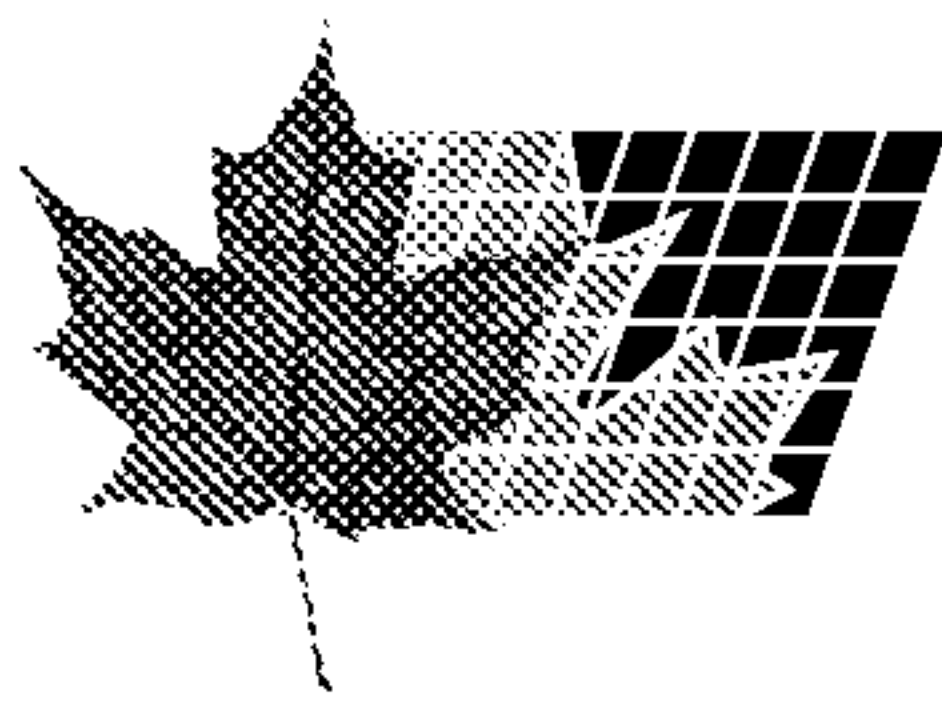




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(54) **DERIVES DE PROTEINES PNEUMOCOCCIQUES DE LIAISON
DE CHOLINE DESTINES A DES VACCINS**

(54) **DERIVATIVES OF PNEUMOCOCCAL CHOLINE BINDING
PROTEINS FOR VACCINES**

(57) L'invention concerne des agents bactériens immunogènes destinés à être administrés à l'homme et à des animaux pour stimuler une réponse immune. L'invention concerne en particulier la vaccination d'espèces de mammifères présentant des polypeptides dérivés de pneumocoques qui comprennent une hélice alpha mais ne comprennent pas de région de liaison de choline qui stimule la production d'anticorps protégeant le sujet vacciné contre une infection aux espèces bactériennes pathogènes. Selon un autre aspect, l'invention concerne des anticorps contre ces protéines et les complexes de protéines, lesquels peuvent servir comme agents de diagnostic ou de protection contre des espèces bactériennes pathogènes.

(57) The present invention provides bacterial immunogenic agents for administration to humans and non-human animals to stimulate an immune response. It particularly relates to the vaccination of mammalian species with pneumococcal derived polypeptides that include an alpha helix but exclude a choline binding region as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. In another aspect the invention provides antibodies against such proteins and protein complexes that may be used as diagnostics and/or as protective/treatment agents for pathogenic bacterial species.

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(54) Title: DERIVATIVES OF PNEUMOCOCCAL CHOLINE BINDING PROTEINS FOR VACCINES (57) Abstract The present invention provides bacterial immunogenic agents for administration to humans and non-human animals to stimulate an immune response. It particularly relates to the vaccination of mammalian species with pneumococcal derived polypeptides that include an alpha helix but exclude a choline binding region as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. In another aspect the invention provides antibodies against such proteins and protein complexes that may be used as diagnostics and/or as protective/treatment agents for pathogenic bacterial species.			

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DERIVATIVES OF PNEUMOCOCCAL CHOLINE BINDING PROTEINS FOR VACCINES

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This application claims the benefit of U.S. Prov. Appl'n Serial No. 60/085,743, filed May 15, 1998 and U.S. Prov. Appl'n Serial NO 60/080,878, filed April 7, 1998.

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This invention relates generally to the field of bacterial antigens and their use, for example, as immunogenic agents in humans and animals to stimulate an immune response. More specifically, it relates to the vaccination of mammalian species with a polypeptide comprising an alpha helix-forming polypeptide obtained from a choline binding polypeptide as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by pathogenic bacterial species. Further, the invention relates to antibodies and antagonists against such polypeptides useful in diagnosis and passive immune therapy with respect to diagnosing and treating such pneumococcal infections.

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In a particular aspect, the present invention relates to the prevention and treatment of pneumococcal infections such as infections of the middle ear, nasopharynx, lung and bronchial areas, blood, CSF, and the like, that are caused by pneumococcal bacteria. In this regard, certain types of *Streptococcus pneumoniae* are of particular interest.

S. pneumoniae is a gram positive bacteria which is a major causative agent in invasive infections in animals and humans, such as sepsis, meningitis, otitis media and lobar pneumonia (Tuomanen, et al. NEJM 322:1280-1284 (1995)). As part of the infective process, pneumococci

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readily bind to non-inflamed human epithelial cells of the upper and lower respiratory tract by binding to eukaryotic carbohydrates in a lectin-like manner (Cundell et al., Micro. Path. 17:361-374 (1994)). Conversion to invasive pneumococcal infections for bound bacteria may involve the local generation of inflammatory factors which may activate the epithelial cells to change the number and type of receptors on their surface (Cundell, et al., Nature, 377:435-438 (1995)). Apparently, one such receptor, platelet activating factor (PAF) is engaged by the pneumococcal bacteria and within a very short period of time (minutes) from the appearance of PAF, pneumococci exhibit strongly enhanced adherence and invasion of tissue. Certain soluble receptor analogs have been shown to prevent the progression of pneumococcal infections (Idanpaan-Heikkila et al., J. Inf. Dis., 176:704-712 (1997)).

A family of choline binding proteins (CBPs), which are non-covalently bound to phosphorylcholine, are present on the surface of pneumococci and have a non-covalent association with teichoic acid or lipoteichoic acid. An example of such family is choline binding protein A (CbpA), an approximately 75kD weight type of CBP which includes a unique N-terminal domain, a proline rich region, and a C-terminal domain comprised of multiple 20 amino acid repeats responsible for binding to choline. A segment of the N-terminal portion of CbpA protein forms an alpha helix as part of its three-dimensional structure.

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Accordingly, it is an object of the present invention to provide a polypeptide having broad protection against pneumococcal infections.

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Definitions

In order to facilitate understanding of the description below and the examples which follow certain

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frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded
5 and/or followed by capital letters and/or numbers. The
starting plasmids herein are either commercially
available, publicly available on an unrestricted basis, or
can be constructed from available plasmids in accord with
published procedures. In addition, equivalent plasmids to
10 those described are known in the art and will be apparent
to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of
the DNA with a restriction enzyme that acts only at
15 certain sequences in the DNA. The various restriction
enzymes used herein are commercially available and their
reaction conditions, cofactors and other requirements were
used as would be known to the ordinarily skilled artisan.

For analytical purposes, typically 1 μ g of plasmid or DNA
20 fragment is used with about 2 units of enzyme in about 20
 μ l of buffer solution. For the purpose of isolating DNA
fragments for plasmid construction, typically 5 to 50 μ g
of DNA are digested with 20 to 250 units of enzyme in a
larger volume. Appropriate buffers and substrate amounts
25 for particular restriction enzymes are specified by the
manufacturer. Incubation times of about 1 hour at 37°C are
ordinarily used, but may vary in accordance with the
supplier's instructions. After digestion the reaction is
electrophoresed directly on a polyacrylamide gel to
30 isolate the desired fragment.

Size separation of the cleaved fragments is
performed using 8 percent polyacrylamide gel described by
Goeddel, D. et al., Nucleic Acids Res., 8:4057 (1980).
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"Oligonucleotides" refers to either a single
stranded polydeoxynucleotide or two complementary
polydeoxynucleotide strands which may be chemically

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synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide
5 will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic
10 acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

15 "HPS portion" as used herein refers to an amino acid sequence as set forth in SEQ ID NO:2 for a choline binding protein ("CBP") of a pneumococcal bacteria that may be located amino terminal with respect to the proline rich
20 portion of the overall amino acid sequence for such CBP.

The terms "identity", "% identity" or "percent identity" as utilized in this application refer to a calculation of differences between two contiguous
25 sequences which have been aligned for "best fit" (to provide the largest number of aligned identical corresponding sequence elements, wherein elements are either nucleotides or amino acids) and all individual differences are considered as individual difference with
30 respect to the identity. In this respect, all individual element gaps (caused by insertions and deletions with respect to an initial sequence ("reference sequence")) over the length of the reference sequence and individual substitutions of different elements (for individual
35 elements of the reference sequence) are considered as individual differences in calculating the total number of differences between two sequences. Individual differences may be compared between two sequences where an initial

sequences (reference sequence) has been varied to obtain a variant sequence (comparative sequence) or where a new sequence (comparative sequence) is simply aligned and compared to such a reference sequence. When two aligned
5 sequences are compared all of the individual gaps in BOTH sequences that are caused by the "best fit" alignment over the length of the reference sequence are considered individual differences for the purposes of identity. If an alignment exists which satisfies the stated minimum
10 identity, then a sequence has the stated minimum identity to the reference sequence. For example, the following is a hypothetical comparison of two sequences having 100 elements each that are aligned for best fit wherein one sequence is regarded as the "reference sequence" and the
15 other as the comparative sequence. All of the individual alignment gaps in both sequences are counted over the length of the reference sequence and added to the number of individual element substitution changes (aligned elements that are different) of the comparative sequence
20 for the total number of element differences. The total number of differences (for example 7 gaps and 3 substitutions) is divided by the total number of elements in the length of the reference sequence (100 elements) for the "percentage difference" (10/100). The resulting
25 percentage difference (10%) is subtracted from 100% identity to provide a "% identity" of 90% identity. For the identity calculation all individual differences in both sequences are considered in the above manner over a discrete comparison length (the length of the reference
30 sequence) of two best fit aligned sequences to determine identity. Thus, no algorithm is necessary for such an identity calculation.

"Isolated" in the context of the present invention
35 with respect to polypeptides and/or polynucleotides means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living organism

is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such
5 polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and
10 preferably are purified to homogeneity.

Summary of the Invention

In one aspect the present invention relates to a vaccine for treating or preventing pneumococcal bacterial
15 infections which utilizes as an immunogen at least one polypeptide truncate of a pneumococcal surface-binding protein, analog, or variant having a highly conserved immunogenic alpha-helical portion (corresponding generally to a "consensus" amino acid sequence as set forth in SEQ
20 ID NO:1) with respect to different types of pneumococcal bacteria, which polypeptide does not include a choline-binding portion. Preferably, the C-terminal choline-binding portion is absent from such polypeptides. More preferred are such polypeptides wherein the HPS amino acid
25 sequence is also absent. Even further preferred are polypeptides wherein the highly conserved immunogenic alpha-helical portion corresponding generally to a "consensus" amino acid sequence as set forth in SEQ ID NO:1 also corresponds generally to the amino acid sequence
30 as set forth in SEQ ID NO:19 (amino acids 1 to 103 of SEQ ID NO:19 are identical to amino acids 1 to 103 of SEQ ID NO:1). Also preferred as vaccines are recombinantly-produced, isolated polypeptides that are missing both an HPS portion and the choline-binding portion.

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More preferred as vaccines are one or more polypeptide truncates of pneumococcal surface-binding proteins, analogs or variants including a single highly conserved alpha-helix immunogenic portion with respect to

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different types of pneumococci, which polypeptides do not include a C-terminal choline-binding portion. Further preferred are isolated recombinantly produced polypeptides having such structure. Also preferred are such polypeptides that do not include either a C-terminal choline-binding portion or a HPS portion.

The present invention further provides a vaccine comprising a polypeptide including an immunogenic portion that is capable of forming an alpha helix, which polypeptide includes a sequence that has at least 85% identity and preferably at least 87% identity to the amino acid sequence of SEQ ID NO:1, wherein the isolated polypeptide does not include a C-terminal choline-binding portion. Further preferred are such polypeptides that comprise a polypeptide sequence that has at least 85% identity and preferably at least 87% identity to an amino acid sequence according SEQ ID NO:19. Preferably, the sequence of the isolated polypeptide includes neither an HPS portion (SEQ ID NO:2) nor a C-terminal choline-binding portion. Further preferred are isolated recombinantly produced polypeptides having such structure. In particular, such polypeptides corresponding to alpha helical structures of different types of *S. pneumoniae* bacteria are contemplated. Particularly preferred are the serotypes 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F of such *S. pneumoniae* bacteria. Examples of such serotypes of bacteria are readily available from standard ATCC catalogs.

In an additional aspect, the present invention further provides a vaccine against *S. pneumoniae* comprising a synthetic or recombinant polypeptide comprising a plurality of alpha-helical portions, each derived from different naturally occurring *S. pneumoniae* choline-binding polypeptides wherein such alpha-helical portions have at least 85% identity to the amino acid sequence of SEQ ID NO:1, and wherein the isolated

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polypeptide does not include a choline-binding portion. Further preferred are those wherein the amino acid sequence for the alpha-helix areas is at least 85% identical to the amino acid sequence of SEQ ID NO:19.

5 Preferably, such synthetic polypeptide includes neither a HPS portion nor a choline-binding portion. Analogs and variants of such chain structure polypeptides wherein such alpha helical portions may be synthetic variant amino acid sequences (or may be a mixture of naturally occurring and

10 variant sequences) are also contemplated and embraced by the present invention. In a preferred aspect, chain vaccines polypeptides having at least ten different alpha helical structures corresponding to *S. pneumoniae* serotypes 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14,

15 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F are provided. Further preferred are polypeptides including at least fifteen of such alpha-helical structures, more preferred are polypeptides including at least 20 such alpha-helical structures and more preferred are

20 polypeptides including at least one alpha-helical structure corresponding to each of the *S. pneumoniae* serotypes 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F. Another preferred polypeptide comprises each of the alpha helical

25 structures from the amino acid sequences of SEQ ID NOS:3-18 which correspond to SEQ ID NO:1.

In another aspect, the invention relates to passive immunity vaccines formulated from antibodies against a

30 polypeptide including a highly conserved immunogenic portion with respect to different types of pneumococcal bacteria which portion is capable of forming an alpha-helix having the hereinbefore described identity to the amino acid sequence of SEQ ID NO:1, which polypeptide does

35 not include a C-terminal choline-binding portion, wherein said antibodies will bind to at least one *S. pneumoniae* species. Preferably, if such polypeptide is a truncate of a native pneumococcal surface-binding protein both its HPS portion (where applicable) and its choline-binding portion

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are absent from such polypeptide. Such passive immunity vaccines can be utilized to prevent and/or treat pneumococcal infections in immunocompromised patients, patients having an immature immune system (such as young
5 children) or patients who already have an ongoing infection. In this manner, according to a further aspect of the invention, a vaccine can be produced from a synthetic or recombinant polypeptide wherein the polypeptide includes the conserved alpha helical portions
10 of two or more different choline binding polypeptides of *S. pneumoniae*.

This invention also relates generally to the use of an isolated polypeptide having a highly conserved
15 immunogenic portion with respect to different types of pneumococcal bacteria which portion is capable of forming an alpha-helix (corresponding generally to SEQ ID NO:1 or to SEQ ID NO:19) wherein the isolated polypeptide does not include a choline-binding portion, to raise antibodies in
20 non-human mammalian species useful, for example, as diagnostic reagents and vaccines.

In yet another aspect, the present invention relates to the production of a polypeptide including a highly
25 conserved immunogenic portion with respect to different types of pneumococcal bacteria which portion is capable of forming an alpha-helix whose sequence corresponds generally to the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:19, wherein the isolated polypeptide does not
30 include a choline-binding portion. Preferably, such recombinant production is of a truncated native pneumococcal surface-binding polypeptide wherein both the HPS portion (where applicable) and the choline-binding portion are absent.

35

In still another aspect, the present invention provides an isolated choline-binding polypeptide, wherein the non-choline binding region of such polypeptide has at least 90% identity to the corresponding amino acid

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sequence portion of a naturally occurring pneumococcal surface-binding protein which is a member selected from the group consisting of SEQ ID NOS:3-18. The invention relates to fragments of such polypeptides which include at least the conserved alpha-helical portion corresponding generally to SEQ ID NO:1, and which has at least 85% identity thereto, wherein the isolated polypeptide preferably is free of a choline binding region.

10 In another aspect the present invention provides an isolated polypeptide comprising an amino acid sequence which has at least 90% identity to one of the amino acid sequences selected from the group consisting of SEQ ID NO:3-18. Preferably, such isolated polypeptide comprises
15 an amino acid sequence which has at least 95% identity, and more preferably 97% identity, to one of the amino acid sequences selected from the group consisting of SEQ ID NO:3-18. The invention further relates to fragments of such polypeptides.

20 In a yet further aspect, the present invention provides a *S. pneumoniae* CBP polypeptide encoded by a polynucleotide that will hybridize under highly stringent conditions to the complement of a polynucleotide encoding
25 a polypeptide having an amino acid selected from the group consisting of SEQ ID NOS:1 and 3-18. Particularly preferred are polypeptides comprising an amino acid sequence segment that is at least 90% identical to the amino acid sequence of SEQ ID NO:1. Further preferred are
30 such polypeptides comprising a contiguous amino acid sequence that has at least 95% identity with respect to the amino acid sequence of SEQ ID NO:1. And, even more preferred are polypeptides comprising an amino acid sequence that has at least 97% identity with respect to
35 the amino acid sequence of SEQ ID NO:1.

In another aspect the present invention provides polynucleotides which encode the hereinabove described polypeptides of the invention. The polynucleotide of the

present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The polynucleotides which encode polypeptides including the amino acid sequences of at least one of SEQ ID NOS:3-18 (or polypeptides that have at least 90% identity to the amino acid sequences of such polypeptides) may be one of the coding sequences shown in SEQ ID NOS:20-35 or may be of a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptides as the DNA of SEQ ID NOS:20-35.

15

The polynucleotides which encode the polypeptides of SEQ ID NOS:3-18 may include: only the coding sequence for the polypeptide; the coding sequence for the polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the polypeptide. The polypeptides encoded may comprise just a single alpha-helical portion or multiple alpha-helical portion and may independently or collectively include N-terminal sequences 5' of such alpha helical areas and/or sequences corresponding to the "X" structures or proline rich areas (as set forth in Figure 1, for example).

