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(54) Title: STREPTOCOCCUS PYOGENES ANTIGENS AND CORRESPONDING DNA FRAGMENTS

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   1 ATGAAAAAGA CATTAACTTT GCTACTGGCA CTCTTTGCCA TCGGGGTAAC TAGTAGCGTC
  61 AGAGCGGAGG ATGAACAAAG TAGTACACAA AAGCCAGTAA AATTTGATTT GGATGGACCT
 121 CAACAAAAAA TTAAAGATTA TAGTGGCAAC ACAATCACTC TAGAAGACTT ATATGTTGGT
 181 AGTAAAGTAG TAAAAATATA TATCCCTCAA GGATGGTGGG TATATCTTTA CAGACAATGT
 241 GATCATAACA GTAAAGAACG AGGAATTTTA GCTAGTCCTA TTCTCGAAAA AAATATAACA
 301 AAAACAGATC CTTATCGTCA ATATTATACA GGAGTACCTT ATATTCTTAA CTTAGGAGAA
 361 GATCCTTTGA AGAAAGGAGA AAAATTAACT TTCTCATTTA AAGGAGAAGA CGGATTTTAT
 421 GTCGGTAGCT ATATCTATAG AGACTCTGAT ACTATAAAAA AACAAAAAGA AGCTGAAGAA
 481 GCACTTCAAA AAAAGGAAGA GGAAAAGCAA CAAAAACAGC TAGAAGAAAG CATGCTAAAG
 541 CAGATAAGAG AAGAAGACCA TAAACCTTGG CATCAGCGGT TAAGTGAGAG CATCCAAGAT
 601 CAGTGGTGGA ACTTTAAGGG ACTGTTTCAG TGA
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(57) Abstract: The present invention relates to antigens, more particularly antigens of Streptococcus pyogenes (also called group A Streptococcus (GAS)) bacterial pathogen which are useful as vaccine component for therapy and/or prophylaxis.

WO 02/050107 A3

STREPTOCOCCUS PYOGENES ANTIGENS AND CORRESPONDING DNA FRAGMENTS

FIELD OF THE INVENTION

The present invention is related to antigens, more particularly BVH-P2, BVH-P3, BVH-P4, BVH-P5, and BVH-P6 antigens of Group A Streptococcus (S. pyogenes) bacterial pathogen which may be used to prevent, diagnose and/or treat streptococcal infections.

BACKGROUND OF THE INVENTION

Streptococci are gram (+) bacteria which are differentiated by group specific carbohydrate antigens A through O which are found at the cell surface. S. pyogenes isolates are further distinguished by type-specific M protein antigens. M proteins are important virulence factors which are highly variable both in molecular weights and in sequences. Indeed, more than 80-M protein types have been identified on the basis of antigenic differences.

S. pyogenes is responsible for many diverse infection types, including pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis. A resurgence of invasive disease in recent years has been documented in many countries, including those in North America and Europe. Although the organism is sensitive to antibiotics, the high attack rate and rapid onset of sepsis results in high morbidity and mortality.

To develop a vaccine that will protect hosts from S. pyogenes infection, efforts have focused on virulence factors such as the type-specific M proteins. However, the amino-terminal portion of M proteins was found to induce cross-reactive antibodies which reacted with human myocardium, tropomyosin, myosin, and vimentin, which might be implicated in autoimmune diseases. Others have used recombinant techniques to produce complex hybrid proteins containing amino-terminal peptides of M proteins

from different serotypes. However, a safe vaccine containing all *S. pyogenes* serotypes will be highly complex to produce and standardize.

In addition to the serotype-specific antigens, other *S. pyogenes* proteins have generated interest as potential vaccine candidates. The C5a peptidase, which is expressed by at least *S. pyogenes* 40 serotypes, was shown to be immunogenic in mice, but its capacity to reduce the level of nasopharyngeal colonization was limited. Other investigators have also focused on the streptococcal pyrogenic exotoxins which appear to play an important role in pathogenesis of infection. Immunization with these proteins prevented the deadly symptoms of toxic shock, but did not prevent colonization.

The University of Oklahoma has set up a genome sequencing project for *S. pyogenes* strain M1 GAS (http://dnal. chem. ou. edu/strep. html).

