGSTEFVIGSTLNPDITESLPLGCVTYSLRFQNKITA
KDFQSAISAAARNEIQRISKNMREGWDFAVGTSKSAKSIRDVLAAEMPQEADITYKGMRA
30 LAERIEAGSVKKAKFENLKPERIEVFAGGLAVMMAAFEEMKLDGMTVTEALRDGVFYD
LIGRGLNEDMRGQTVAEFQHRYHVSINQAKRTAETAQTFMDSLCHAKNVTVQELALWQOY
LGRAAALHEIGLDIAHTGYHKHSAYILENADMPGFSRKEQTILAQLVIGHRGDMKKMSGI
IGTNEMLWYAVLSRLAALFCRSRQDLSPKQNMQLRDTESCGFILRIDREWLERHPLIA
DALEYESVQWQKINMPFKVEAV

35

NMB2056 HemK

ATGAACGGTAAATACTACTACGGCACAGGCCCGCCGCAAAAGTTCAAGTGGCTCGTGATTTC
CTGATTAAAGGTACAGGTCAAATCATCGTAAACGGTCGTCCCGTTGACGAATTCTTCGCA
40 CGGGAAACCAGCCGAATGGTTGTTTCGCCAACCCCTTGGTTCTGACTGAAAACGCCGAATCT
TTCGACATCAAAGTCAATGTTGTTGGCGGCGGCGAAAACCGCCAGTCCGGCGCAATCCGC
CACGGCATTACCCGTGCCCTGATCGACTTCGATGCCGCGTTGAAACCCGCCTTGCTCAA
GCTGGTTTTGTTACCCGCGATGCCGCGAAGTCGAACGTAAAAAACCGGTCTGCGCAA
GCACGCCGTGCAAAACAATTCTCCAAACGTTAA

45

NMB2056 Protein sequence

MNGKYYYGTGRRKSSVARVFLIKGTGQIIVNGRPVDEFFARETSRMVVRQPLVLTEAES
FDIKVNVVGGGETGQSGAIRHGITRALIDFDAALKPALSQAGFVTRDAREVERKKPGLRK
ARRAKQFSKR

50

NMB0808 DNA sequence

ATGTCCGCCCTCTCCCATCATCAACCGCTGATTCTGCAAAGCCCGGACAGCCGCTCG
GAACTTGCCGCCTTTGCAGGCAAAACACTGACCCTGAACATTGCCGGGCTGAAACTGGCG
GGACGCATCACGGAAGACGGTTTGCTCTCGCGGGGAAACGGCTTTGCAGACACCGAAATT
55 ACCTTCCGCAACAGCGCGGTACAGAAAATCCTCCAAGGAGGCGAACC CGGGGCGGGCGAC
ATCGGGCTCGAAGGCGACCTCATCCTCGGCATCGCGGTACTGTCCCTGCTCGGCAGCCTG
CGTTCCCGCGCATCGGACGAATTGGCACGGATTTTCGGCACGCAGGCAGACATCGGCAGC
CGTGCCGCCGACATCGGACACGGCATCAAACAAATCGGCAGGAACATCGCCGAACAAATC

GGCGGATTTTCCCGCGAATCCGAGTCCGCAAACATCGGCAACGAAGCCCTTGCCGACTGC
CTCGACGAAATAAGCAGACTGCGCGACGGCGTGGAACGCCTCAACGAACGCCTCGACCGG
CTCGAACGCGACATTTGGATAGACTAA

5 NMB0808 Protein sequence

MSALLPIINRLILQSPDSRSELAAFAGKTLTLNLAGLKLGRITEDGLLSAGNGFADTEI
TFRNSAVQKILQGGEPGAGDIGLEGDLILGIAVLSSLGSLRSRASDELARIFGTQADIGS
RAADIGHGIKQIGRNIAEQIGGFSRESESANIGNEALADCLDEISRLRDGVERLNERLDR
LERDIWID