20
25

The invention further relates to a polynucleotide comprising a polynucleotide sequence that has at least 95% identity and preferably at least 97% identity to a polynucleotide encoding one of the polypeptides comprising SEQ ID NO:3-18. The invention further relates to fragments of such polynucleotides which include at least the portion of the polynucleotide encoding the polypeptide sequence corresponding to SEQ ID NO:1.

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Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes

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only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence. In particular, the polypeptides may include any or all of the types of structures set forth
5 schematically in Figure 1.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides
10 including the amino acid sequences of SEQ ID NOS:3-18. The variants of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides. Complements to such coding polynucleotides may be utilized
15 to isolate polynucleotides encoding the same or similar polypeptides. In particular, such procedures are useful to obtain alpha helical coding segments from different serotypes of *S. pneumoniae*, which is especially useful in the production of "chain" polypeptide vaccines containing
20 multiple alpha helical segments.

Thus, the present invention includes polynucleotides encoding polypeptides including the same polypeptides as shown in the Sequence Listing as SEQ ID NOS:3-18 as well
25 as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of SEQ ID NOS:3-18. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in the Sequence Listing as SEQ ID NOS:20-35. As known in the art, an allelic variant is an alternate form of a
30 polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.
35

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be, for example, a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used.

10 The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to

15 polynucleotides (hybridization target sequences) which hybridize to the complements of the hereinabove-described sequences if there is at least 70% and preferably 80% identity between the target sequence and the complement of the sequence to which the target sequence hybridizes,

20 preferably at least 85% identity. More preferred are such sequences having at least 90% identity, preferably at least 95% and more preferably at least 97% identity between the target sequence and the sequence of complement of the polynucleotide to which it hybridizes. The

25 invention further relates to the complements to both the target sequence and to the polynucleotide sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NOS:3 to 18. The present invention particularly relates to polynucleotides which hybridize

30 under stringent conditions to the complements of the hereinabove-described polynucleotides as well as to those complements. As herein used, the term "stringent conditions" means hybridization will occur with the complement of a polynucleotide and a corresponding

35 sequence only if there is at least 95% and preferably at least 97% identity between the target sequence and the sequence of complement of the polynucleotide to which it hybridizes. The polynucleotides which hybridize to the complements of the hereinabove described polynucleotides

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in a preferred embodiment encode polypeptides which retain an immunogenic portion that will cross-react with an antibody to at least one of the polypeptides having a sequence according to SEQ ID NOS:3-18, or to a polypeptide
5 that includes an amino acid sequence which has at least 85% identity to that of SEQ ID NO:1.

In a still further aspect, the present invention provides for the production of such polypeptides and
10 vaccines as set forth above having a histidine label (or other suitable label) such that the full-length proteins, truncates, analogs or variant discussed above can be isolated due to their label.

15 In another aspect the present invention relates to a method of prophylaxis and/or treatment of diseases that are mediated by pneumococcal bacteria that have surface-binding CBP proteins. In particular, the invention relates to a method for the prophylaxis and/or treatment
20 of infectious diseases that are mediated by *S. pneumoniae* that have a CBP surface-binding protein that forms an alpha helix (comprising a sequence that has at least an 85% identity to the amino acid sequence of SEQ ID NO:1). In a still further preferred aspect, the invention relates
25 to a method for the prophylaxis and/or treatment of such infections in humans.

In still another aspect the present invention relates to a method of using one or more antibodies
30 (monoclonal, polyclonal or sera) to the polypeptides of the invention as described above for the prophylaxis and/or treatment of diseases that are mediated by pneumococcal bacteria that have CBP surface-binding proteins. In particular, the invention relates to a
35 method for the prophylaxis and/or treatment of infectious diseases that are mediated by *S. pneumoniae* CBP proteins which include an alpha helical portion having the hereinbefore described identity to the consensus sequence of SEQ ID NO:1. In a still further preferred aspect, the

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invention relates to a method for the prophylaxis and/or treatment of otitis media, nasopharyngeal, bronchial infections, and the like in humans by utilizing antibodies to the alpha-helix containing immunogenic polypeptides of
5 the invention as described above.

Brief Description of Drawings

Figure 1 is a diagram of a pneumococcal CBP protein which shows from the N-terminal to the C-terminal, respectively, (a) a N-terminal sequence, (b) one of a potential alpha-helical forming area conserved segment (R1) that may not be present in some CBP polypeptides, (c) an optional small bridging sequence of amino acids that may bridge two conserved alpha-helical segments (X), (d) a second of a potential alpha-helical forming area consensus sequence (R2) related to the first consensus sequence (which corresponds to SEQ ID NO:1), (e) a proline rich area sequence, (f) a choline binding repeats area, and (g) a C-terminal tail sequence. Where relevant, an optional HPS sequence may naturally occur 5' of the proline rich sequence and 3' of the R1, X, and/or R2 areas.

Figure 2 reports the results for passive immunity protection against 1600 cfu virulent serotype 6B *S. pneumoniae* SP317 (in mice) that was provided by day 31 rabbit antisera to a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). Eighty percent of the mice immunized with the truncate antisera prior to challenge survived the 14 day observation period. By contrast, all mice immunized with a control sera (pre-immune rabbit sera) were dead by day 7.

30

Figure 3 reports the results for passive immunity protection against 3450 cfu virulent serotype 6B *S. pneumoniae* SP317 (in mice) that was provided by day 52 rabbit antisera to a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). One hundred percent of the mice immunized with the truncate antisera prior to challenge survived the 10 day observation period. By

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contrast, ninety percent of the mice immunized with a control sera (pre-immune rabbit sera) were dead at day 10.

Figure 4 reports the results for passive immunity protection against 580 cfu virulent serotype 6B *S. pneumoniae* SPSJ2 (in mice) that was provided by day 31 rabbit antisera to a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). Fifty percent of the mice immunized with the truncate antisera prior to challenge survived the 10 day observation period. By contrast, all mice immunized with a control sera (pre-immune rabbit sera) were dead by day 8.

15

Figure 5 reports the results for active immunity protection against 560 cfu virulent serotype 6B *S. pneumoniae* SPSJ2 (in mice) that was provided by immunization with a pneumococcal CBP truncate polypeptide, NR1X (truncate missing the second conserved alpha-helical area R2, as well as both the proline and the choline binding areas). Eighty percent of the mice actively immunized with the NR1X CBP truncate prior to challenge survived the 14 day observation period. By contrast, all mice immunized with a control (sham mice) of PBS and adjuvant were dead by day 8.

25

Figure 6 reports the results for active immunity protection against 680 cfu virulent serotype 6B *S. pneumoniae* SPSJ2 (in mice) that was provided by immunization with a pneumococcal CBP truncate polypeptide, NR1XR2 (truncate missing both the proline and the choline binding areas, but including two conserved alpha-helical areas R1 and R2). Fifty percent of the mice actively immunized with the NR1XR2 CBP truncate prior to challenge survived the 14 day observation period. By contrast, all mice immunized with a control (SP90) protein and adjuvant were dead by day 9.

30

35

Figure 7 is an alignment report of the amino terminus of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The
5 consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:36). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

10

Figure 8 shows the sequence pair distances for the amino acid sequences as described for Figure 7 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a
15 comparison is reported for the amino acid sequences set forth in Figure 7.

Figure 9 is an alignment report for a first helical region in the amino acid sequences of CBP polypeptides
20 from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:38). One letter codes are utilized to represent the
25 sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 10 shows the sequence pair distances for the
30 amino acid sequences as described for Figure 9 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 9.

35

Figure 11 is an alignment report for the region X in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the

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comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:37). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 12 shows the sequence pair distances for the amino acid sequences as described for Figure 11 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 11.

Figure 13 is an alignment report for the second helical region A in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:1). One letter codes are utilized to represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

Figure 14 shows the sequence pair distances for the amino acid sequences as described for Figure 13 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set forth in Figure 13.

Figure 15 is an alignment report for the second helical region B in the amino acid sequences of CBP polypeptides from various types of *S. pneumoniae* and a consensus sequence is reported at the top of each row (sets of lines) of the comparison. The consensus sequence for the comparison is listed as the "Majority" sequence (SEQ ID NO:19). One letter codes are utilized to

represent the sequences which are aligned for a "best fit" comparison wherein dashes in a sequence indicate spacing gaps of the contiguous sequence.

5 Figure 16 shows the sequence pair distances for the amino acid sequences as described for Figure 15 and set forth therein. A Clustal method with identity residue weight table is used. The percent similarity for such a comparison is reported for the amino acid sequences set
10 forth in Figure 15.

Detailed Description of the Invention

15 In accordance with an aspect of the present invention there is provided a vaccine to produce a protective response against *S. pneumoniae* infections which employs a polypeptide which comprises a member selected from the group consisting of:

20 (a) an amino acid sequence which produces an alpha helical structure and which is at least 85% identical to the amino acid sequence of SEQ ID NO:1 and which is free of a choline binding region, and

 (b) an isolated truncate of a naturally occurring
25 *S. pneumoniae* polypeptide that comprises an alpha helical portion that has at least 85% identity to the amino acid sequence of SEQ ID NO:1 and is free of a choline binding region,

 (c) an isolated truncate of a naturally occurring
30 *S. pneumoniae* polypeptide that comprises an alpha helical portion that has at least 90% identity to the amino acid sequence of SEQ ID NO:19 and is free of a choline binding region. In a preferred aspect, such isolated truncate polypeptide is a member selected from the group consisting
35 of SEQ ID NOS:3-18 and said isolated polypeptide is free of a choline binding region and, if relevant, a HPS region; or a fragment thereof which includes at least the alpha helical segment which corresponds to the consensus sequence of SEQ ID NO:1. Particularly preferred are

vaccines which utilize such truncate polypeptides that include at least such alpha helical area or utilize a recombinant immunogen polypeptide comprising at least two of such alpha-helical segments. Such polypeptide may be
5 a recombinant polypeptide containing multiple alpha-helical areas from one or more truncates. Further preferred are recombinant immunogen polypeptides comprising at least two alpha-helical areas corresponding to the alpha helical areas of two or more truncates from
10 different types of pneumococcal bacteria. Such polypeptide may be a recombinant polypeptide containing multiple alpha-helical areas from one or more different types of pneumococcal bacteria.

15 In accordance with the present invention, there is provided an isolated polypeptide comprising a truncated surface-binding polypeptide derived from *S. pneumoniae*, said isolated polypeptide containing an alpha-helical area whose amino acid sequence corresponds generally to the
20 amino acid sequence of SEQ ID NO:1, but free of a choline binding area. Preferably, said isolated polypeptide also omits any naturally occurring repeats of the alpha-helical forming area and omits any HPS amino acid sequence that may be present.

25

It is an object of the present invention to utilize as immunogenic composition for a vaccine (or to produce antibodies for use as a diagnostic or as a passive vaccine) comprising an immunogenic polypeptide comprising
30 a pneumococcal surface-binding polypeptide with an alpha helical portion from which a choline binding region has been omitted. In one embodiment, such truncated proteins (naturally or recombinantly produced, as well as functional analogs) from *S. pneumoniae* bacteria are contemplated.
35 Even more particularly, *S. pneumoniae* polypeptides having a single alpha helical portion that omit any HPS areas that occur and choline binding areas of the native protein are contemplated.

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A particularly preferred embodiment of such an immunogenic composition is for use as a vaccine (or as an immunogen for producing antibodies useful for diagnostics or vaccines) wherein the active component of the immunogenic composition is an isolated polypeptide comprising at least one member selected from the group consisting of:

(a) an amino acid sequence which is selected from SEQ ID NOS:3-19,

(b) a polypeptide which has at least 90% identity to (a), preferably at least 95% identity to (a), and even more preferred at least 97% identity to (a), or

(c) a fragment of (a) or (b) wherein such fragment includes at least one alpha helical portion that corresponds to the consensus sequence which is SEQ ID NO:1 and said fragment does not comprise a choline binding region. Preferably, such vaccines utilize a polypeptide that contains neither a choline binding region nor an HPS region that occurs as part of the amino acid sequences in the native proteins.

In another preferred embodiment, there is provided a vaccine which includes at least one isolated polypeptide which includes an amino acid sequence which has at least 85% identity (preferably 87% identity and more preferably at least 90% identity) to SEQ ID NO:1, which isolated polypeptide is free of a choline binding portion and, where applicable, is also preferably free of an HPS portion. The preferred polypeptide may also include one or more of the N-terminal sequences that are located 5' of the alpha helical areas in the polypeptides having an amino acid sequence selected from the group consisting of SEQ ID NOS:3-18, or the like. The polypeptide truncate may also include one or more of the proline regions (region "P" in Figure 1) and/or the spanning region (region "X" in Figure 1).

In another aspect of the invention, such an immunogenic composition may be utilized to produce

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antibodies to diagnose pneumococcal infections, or to produce vaccines for prophylaxis and/or treatment of such pneumococcal infections as well as booster vaccines to maintain a high titer of antibodies against the immunogen(s) of the immunogenic composition.

While other antigens have been contemplated to produce antibodies for diagnosis and for the prophylaxis and/or treatment of pneumococcal infections, there is a need for improved or more efficient vaccines. Such vaccines should have an improved or enhanced effect in preventing bacterial infections mediated pneumococci having surface-binding polypeptides. Further, to avoid unnecessary expense and provide broad protection against a range of pneumococcal serotypes there is a need for polypeptides that comprise an immunogenic amino acid sequence corresponding to a portion of pneumococcal surface-binding polypeptides that is a highly conserved portion among various types of pneumococci. Preferably, such polypeptides avoid the inclusion of amino acid sequences corresponding to other portions of the native polypeptides, such as the choline binding region and/or the HPS region.

There is a need for improved antigenic compositions comprising highly conserved portions of polypeptides that bind to the surface of pneumococcal bacteria for stimulating high-titer specific antisera to provide protection against infection by pathogenic pneumococcal bacteria and also for use as diagnostic reagents.

In such respect, truncated polypeptides, functional variant analogs, and recombinantly produced truncated polypeptides of the invention are useful as immunogens for preparing vaccine compositions that stimulate the production of antibodies that can confer immunity against pathogenic species of bacteria. Further, preparation of vaccines containing purified proteins as antigenic ingredients are well known in the art.

Generally, vaccines are prepared as injectables, in the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling.

5 Solid forms which are dissolved or suspended prior to use may also be formulated. Pharmaceutical carriers are generally added that are compatible with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to,

10 water, saline solutions, dextrose, or glycerol. Combinations of carriers may also be used.

Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as

15 adjuvants, wetting agents, or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

Vaccines are generally formulated for parenteral administration and are injected either subcutaneously or

20 intramuscularly. Such vaccines can also be formulated as suppositories or for oral administration, using methods known in the art.

The amount of vaccine sufficient to confer immunity

25 to pathogenic bacteria is determined by methods well known to those skilled in the art. This quantity will be determined based upon the characteristics of the vaccine recipient and the level of immunity required. Typically, the amount of vaccine to be administered will be

30 determined based upon the judgment of a skilled physician. Where vaccines are administered by subcutaneous or intramuscular injection, a range of 50 to 500 μ g purified protein may be given.

35 The term "patient in need thereof" refers to a human that is infected with, or likely, to be infected with, pathogenic pneumococcal bacteria that produce CbpA, or the like, preferably *S. pneumoniae* bacteria (however a mouse

model can be utilized to simulate such a patient in some circumstances).

5 In addition to use as vaccines, the polypeptides of the present invention can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

10

The polynucleotides encoding the immunogenic polypeptides described above may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be, for example, a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

25 The identification of multiple coil structures of alpha helical amino acid segments in the *S. pneumoniae* polypeptides according to the invention may be determined by the location of proline rich areas with respect to one another. Further the "X" area optionally located between two or more alpha-helical structures can play a part in the formation of a coil within a coil structure. Standard three-dimensional protein modeling may be utilized for determining the relative shape of such structures. An example of a computer program, the Paircoil Scoring Form Program ("PairCoil program"), useful for such three-dimensional protein modelling is described by Berger et al. in the Proc. Natl. Acad. of Sci. (USA), 92:8259-8263 (August 1995). The PairCoil program is described as a computer program that utilizes a mathematical algorithm to

30
35

predict locations of coiled-coil regions in amino acid sequences. A further example of such a computer program is described by Wolf et al., Protein Science 6:1179-1189 (June 1997) which is called the Multicoil Scoring Form
5 Program ("Multicoil program"). The MultiCoil program is based on the PairCoil algorithm and is useful for locating dimeric and trimeric coiled coils .

In a preferred aspect, the invention provides for
10 recombinant production of such polypeptides in a host bacterial cell other than a *S. pneumoniae* species host to avoid the inclusion of native surface-binding polypeptides that have a choline binding region. A preferred host is a species of such bacteria that can be cultured under
15 conditions such that the polypeptide of the invention is secreted from the cell.

The present invention also relates to vectors which include polynucleotides encoding one or more of the
20 polypeptides of the invention that include the highly conserved alpha-helical amino acid sequence in the absence of an area encoding a choline binding amino acid sequence, host cells which are genetically engineered with vectors of the invention and the production of such immunogenic
25 polypeptides by recombinant techniques in an isolated and substantially immunogenically pure form.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors comprising a
30 polynucleotide encoding a polypeptide comprising the highly conserved alpha-helical region but not having a choline binding region, or the like of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the
35 form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the polynucleotides which encode such polypeptides. The

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culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

5

Vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

15

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

20

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

25

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In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampi-

cillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the proteins.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen, Inc.), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired

gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and TRP. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

10

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides

of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

10

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas,

Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful
5 expression vectors for bacterial use can comprise a
selectable marker and bacterial origin of replication
derived from commercially available plasmids comprising
genetic elements of the well known cloning vector pBR322
(ATCC 37017). Such commercial vectors include, for
10 example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala,
Sweden) and GEM1 (Promega Biotec, Madison, WI, USA).
These pBR322 "backbone" sections are combined with an
appropriate promoter and the structural sequence to be
expressed.

15
Following transformation of a suitable host strain
and growth of the host strain to an appropriate cell
density, the selected promoter is induced by appropriate
means (e.g., temperature shift or chemical induction) and
20 cells are cultured for an additional period.

Cells are typically harvested by centrifugation,
disrupted by physical or chemical means, and the resulting
crude extract retained for further purification.

25
Microbial cells employed in expression of proteins
can be disrupted by any convenient method, including
freeze-thaw cycling, sonication, a french press,
mechanical disruption, or use of cell lysing agents, such
30 methods are well known to those skilled in the art.
However, preferred are host cells which secrete the
polypeptide of the invention and permit recovery of the
polypeptide from the culture media.

35
Various mammalian cell culture systems can also be
employed to express recombinant protein. Examples of
mammalian expression systems include the COS-7 lines of
monkey kidney fibroblasts, described by Gluzman, Cell,
23:175 (1981), and other cell lines capable of expressing

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a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

10

The polypeptides can be recovered and/or purified from recombinant cell cultures by well-known protein recovery and purification methods. Such methodology may include ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. In this respect, chaperones may be used in such a refolding procedure. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides that are useful as immunogens in the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Particularly preferred immunogens are truncated pneumococcal polypeptides that comprise a single highly conserved alpha helical area, but do not comprise a choline binding region or a HPS region. Therefore, antibodies against such polypeptides should bind to other pneumococcal bacterial species (in addition to the *S. pneumoniae* species from

which such polypeptides were derived) and vaccines against such *S. pneumoniae* should give protection against other pneumococcal bacterial infections.