Therefore there remains an unmet need for S. pyogenes antigens that may be used vaccine components for the prophylaxis and/or therapy of *S. pyogenes* infection.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided an isolated polynucleotide comprising:

(a) a polynucleotide encoding a polypeptide that comprises an amino acid sequence at least 70% identical to the amino acid sequence set forth in SEQ ID NO:10, wherein

the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(b) a polynucleotide encoding a polypeptide that comprises an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(c) a polynucleotide encoding a polypeptide that comprises an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(d) a polynucleotide encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(e) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 10; or

(f) a polynucleotide encoding a polypeptide comprising an immunogenic fragment consisting of at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*.

According to a second aspect of the invention there is provided an isolated polynucleotide that is complementary to the polynucleotide of the first aspect.

2002216863 12 Feb 2008

According to a third aspect of the invention there is provided an isolated polynucleotide that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 10.

According to a fourth aspect of the invention there is provided a vector comprising the polynucleotide of any one of the first, second or third aspects, wherein the polynucleotide is operably linked to an expression control region.

According to a fifth aspect of the invention there is provided a host cell transfected with the vector of the fourth aspect.

According to a sixth aspect of the invention there is provided a process for producing the polypeptide encoded by the polynucleotide of the first aspect, said process comprising culturing the host cell according to the fifth aspect under conditions suitable for expression of the polypeptide.

According to a seventh aspect of the invention there is provided an isolated polypeptide comprising:

(a) a polypeptide comprising an amino acid sequence at least 70% identical to the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(b) a polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(c) a polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(d) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(e) a polypeptide capable of generating antibodies having binding specificity for a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 10;

(f) a polypeptide comprising an immunogenic fragment consisting of at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(g) the polypeptide of (a), (b), (c), or (d) wherein the N-terminal Met residue is deleted; or

(h) the polypeptide of (a), (b), (c), or (d) wherein the secretory amino acid sequence is deleted.

According to an eighth aspect of the invention there is provided a chimeric polypeptide comprising two or more immunogenic polypeptide fragments, wherein each of the two or more immunogenic polypeptide fragments comprise at least 10 contiguous amino acid residues from a polypeptide consisting of SEQ ID NO: 10, provided that the two or more immunogenic polypeptide fragments are linked to form a chimeric polypeptide.

According to a ninth aspect of the invention there is provided a pharmaceutical composition comprising the polypeptide according to the seventh aspect and a pharmaceutically acceptable carrier or diluent.

According to a tenth aspect of the invention there is provided a pharmaceutical composition comprising the chimeric polypeptide according to the eighth aspect and a pharmaceutically acceptable carrier or diluent.

According to an eleventh aspect of the invention there is provided an isolated antibody, or antigen-binding fragment thereof, that specifically binds to the polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:10.

According to a twelfth aspect of the invention there is provided a method for therapeutic or prophylactic treatment of pharyngitis, erysipelas, impetigo, scarlet fever, bacteremia, necrotizing fasciitis, or toxic shock in a host susceptible to pharyngitis, erysipelas, impetigo, scarlet fever, bacteremia, necrotizing fasciitis, or toxic shock, said method comprising administering to the host a therapeutic or prophylactic amount of the pharmaceutical composition according to the ninth or tenth aspect.

According to a thirteenth aspect of the invention there is provided a method for therapeutic or prophylactic treatment of *Streptococcus pyogenes* bacterial infection in a host susceptible to *Streptococcus pyogenes* infection, said method comprising administering to the host a therapeutic or

2002216863 12 Feb 2008

prophylactic amount of the pharmaceutical composition according to the ninth or tenth aspect.

According to a fourteenth aspect of the invention there is provided a method for diagnosing a streptococcal infection in a host susceptible to streptococcal infection comprising:

(a) obtaining a biological sample from the host;

(b) incubating an antibody, or antigen-binding fragment thereof, that specifically binds to the polypeptide according to the seventh aspect with the biological sample to form a mixture; and

(c) detecting specifically bound antibody or bound antigen-binding fragment in the mixture which indicates the presence of a streptococcal infection.