10

NMB0774 upp DNA sequence

ATGAACGTTAATGTTATCAACCATCCGCTCGTCCGCCACAAATTAACCCTGATGAGGGAG
GCGGATTGCAGCACTACAAATTCGGGACGCTTGCCACCGAGCTGGCGCGCCTGATGGCA
TACGAGGCAAGCCGTGATTTTGAATCGAAAAATACCTTATCGACGGATGGTGCGGTGAG
15 ATTGAAGGCGACCGCATCAAGGGCAAAACATTGACCGTCGTTCCCATACTGCGTGCAGGT
TTGGGTATGCTTGACGGTGTGCTCGACCTGATTCCGACTGCCAAAATCAGTGTAGTCGGA
CTGCAGCGCGACGAAGAAACGCTGAAGCCTATTTCTATTTTGAGAAATTTGTGGACAGT
ATGGACGAACGTCCGGCTTTGATTATCGATCCTATGCTGGCGACAGGCGGTTTCGATGGTT
GCCACCATCGACCTTTTGAAAGCCAAGGGCTGCAAAAATATCAAGGCACTGGTGCTGGTT
20 GCCGCGCCCGAGGGTGTGAAGGCGGTCAACGACGCGCACCCCTGACGTTACGATTTACACC
GCCGCGCTCGACAGCCACTTGAACGAGAACGGCTACATCATCCCCGGCTTGGGCGATGCG
GGCGACAAGATTTTCGGCACGCGCTAA

NMB0774 Protein sequence

25 MNVNVINHPLVRHKLTLMREADCSTYKFRTLATELARLMAYEASRDFFEIEKYLIDGWCGQ
IEGDRIKGTLLTVVPILRAGLGMLDGVLDLIPTAKISVVG LQRDEETLKPI SYFEK FVDS
MDERPALIIDPLATGGSMVATIDLLKAKGCKNIKALVLVAAPGKAVNDAHPDVTIYT
AALDSLHNENGYIIPGLGDAGDKIFGTR

30 NMA0078 putative integral membrane protein DNA sequence

TTGGCGTTTACTTTAATGCGTCGCGCCATGATACGTAAAATGCCCTATACGGAAGATATG
CGCCCAGGCGATACCGCTAATCCTTATGGTGCGTCCAAAGCGATGGTGGAACGGATGTTA
ACCGACATCCAAAAAGCCGATCCGCGCTGGAGCATGATTTTGTGCGTTATTTCAATCCG
ATTGGCGCGCATGAAAGCGGCTTGATTGGCGAGCAGCCAAACGGCATCCCGAATAATTTG
35 TTGCCTTATATCTGCCAAGTGGCGGCGAGGCAAACTGCCGCAATTGGCGGTATTTGGCGAT
GACTACCTTACCCCCGACGGCACGGGGATGCGTGACTATATTCATGTGATGGATTTGGCA
GAAGGCCATGTCGCGGCTATGCAGGCAAAAAGTAATGTAGCAGGCACGCATTTGCTGAAC
TTAGGCTCCGCGCGCGCTTCTTCGGTGTGGAATCATCCGCGCATTTGAAGCAGCTTCG
GGTTTGACGATTCGGTATGAAGTCAAACCGCGCGGTGCCGGTGATTTGGCGTGCTTCTAT
40 GCCGACCTTCTTATACAAAGGCGCAAAATCGGCTGGCAAACCCAGCGTGATTTAACCCAA
ATGATGGAAGACTCATGGCGCTGGGTGAGTAATAATCCGAATGGCTACGACGATTAA

NMA0078 Protein sequence

45 MAFTLMRRAMIRKMPYTEDMRPGDTANPYGASKAMVERMLTDIQKADPRWSMILLRYFNP
IGAHESLIGEQPNGIPNNLLPYICQVAAGKLPQLAVFGDDYPTPDGTGMRDYIHVMDLA
EGHVAAMQAKSNVAGTHLLNLGSGRASSVLEIIRAFEAAAGSLTIPYEVKPRRAGDLACFY
ADPSYTKAQIGWQTQORDLTQMMEDSWRVWSNNPNGYDD

NMB0337 Branched-chain amino acid aminotransferase DNA sequence

50 ATGAGCAGACCCGTACCCGCCGTATTCGGCAGCGTTTTTCACAGTCAAATGCCCGTCCTC
GCCTACCGCGAAGGCAAATGGCAGCCGACCGAATGGCAATCTTCCCAAGACCTCTCCCTC
GCACCGGGCGCGCAGCCCTGCACTACGGCAGCGAATGTTTCGAGGGACTGAAAGCCTTC
CGTCAGGCAGCAGGCAAAATCGTGCTGTTCCGTCGACTGCCAATATCGCGCGTATGCGG
CAAAGTCGCGACATTTTGCACCTGCCGCGCCCCGAAACCGAAGCTTATCTTGACGCGCTA
55 ATCAAATTGGTCAAACGTGCCGCCGATGAAATTCCTGATGCGCCTGCCGCCCTGTACCTG
CGTCCGACCTTAATCGGTACCGATCCCGTTATCGGCAAGGCCGGTTCTCCTTCGGAAACC