5 Procedures for the isolation of the individually expressed alpha-helical containing polypeptides may be isolated by recombinant expression/isolation methods that are well-known in the art. Typical examples for such isolation may utilize an antibody to a conserved area of
10 the protein or to a His tag or cleavable leader or tail that is expressing as part of the protein structure.

 The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them
15 can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab
20 expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

 Antibodies generated against the polypeptides
25 corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner,
30 even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides.

 For preparation of monoclonal antibodies, any
35 technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today

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4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

5

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

10

In order to facilitate understanding of the above description and the examples which follow below, as well as the Figures included herewith, Table 1 below sets forth the bacterial source for the polypeptides of SEQ ID NOS:3-18 and the polynucleotides encoding them (SEQ ID NOS:20-35, respectively). The name and/or type of bacteria is specified and a credit or source is named. The sequences from such types of bacteria are for illustrative purposes only since by utilizing probes and/or primers as described herein other sequences of similar type may be readily obtained by utilizing only routine skill in the art.

15

20

25

TABLE 1

SEQ ID NO.	Type Of Pneumococcus	Source Credit or ATCC No.
3	1	ATCC 33400
4	2	SPATCC 11733
5	2	ATCC2 (catalog #6302)
6	4	ATCC4 (catalog #6304)
7	6B	ATCC 6B (catalog #6326)
8	18C	SPATCC 18C (ATCC catalog #10356)

TABLE 1
(Continued)

SEQ ID NO.	Type Of Pneumococcus	Source Credit or ATCC No.
9	4	Norway type 4; Nat'l. Inst. of Public Health, Norway Ingeborg Aagerge
10	noncapsulated	R6X; Rockefeller Univ., Rob Masure (from D39, type 2)
11	6B	SPB 105; Boston Univ., Steve Pelton
12	23F	SPB 328; Boston Univ., Steve Pelton
13	14	SPB 331; Boston Univ., Steve Pelton
14	23F	SPB 365; Boston Univ., Steve Pelton
15	9V	SPR 332; Rockefeller Univ., Rob Masure
16	6B	SPSJ 2p; St. Jude Children's Research Hospital, Pat Flynn (clinical isolate passaged 1x in mice for virulence)
17	14	SPSJ 9; St. Jude Children's Research Hospital, Pat Flynn (clinical isolate - nares, pneumonia)
18	19A	SPSJ 12; St. Jude Children's Research Hospital, Pat Flynn (clinical isolate)

5

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

10

Example 1**Generation of CbpA Truncate Protein Vectors****A. Vector for Full Length CbpA (NR1XR2PC)**

5 A virulent serotype 4 *S. pneumoniae* strain, Norway 4
(obtained from I. Aaberge, National Institute of Public
Health, Oslo, Norway) was used as a source of genomic DNA
template for amplifying the polynucleotide encoding full-
length CbpA. Full length CbpA was amplified with PCR
10 primers SJ533 and SJ537 described below.

The degenerate forward primer SJ533 was designed
based on the CbpA N-terminal sequence XENEG provided by
H.R. Masure (St. Jude Children's Research Hospital,
15 Memphis, TN). The SJ533 primer = 5' GGC GGA TCC ATG
GA(A,G) AA(C,T) GA(A,G) GG 3'. It incorporates both
BamHI and NcoI restriction sites and an ATG start codon.

20 The 3' reverse primer SJ537 = 5' GCC GTC GAC TTA GTT
TAC CCA TTC ACC ATT GGC 3'

This primer incorporates a SalI restriction site for
cloning purposes, and the natural stop codon from CbpA,
and is based on type 4 and R6X sequence generated in-
25 house.

PCR product generated from genomic DNA template with
SJ533 and SJ537 was digested with BamHI and SalI, and
cloned into the pQE30 expression vector (Qiagen, Inc.)
30 digested with BamHI, XbaI, and SmaI. The type R6X
template resulted in full-length vector PMI581 and the
type 4 template DNA resulted in full-length vector
PMI580.

35 B. Vector for CbpA Truncate Protein (NR1XR2)

The naturally occurring PvuII site in the end on the
second amino repeat (nucleic acid 1228 of Type 4
sequence) was exploited to create a truncated version of

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CbpA, containing only the amino terminus of the gene. To create the truncate clone, the full-length clone PMI580 (Type 4) or PMI581 (R6X) was digested with PvuII and XbaI, and the amino terminus along with a portion of the expression vector was isolated by size on an agarose gel. pQE30 was digested with XbaI and SmaI, and the band corresponding to the other half of the vector was also size selected on an agarose gel. The two halves were ligated and clones identified by restriction digest, then expressed. In this instance, the stop codon utilized is in the expression vector, so the protein expressed is larger than the predicted size due to additional amino acids at the 5' and 3' end of the cloning site.

15 C. Vector for CbpA Truncate Protein (NR1X)

A similar strategy was used to express only the first amino repeat of CbpA. Here the naturally occurring XmnI site between the two amino repeats (nucleic acid 856 of Type 4 sequence) was utilized. CbpA full-length clone PMI580 was digested with XmnI and AatII. Expression vector pQE30 was digested with AatII and SmaI. Once again, the two sized fragments were ligated, and clones were screened by restriction digest and expressed.

25 Example 2

Expression of CbpA Truncate Protein From Expression Vectors

All proteins are expressed from the vectors described in Examples 1A-1C in the Qia expressionist System (Qiagen) using the *E. coli* expression vector pQE30, and the amino terminus His6 tagged proteins are detected by western analysis using both anti-Histidine antibodies and gene specific antibodies.

35 The expressed CBP truncates were purified as follows.

A single colony was selected from plated bacteria

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containing the recombinant plasmid and grown overnight at 37° in 6.0 ml LB buffer with 50 ug/ml Kanamycin and 100 ug/ml Ampicillin. This 6.0 ml culture was added to 1L LB with antibiotics at above concentrations. The culture
5 was shaken at 37°C until $A_{600} = \sim 0.400$. 1M IPTG was added to the 1L culture to give a final concentration of 1mM. The culture was then shaken at 37°C for 3-4 h. The 1L culture was spun 15 min. in 250 ml conical tubes at 4000 rpm in a model J-6B centrifuge. The supernatant was
10 discarded and the pellet stored at -20°C until use.

The 1L pellet was resuspended in 25 ml 50 mM NaH_2PO_4 , 10mM Tris, 6M GuCl , 300mM NaCl , pH 8.0 (Buffer A). This mixture was then rotated at room temperature
15 for 30 minutes. The mixture was then subjected to sonication (VibraCell Sonicator, Sonics and Materials, Inc., Danbury, CT) using the microtip, two times, for 30 sec., at 50% Duty Cycle and with the output setting at 7.

The mixture was spun 5 min. at 10K in a JA20 rotor and
20 the supernatant removed and discarded. The supernatant was loaded on a 10 ml Talon (Clonetech, Palo Alto, CA) resin column attached to a GradiFrac System (Pharmacia Biotech, Upsala, Sweden). The column was equilibrated with 100 ml Buffer A and washed with 200 ml of this
25 buffer. A volume based pH gradient using 100% 50 mM NaH_2PO_4 , 8M Urea, 20mM MES, pH 6.0 (Buffer B) as the final target buffer was run over a total volume of 100 ml. Protein eluted at ~ 30% Buffer B. Eluted peaks were collected and pooled.

30

For refolding, dialysis was carried out with a 2L volume of PBS at room temperature for approximately 3 hr. using dialysis tubing with a molecular weight cutoff of 14,000. The sample was then dialyzed overnight in 2L of
35 PBS at 4°C. Additional buffer exchange was accomplished during the concentration of the protein using Centriprep-30 spin columns by adding PBS to the spun retentate and

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respinning. The protein concentration was determined using the BCA protein assay and the purity visualized using a Coomassie stained 4-20% SDS-PAGE gel.

5

Example 3

Passive Protection with Anti-CbpA Truncate NR1XR2 Antisera

10

A. Generation of Rabbit Immune Serum

Rabbit immune serum against CbpA truncate was generated at Covance (Denver, PA). Following collection of preimmune serum, a New Zealand white rabbit (#ME101) was immunized with 250 µg CbpA truncate NR1XR2 (containing both alpha helix I and alpha helix II amino acid N-terminal repeats that are prepared from 483:58) in Complete Freund's Adjuvant. The rabbit was given a boost of 125 µg CbpA truncate in Incomplete Freund's Adjuvant on day 21 and bled on days 31 and 52.

20

B. Passive Protection in Mice

C3H/HeJ mice (5 mice/group) were passively immunized intraperitoneally with 100 µl of a 1:2 dilution of rabbit sera in sterile PBS (preimmune or day 31 immune sera). One hour after administration of serum, mice were challenged with 1600 cfu virulent serotype 6B *S. pneumoniae*, strain SP317 (obtained from H.R. Masure). Mice were monitored for 14 days for survival.

30

Eighty percent of the mice immunized with rabbit immune serum raised against CbpA truncate NR1XR2 protein survived the challenge for 14 days (Figure 2). All mice immunized with preimmune rabbit serum were dead by day 7.

35

C. Passive Protection in Mice (Higher Challenge Dose)

C3H/HeJ mice (10 mice/group) were passively immunized intraperitoneally with 100 µl of a 1:2 dilution of rabbit sera in sterile PBS (preimmune or day 52 immune sera). One hour after administration of serum, mice were
5 challenged with 3450 cfu virulent serotype 6B *S. pneumoniae*, strain SP317. Mice were monitored for 10 days for survival.

One hundred percent of the mice immunized with
10 rabbit immune serum raised against CbpA truncate NR1XR2 protein survived the challenge for ten days (Figure 3). Ninety percent of the mice immunized with preimmune rabbit serum were dead at day 10.

15 D. Passive Protection in Mice (Against High Virulence)

C3H/HeJ mice (10 mice/group) were passively immunized intraperitoneally with 100 µl of a 1:2 dilution of rabbit sera in sterile PBS (preimmune or day 52 immune
20 sera). One hour after administration of serum, mice were challenged with 580 cfu virulent serotype 6B *S. pneumoniae*, strain SPSJ2 (provided by P. Flynn, St. Jude Children's Research Hospital, Memphis, TN). Mice were monitored for 10 days for survival.

25 Fifty percent of the mice immunized with rabbit immune serum raised against CbpA truncate NR1XR2 protein survived the challenge for 10 days (Figure 4). All of the mice immunized with preimmune rabbit serum were dead
30 at day 8.

These data demonstrate that antibodies specific for CbpA are protective against systemic pneumococcal infection. The data further indicate that the choline-
35 binding region is not necessary for protection, as antibody specific for truncated protein NR1XR2, lacking the choline-binding repeats, was sufficient for protection. In addition, serum directed against

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recombinant CbpA protein based on a serotype 4 sequence, was still protective against challenge with two different strains of serotype 6B.

5

Example 4**Active Protection with Anti-CbpA Truncates NR1X and NR1XR2**

10 A. Active Protection With NR1X Truncate Vaccination
C3H/HeJ mice (10/group) were immunized
intraperitoneally with CbpA truncate protein NR1X (15µg
in 50 µl PBS, plus 50 µl Complete Freund's Adjuvant). A
group of 10 sham immunized mice received PBS and
adjuvant. A second immunization was administered four
15 weeks later, 15 µg protein i.p. with Incomplete Freund's
Adjuvant (sham group received PBS plus IFA). Blood was
drawn (retro-orbital bleed) at weeks 3, 6, and 9 for
analysis of immune response. The ELISA end point anti-
CbpA truncate titer of pooled sera from the 10 CbpA
20 immunized mice at 9 weeks was 4,096,000. No antibody was
detected in sera from sham immunized mice. Mice were
challenged at week 10 with 560 CFU serotype 6B *S.*
pneumoniae strain SPSJ2. Mice were monitored for 14 days
for survival.

25

Eighty percent of the mice immunized with CbpA
truncate protein NR1X survived the challenge for 14 Days
(results shown in Figure 5). All sham immunized mice
were dead by day 8.

30

**B. Active Protection With NR1XR2 Truncate
Vaccination**

C3H/HeJ mice (10/group) were immunized
intraperitoneally with CbpA truncate protein NR1XR2 (15µg
35 in 50 µl PBS, plus 50 µl Complete Freund's Adjuvant). A
group of 10 control immunized mice received pneumococcal
recombinant protein SP90 and adjuvant. A second

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immunization was administered four weeks later, 15µg protein i.p. with Incomplete Freund's Adjuvant. Blood was drawn (retro-orbital bleed) at weeks 3, 6, and 9 for analysis of immune response. The ELISA end point anti-
5 CbpA truncate titer of pooled sera from the 10 CbpA immunized mice at 9 weeks was 4,096,000. Mice were challenged at week 10 with 680 CFU serotype 6B *S. pneumoniae* strain SPSJ2. Mice were monitored for 14 days for survival.

10

Fifty percent of the mice immunized with CbpA truncate protein NR1XR2 survived the challenge for 14 days (results shown in Figure 6). All control immunized mice were dead by day 9.

15

These data demonstrate that immunization with recombinant CbpA truncate proteins elicit production of specific antibodies capable of protecting against systemic pneumococcal infection and death. The data
20 further indicate that the choline-binding region is not necessary for protection, as the immunogens were truncated proteins NR1X and NR1XR2. Additionally, the results suggest that a single amino terminal repeat may be sufficient to elicit a protective response. Cross
25 protection is demonstrated as the recombinant pneumococcal protein was generated based on serotype 4 DNA sequence and protection was observed following challenge with a serotype 6B isolate.

30

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as
35 particularly described.

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Koenig, Scott
Johnson, Leslie S

<120> Derivatives of Choline Binding Proteins for Vaccines

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<151> 1998-05-15

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35 40 45

Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser Arg Asn
50 55 60

Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser Lys Lys Ala
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 35 40 45
 Asp Glu Ser Asp Ser Glu Asp Tyr Leu Lys Glu Gly Leu Arg Ala Pro
 50 55 60
 Leu Gln Ser Lys Leu Asp Thr Lys Lys Ala Lys Leu Ser Lys Leu Glu
 65 70 75 80
 Glu Leu Ser Asp Lys Ile Asp Glu Leu Asp Ala Glu Ile Ala Lys Leu
 85 90 95
 Glu Val Gln Leu Lys Asp Ala Glu Gly Asn Asn Asn Val Glu Ala Tyr
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 35 40 45
 Leu Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu Arg
 50 55 60
 Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Lys Glu Glu Leu Thr Ser
 65 70 75 80
 Lys Thr Lys Lys Glu Ile Asp Ala Ala Phe Glu Gln Phe Asn Lys Asp
 85 90 95
 Thr Leu Lys Pro Gly Glu Lys Val Glu Glu Ala Glu Lys Lys Val Glu
 100 105 110
 Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp His Arg Asn
 115 120 125

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

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 210 215 220
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 260 265 270
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Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln					
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Pro Gln Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro Ala Pro Lys Asp					
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 Lys Pro Lys Asn Lys Arg Arg Val Thr Ala Ala Phe Glu Gln Phe Lys
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Glu	Arg	Asp	Lys	Ala	Arg	Lys	Glu	Val	Glu	Glu	Tyr	Val	Lys	Lys	Ile
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Val	Gly	Glu	Ser	Tyr	Ala	Lys	Ser	Thr	Lys	Lys	Arg	His	Thr	Ile	Thr
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Val	Ala	Leu	Val	Asn	Glu	Leu	Asn	Asn	Ile	Lys	Asn	Glu	Tyr	Leu	Asn
	65			70						75					80
Lys	Ile	Val	Glu	Ser	Thr	Ser	Glu	Ser	Gln	Leu	Gln	Ile	Leu	Met	Met
				85					90					95	
Glu	Ser	Arg	Ser	Lys	Val	Asp	Glu	Ala	Val	Ser	Lys	Phe	Glu	Lys	Asp
			100					105					110		
Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Asp	Ser	Ser	Thr	Lys	Pro	Glu	Ala
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Asp	Thr	Ala	Lys	Pro	Asn	Lys	Pro	Thr	Glu	Pro	Gly	Glu	Lys	Val	Ala
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Lys	Glu	Glu	Asp	Arg	Arg	Asn	Tyr	Pro	Thr	Ile	Thr	Tyr	Lys	Thr	Leu
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Glu	Leu	Glu	Ile	Ala	Glu	Ser	Asp	Val	Glu	Val	Lys	Lys	Ala	Glu	Leu
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	260	265 270
Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro		
	275	280 285
Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu		
	290	295 300
Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn		
305	310	315 320
Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser		
	325	330 335
Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala		
	340	345 350
Lys Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val		
	355	360 365
Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp		
	370	375 380
Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp		
385	390	395 400
Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro		
	405	410 415
Lys Ala Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu		
	420	425 430
Gln Pro Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu		
	435	440 445

<210> 10

<211> 414

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of *Streptococcus pneumoniae*

<400> 10

Thr	Glu	Asn	Glu	Gly	Ser	Thr	Gln	Ala	Ala	Thr	Ser	Ser	Asn	Met	Ala
1				5				10					15		

Lys	Thr	Glu	His	Arg	Lys	Ala	Ala	Lys	Gln	Val	Val	Asp	Glu	Tyr	Ile
		20					25					30			