According to a fifteenth aspect of the invention there is provided a method for detecting an antibody that specifically binds to *Streptococcus pyogenes* in a host susceptible to streptococcal infection comprising:

(a) obtaining a biological sample from a host;

(b) incubating one or more polypeptides according to the seventh aspect with the biological sample to form a mixture; and

(c) detecting specifically bound polypeptide in the mixture which indicates the presence of an antibody specific to that specifically binds to *Streptococcus pyogenes*.

According to a sixteenth aspect of the invention there is provided use of the pharmaceutical composition according to the ninth or tenth aspect for the preparation of a medicament for the prophylactic or therapeutic treatment of pharyngitis, erysipelas, impetigo, scarlet fever,

2002216863 12 Feb 2008

bacteremia, necrotizing fasciitis, or toxic shock in a host susceptible to pharyngitis, erysipelas, impetigo, scarlet fever, bacteremia, necrotizing fasciitis, or toxic shock.

According to a seventeenth aspect of the invention there is provided use of the pharmaceutical composition according to the ninth or tenth aspect for preparation of a medicament for the prophylactic or therapeutic treatment of a streptococcal infection in a host susceptible to the streptococcal infection, wherein the streptococcal infection is a *Streptococcus pyogenes* infection.

According to an eighteenth aspect of the invention there is provided a kit comprising the isolated polypeptide according to the seventh aspect for detection or diagnosis of a streptococcal infection.

Disclosed herein is an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID Nos : 2,4,6,8,10,12,14 and 16 or fragments or analogs thereof.

Also disclosed herein are polypeptides which comprise an amino acid sequence chosen from SEQ ID Nos : 2,4,6,8,10,12,14 and 16 or fragments or analogs thereof.

Also disclosed herein are polypeptides encoded by polynucleotides of the invention, pharmaceutical compositions,

vectors comprising polynucleotides of the invention operably linked to an expression control region, as well as host cells transfected with said vectors and methods of producing polypeptides comprising culturing said host cells under conditions suitable for expression.

BRIEF DESCRIPTION OF THE DRAWINGS

In Figures 1, 3, 5, 7, 9, the underlined portion of the sequence represents the region coding for the leader peptide. In Figures 2, 4, 6, 8, 10, the underlined portion of the sequence represents the leader peptide.

Figure 1 represents the DNA sequence of BVH-P2 gene from serotype M3 S. pyogenes strain ATCC12384; SEQ ID NO: 1.

Figure 2 represents the amino acid sequence BVH-P2 polypeptide from serotype 3 S. pyogenes strain ATCC12384; SEQ ID NO: 2.

Figure 3 represents the DNA sequence of BVH-P3 gene from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 3.

Figure 4 represents the amino acid sequence BVH-P3 polypeptide from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 4.

Figure 5 represents the DNA sequence of BVH-P4 gene from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 5.

Figure 6 represents the amino acid sequence BVH-P4 polypeptide from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 6.

Figure 7 represents the DNA sequence of BVH-5 gene from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 7.

Figure 8 represents the amino acid sequence BVH-P5 polypeptide from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 8.

Figure 9 represents the DNA sequence of BVH-P6 gene from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 9.

Figure 10 represents the amino acid sequence BVH-P6 polypeptide from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 10.

Figure 11 represents the DNA sequence of BVH-P4 gene from serotype M3 S. pyogenes strain ATCC123834; SEQ ID NO: 11.

Figure 12 represents the amino acid sequence BVH-P4 polypeptide from serotype M3 S. pyogenes strain ATCC12384; SEQ ID NO: 12.

Figure 13 represents the DNA sequence of BVH-P4 gene from serotype M6 S. pyogenes strain SPY67; SEQ ID NO: 13.

Figure 14 represents the amino acid sequence BVH-P4 polypeptide from serotype M3 S. pyogenes strain SPY67; SEQ ID NO: 14.

Figure 15 represents the DNA sequence of BVH-P4 gene from serotype S. pyogenes strain B514; SEQ ID NO: 15.

Figure 16 represents the amino acid sequence BVH-P4 polypeptide from serotype S. pyogenes strain B514; SEQ ID NO: 16.