GCCCTGCTGTATATTTTGGCTTCCCCCGTCGGCGACTATTTCAAAGTCGGATCGCCCGTC
 AAAATTTTGGTGGAAACCGAACACATCCGCTGCGCCCCGCATATGGGCCGCGTCAAATGC
 GCGGCAACTACGCTTCCGCCATGCACTGGGTGCTGAAGGCGAAAGCCGAATATGGCGCA
 AATCAAGTCCTGTTCTGCCCCGAACGGCGACGTGCAGGAAACCGGCGCGTCCAACTTTATC
 5 CTGATTAACGGCGATGAAATCATTACCAAACCGCTGACCGACGAGTTTTTGCACGGCGTA
 ACCCGCGATTCCGTA CTGACGGTTGCCAAAGATTTGGGCTATACCGTCAGCGAACGCAAT
 TTCACGGTTGACGAACTCAAAGCTGCGGTGGAAAACGGTGCGGAAGCCATTTTGACCGGT
 ACGGCAGCCGTCATCTCGCCCGTTACTTCCTTCGT CATCGGCGGCAAAGAAATCGAAGTG
 AAAAGCCAAGAACGCGGCTATGCCATCCGTAAGGCGATTACCGACATCCAGTATGGTTTG
 10 GCGGAAGACAAATACGGCTGGCTGGTTGAAGTGTGCTGA

NMB0337 Protein sequence

MSRPVPAVFGSVFHSQMPVLAYREGKWQPTTEWQSSQDLSLAPGAHALHYGSECFEGLKAF
 RQADGKIVLFRPTANIARMROSADILHLPRPETEAYLDALIKLVKRADEIPDAPAALYL
 15 RPTLIGTDPVIGKAGSPSETALLYILASPVGDYFKVGSVPVKILVETEHIRCAPHMGRVKC
 GGNYSAMHWWLKAKEYGANQVLFPCPNGDVQETGASNFILINGDEIITKPLTDEFLHGV
 TRDSVLTVAKDLGYTVSERNFTVDELKAAVENGAEAILTGTAAVISPVTSFVIGGKEIEV
 KSQERGYAIRKAITDIQYGLAEDKYGWLVEVC

20 NMB0191 ParA family protein DNA sequence

ATGAGTGCGAACATCCTTGCCATCGCCAATCAGAAGGGCGGTGTGGGCAAAACGACGACG
 ACGGTAAATTTGGCGGCTTCGCTGGCATCGCGCGGCAAACGCGTGCTGGTGGTCGATTTG
 GATCCGCAGGGCAATGCGACGACGGGCAGCGGCATCGACAAGGCGGGTTTGCAGTCCGGC
 GTTTATCAGGTCTTATTGGGCGATGCGGACGTGCAGTCGGCGGCGGTACGCAGCAAAGAG
 25 GCGGATACGCTGTGTTGGGTGCGAACCGCGCGCTGGCCGGCGCGGAAATCGAACTGGTG
 CAGGAAATCGCCCGGGAAGTGCGTTTGAAAAACGCGCTCAAGGCAGTGGAAGAAGATTAC
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 GATTTGATTGCGACCGTGCGCAAATCCGTCAGGCGGTCAATCCCGATTTGGACATCACG
 30 GGCATCGTGCGCACGATGTACGACAGCCGACGAGGCTGGTTGCCGAAGTCAGCGAACAG
 TTGCGCAGCCATTTCCGGGATTTGCTTTTTGAAAACCGTCATCCCGCGCAATATCCGCCTT
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 AAGGCGTATCTTGCCTTGGCGGACGAGCTGGCGGCGAGGGTGTTCGGGGAAATAG

35 NMB0191 Protein sequence

MSANILAIANQKGGVGGKTTTTVNLAASLASRGKRVLVVDLDPQGNATTGSGIDKAGLQSG
 VYQVLLGDADVQSAAVRSKEGGYAVLGANRALAGAEIELVQEIAREVRLKNALKAVEEDY
 DFILIDCPPSLTLLTLNGLVAAGGVIVPMLCEYYALEGISDLIATVRKIRQAVNPDLIT
 GIVRTMYDSRSLVAEVSEQLRSHFGDLLFETVIPRNLRLAEAPSHGMPVMAYDAQAGT
 40 KAYLALADELAARVSGK