Glu	Lys	Met	Leu	Arg	Glu	Ile	Gln	Leu	Asp	Arg	Arg	Lys	His	Thr	Gln
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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35					40					45					
Asn	Val	Ala	Leu	Asn	Ile	Lys	Leu	Ser	Ala	Ile	Lys	Thr	Lys	Tyr	Leu
50						55					60				
Arg	Glu	Leu	Asn	Val	Leu	Glu	Glu	Lys	Ser	Lys	Asp	Glu	Leu	Pro	Ser
65					70					75					80
Glu	Ile	Lys	Ala	Lys	Leu	Asp	Ala	Ala	Phe	Glu	Lys	Phe	Lys	Lys	Asp
				85					90					95	
Thr	Leu	Lys	Pro	Gly	Glu	Lys	Val	Ala	Glu	Ala	Lys	Lys	Lys	Val	Glu
			100					105					110		
Glu	Ala	Lys	Lys	Lys	Ala	Glu	Asp	Gln	Lys	Glu	Glu	Asp	Arg	Arg	Asn
		115					120					125			
Tyr	Pro	Thr	Asn	Thr	Tyr	Lys	Thr	Leu	Glu	Leu	Glu	Ile	Ala	Glu	Phe
	130					135					140				
Asp	Val	Lys	Val	Lys	Glu	Ala	Glu	Leu	Glu	Leu	Val	Lys	Glu	Glu	Ala
145					150					155					160
Lys	Glu	Ser	Arg	Asn	Glu	Gly	Thr	Ile	Lys	Gln	Ala	Lys	Glu	Lys	Val
				165					170					175	
Glu	Ser	Lys	Lys	Ala	Glu	Ala	Thr	Arg	Leu	Glu	Asn	Ile	Lys	Thr	Asp
			180					185					190		
Arg	Lys	Lys	Ala	Glu	Glu	Glu	Ala	Lys	Arg	Lys	Ala	Asp	Ala	Lys	Leu
		195					200					205			
Lys	Glu	Ala	Asn	Val	Ala	Thr	Ser	Asp	Gln	Gly	Lys	Pro	Lys	Gly	Arg
	210					215					220				
Ala	Lys	Arg	Gly	Val	Pro	Gly	Glu	Leu	Ala	Thr	Pro	Asp	Lys	Lys	Glu
225				230						235					240
Asn	Asp	Ala	Lys	Ser	Ser	Asp	Ser	Ser	Val	Gly	Glu	Glu	Thr	Leu	Pro
				245					250					255	
Ser	Ser	Ser	Leu	Lys	Ser	Gly	Lys	Lys	Val	Ala	Glu	Ala	Glu	Lys	Lys
			260					265					270		
Val	Glu	Glu	Ala	Glu	Lys	Lys	Ala	Lys	Asp	Gln	Lys	Glu	Glu	Asp	Arg
	275						280					285			
Arg	Asn	Tyr	Pro	Thr	Asn	Thr	Tyr	Lys	Thr	Leu	Asp	Leu	Glu	Ile	Ala
	290					295					300				
Glu	Ser	Asp	Val	Lys	Val	Lys	Glu	Ala	Glu	Leu	Glu	Leu	Val	Lys	Glu
305					310					315					320
Glu	Ala	Lys	Glu	Pro	Arg	Asp	Glu	Glu	Lys	Ile	Lys	Gln	Ala	Lys	Ala
				325					330					335	
Lys	Val	Glu	Ser	Lys	Lys	Ala	Glu	Ala	Thr	Arg	Leu	Glu	Asn	Ile	Lys
			340					345					350		
Thr	Asp	Arg	Asp	Asp	Ala	Glu	Glu	Glu	Ala	Lys	Arg	Lys	Ala	Ala	Glu
	355						360					365			

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Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro
 370 375 380

Ala Thr Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu
 385 390 395 400

Gln Pro Lys Ala Glu Lys Thr Asp Asp Gln Gln Ala Glu Glu
 405 410

<210> 11

<211> 425

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of
 Streptococcus pneumoniae

<400> 11

Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala
 1 5 10 15

Asn Glu Ser Gln Ala Gly His Arg Lys Ala Ala Glu Gln Phe Asp Glu
 20 25 30

Tyr Ile Lys Thr Met Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
 35 40 45

Phe Ala Leu Asn Ile Lys Leu Ser Arg Ile Lys Thr Glu Tyr Leu Arg
 50 55 60

Lys Leu Asn Val Leu Glu Glu Lys Ser Lys Ala Glu Leu Pro Ser Glu
 65 70 75 80

Thr Lys Lys Glu Ile Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
 85 90 95

Asn Arg Thr Lys Lys Thr Val Ala Glu Ala Glu Lys Lys Val Glu Glu
 100 105 110

Ala Lys Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp His Arg Asn Tyr
 115 120 125

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
 130 135 140

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
 145 150 155 160

Glu Ser Arg Asp Asp Glu Lys Ile Lys Gln Ala Glu Ala Lys Val Glu
 165 170 175

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
 180 185 190

Glu Lys Ala Glu Glu Glu Ala Lys Arg Arg Ala Glu Ala Lys Leu Lys
 195 200 205

Glu Ala Val Glu Lys Asn Val Ala Thr Ser Glu Gln Asp Lys Pro Lys
 210 215 220

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Gly Arg Arg Lys Arg Gly Val Pro Gly Glu Gln Ala Thr Pro Asp Lys
 225 230 235 240
 Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Ala
 245 250 255
 Leu Pro Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu
 260 265 270
 Lys Lys Val Ala Glu Ala Glu Lys Lys Ala Lys Ala Gln Lys Glu Glu
 275 280 285
 Asp Arg Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu
 290 295 300
 Ile Ala Glu Ser Asp Val Lys Val Lys Glu Ser Glu Leu Glu Leu Val
 305 310 315 320
 Lys Glu Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Val Asn Gln Ala
 325 330 335
 Lys Ala Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys
 340 345 350
 Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala
 355 360 365
 Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro
 370 375 380
 Ala Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro
 385 390 395 400
 Ala Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys Ala Glu
 405 410 415
 Lys Thr Asp Asp Gln Gln Ala Glu Glu
 420 425

<210> 12

<211> 426

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of
Streptococcus pneumoniae

<400> 12

Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Lys Ala
 1 5 10 15
 Asn Lys Ser Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
 20 25 30
 Tyr Ile Lys Lys Lys Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
 35 40 45
 Val Gly Leu Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His
 50 55 60

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

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Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala
 405 410 415

Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
 420 425

<210> 13

<211> 425

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of
Streptococcus pneumoniae

<400> 13

Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Lys Ala
 1 5 10 15

Asn Lys Ser Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
 20 25 30

Tyr Ile Lys Lys Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
 35 40 45

Val Gly Leu Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His
 50 55 60

Gly Leu Ser Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu
 65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
 85 90 95

Leu Pro Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val
 100 105 110

Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg
 115 120 125

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu
 130 135 140

Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu
 145 150 155 160

Ala Lys Glu Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys
 165 170 175

Val Glu Asn Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr
 180 185 190

Asp Arg Glu Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu
 195 200 205

Gln Glu Ala Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg
 210 215 220

Ala Lys Arg Glu Val Phe Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
 225 230 235 240

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Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr
 245 250 255
 Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
 260 265 270
 Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg
 275 280 285
 Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
 290 295 300
 Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu
 305 310 315 320
 Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala
 325 330 335
 Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
 340 345 350
 Thr Asp Arg Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala
 355 360 365
 Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala
 370 375 380
 Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala
 385 390 395 400
 Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala
 405 410 415
 Glu Lys Pro Ala Asp Gln Gln Ala Glu
 420 425

<210> 14

<211> 424

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of
Streptococcus pneumoniae

<400> 14

Thr Glu Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala
 1 5 10 15
 Asn Lys Ser Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
 20 25 30
 Tyr Ile Lys Lys Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
 35 40 45
 Val Gly Leu Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His
 50 55 60
 Gly Leu Ser Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu
 65 70 75 80

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Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr
 85 90 95
 Leu Pro Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val
 100 105 110
 Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg
 115 120 125
 Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu
 130 135 140
 Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu
 145 150 155 160
 Ala Lys Glu Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys
 165 170 175
 Val Glu Asn Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr
 180 185 190
 Asp Arg Glu Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu
 195 200 205
 Gln Glu Ala Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg
 210 215 220
 Ala Lys Arg Glu Val Leu Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
 225 230 235 240
 Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr
 245 250 255
 Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
 260 265 270
 Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg
 275 280 285
 Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
 290 295 300
 Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu
 305 310 315 320
 Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala
 325 330 335
 Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
 340 345 350
 Thr Asp Arg Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala
 355 360 365
 Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala
 370 375 380
 Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala
 385 390 395 400
 Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala

405410415

Glu Lys Pro Ala Asp Gln Gln Ala
420

<210> 15
<211> 419
<212> PRT
<213> Artificial Sequence

<220>
<223> Description: Amino acid sequence derived from a cDNA from
Streptococcus pneumoniae

<400> 15

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

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245								250				255			
Leu	Lys	Pro	Gly	Lys	Lys	Val	Ala	Glu	Ala	Glu	Lys	Lys	Val	Glu	Glu
			260					265					270		
Ala	Asp	Lys	Lys	Ala	Lys	Ala	Gln	Lys	Glu	Glu	Asp	Arg	Arg	Asn	Tyr
		275					280					285			
Pro	Thr	Asn	Thr	Tyr	Lys	Thr	Leu	Glu	Leu	Glu	Ile	Ala	Glu	Ser	Asp
	290					295					300				
Val	Lys	Val	Lys	Glu	Ala	Glu	Leu	Glu	Leu	Val	Lys	Glu	Glu	Ala	Lys
305					310					315					320
Glu	Ser	Arg	Asn	Glu	Glu	Lys	Ile	Lys	Gln	Ala	Lys	Ala	Lys	Val	Glu
			325						330					335	
Ser	Lys	Lys	Ala	Glu	Ala	Thr	Arg	Leu	Glu	Lys	Ile	Lys	Thr	Asp	Arg
			340					345					350		
Lys	Lys	Ala	Glu	Glu	Glu	Ala	Lys	Arg	Lys	Ala	Ala	Glu	Glu	Asp	Lys
		355					360					365			
Val	Lys	Glu	Lys	Pro	Ala	Glu	Gln	Pro	Gln	Pro	Ala	Pro	Ala	Pro	Gln
	370					375					380				
Pro	Glu	Lys	Pro	Ala	Glu	Glu	Pro	Glu	Asn	Pro	Val	Pro	Ala	Pro	Lys
385					390					395					400
Pro	Glu	Asn	Pro	Ala	Glu	Gln	Pro	Lys	Ala	Glu	Lys	Pro	Ala	Asp	Gln
			405						410					415	

Gln Ala Glu

<210> 16

<211> 414

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of *Streptococcus pneumoniae*

<400> 16

Thr	Glu	Asn	Glu	Gly	Ser	Thr	Gln	Ala	Ala	Thr	Ser	Ser	Asn	Met	Ala
1				5				10						15	

Lys	Thr	Glu	His	Arg	Lys	Ala	Ala	Lys	Gln	Val	Val	Asp	Glu	Tyr	Ile
		20					25						30		

Glu	Lys	Met	Leu	Arg	Glu	Ile	Gln	Leu	Asp	Arg	Arg	Lys	His	Thr	Gln
		35					40					45			

Asn	Val	Ala	Leu	Asn	Ile	Lys	Leu	Ser	Ala	Ile	Lys	Thr	Lys	Tyr	Leu
	50					55					60				

Arg	Glu	Leu	Asn	Val	Leu	Glu	Glu	Lys	Ser	Lys	Asp	Glu	Leu	Pro	Ser
65					70					75					80

Glu	Ile	Lys	Ala	Lys	Leu	Asp	Ala	Ala	Phe	Glu	Lys	Glu	Lys	Lys	Asp
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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85					90					95					
Thr	Leu	Lys	Pro	Gly	Glu	Lys	Val	Ala	Glu	Ala	Lys	Lys	Lys	Val	Glu
			100					105					110		
Glu	Ala	Lys	Lys	Lys	Ala	Glu	Asp	Gln	Lys	Glu	Glu	Asp	Arg	Arg	Asn
		115					120					125			
Tyr	Pro	Thr	Asn	Thr	Tyr	Lys	Thr	Leu	Glu	Leu	Glu	Ile	Ala	Glu	Phe
	130					135					140				
Asp	Val	Lys	Val	Lys	Glu	Ala	Glu	Leu	Glu	Leu	Val	Lys	Glu	Glu	Ala
145						150					155				160
Lys	Glu	Ser	Arg	Asn	Glu	Gly	Thr	Ile	Lys	Gln	Ala	Lys	Glu	Lys	Val
				165					170					175	
Glu	Ser	Lys	Lys	Ala	Glu	Ala	Thr	Arg	Leu	Glu	Asn	Ile	Lys	Thr	Asp
			180					185					190		
Arg	Lys	Lys	Ala	Glu	Glu	Glu	Ala	Lys	Arg	Lys	Ala	Asp	Ala	Lys	Leu
		195					200					205			
Lys	Glu	Ala	Asn	Val	Ala	Thr	Ser	Asp	Gln	Gly	Lys	Pro	Lys	Gly	Arg
	210					215					220				
Ala	Lys	Arg	Gly	Val	Pro	Gly	Glu	Leu	Ala	Thr	Pro	Asp	Lys	Lys	Glu
225						230					235				240
Asn	Asp	Ala	Lys	Ser	Ser	Asp	Ser	Ser	Val	Gly	Glu	Glu	Thr	Leu	Pro
				245					250					255	
Ser	Ser	Ser	Leu	Lys	Ser	Gly	Lys	Lys	Val	Ala	Glu	Ala	Glu	Lys	Lys
			260					265					270		
Val	Glu	Glu	Ala	Glu	Lys	Lys	Ala	Lys	Asp	Gln	Lys	Glu	Glu	Asp	Arg
		275					280					285			
Arg	Asn	Tyr	Pro	Thr	Asn	Thr	Tyr	Lys	Thr	Leu	Asp	Leu	Glu	Ile	Ala
	290					295					300				
Glu	Ser	Asp	Val	Lys	Val	Lys	Glu	Ala	Glu	Leu	Glu	Leu	Val	Lys	Glu
305						310					315				320
Glu	Ala	Lys	Glu	Pro	Arg	Asp	Glu	Glu	Lys	Ile	Lys	Gln	Ala	Lys	Ala
				325					330					335	
Lys	Val	Glu	Ser	Lys	Lys	Ala	Glu	Ala	Thr	Arg	Leu	Glu	Asn	Ile	Lys
			340					345					350		
Thr	Asp	Arg	Lys	Lys	Ala	Glu	Glu	Glu	Ala	Lys	Arg	Lys	Ala	Ala	Glu
		355					360					365			
Glu	Asp	Lys	Val	Lys	Glu	Lys	Pro	Ala	Glu	Gln	Pro	Gln	Pro	Ala	Pro
	370					375					380				
Ala	Thr	Gln	Pro	Glu	Lys	Pro	Ala	Pro	Lys	Pro	Glu	Lys	Pro	Ala	Glu
385						390					395				400
Gln	Pro	Lys	Ala	Glu	Lys	Thr	Asp	Asp	Gln	Gln	Ala	Glu	Glu		
				405					410						

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Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp His Arg Asn Tyr Pro
 275 280 285
 Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val
 290 295 300
 Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Gly
 305 310 315 320
 Ser Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser
 325 330 335
 Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys
 340 345 350
 Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val
 355 360 365
 Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Gln Pro
 370 375 380
 Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro
 385 390 395 400
 Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
 405 410

<210> 18

<211> 406

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of
Streptococcus pneumoniae

<400> 18

Thr Glu Asn Glu Gly Thr Thr Gln Ala Pro Thr Ser Ser Asn Arg Gly
 1 5 10 15
 Asn Glu Ser Gln Ala Glu His Met Lys Ala Ala Lys Gln Val Asp Glu
 20 25 30
 Tyr Ile Glu Lys Met Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
 35 40 45
 Val Gly Leu Leu Thr Lys Leu Gly Ala Ile Lys Thr Glu Tyr Leu Arg
 50 55 60
 Gly Leu Ser Val Ser Lys Glu Lys Ser Thr Ala Glu Leu Pro Ser Glu
 65 70 75 80
 Ile Lys Glu Lys Leu Thr Ala Ala Phe Lys Gln Phe Lys Lys Asp Thr
 85 90 95
 Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Ala Glu
 100 105 110
 Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
 115 120 125

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Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
 130                               135                       140

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Val Lys Ala Asn
145                               150                       155                       160

Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Glu Ala Glu Val Glu
                               165                       170                       175

Ser Lys Lys Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg
                               180                       185                       190

Glu Lys Ala Glu Glu Glu Ala Lys Arg Arg Val Asp Ala Lys Glu Gln
                               195                       200                       205

Asp Glu Ser Ser Lys Arg Arg Lys Ser Arg Val Lys Arg Gly Asp Leu
210                               215                       220

Gly Glu Gln Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser
225                               230                       235                       240

Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys Pro
                               245                       250                       255

Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Asp Lys
                               260                       265                       270

Lys Ala Lys Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn
                               275                       280                       285

Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val
290                               295                       300

Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Pro Arg
305                               310                       315                       320

Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser Lys Lys
                               325                       330                       335

Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala
                               340                       345                       350

Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu
355                               360                       365

Lys Pro Ala Glu Gln Pro Lys Pro Ala Pro Ala Pro Gln Pro Glu Lys
370                               375                       380

Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro Lys Ala Glu Lys
385                               390                       395                       400

Pro Ala Asp Gln Gln Ala
                               405

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<210> 19

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from a cDNA from the genome of

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Streptococcus pneumoniae

<400> 19

Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys Lys Lys
 1 5 10 15

Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr
 20 25 30

Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys
 35 40 45

Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser Arg Asn
 50 55 60

Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser Lys Lys Ala
 65 70 75 80

Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala Glu
 85 90 95

Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys
 100 105 110

Pro Ala

<210> 20

<211> 1295

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA derived from the genome *S. pneumoniae*

<400> 20

acagagaagg	aggtaactac	cccagtagcc	acttcttcta	ataaggcaaa	taaaagtcag	60
acagaacata	tgaaagctgc	tgaacaagtc	gatgaatata	taaacaaaat	gatccaatta	120
gataaaagaa	aacataccca	aatctcgc	ttaaacataa	agttgagcgc	aattaaaacg	180
aagtatttgc	gtgaattaaa	tgttttagaa	gagaagtcga	aaaaagaaga	gttgacgtca	240
aaaacaaaaa	aagagataga	cgcagctttt	gagcagttta	acaaagatac	attgaaacca	300
ggagaaaagg	ttgaagaagc	tgagaagaag	gttgaagaag	ctgagaaaaa	agccaaggat	360
caaaaagaag	aagatcaccg	taactaccca	accattactt	acaaaacgct	tgaacttgaa	420
attgctgagt	ccgatgtgga	agttaaaaaa	gcggagcttg	aactagtaaa	agaggaagct	480
aagggatctc	gaaacgagga	aaaaattaag	aaagcaaaag	cggaagttga	gagtaaaaaa	540
gctgaggcta	caaagttaga	agaaatcaag	acagaacgta	aaaaagcaga	agaagaagct	600
aaacgaaaag	cagaagcaga	agaagaagtt	aaaaataaac	taaagaagcg	gacaaaacga	660
ggagcttttg	gagagccagc	aacacctgat	aaaaaagaaa	atgatgcgaa	gtcttcagat	720
tctagcgtgg	tgaagaaatc	ttccaagccc	atcctgaaat	cagaaaaaaa	agtagcagaa	780
gctgagaaga	aggttgcaga	agctgagaag	aaggttgcag	aagctgagaa	aaaagccaag	840
gatcaaaaag	aagaagatcg	ccgtaactac	ccaaccaata	cttacaaaac	gcttgaactt	900
gaaattgctg	agtccgatgt	gaaagttaaa	gaagcggagc	ttgaactagt	aaaagaggaa	960
gctaaggaac	ctcaaaacga	ggaaaaaatt	aagcaagcaa	aagcgaaagt	tgagagtaaa	1020
aaagctgagg	ctacaagggt	agaaaaaatc	aagacagatc	gtaaaaaagc	agaagaagct	1080
aaacgaaaag	tagcagaaga	agataaagtt	aaagaaaaac	cagctgaaca	accacaacca	1140
gctcctgcac	caaaaccagc	gccggctcct	caaccagaaa	aaccagctga	acaaccaaaa	1200
gcagaaaaac	cagctgatca	acaagctgaa	gaagactatg	ctcgtagatc	agaagaagaa	1260
tataaccgcg	ttgacttaac	agcaccggca	aaagc			1295