Figure 17 depicts the comparison of the nucleotide sequences of the BVH-P4 genes from the S. pyogenes serotype M1 ATCC700294, serotype M3 ATCC12384, serotype M6 SPY77 strains and the mouse isolate B514 by using the program Clustal W from MacVector sequence analysis software (version 6.5). Identical nucleotides are presented as * and differences are indicated by blank spaces.

Figure 18 depicts the comparison of the predicted amino acid sequences of the BVH-P4 partial open reading frames from the S. pyogenes serotype M1 ATCC700294, serotype M3 ATCC12384, serotype M6 SPY77 strains and the mouse isolate B514 by using the program Clustal W from MacVector sequence analysis software (version 6.5). Underneath the alignment, there is a consensus line.

Identical amino acid are illustrated with a * while differences are indicated by periods.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides purified and isolated DNA molecules, which encode Streptococcal polypeptides that can be used to prevent, treat, and/or diagnose Streptococcal infection.

Those skilled in the art will appreciate that the invention includes DNA molecules that encode analogs such as mutants, variants, homologues and derivatives of such polypeptides, as described herein in the present patent application. The invention also includes RNA molecules corresponding to the DNA molecules of the invention. In addition to the DNA and RNA molecules, the invention includes the corresponding polypeptides and monospecific antibodies that specifically bind to such polypeptides.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides a polynucleotide encoding a polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a polypeptide having a sequence chosen from: **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs or thereof.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide having a sequence chosen from **SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16.**

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16.**

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16.**

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16.**

According to one aspect, the present invention provides a polynucleotide encoding a polypeptide comprising a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs or thereof.

According to one aspect, the present invention provides a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16.**

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a polypeptide having a sequence chosen from: **SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16.**

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide having a sequence chosen from **SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14 or 16.**

In accordance with the present invention, all polynucleotides encoding polypeptides are within the scope of the present invention.

According to one aspect, the present invention relates to polypeptides having at least 70% identity to a second polypeptide having an amino acid sequence chosen from: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides having at least 95% identity to a second polypeptide having an amino acid sequence chosen from: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising sequences from SEQ ID Nos : 2,4,6,8,10,12,14,16 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 or fragments or analogs thereof.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide having a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides having at least 70% identity to a second polypeptide having an amino acid sequence chosen from: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16,.

According to one aspect, the present invention relates to polypeptides having at least 95% identity to a second polypeptide having an amino acid sequence chosen from: SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16,.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising sequences from SEQ ID Nos : 2,4,6,8,10,12,14,16.

According to one aspect, the present invention relates to

polypeptides capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide having a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16.

In a further embodiment, the polypeptides in accordance with the present invention are antigenic.

In a further embodiment, the polypeptides in accordance with the present invention are immunogenic.

In a further embodiment, the polypeptides in accordance with the present invention can elicit an immune response in a host.

In a further embodiment, the present invention also relates to polypeptides which are able to raise antibodies having binding specificity to the polypeptides of the present invention as defined above.

An antibody that "has binding specificity" is an antibody that recognizes and binds the selected polypeptide but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, which naturally includes the selected peptide. Specific binding can be measured using an ELISA assay in which the selected polypeptide is used as an antigen.

In accordance with the present invention, "protection" in the biological studies is defined by a significant increase in the survival curve, rate or period. Statistical analysis using the Log rank test to compare survival curves, and Fisher exact test to compare survival rates and numbers of days to death, respectively, might be useful to calculate P values and determine whether the difference between the two groups is statistically significant. P values of 0.05 are regarded as not significant.

In accordance with the present invention, there is provided a consensus nucleotide sequence for BVH-P4 depicted in Figure 17. As can be seen by the alignement, the polynucleotide encoding the polypeptide of the invention is well conserved. Without restricting the scope of the invention, the following table A shows the possible modifications:

Position on alignement in Figure 17	Possible nucleotide
74	G or T
130	C or T
253	C or T
274	G or A
412	C or T
445	A or G
841	T or C
868	G or A
917	C or T

In accordance with the present invention, there is provided a consensus amino acid sequence for BVH-P4 depicted in Figure 18. As can be seen by the alignement, the polypeptide of the invention is well conserved. Without restricting the scope of the invention, the following table B shows the possible modifications:

Position on alignement in Figure 18	Possible amino acid
25	S or A

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the polypeptides of the invention, or of analogs thereof.

The fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide or analog thereof as described herein. The present invention further provides fragments having at least 10 contiguous amino acid residues from the polypeptide sequences of the present invention. In one embodiment, at least 15 contiguous amino acid residues. In one embodiment, at least 20 contiguous amino acid residues.

The skilled person will appreciate that analogs of the polypeptides of the invention will also find use in the context of the present invention, i.e. as antigenic/immunogenic material. Thus, for instance proteins or polypeptides which include one or more additions, deletions, substitutions or the like are encompassed by the present invention.

These substitutions are those having a minimal influence on the secondary structure and hydropathic nature of the polypeptide. Preferred substitutions are those known in the art as conserved, i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups. These include substitutions such as those described by Dayhoff, M. in Atlas of Protein Sequence and Structure 5, 1978 and by Argos, P. in EMBO J. 8, 779-785, 1989. For example, amino acids, either natural or unnatural, belonging to one of the following groups represent conservative changes:

ala, pro, gly, gln, asn, ser, thr, val;
cys, ser, tyr, thr;
val, ile, leu, met, ala, phe;
lys, arg, orn, his;
and phe, tyr, trp, his.

The preferred substitutions also include substitutions of D-enantiomers for the corresponding L-amino acids.

The percentage of homology is defined as the sum of the percentage of identity plus the percentage of similarity or conservation of amino acid type.

In an alternative approach, the analogs could be fusion proteins, incorporating moieties which render purification easier, for example by effectively tagging the desired polypeptide. It may be necessary to remove the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

Thus, what is important for analogs, derivatives and fragments is that they possess at least a degree of the antigenicity/immunogenic of the protein or polypeptide from which they are derived.

As used herein, "fragments", "analogs" or "derivatives" of the polypeptides of the invention include those polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably conserved) and which may be natural or unnatural.

In one embodiment, analogs of polypeptides of the invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 75% homology. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

In a further embodiment, polypeptides will have greater than 70% homology. In a further embodiment, polypeptides will have greater than 75% homology. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, derivatives and analogs of polypeptides of the invention will have less than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10. Preferred substitutions are those known in the art as conserved i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups.

One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are contemplated in the present invention.

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the polypeptides of the invention, or of analogs thereof.

For fragments of the polypeptides described herein, or of analogs thereof, the situation is slightly different from native protein. It is well known that it is possible to screen an antigenic polypeptide to identify epitopic regions, i.e. those regions which are responsible for the polypeptide's antigenicity or immunogenicity. Methods for carrying out such screening are

well known in the art. Thus, the fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide, analog as described herein.

Also included are polypeptides which have fused thereto other compounds which alter the polypeptides biological or pharmacological properties i.e. polyethylene glycol (PEG) to increase half-life; leader or secretory amino acid sequences for ease of purification; prepro- and pro- sequences; and (poly)saccharides.

Furthermore, in those situations where amino acid regions are found to be polymorphic, it may be desirable to vary one or more particular amino acids to more effectively mimic the different epitopes of the different streptococcus strains.

Moreover, the polypeptides of the present invention can be modified by terminal -NH_2 acylation (eg. by acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g. with ammonia or methylamine) to provide stability, increased hydrophobicity for linking or binding to a support or other molecule.

Also contemplated are hetero and homo polypeptide multimers of the polypeptide fragments and analogues. These polymeric forms include, for example, one or more polypeptides that have been cross-linked with cross-linkers such as avidin/biotin, gluteraldehyde or dimethylsuperimidate. Such polymeric forms also include polypeptides containing two or more tandem or inverted contiguous sequences, produced from multicistronic mRNAs generated by recombinant DNA technology. In a further embodiment, the present invention also relates to chimeric polypeptides which comprise one or more polypeptides or

fragments or analogs thereof as defined in the figures of the present application.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 or fragments or analogs thereof; provided that the polypeptides are linked as to formed a chimeric polypeptide.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14 or 16 provided that the polypeptides are linked as to formed a chimeric polypeptide.