NMB1710 Glutamate dehydrogenase(gdhA) DNA sequence

ATGACTGACCTGAACACCCTGTTTGCCAACCTCAAACAACGCAATCCCAATCAGGAGCCG
 TTCCATCAGGCGGTTGAAGAAGTCTTCATGAGTCTCGATCCGTTTTTGGCAAAAAATCCG
 45 AAATACACCCAGCAAAGCCTGCTGGAACGCATCGTCGAACCCGAACGCGTCGTGATGTTT
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 ATGAGTTCCGCCATCGGTCTTACAAAGGCGGCTGCGCTTCCATCCGACCGTCGATTTG
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 GCGCAAGGCTTCTAA

NMB1710 Protein sequence

10 MTDLNTLFANLKQRNPNQEPFHQAVEEVFMSLDPFLAKNPKYTQOSLLERIVEPERVVMF
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 MGGGKGGSDFDPKGKSDAEVMRFCQAFMTELYRHI GADTDVPAGDIGVGGREIGYLFQGY
 KKI RNEFSSVLTGKGLEWGGSLIRPEATGYGCVYFAQAMLQTRNDSFEGKRVLISGSGNV
 AQYAAEKAIQLGAKVLTVSDSNGFVLFDPDSGMTAQLAALIELKEVRRERVATYAKEQGL
 15 QYFEKQKPWGVAAEIALPCATQNELDEEAAKTL LANGCYVVAEGANMPSTLGAVEQFIKA
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 VNYVNGANIAGFVKVADAMLAQGF

NMB0062 Glucose-1-phosphate thymidyltransferase(rfbA-1) DNA sequence

20 ATGAAAGGCATCATACTGGCAGGCGGCAGCGGCACGCGCCTCTACCCCATCACGCGCGGC
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 25 AATGTTTGCTTGGTTTTGGGCGACAATATTTTTTACGGTCAGTCGTTTACGCAAACATTG
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 TATTTGCTGCGCCTGTTGAAAAAATAA

NMB0062 Protein sequence

MKGIILAGGSGTRLYPITRGVSKQLLPVYDKPMIYYPLSVLMLAGIRDILVITAPEDNAS
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 KQAAAQTHGATVFAYQVKNPERFGVVEFNENFRAVSIIEKPQRPKSDWAVTGLYFYDNRA
 40 VEFKQLKPSARGELEITDLNRMYLEDGSLSVQILGRGFAWLDGTTHESLHEAASFVQTV
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NMB1583 Imidazoleglycerol-phosphate dehydratase(hisB) DNA sequence

45 ATGAATTTGACTAAAACACAACGCCAACTGCACAACCTTCTGACCCCTCGCCCAAGAAGCA
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 50 GCCGAAACCGCATCTGCTGCCGGCTGCCGAGCGTTACCGTCAACCGCAATACCTGCGAA
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 55 CCCGGACTCGTGTAACATCGAATTTACCCGCGCACTAATCGGACGTTTCGATGTGCGAT
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GGCACGCTGACCGCATAA

NMB1583 Protein sequence

5 MNLTKTQRQLHNFLTLAQEAGSLSKLAKLCGYRTPVALYKLKQRLEKQAEDPDARGIRPS
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 TQITVSINLDGSGKSRLDTGVFPFLEHMIDQIARHG MIDIDISCKGDLHIDDHHTAEDIGI
 TLGQAIRQALGDKKGIRRYGHSYVPLDEALSRVVIDLSGRPGLVYNIEFTRALIGREFVD
 LFEEFFHGIVNHSMMTLHIDNLSGKNAHHQAETVFKAFGRALRMAVEHDPDMAGQTPSTK
 10 GTLTA

Schedule of SEQ ID Nos

SEQ ID No	Sequence
1	NMB0341 DNA
2	NMB0341 Protein
3	NMB0338 DNA
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5	NMB1345 DNA
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12	NMB0279 Protein
13	NMB2050 DNA
14	NMB2050 Protein
15	NMB1335 DNA
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42	NMB0808 Protein
43	NMB0774 DNA
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45	NMA0078 DNA
46	NMA0078 Protein
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48	NMB0337 Protein
49	NMB0191 DNA
50	NMB0191 Protein
51	NMB1710 DNA
52	NMB1710 Protein
53	NMB0062 DNA
54	NMB0062 Protein
55	NMB1583 DNA

CLAIMS

1. A method for identifying a polypeptide of a microorganism which polypeptide is associated with an immune response in an animal which has
5 been subjected to the microorganism, the method comprising the steps of
- (1) providing a plurality of different mutants of the microorganism;
- (2) contacting the plurality of mutant microorganisms with antibodies
10 from an animal which has raised an immune response to the microorganism or a part thereof, under conditions whereby if the antibodies bind to the mutant microorganism the mutant microorganism is killed;
- (3) selecting surviving mutant microorganisms from step (2);
- 15 (4) identifying the gene containing the mutation in any surviving mutant microorganism; and
- (5) identifying the polypeptide encoded by the gene.
- 20
2. A method according to Claim 1 wherein the microorganism is a pathogenic microorganism.
- 25 3. A method according to Claim 1 or 2 wherein the animal subjected to the microorganism is a host of the microorganism.
4. A method according to any of Claims 1 to 3 wherein the animal subjected to the microorganism is a human who is or has been infected with the
30 microorganism.