<210> 21

<211> 755

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<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA from *Streptococcus pneumoniae*

<400> 21

acagagaacg	agggaagtac	ccaagcagcc	acttcttcta	atatggcaaa	gacagaacat	60
aggaaagctg	ctaaacaagt	cgtcgatgaa	tatatagaaa	aaatgttgag	ggagattcaa	120
ctagatagaa	gaaaacatac	ccaaaatgtc	gccttaaaca	taaagttgag	cgcaattaaa	180
acgaagtatt	tgcgtgaatt	aaatgtttta	gaagagaagt	cgaaagatga	gttgccgtca	240
gaaataaaag	caaagttaga	cgcagctttt	gagaagttta	aaaaagatac	attgaaacca	300
ggagaaaagg	tagcagaagc	taagaagaag	gttgaagaag	ctaagaaaaa	agccgaggat	360
caaaaagaag	aagatcgctg	taactaccca	accaatactt	acaaaacgct	tgaacttgaa	420
attgctgagt	tcgatgtgaa	agttaaagaa	gcggagcttg	aactagtaaa	agaggaagct	480
aaagaatctc	gaaacgaggg	cacaattaag	caagcaaaag	agaaagttga	gagtaaaaaa	540
gctgaggcta	caaggttaga	aaacatcaag	acagatcgta	aaaaagcaga	agaagaagct	600
aaacgaaaag	cagcagaaga	agataaagtt	aaagaaaaac	cagctgaaca	accacaacca	660
gcgccggcta	ctcaaccaga	aaaaccagct	ccaaaaccag	agaagccagc	tgaacaacca	720
aaagcagaaa	aaacagatga	tcaacaagct	gaaga			755

<210> 22

<211> 1239

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA from *Streptococcus pneumoniae*

<400> 22

acagagaagg	aggtaactac	ccaagtaccc	acttattcta	atatggcaaa	gacagaacat	60
aggaaagctg	ctaaacaagt	cgtcgatgaa	tatatagaaa	aaatgttgag	ggagattcaa	120
ttagatagaa	gaaaacatac	ccaaaatttc	gccttcaaca	tgaagttgag	cgcaattaaa	180
acggagtatt	tgtatggatt	aaaagagaag	tcggaagctg	agttgccgtc	agaagtaaaa	240
gcaaagttag	acgcagcttt	tgagcagttt	aaaaaagata	cattgaaact	aggagaaaag	300
gtagcagaag	ctgagaagaa	ggttgcagaa	gctgagaaaa	aagccaaggc	tcaaaaagaa	360
gaagatcgcc	gtaactaccc	aaccaatact	tacaaaacgc	ttgaacttga	aattgctgag	420
tccgatgtgg	aagttaaaaa	agcggagctt	gaactattga	aagaggaagc	taaaactcga	480
aacgaggaca	caattaacca	agcaaaagcg	aaagttgaga	gtaaaaaagc	tgaggctaca	540
aagttagaag	aatcaagac	agatcgtaaa	aaagcagaag	aagaagctaa	acgaaaagca	600
gaagcagaag	aagataaagt	taaagataaa	ctaaagaggc	ggacaaaacg	agcagttcct	660
ggagagccag	caacacctga	taaaaaagaa	aatgatgcga	agtcttcaga	ttctagcgta	720
ggtgaagaaa	ctcttccaag	cccatccctg	aaatcaggaa	aaaaggtagc	agaagctgag	780
aagaagggtt	cagaagctga	gaaaaaagcc	aaggatcaaa	aagaagaaga	tcgccgtaac	840
taccaacca	atacttacia	aacgcttgac	cttgaaattg	ctgagtcgga	tgtgaaagtt	900
aaagaagcgg	agcttgaact	agtaaaagag	gaagctaagg	gatctcgaaa	cgaggaaaaa	960
attaaccaag	caaaagcgga	agttgagagt	aaaaaagctg	aggctacaag	gctagaaaaa	1020
atcaagacag	atcgtaaaaa	agcagaagaa	gaagctaaac	gaaaagcagc	agaagaagat	1080
aaagttaaag	aaaaaccagc	tgaacaacca	caaccagcgc	cggctcctca	accagaaaaa	1140
ccaactgaag	agcctgagaa	tccagctcca	gctccaaaac	cagagaagcc	agctgaacaa	1200
ccaaaagcag	aaaaaacaga	tgatcaacaa	gctgaagaa			1239

<210> 23

<211> 1338

<212> DNA

<213> Artificial Sequence

<220>

<213> Description: cDNA from *Streptococcus pneumoniae*

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<400> 23

acagagaacg	agggagctac	ccaagtaccc	acttcttcta	atagggcaaa	tgaaagtcag	60
gcagaacaag	gagaacaacc	taaaaaactc	gattcagaac	gagataaggc	aaggaaagag	120
gtcgaggaat	atgtaaaaaa	aatagtgggt	gagagctatg	caaaatcaac	taaaaagcga	180
catacaatta	ctgtagctct	agttaacgag	ttgaacaaca	ttaagaacga	gtatttgaat	240
aaaatagttg	aatcaacctc	agaaagccaa	ctacagatac	tgatgatgga	gagtcgatca	300
aaagtagatg	aagctgtgtc	taagtttgaa	aaggactcat	cttcttcgtc	aagttcagac	360
tcttccacta	aaccggaagc	ttcagataca	gcgaagccaa	acaagccgac	agaaccagga	420
gaaaaggtag	cagaagctaa	gaagaagggt	gaagaagttg	agaaaaaagc	caaggatcaa	480
aaagaagaag	atcgctgtaa	ctaccaaac	aattacttac	aaacgcttga	acttgaaatt	540
gctgagtcg	atgtggaagt	taaaaaagcg	gagcttgaac	tagtaaaagt	gaaagctaac	600
gaacctcgag	acaagcaaaa	aattaagcaa	gcagaagcgg	aagttgagag	taaacaagct	660
gaggctacaa	ggttaaaaaa	aatcaagaca	gatcgtgaag	aagcagaaga	agaagctaaa	720
cgaagagcag	atgctaaaga	gcaaggtaaa	ccaaaggggc	ggccaaaacg	aggagttcct	780
ggagagctag	caacacctga	taaaaaagaa	aatgatgcga	agtcttcaga	ttctagcgta	840
ggtgaagaaa	ctcttccaag	cccatccctg	aaaccagaaa	aaaaggtagc	agaagctgag	900
aagaagggtg	aagaagctaa	gaaaaaagcc	gaggatcaaa	agaagaaga	tcgccgtaac	960
taccaacca	atacttacia	aacgcttgaa	cttgaaattg	ctgagtcgga	tgtggaagtt	1020
aaaaaagcgg	agcttgaact	agtaaaagag	gaagctaagg	aacctcgaaa	cgaggaaaaa	1080
gttaagcaag	caaaagcgga	agttgagagt	aaaaaagctg	aggctacaag	gttagaaaaa	1140
atcaagacag	atcgtaaaaa	agcagaagaa	gaagctaaac	gaaaagcagc	agaagaagat	1200
aaagttaaag	aaaaaccagc	tgaacaacca	caaccagcgc	cggctccaaa	aacagaaaaa	1260
ccagctccag	ctccaaaacc	agagaatcca	gctgaacaac	caaaagcaga	aaaaccagct	1320
gatcaacaag	ctgaagaa					1338

<210> 24

<211> 1284

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA prepared from Streptococcus pneumoniae genome

<400> 24

gaaggggtta	gaagtgggaa	taactccacg	gttacatcta	gtgggcaaga	tatatcgaag	60
aagtatgctg	atgaagtcga	gtcgcaccta	caaagtatat	tgaaggatgt	caataaaaat	120
ttgaagaaag	ttcaacatac	ccaaaatgcc	gacttcaaca	aaaagttgag	caaaatttaa	180
acgaagtatt	tgtatgaatt	aaatgtttta	gaagagaagt	cggaagctga	gttgacgtca	240
aaaacaaaag	aaacaaaaga	agagttaacc	gcagcttttg	agcagtttaa	aaaagataca	300
ttatcaacag	aaccagaaaa	aaaggtagca	gaagctaaga	agaagggttg	agaagctaag	360
aaaaaagccg	aggatcaaaa	agaaaaagat	cgccgtaact	acccaaccat	tacttacaaa	420
acgcttgaac	ttgaaattgc	tgagtccgat	gtggaagtta	aaaaagcgga	gcttgaacta	480
gtaaaagtga	aagctaacga	acctcgagac	gaggaaaaaa	ttaagcaagc	agaagcgaaa	540
gttgagagta	aacaagctga	ggctacaagg	ttaaaaaaa	tcaagacaga	tcgtgaacaa	600
gctgaggcta	caagggttag	aaacatcaag	acagatcgtg	aacaagcaga	agaagaagct	660
aaagttaaag	atgaaccaa	gaagcggaca	aaacgaggag	ttcttgagga	gccagcaaca	720
cctgataaaa	aagaaaatga	tgcgaaagtct	tcagattcta	gcgtaggtga	agaaactctt	780
ccaagcccat	ccctgaaacc	agaaaaaaag	gttgacagaag	ctgagaagaa	ggttgaagaa	840
gctaagaaaa	aagccgagga	tcaaaaagaa	gaagatcgct	gtaactacc	aaccaatact	900
tacaaaacgc	ttgaacttga	aattgctgag	tccgatgtgg	aagttaaaaa	agcggagctt	960
gaactagtaa	aagaggaagc	taaggaacct	cgaaacgagg	aaaaagttta	gcaagcaaaa	1020
gcggaagttg	agagtaaaca	agctgaggct	acaagggttag	aaaacatcaa	gacagatcgt	1080
aaaaaagcag	aagaagaagc	taaacgaaaa	gcagcagaag	aagataaagt	taaagaaaaa	1140
ccagctgaac	aaccacaacc	agcgccggct	cctcaaccag	aaaaaccagc	tccaaaacca	1200
gaaaaaccag	ctccagctcc	aaaaccagag	aatccagctg	aacaaccaa	agcagaaaaa	1260
ccagctgatc	aacaagctga	agaa				1284

<210> 25

<211> 658

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<212> DNA

<213> Artificial Sequence

<220>

<213> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 25

gaaggggtta	gaagtgggaa	taactccacg	gttacatcta	gtgggcaaga	tatatcgaag	60
aagtatgctg	atgaagtcga	gtcgcaccta	caaagtatat	tgaaggatgt	caataaaaat	120
ttgaaaaaag	ttcaacatac	ccaaaatgcc	gacttcaaca	aaaagttgag	caaaattaaa	180
ccgaagtatt	tgtatgaatt	aaagtgttta	gaagagaagt	cggaagctga	gttgacgtca	240
aaaccaaaga	acaaaagaag	agttaccgca	gcttttgagc	agtttaaaaa	agatacatta	300
tcaacagaac	cagaaaaaaa	ggtagcagaa	gctaagaaga	aggttgaaga	agctaagaaa	360
aaagccgagg	atcaaaaaga	aaaagatcgc	cgtaactacc	caaccattac	ttacaaaacg	420
cttgaacttg	aaattgctga	gtccgatgtg	gaagttaaaa	aagcggagct	tgaactagta	480
aaagaggaag	ctaaggaacc	tcgaaacgag	gaaaaagtta	agcaagcaaa	agcgggaagtt	540
gagagtaaac	aagctgaggc	tacaagggtta	gaaaaaatca	agacagatcg	taaaaaagca	600
gaagaagaag	ctaaacgaaa	agcagcagaa	gaagataaag	ttaaagaaaa	accagctg	658

<210> 26

<211> 1338

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA derived from genome of Streptococcus pneumoniae

<400> 26

acagagaacg	agggagctac	ccaagtaccc	acttcttcta	atagggcaaa	tgaaagtacg	60
gcagaacaag	gagaacaacc	taaaaaactc	gattcagaac	gagataaggc	aaggaaagag	120
gtcgaggaat	atgtaaaaaa	aatagtgggt	gagagctatg	caaaatcaac	taaaaagcga	180
catacaatta	ctgtagctct	agttaacgag	ttgaacaaca	ttaagaacga	gtatttgaat	240
aaaatagttg	aatcaacctc	agaaagccaa	ctacagatac	tgatgatgga	gagtcgatca	300
aaagtagatg	aagctgtgtc	taagtttgaa	aaggactcat	cttcttcgtc	aagttcagac	360
tcttccacta	aaccggaagc	ttcagataca	gcgaagccaa	acaagccgac	agaaccagga	420
gaaaaggtag	cagaagctaa	gaagaagggt	gaagaagctg	agaaaaaagc	caaggatcaa	480
aaagaagaag	atcgtcgtaa	ctacccaacc	attacttaca	aaacgcttga	acttgaaatt	540
gctgagtcg	atgtggaagt	taaaaaagcg	gagcttgaac	tagtaaaagt	gaaagctaac	600
gaacctcgag	acgagcaaaa	aattaagcaa	gcagaagcgg	aagttgagag	taaacaagct	660
gaggctacaa	ggttaaaaaa	aatcaagaca	gatcgtgaag	aagcagaaga	agaagctaaa	720
cgaagagcag	atgctaaaga	gcaaggtaaa	ccaaaggggc	gggcaaaacg	aggagttcct	780
ggagagctag	caacacctga	taaaaaagaa	aatgatgcga	agtcttcaga	ttctagcgta	840
ggtgaagaaa	ctcttccaag	cccatccctg	aaaccagaaa	aaaaggtagc	agaagctgag	900
aagaagggtg	aagaagctaa	gaaaaaagcc	gaggatcaaa	aagaagaaga	tcgccgtaac	960
taccaacca	atacttacia	aacgcttgaa	cttgaaattg	ctgagtcgga	tgtggaagtt	1020
aaaaaagcgg	agcttgaact	agtaaaagag	gaagctaagg	aacctcgaaa	cgaggaaaaa	1080
gttaagcaag	caaaagcggg	agttgagagt	aaaaaagctg	aggctacaag	gttagaaaaa	1140
atcaagacag	atcgtaaaaa	agcagaagaa	gaagctaaac	gaaaagcagc	agaagaagat	1200
aaagttaaag	aaaaaccagc	tgaacaacca	caaccagcgc	cggctccaaa	agcagaaaaa	1260
ccagctccag	ctccaaaacc	agagaatcca	gctgaacaac	caaaagcaga	aaaaccagct	1320
gatcaacaag	ctgaagaa					1338

<210> 27

<211> 1242

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA derived from genome of Streptococcus pneumoniae

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<400> 27

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acagaaaacg aaggaagtac ccaagcagcc acttcttcta atatggcaaa gacagaacat    60
aggaaagctg ctaaacaagt cgtcgatgaa tatatagaaa aaatgttgag ggagattcaa   120
ctagatagaa gaaaacatac ccaaaatgtc gccttaaaca taaagttgag cgcaattaaa   180
acgaagtatt tgcgtgaatt aaatgtttta gaagagaagt cgaaagatga gttgccgtca   240
gaaataaaaag caaagttaga cgcagctttt gagaagttta aaaaagatac attgaaacca   300
ggagaaaagg tagcagaagc taagaagaag gttgaagaag ctaagaaaaa agccgaggat   360
caaaaagaag aagatcgtcg taactacca accaatactt acaaaacgct tgaacttgaa   420
attgctgagt tcgatgtgaa agttaaagaa gcggagcttg aactagtaaa agaggaagct   480
aaagaatctc gaaacgaggg cacaattaag caagcaaaag agaaagttga gagtaaaaaa   540
gctgaggcta caaggttaga aaacatcaag acagatcgta aaaaagcaga agaagaagct   600
aaacgaaaag cagatgctaa gttgaaggaa gctaattgtag cgacttcaga tcaaggtaaa   660
ccaaaggggc gggcaaaacg aggagttcct ggagagctag caacacctga taaaaaagaa   720
aatgatgcga agtcttcaga ttctagcgta ggtgaagaaa ctcttccaag ctcacccctg   780
aatcaggaa aaaaggtagc agaagctgag aagaaggttg aagaagctga gaaaaaagcc   840
aaggatcaaa aagaagaaga tcgccgtaac tacccaacca atacttaca aacgcttgac   900
cttgaaattg ctgagtccga tgtgaaagtt aaagaagcgg agcttgaact agtaaaagag   960
gaagctaagg aacctcgaga cgaggaaaaa attaagcaag caaaagcgaa agttgagagt  1020
aaaaaagctg aggctacaag gttagaaaac atcaagacag atcgtaaaaa agcagaagaa  1080
gaagctaaac gaaaagcagc agaagaagat aaagttaaag aaaaaccagc tgaacaacca  1140
caaccagcgc cggctactca accagaaaaa ccagctccaa aaccagagaa gccagctgaa  1200
caacccaaaag cagaaaaaac agatgatcaa caagctgaag aa                    1242

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<210> 28

<211> 1275

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA derived from genome of *Streptococcus pneumoniae*

<400> 28

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acagagaagg aggtaactac ccaagtagcc acttcttcta atagggcaaa tgaaagtcag    60
gcaggacata ggaaagctgc tgaacaattc gatgaatata taaaacaat gatccaatta   120
gatagaagaa aacataccca aaatttcgcc ttaaacataa agttgagcag aattaaaacg   180
gagtatttgc gtaaattaaa tgttttagaa gagaagtcga aagctgagtt gccgtcagaa   240
acaaaaaaag agatagacgc agcttttgag cagtttaaaa aagataccaa cagaacccaa   300
aaaacggtag cagaagctga gaagaaggtt gaagaagcta agaaaaaagc caaggctcaa   360
aaagaagaag atcaccgtaa ctaccaacc aatacttaca aaacgcttga acttgaaatt   420
gctgagtcgg atgtggaagt taaaaaagcg gagcttgaa tagtaaaaga ggaagctaag   480
gaatctcgag acgatgaaaa aattaagcaa gcagaagcga aagttgagag taaaaaagct   540
gaggctacaa ggttagaaaa catcaagaca gatcgtgaaa aagcagaaga agaagctaaa   600
cgaagagcag aagctaagtt gaaggaagct gttgaaaaga atgtagcgac ttcagagcaa   660
gataaaccaa aggggcggag aaaacgagga gttcctggag agcaagcaac acctgataaa   720
aaagaaaatg atgcgaagtc ttcagattct agcgtaggtg aagaagctct tccaagccca   780
tccttgaaac cagaaaaaaa ggttgagaaa gctgagaaga aggttgagaa agctgagaaa   840
aaagccaagg ctcaaaaaga agaagatcgc cgtaactacc caaccaatac ttacaaaacg   900
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aaagaggaag ctaaggaatc tcgaaacgag gaaaaagtta atcaagcaaa agcgaaagtt  1020
gagagtaaaa aagctgaggg tacaaggtta gaaaaaatca agacagatcg taaaaaagca  1080
gaagaagaag ctaaacgaaa agcagcagaa gaagataaag ttaagaaaaa accagctgaa  1140
caaccacaac cagcgccggc tcctcaacca gaaaaaccaa ctgaagagcc tgagaatcca  1200
gctcccgcac caaaaccaga gaagccagct gaacaaccaa aagcagaaaa aacagatgat  1260
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<210> 29