In order to achieve the formation of antigenic polymers (i.e. synthetic multimers), polypeptides may be utilized having bishaloacetyl groups, nitroarylhalides, or the like, where the reagents being specific for thio groups. Therefore, the link between two mercapto groups of the different polypeptides may be a single bond or may be composed of a linking group of at least two, typically at least four, and not more than 16, but usually not more than about 14 carbon atoms.

In a particular embodiment, polypeptide fragments and analogs of the invention do not contain a starting residue, such as methionine (Met) or valine (Val).

Preferably, polypeptides will not incorporate a leader or secretory sequence (signal sequence). The signal portion of a polypeptide of the invention may be determined according to established molecular biological techniques. The polypeptide of interest may be isolated from a streptococcal culture and subsequently sequenced to determine the initial residue of the mature protein and therefore the sequence of the mature polypeptide.

It is understood that polypeptides can be produced and/or used without their start codon (methionine or valine) and/or without their leader peptide to favor production and purification of recombinant polypeptides. It is known that cloning genes without sequences encoding leader peptides will restrict the polypeptides to the cytoplasm of E. coli and will facilitate their recovery (Glick, B.R. and Pasternak, J.J. (1998) Manipulation of gene expression in prokaryotes. In "Molecular biotechnology: Principles and applications of recombinant DNA", 2nd edition, ASM Press, Washington DC, p.109-143).

The polypeptides may be expressed with or without a leader or secretion sequence. In the former case, the leader may be removed using post-translational processing (see US 4 431 739, US 4 425 437 and US 4 338 397 incorporated herein by reference) or be chemically removed subsequent to purifying the expressed polypeptide.

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polypeptide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iii) a vaccine comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iv) a method for inducing an immune response against Streptococcus, in a host, by administering to the host, an immunogenically effective amount of a polypeptide of the invention to elicit an immune response, e.g., a protective immune response to Streptococcus; and particularly, (v) a method for preventing and/or treating a Streptococcus infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to a host in need.

Before immunization, the polypeptides of the invention can also be coupled or conjugated to carrier proteins such as tetanus toxin, diphtheria toxin, hepatitis B virus surface antigen, poliomyelitis virus VP1 antigen or any other viral or bacterial toxin or antigen or any suitable proteins to stimulate the

development of a stronger immune response. This coupling or conjugation can be done chemically or genetically. A more detailed description of peptide-carrier conjugation is available in Van Regenmortel, M.H.V., Briand J.P., Muller S., Plaué S., «Synthetic Polypeptides as antigens» in Laboratory Techniques in Biochemistry and Molecular Biology, Vol.19 (ed.) Burdou, R.H. & Van Knippenberg P.H. (1988), Elsevier New York.

According to another aspect, there are provided pharmaceutical compositions comprising one or more Streptococcal polypeptides of the invention in a mixture with a pharmaceutically acceptable adjuvant. Suitable adjuvants include (1) oil-in-water emulsion formulations such as MF59™, SAF™, Ribi™ ; (2) Freund's complete or incomplete adjuvant; (3) salts i.e. $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)_2$, $Al(OH)_3$, $AlPO_4$, silica, kaolin; (4) saponin derivatives such as Stimulon™ or particles generated therefrom such as ISCOMs (immunostimulating complexes); (5) cytokines such as interleukins, interferons, macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF) ; (6) other substances such as carbon polynucleotides i.e. poly IC and poly AU, detoxified cholera toxin (CTB)and E.coli heat labile toxin for induction of mucosal immunity. A more detailed description of adjuvant is available in a review by M.Z.I Khan et al. in Pharmaceutical Research, vol.11, No.1 (1994) pp2-11, and also in another review by Gupta et al., in Vaccine, Vol.13, No14, pp1263-1276 (1995) and in WO 99/24578, which are herein incorporated by reference. Preferred adjuvants include QuilA™, QS21™, Alhydrogel™ and Adjuphos™.

In a further embodiment, there is provided a method of manufacturing a pharmaceutical composition comprising admixing a polypeptide of the invention with a pharmaceutically acceptable diluent, excipient or adjuvant.