5. A method according to any of the preceding claims wherein the microorganism is a bacterium.
6. A method according to Claim 5 wherein the bacterium is *Neisseria meningitidis*.
7. A method according to any of the preceding claims wherein the mutant microorganisms have insertional mutations.
8. A method according to any of the preceding claims wherein any surviving mutant selected in step (3) is backcrossed into a parental strain of the microorganism and it is determined whether the resulting cross is resistant to killing under conditions as set out in step (2).
9. A method according any one of the preceding claims wherein in step (2) complement mediates killing of the microorganisms to which the antibodies are bound.
10. A method of identifying a gene encoding a polypeptide of a microorganism which polypeptide is associated with an immune response in an animal which has been subjected to the microorganism, the method comprising carrying out steps (1) to (4) as defined in Claim 1.
11. A method of selecting a microorganism mutated in a gene encoding a polypeptide which polypeptide is associated with an immune response in an animal which has been subjected to the microorganism, the method comprising carrying out steps (1) to (3) as defined in Claim 1.
12. A method for making an antigen the method comprising carrying out the method according to any of Claims 1 to 9 and synthesising the polypeptide identified in step (5) or an antigenic fragment or variant thereof, or fusion of such polypeptide or fragment or variant.

13. A method according to Claim 12 wherein the variant is a homologous polypeptide from a related microorganism.
- 5 14. A method for making a vaccine for combating a microorganism the method comprising making an antigen according to the method of Claim 12 or 13 or polynucleotide encoding said antigen and combining the antigen or polynucleotide, with a suitable carrier.
- 10 15. A method according to Claim 14 wherein the antigen or polynucleotide is combined with an adjuvant.
16. An antigen obtainable according to the method of Claim 12 or 13 or a polynucleotide encoding said antigen.
- 15 17. A vaccine obtainable by the method of Claims 14 or 15.
18. An antigen or polynucleotide according to Claim 16 for use in a vaccine.
- 20 19. A method of vaccinating an individual against a microorganism, the method comprising administering an antigen or polynucleotide according to Claim 16, or a vaccine according to Claim 17, to the individual.
- 25 20. Use of an antigen or polynucleotide according to Claim 16, or a vaccine according to Claim 17 in the manufacture of a vaccine for vaccinating an individual against a microorganism.
- 30 21. A method for making a polynucleotide the method comprising carrying out the steps of Claim 10 and isolating or synthesising the identified gene or a variant or fragment thereof or a fusion of such gene or variant or fragment.
22. A polynucleotide obtainable by the method of Claim 20.

23. A mutant microorganism obtainable by the method of Claim 11.
24. Any novel method for identifying antigens of microorganisms as herein
5 described.
25. A polypeptide comprising the amino acid sequence selected from any one
of SEQ ID Nos 4, 2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34,
36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56;
10 or a fragment or variant thereof or a fusion of such a fragment or variant.
26. A polynucleotide encoding a polypeptide according to Claim 25.
- 15 27. A polypeptide according to Claim 25 or polynucleotide according to Claim
26 for use in medicine.
28. A polypeptide according to Claim 25 or polynucleotide according to Claim
26 for use in a vaccine.
- 20 29. A method for making a polypeptide according to Claim 25, the method
comprising expressing the polynucleotide of Claim 26 in a host cell and
isolating said polypeptide.
- 25 30. A method for making a polypeptide according to Claim 26 comprising
chemically synthesising said polypeptide.
31. A method of vaccinating an individual against *Neisseria meningitidis*, the
method comprising administering to the individual a polypeptide according
30 to Claim 25 or a polynucleotide according to Claim 26.

32. Use of a polypeptide according to Claim 25 or a polynucleotide according to Claim 26 in the manufacture of a vaccine for vaccinating an individual against *Neisseria meningitidis*.
- 5 33. Any novel *Neisseria meningitidis* vaccine as herein disclosed.