<211> 1278

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA derived from genome of *Streptococcus pneumoniae*

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<400> 29

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gatagaagaa	aacataccca	aaatgtcggc	ttactcacaa	agttgggcgt	aattaaaacg	180
gagtatttgc	atggattaag	tgtttcaaaa	aagaagtcgg	aagctgagtt	gccgtcagaa	240
ataaaagcaa	agttagacgc	agctttttgag	cagtttaaaa	aagatacatt	accaacagaa	300
ccaggaaaaa	aggtagcaga	agctgagaag	aagggttgaag	aagctaagaa	aaaagccgag	360
gatcaaaaag	aaaaagatct	ccgtaactac	ccaaccaata	cttacaaaac	gcttgaactt	420
gacattgctg	agtccgatgt	ggaagttaaa	aaagcggagc	ttgaactagt	aaaagaggaa	480
gctaagggaat	ctcgagacga	gaaaaaaatt	aatcaagcaa	aagcgaaagt	tgagaataaa	540
aaagctgagg	ctacaagggt	aaaaaacatc	aagacagatc	gtgaaaaagc	agaagaagct	600
aaacgaagag	cagatgctaa	gttgcaggaa	gctaattgtag	cgacttcaga	gcaagataaa	660
tcaaagaggc	gggcaaaacg	agaagttctt	ggagagctag	caacacctga	taaaaaagaa	720
aatgatgcga	agtcttcaga	ttctagcgta	ggtgaagaaa	ctcttacaag	cccatccctg	780
aaaccagaaa	aaaaggtagc	agaagctgag	aagaagggtt	aagaagctaa	gaaaaaagcc	840
gaggatcaaa	aagaagaaga	tcgtcgtaac	taccaacca	atacttaca	aacgcttgaa	900
cttgaaattg	ctgagtccga	tgtggaagtt	aaaaaagcgg	agcttgaact	agtaaaagag	960
gaagctaagg	aatctcgaaa	cgaggaaaaa	attaagcaag	taaaagcgaa	agttgagagt	1020
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gaagaagcta	aacgaagagc	agcagaagaa	gataaagtta	aagaaaaacc	agctgaacaa	1140
ccacaaccag	cgccggctcc	tcaaccagaa	aaaccaactg	aagagcctga	gaatccagct	1200
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<210> 30

<211> 1276

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: cDNA derived from genome of *Streptococcus pneumoniae*

<400> 30

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ccaggaaaaa	aggtagcaga	agctgagaag	aagggttgaag	aagctaagaa	aaaagccgag	360
gatcaaaaag	aaaaagatct	ccgtaactac	ccaaccaata	cttacaaaac	gcttgaactt	420
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aatgatgcga	agtcttcaga	ttctagcgta	ggtgaagaaa	ctcttacaag	cccatccctg	780
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cttgaaattg	ctgagtccga	tgtggaagtt	aaaaaagcgg	agcttgaact	agtaaaagag	960
gaagctaagg	aatctcgaaa	cgaggaaaaa	attaagcaag	taaaagcgaa	agttgagagt	1020
aaaaaagctg	aggctacaag	gctagaaaac	atcaagacag	atcgtaaaaa	agcagaagaa	1080
gaagaagcta	aacgaagagc	agcagaagaa	gataaagtta	aagaaaaacc	agctgaacaa	1140
ccacaaccag	cgccggctcc	tcaaccagaa	aaaccaactg	aagagcctga	gaatccagct	1200
ccagctccag	ctccaaaacc	agagaatcca	gctgaaaaac	caaaagcaga	aaagccagct	1260
gatcaacaag	ctgaag					1278

<210> 31

<211> 1272

<212> DNA

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<213> Artificial Sequence

<220>

<223> Description: cDNA derived from genome of *Streptococcus pneumoniae*

<400> 31

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gatagaagaa	aacataccca	aaatgtcggc	ttactcacia	agttgggctg	aattaaaacg	180
gagtatttgc	atggattaag	tgtttcaaaa	aagaagtcgg	aagctgagtt	gccgtcagaa	240
ataaaagcaa	agttagacgc	agctttttgag	cagtttataa	aagatacatt	accaacagaa	300
ccaggtaaaa	aggtagcaga	agctgagaag	aagggtgaag	aagctaagaa	aaaagccgag	360
gatcaaaaag	aaaaagatct	ccgtaactac	ccaaccaata	cttacaaaac	gcttgaactt	420
gacattgctg	agtcgcatgt	ggaagttaaa	aaagcggagc	ttgaactagt	aaaagaggaa	480
gctaaggaat	ctcgagacga	gaaaaaaatt	aatcaagcaa	aagcgaaagt	tgagaataaa	540
aaagctgagg	ctacaagggt	aaaaaacatc	aagacagatc	gtgaaaaagc	agaagaagct	600
aaacgaagag	cagatgctaa	gttgcaggaa	gctaattgtg	cgacttcaga	gcaagataaa	660
tcaaagaggc	gggcaaaacg	agaagttctt	ggagagctag	caacacctga	taaaaaagaa	720
aatgatgcga	agtcttcaga	ttctagcgta	ggtgaagaaa	ctcttacaag	cccatccctg	780
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gaggatcaaa	aagaagaaga	tcgtcgtaac	tacccaacca	atacttacia	aacgcttgaa	900
cttgaaattg	ctgagtcgga	tgtggaagtt	aaaaaagcgg	agcttgaact	agtaaaagag	960
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ccagctccag	ctccaaaacc	agagaatcca	gctgaaaaac	caaaagcaga	aaagccagct	1260
gatcaacaag	ct					1272

<210> 32

<211> 1258

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: Coding strand of cDNA derived from genome of *Streptococcus pneumoniae*

<400> 32

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aaacataccc	aaaatttagc	cttcaacata	cagttgagca	gaattaaaac	ggagtatttg	180
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gcagctttta	agcagtttaa	aaaagataca	ttaccaacag	aaccagaaaa	aaaagtagca	300
gaagctgaga	agaagggtga	agaagctgag	aagaaggtag	cagaagctaa	gaaaaaagcc	360
aaggctcaaa	aagaagaaga	tcaccgtaac	tacccaacca	ttacttacia	aacgcttgac	420
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gctgagtcgg	atgtgaaagt	taaagaagcg	gagcttgaac	tagtaaaaga	ggaagctaa	960
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gaggctacaa	ggttagaaaa	aatcaagaca	gatcgtaaaa	aagcagaaga	agaagctaaa	1080
cgaaaagcag	cagaagaaga	taaagttaaa	gaaaaaccag	ctgaacaacc	acaaccagcg	1140
ccggctcctc	aaccagaaaa	accagctgaa	gagcctgaga	atccagttcc	agctccaaaa	1200
ccagagaatc	cagctgaaca	accaaagca	gaaaaaccag	ctgatcaaca	agctgaag	1258

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<210> 33
 <211> 1242
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description: Coding strand of cDNA derived from genome of Streptococcus pneumoniae

<400> 33
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 ctagatagaa gaaaacatac ccaaaatgtc gccttaaaca taaagttgag cgcaattaaa 180
 acgaagtatt tgcgtgaatt taatgtttta gaagagaagt cgaaggatga gttgccgtca 240
 gaaataaaaag caaagttaga cgcagctttt gagaagttta aaaaagatac attgaaacca 300
 ggagaaaagg tagcagaagc taagaagaag gttgaagaag ctaagaaaaa agccgaggat 360
 caaaaagaag aagatcgtcg taactaccca accaatactt acaaaaacgct tgaacttgaa 420
 attgctgagt tcgatgtgaa agttaaagaa gcggagcttg aactagtaaa agaggaagct 480
 aaagaatctc gaaacgaggg cacaattaag caagcaaaag agaaagttga gagtaaaaaa 540
 gctgaggcta caaggttaga aaacatcaag acagatcgta aaaaagcaga agaagaagct 600
 aaacgaaaag cagatgctaa gttgaaggaa gctaattgtag cgacttcaga tcaaggtaaa 660
 ccaaaggggc gggcaaaacg aggagttcct ggagagctag caacacctga taaaaaagaa 720
 aatgatgcga agtcttcaga ttctagcgta ggtgaagaaa ctcttccaag ctcatccctg 780
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<210> 34
 <211> 1236
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description: Coding strand from cDNA derived from genome of Streptococcus pneumoniae

<400> 34
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 ttagatagaa gtaaacatat caaaactgta aatctaatta acaaatgca agacattaag 180
 agaacgtatt tgtatgaatt aaatgtttta gaagataagt cgaaagctga gttgccgtca 240
 aaaataaaaag cagagttaga cgcagctttt gagcagttta aaaaagatac attaccaaca 300
 gaaccaggaa aaaaggtagc agaagctaag aagaagggtg aagaagctga gaaaaaagcc 360
 aaggctcaaa aagaagaaga ttaccgtaac tacccaacca ttacttacia aacgcttgaa 420
 cttgaaattg ctgagtccga tgtgaaagtt aaagaagcgg agcttgaact agtaaaaaag 480
 gaagctgacg aatctcgaaa cgagggcaca attaaccaag caaaagcgaa agttgagagt 540
 gaacaagctg aggctacaag gttaaaaaaa atcaagacag atcgtgaaaa agcagaagaa 600
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 gtaaaacgag gagatttttg agagccagca acacctgata aaaaagaaaa tgatgcgaag 720
 tcttcagatt ctagcgtagg tgaagaaact cttccaagcc catccctgaa accaggaaaa 780
 aaggtagcag aagctgagaa gaaggttgaa gaagctgaga aaaaagccaa ggatcaaaaa 840
 gaagaagatc accgtaacta cccaaccatt acttaciaaa cgcttgaact tgaaattgct 900
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 tctcgaaacg aggaaaaagt taagcaagca aaagcgggaag ttgagagtaa aaaagctgag 1020

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gctacaaggt tagaaaaaat caagacagat cgtaaaaaag cagaagaaga agctaaacga 1080
aaagcagcag aagaagataa agttaaagaa aaaccagctg aacaaccaca accagcgccg 1140
gctcctcaac cagaaaaaacc agtccagct ccaaaaccag agaatccagc tgaacaacca 1200
aaagcagaaa aaccagctga tcaacaagct gaagaa 1236

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<210> 35

<211> 1218

<212> DNA

<213> Artificial Sequence

<220>

<223> Description: Coding strand from cDNA derived from genome of
Streptococcus
pneumoniae

<400> 35

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gatagaagaa aacataccca aaatgtcggc ttactcacia agttgggccc aattaaaacg 180
gagtatttgc gtggattaag tgtttcaaaa gagaagtcga cagctgagtt gccgtcagaa 240
ataaaagaaa agttaaccgc agctttttaag cagtttaaaa aagatacatt gaaaccagaa 300
aaaaaggtag cagaagctga gaagaaggta gcagaagcta agaaaaaagc cgaggatcaa 360
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gaacctcgag acgaggaaaa aattaagcaa gcagaagcgg aagttgagag taaaaaagct 540
gaggctacaa ggttaaaaaa aatcaagaca gatcgtgaaa aagcagaaga agaagctaaa 600
cgaagagtag atgctaaaga gcaagatgaa tcatcaaaga ggcgaaagag tcgggtaaaa 660
cgaggagatc ttggagagca agcaacacct gataaaaaag aaaatgatgc gaagtcttca 720
gattctagcg taggtgaaga aactcttcca agcccatccc tgaaaccagg aaaaaaggta 780
gcagaagctg agaagaaggt tgaagaagct gataaaaaag ccaaggctca aaaagaagaa 840
gatcgccgta actacccaac caatacttac aaaacgcttg aacttgaaat tgctgagtcc 900
gatgtggaag ttaaaaaaagc ggagcttgaa ctagtaaaag aggaagctaa ggaacctcga 960
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gcagaagaag ataaagttaa agaaaaacca gctgaacaac caaaaccagc gccggctcct 1140
caaccagaaa aaccagctcc aaaaccagag aatccagctg aacaaccaaa agcagaaaaa 1200
ccagctgac aacaagct 1218

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<210> 36

<211> 102

<212> PRT

<213> Artificial Sequence

<220>

<223> Description: Amino acid sequence derived from consensus cDNA sequence

<400> 36

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Thr Glu Asn Glu Gly Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala
 1             5             10             15

```

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Asn Gln Thr Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr
      20             25             30

```

```

Ile Lys Lys Met Leu Glu Gln Leu Asp Arg Arg Lys His Thr Gln Asn
      35             40             45

```

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Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Glu Tyr Leu Arg
      50             55             60

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Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Ala Glu Leu Pro Ser Glu

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65		70		75		80									
Ile	Lys	Ala	Lys	Leu	Asp	Ala	Ala	Phe	Glu	Gln	Phe	Lys	Lys	Asp	Thr
				85					90					95	
Leu	Lys	Thr	Glu	Pro	Gly										
			100												

<210> 37
 <211> 55
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description: Amino acid sequence derived from a cDNA consensus sequence from the genome of Streptococcus pneumoniae

<400> 37
Asp Ala Lys Leu Glu Ala Thr Ser Glu Gln Asp Lys Pro Lys Gly Arg
1 5 10 15
Ala Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
20 25 30
Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro
35 40 45
Ser Pro Ser Leu Lys Pro Glu
50 55

<210> 38
 <211> 103
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description: Amino acid sequence derived from consensus sequence of cDNA derived from the genome of Streptococcus pneumoniae

<400> 38
Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys Lys Lys
1 5 10 15
Ala Lys Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr
20 25 30
Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys
35 40 45
Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser Arg Asp
50 55 60
Glu Gly Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser Lys Lys Ala
65 70 75 80
Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg Glu Lys Ala Glu
85 90 95
Glu Glu Ala Lys Arg Arg Ala
100

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WHAT IS CLAIMED IS:

1. A vaccine against bacterial infections comprising an immunogen which is a polypeptide truncate
5 of a pneumococcal surface-binding protein, analog or variant having a highly conserved immunogenic alpha-helical portion with respect to different types of pneumococcal bacteria, which polypeptide does not include a choline-binding portion.
- 10
2. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 75 % identity with respect to the amino acid sequence of SEQ ID NO:1.
- 15
3. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 85 % identity with respect to the amino acid sequence of SEQ ID NO:1.
- 20
4. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has at least 90 % identity with respect to the amino acid sequence of a member consisting of:
- 25
- (a) the amino acid sequence of SEQ ID NO:1, and
(b) the amino acid sequence of SEQ ID NO:19.
5. A vaccine according to claim 1, wherein the amino acid sequence of said alpha-helical portion has
30 at least 95 % identity with respect to the amino acid sequence of a member selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:1, and
(b) the amino acid sequence of SEQ ID NO:19.
- 35
6. A vaccine according to claim 1, wherein said vaccine is for preventing or treating otitis media, sepsis, meningitis and lobar pneumonia infections.

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7. A vaccine according to claim 6, wherein said vaccine is for invasive infections.

8. A vaccine according to claim 6, wherein
5 said vaccine is for otitis media infections caused by *S. pneumoniae*.

9. A vaccine according to claim 1, wherein said polypeptide truncate comprise an amino acid sequence
10 which has at least 90 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

10. A vaccine according to claim 1, wherein
15 said polypeptide truncate comprise an amino acid sequence which has at least 95 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

20 11. An antibody raised against an immunogen which is a polypeptide truncate of a pneumococcal surface-binding protein, analog or variant having a highly conserved immunogenic alpha-helical portion with respect to different types of pneumococcal bacteria,
25 which polypeptide does not include a choline-binding portion.

12. An antibody according to claim 11, wherein the amino acid sequence of said alpha-helical portion has
30 at least 85 % identity with respect to the amino acid sequence of SEQ ID NO:1.

13. An antibody according to claim 11, wherein the amino acid sequence of said alpha-helical portion has
35 at least 90 % identity with respect to the amino acid sequence of a member selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:1,
and

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(b) the amino acid sequence of SEQ ID NO:19.

14. An antibody according to claim 11, wherein the amino acid sequence of said alpha-helical portion has at least 95 % identity with respect to the amino acid sequence of a member selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:1,
and

10 (b) the amino acid sequence of SEQ ID NO:19.

15 15. An antibody according to claim 11, wherein said polypeptide truncate comprise an amino acid sequence which has at least 95 % identity with respect to a member selected from the group consisting of the amino acid sequences of each of SEQ ID NOS:3 to 18.

16. An antibody according to claim 11, wherein said antibody is an antibody that will detect *S. pneumoniae* infections.

17. An antibody according to claim 15, wherein said antibody is effective for the prevention and/or treatment of *S. pneumoniae* infections.

25 18. An antibody according to claim 15, wherein said antibody is effective for the prevention and/or treatment of pneumococcal infections caused by types 1-5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F *S. pneumoniae* bacteria.

19. A method for preventing and/or treating pneumococcal infections in a host comprising immunizing said host with a member selected from the group consisting of:

(a) a vaccine according to claim 2, and
(b) at least one antibody raised against an immunogen which is a polypeptide truncate of a pneumococcal surface-binding protein, analog or variant

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comprising an amino acid sequence that is has at least 90
% identity to the amino acid sequence of a member
selected from the group consisting of SEQ ID NO:3 to 18,
which polypeptide does not include a choline-binding
5 portion.

20. A polypeptide comprising an amino acid
sequence which has at least 90 % identity with respect to
a member selected from the group consisting of the amino
10 acid sequences of each of SEQ ID NOS:3 to 18.

21. An isolated polynucleotide comprising
polynucleotide sequence having at least 90 % identity to
a member selected from the group consisting of:

15 (a) a polynucleotide coding sequence encoding
a polypeptide comprising a member selected from the group
consisting of the amino acid sequences of each of SEQ ID
NOS:3 to 18, and

(b) and the complement of (a).

Figure 1

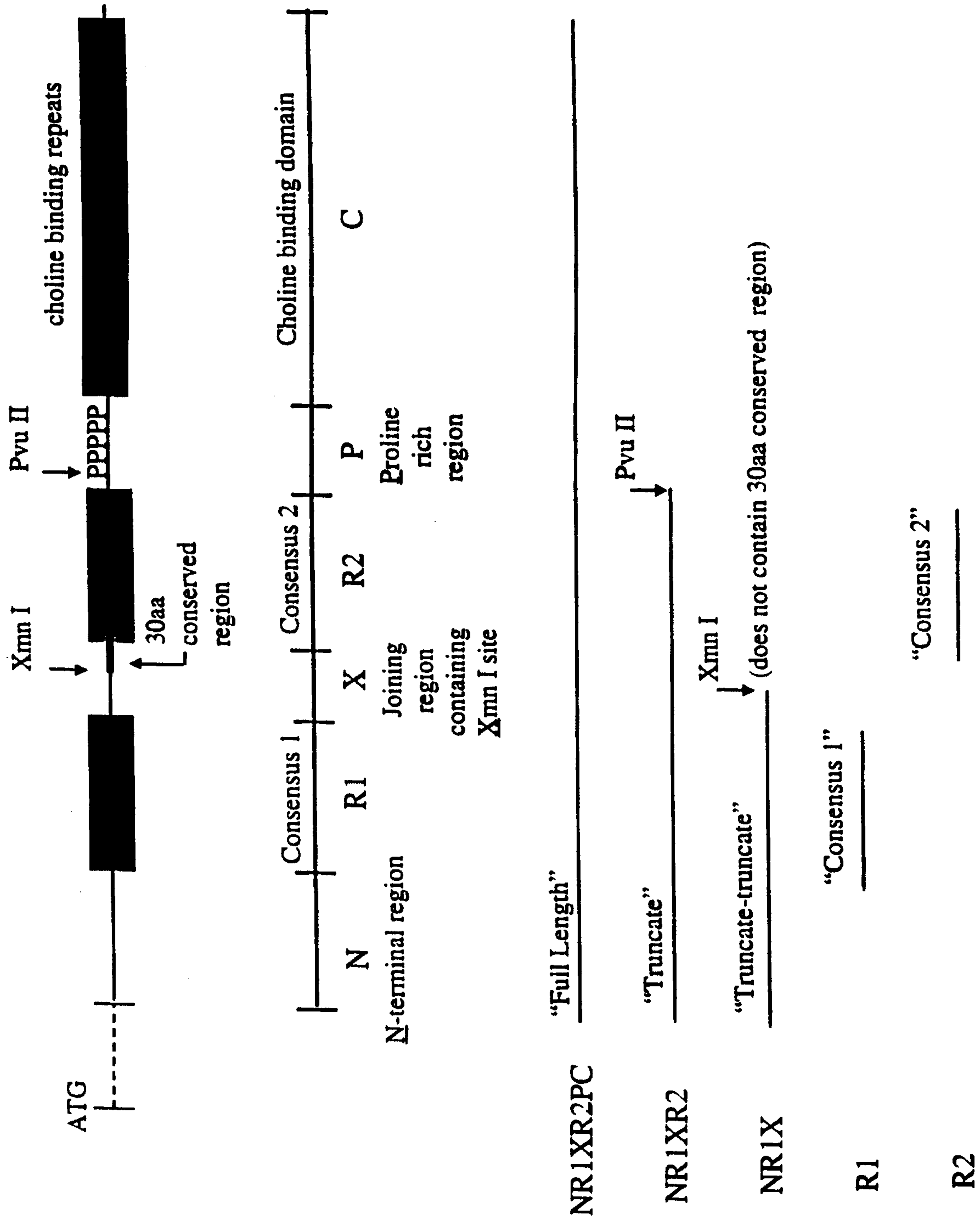
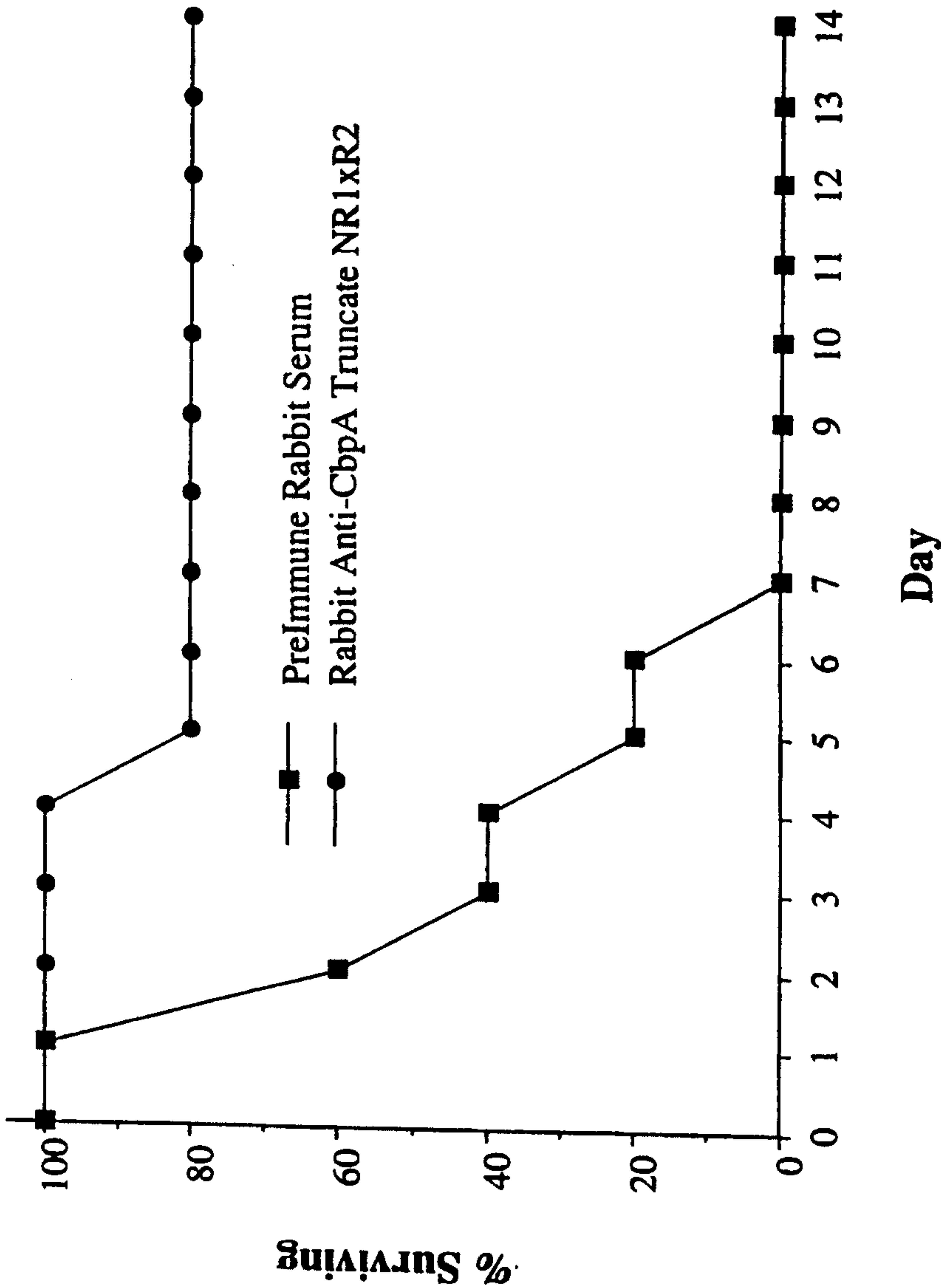
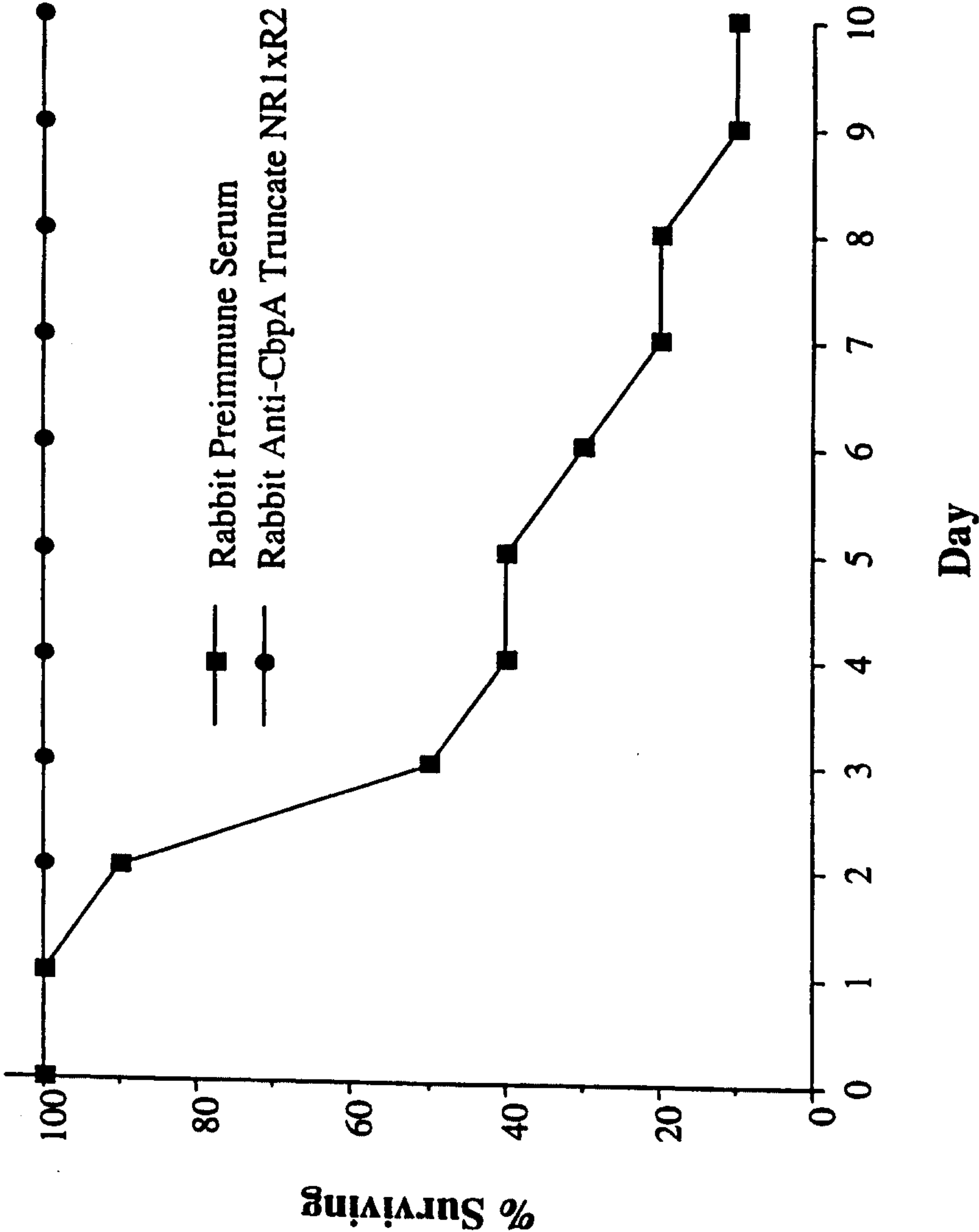


Figure 2



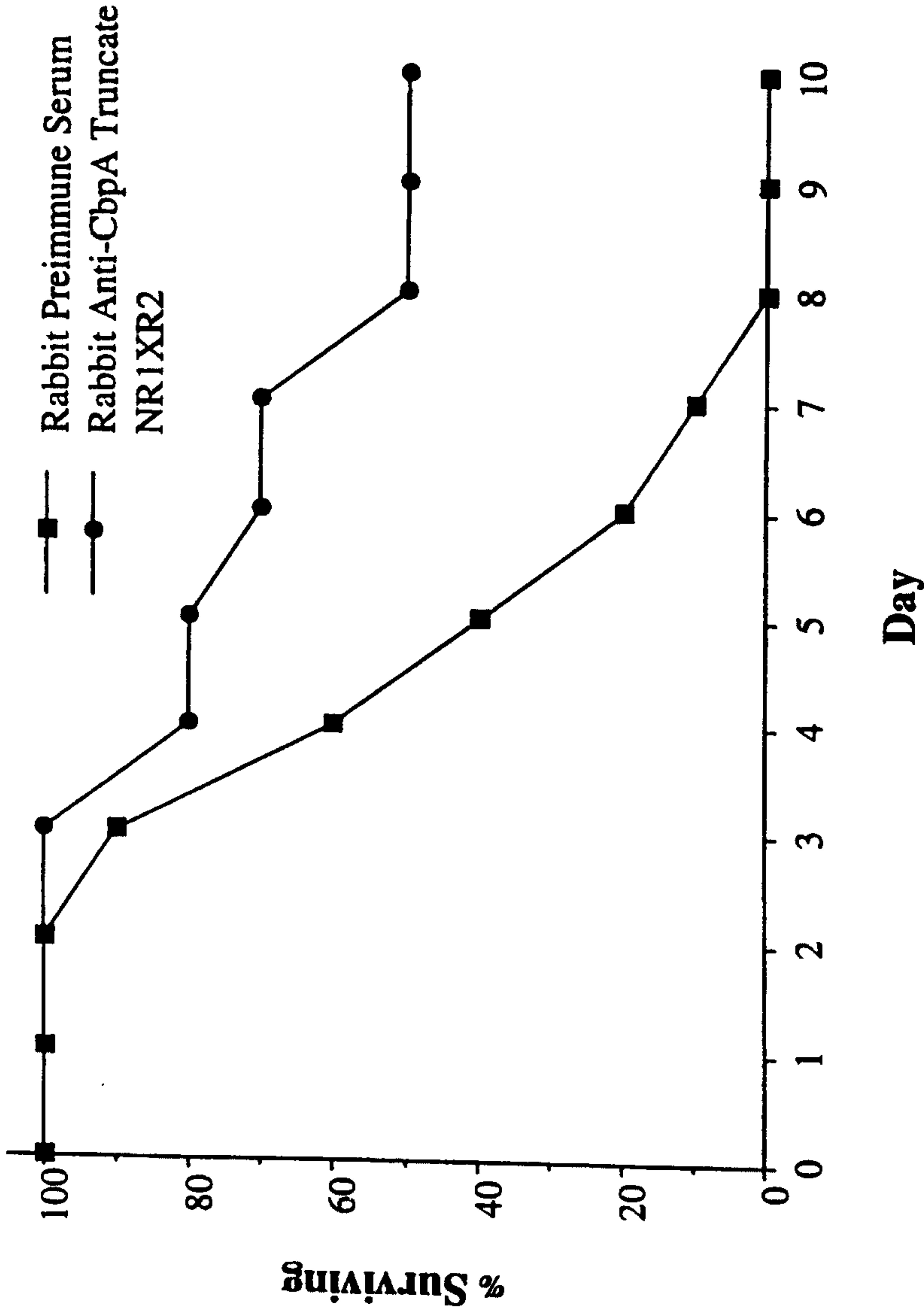
(Sheet 2 of 16)

Figure 3



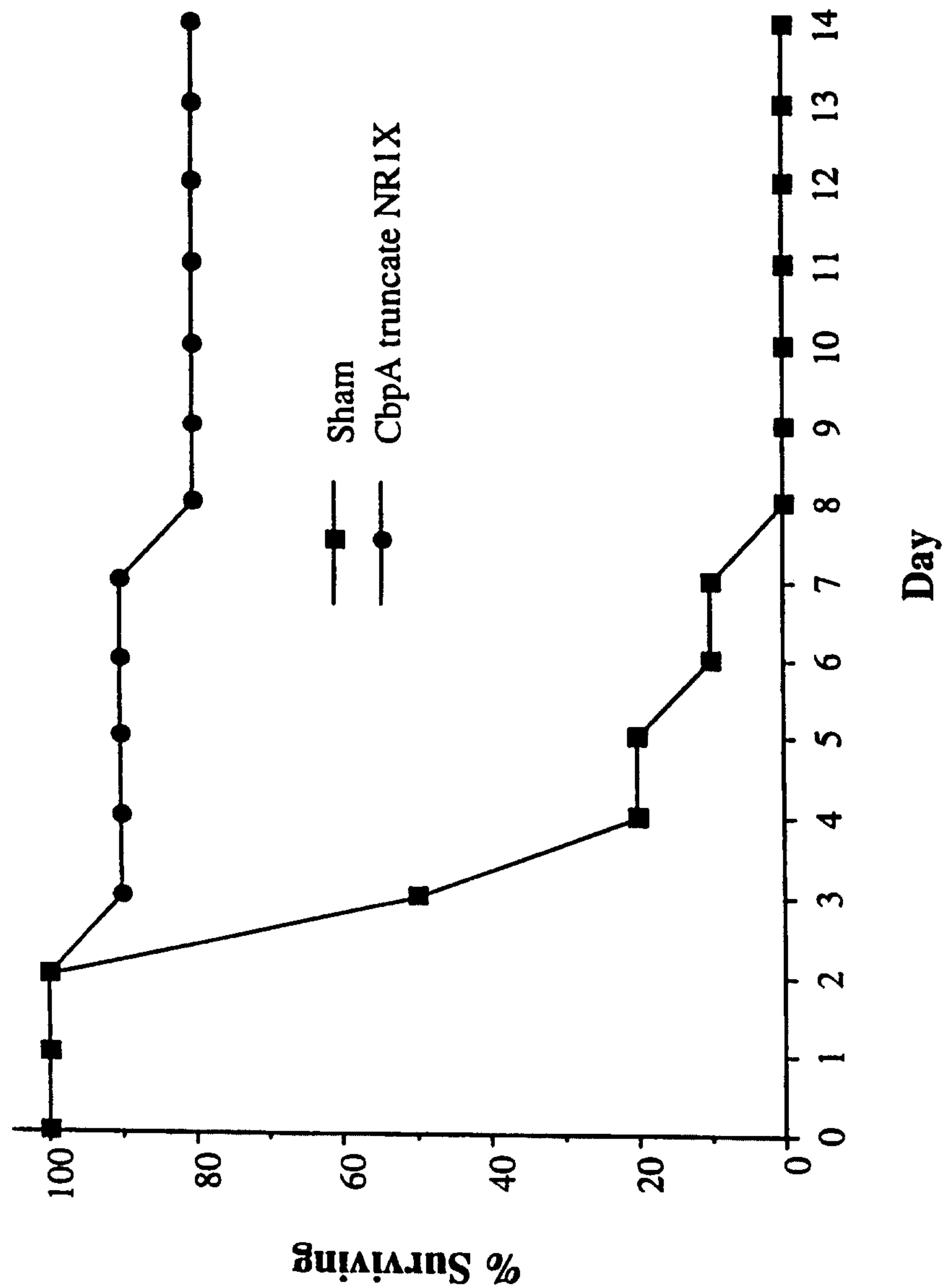
(Sheet 3 of 16)

Figure 4



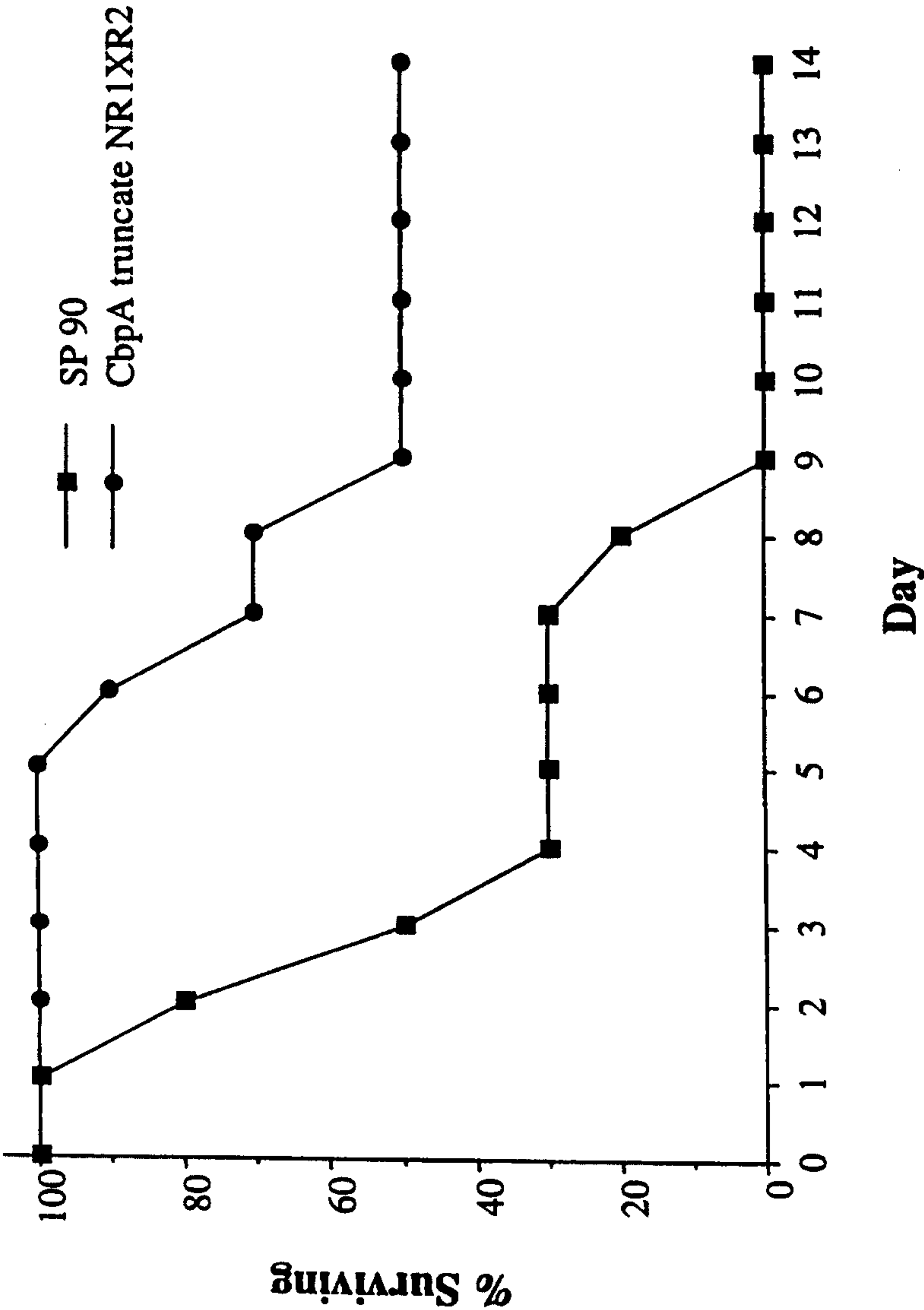
(Sheet 4 of 16)

Figure 5



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Figure 6



(Sheet 6 of 16)

Figure 7

[illegible][illegible]

Decoration 'Decoration #1': Box residues that differ from the Consensus.

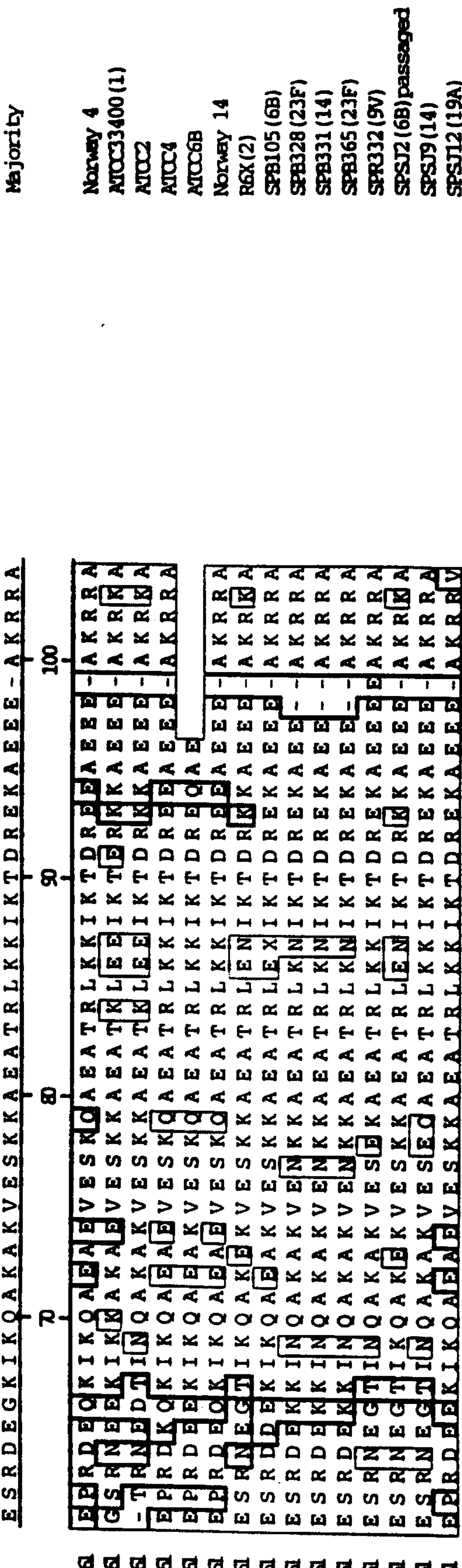
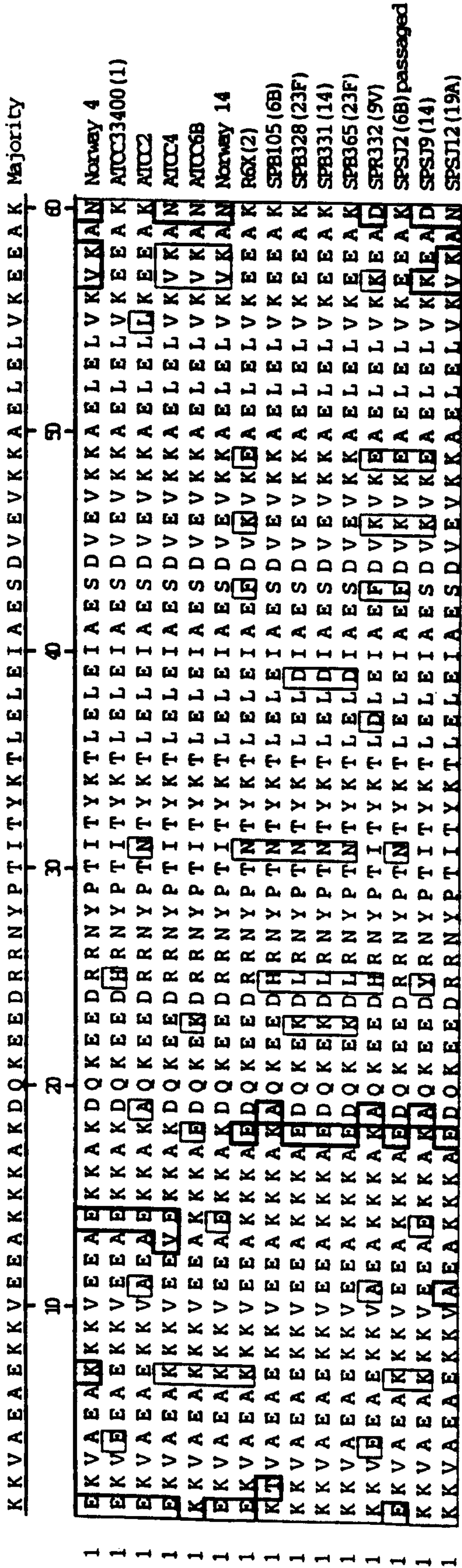
Decoration 'Decoration #3': Box residues that match the Consensus exactly.

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Figure 8

Percent Identity																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1																	Norway4
2																	ATCC33400(1)
3																	ATCC11733(2)
4																	ATCC2
5																	ATCC4
6																	ATCC6B
7																	ATCC18C
8																	R6X(2)
9																	SPB105(6B)
10																	SPB328(23F)
11																	SPB331(14)
12																	SPB365(23F)
13																	SPB609(6B)
14																	SPR332(9V)
15																	SPSJ2(6B)p
16																	SPSJ9(14)
17																	SPSJ12(19A)

Figure 9



Decoration 'Decoration #2': Box residues that differ from the Consensus.

Decoration 'Decoration #3': Box residues that match the Consensus exactly.

Figure 10

Percent Identity															
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	80.8	86.5	76.0	92.7	100.0	78.8	82.7	79.8	79.8	79.8	76.0	78.8	83.7	91.3	1
2			78.8	76.0	80.8	82.7	83.7	78.8	78.8	78.8	76.9	82.7	77.9	79.8	2
3				74.0	77.9	84.6	82.7	79.8	79.8	79.8	78.8	84.6	80.8	77.9	3
4					98.1	76.9	81.7	77.9	77.9	77.9	74.0	76.9	81.7	89.4	4
5						79.2	83.3	84.4	84.4	84.4	76.0	79.2	81.2	93.8	5
6							82.7	79.8	79.8	79.8	76.0	78.8	83.7	91.3	6
7								82.7	82.7	82.7	81.7	100.0	83.7	78.8	7
8									86.5	86.5	80.8	82.7	81.7	85.6	8
9										100.0	78.8	82.7	80.8	83.7	9
10											78.8	82.7	80.8	83.7	10
11												78.8	80.8	83.7	11
12													91.3	79.8	12
13														78.8	13
14															14
15															15

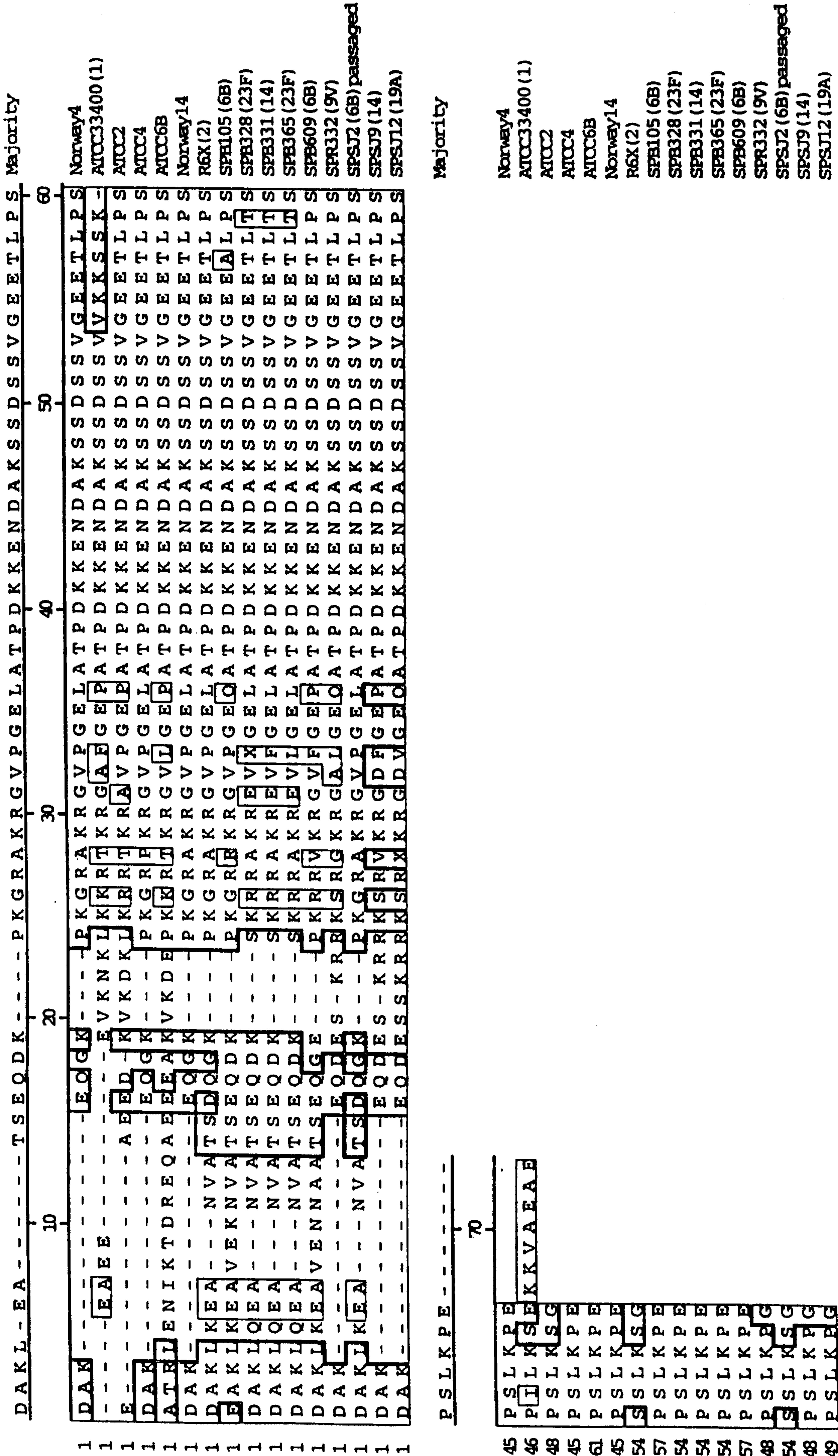
Norway 4
ATCC33400(1)
ATCC2
ATCC4
ATCC6B
Norway 14
R6X(2)
SPB105(6B)
SPB328(23F)
SPB331(14)
SPB365(23F)
SPR332(9V)
SPSJ2(6B)passed
SPSJ9(14)
SPSJ12(19A)

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Figure 11



Decoration 'Decoration #1': Box residues that differ from the Consensus.

Decoration 'Decoration #2': Box residues that match the Consensus exactly.

Figure 12

Percent Identity																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		52.0	76.0	98.0	82.0	100.0	86.0	86.0	82.0	82.0	82.0	84.0	76.0	86.0	76.0	76.0
2			58.5	52.0	55.2	52.0	43.1	44.8	43.1	44.8	43.1	48.3	49.1	43.1	50.9	46.3
3				76.0	81.1	76.0	71.7	71.7	69.8	69.8	69.8	69.8	69.8	71.7	71.7	69.8
4					82.0	98.0	84.0	86.0	80.0	80.0	80.0	84.0	76.0	84.0	76.0	76.0
5						82.0	62.7	64.5	64.4	64.4	66.1	67.7	71.7	62.7	71.7	70.4
6							86.0	86.0	82.0	82.0	82.0	84.0	76.0	86.0	76.0	76.0
7								79.7	81.4	81.4	81.4	78.0	66.0	100.0	66.0	66.7
8									78.0	78.0	78.0	83.9	69.8	79.7	67.9	66.7
9										98.3	98.3	78.0	67.9	81.4	67.9	64.8
10											98.3	79.7	67.9	81.4	69.8	64.8
11												78.0	69.8	81.4	67.9	64.8
12													69.8	78.0	75.5	66.7
13														66.0	92.5	88.7
14															66.0	66.7
15																88.7
16																

Norway4

ATCC33400(1)

ATCC2

ATCC4

ATCC6B

Norway14

R6X(2)

SPB105(6B)

SPB328(23F)

SPB331(14)

SPB365(23F)

SPB609(6B)

SPR332(9V)

SPSJ2(6B)passed

SPSJ9(14)

SPSJ12(19A)

Figure 13

	10	20	30	40	50	60	K	V	A	E	A	E	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K		
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	A	E	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	A	E	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
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1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
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1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
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1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
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1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K
1	K	K	V	A	E	A	E	K	K	V	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	E	I	A	E	S	D	V	E	V	K	K	A	E	L								

[illegible]

Decoration 'Decoration 1': Box residues that differ from the Consensus.

Figure 14

[illegible]

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Figure 15

1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	Majority
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	Norway4
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	ATOC33400(1)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	ATOC11733(2)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	ATOC2
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	ATOC4
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	ATOC6B
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	ATOC18C-3
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	Norway14
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	R6X(2)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPB105(6B)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPB328(23F)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPB331(14)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPB365(23F)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPR332(9V)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPSJ2(6B)passed
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPSJ9(14)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	SPSJ12(19A)
1	K	K	V	A	E	A	E	K	K	V	E	E	A	K	K	K	A	E	D	Q	K	E	E	D	R	R	N	Y	P	T	N	T	Y	K	T	L	E	I	A	E	S	D	V	E	V	K	K	A	E	L	E	L	V	K	E	E	A	K	Consensus B

15 / 16

61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	Majority
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	Norway4
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	ATOC33400(1)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	ATOC11733(2)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	ATOC2
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	ATOC4
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	ATOC6B
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	ATOC18C-3
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	Norway14
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	R6X(2)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPB105(6B)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPB328(23F)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPB331(14)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPB365(23F)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPR332(9V)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPSJ2(6B)passed
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPSJ9(14)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	SPSJ12(19A)
61	E	P	R	N	E	E	K	I	K	Q	A	K	A	K	V	E	S	K	K	A	E	A	T	R	L	E	K	I	K	T	D	R	K	K	A	E	E	E	-	A	K	R	K	A	A	E	E	D	K	V	K	E	K	P	A	Consensus B

Decoration 'Decoration #1': Box residues that differ from the Consensus.

Decoration '': Box residues that match the Consensus exactly.

(Sheet 15 of 16)

Figure 16

Percent Identity																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1		89.4	89.5	91.2	100.0	98.2	96.5	100.0	92.1	92.1	91.2	92.1	91.2	93.0	92.1	94.7	97.4	98.2	1
2			85.8	91.2	89.4	87.6	85.8	89.4	92.0	92.0	86.7	87.6	86.7	92.0	92.0	87.6	89.4	91.2	2
3				87.7	89.5	89.5	87.7	89.5	90.4	90.4	87.7	88.6	87.7	91.2	90.4	86.0	86.8	91.2	3
4					91.2	89.5	87.7	91.2	93.9	93.9	86.0	86.8	86.0	93.9	93.9	93.0	91.2	91.2	4
5						98.2	96.5	100.0	92.1	92.1	91.2	92.1	91.2	93.0	92.1	94.7	97.4	98.2	5
6							96.5	98.2	92.1	92.1	91.2	92.1	91.2	91.2	92.1	93.0	95.6	96.5	6
7								96.5	88.6	88.6	87.7	88.6	87.7	89.5	88.6	93.0	93.9	94.7	7
8									92.1	92.1	91.2	92.1	91.2	93.0	92.1	94.7	97.4	98.2	8
9										100.0	88.6	89.5	88.6	94.7	100.0	90.4	92.1	93.9	9
10											88.6	89.5	88.6	94.7	100.0	90.4	92.1	93.9	10
11												99.1	100.0	89.5	88.6	87.7	88.6	93.0	11
12													99.1	90.4	89.5	88.6	89.5	93.9	12
13														89.5	88.6	87.7	88.6	93.0	13
14															94.7	92.1	95.6	94.7	14
15																90.4	92.1	93.9	15
16																	94.7	93.0	16
17																		95.6	17
18																			18
			</																