In a further aspect, the invention provides a method for prophylactic or therapeutic treatment of Streptopcoccal bacterial infection in a host susceptible to Streptococcal

infection comprising administering to a host a therapeutic or prophylactic amount of a composition of the invention.

Pharmaceutical compositions of the invention may be administered parenterally by injection, rapid infusion, nasopharyngeal absorption, dermoabsorption, or bucal or oral. Pharmaceutically acceptable carriers also include tetanus toxoid.

Pharmaceutical compositions of the invention are used for the treatment or prophylaxis of streptococcal infection and/or diseases and symptoms mediated by streptococcal infection as described in P.R. Murray (Ed, in chief),E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken. Manual of Clinical Microbiology, ASM Press, Washington, D.C. sixth edition, 1995, 1482p which are herein incorporated by reference. In one embodiment, pharmaceutical compositions of the present invention are used for the treatment or prophylaxis of pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis and also toxic shock. In one embodiment, pharmaceutical compositions of the invention are used for the treatment or prophylaxis of streptococcus infection and/or diseases and symptoms mediated by streptococcus infection, in particular group A streptococcus (S. pyogenes), group B streptococcus (GBS or S.agalactiae), S.pneumoniae, S.dysgalactiae, S.uberis, S.nocardia as well as Staphylococcus aureus. In a further embodiment, the streptococcus infection is Streptococcus pyogenes.

In a particular embodiment, pharmaceutical compositions are administered to those host at risk of streptococcus infection such as infants, elderly and immunocompromised hosts.

According to a further aspect, the streptococcal polypeptides of the invention may be used in a kit comprising the polypeptides of the invention for detection or diagnosis of streptococcal infection.

As used in the present application, the term " host" include mammals. In a further embodiment, the mammal is human.

Pharmaceutical compositions are preferably in unit dosage form of about 0.001 to 100 µg/kg (antigen/body weight) and more preferably 0.01 to 10 µg/kg and most preferably 0.1 to 1 µg/kg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

Pharmaceutical compositions are preferably in unit dosage form of about 0.1 µg to 10 mg and more preferably 1µg to 1 mg and most preferably 10 to 100 µg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

In one embodiment, polynucleotides are those illustrated in **SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15** which may include the open reading frames (ORF), encoding the polypeptides of the invention.

It will be appreciated that the polynucleotide sequences illustrated in the figures may be altered with degenerate codons yet still encode the polypeptides of the invention. Accordingly the present invention further provides polynucleotides which hybridize to the polynucleotide sequences herein above described (or the complement sequences thereof) having 50% identity between sequences. In one embodiment, at least 70% identity between sequences. In one embodiment, at least 75% identity between sequences. In one embodiment, at least 80% identity between sequences. In one embodiment, at least 85% identity between sequences. In one embodiment, at least 90% identity between sequences. In a further embodiment, polynucleotides are hybridizable under stringent conditions i.e. having at least 95% identity. In a further embodiment, more than 97% identity.

Suitable stringent conditions for hybridation can be readily determined by one of skilled in the art (see for example Sambrook *et al.*, (1989) Molecular cloning: A Laboratory Manual,

2nd ed, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology, (1999) Edited by Ausubel F.M. *et al.*, John Wiley & Sons, Inc., N.Y.).

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises **SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises **SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16**.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising **SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16** or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising **SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16**.

In a further embodiment, polynucleotides are those illustrated in **SEQ ID NOs : 1, 3, 5, 7, 9, 11, 13, 15** encoding polypeptides of the invention.

As will be readily appreciated by one skilled in the art, polynucleotides include both DNA and RNA.

The present invention also includes polynucleotides complementary to the polynucleotides described in the present application.

In a further aspect, polynucleotides encoding polypeptides of the invention, or fragments, analogs or derivatives thereof, may be used in a DNA immunization method. That is, they can be incorporated into a vector which is replicable and expressible upon injection thereby producing the antigenic polypeptide in vivo. For example polynucleotides may be incorporated into a plasmid vector under the control of the CMV promoter which is functional in eukaryotic cells. Preferably the vector is injected intramuscularly.

According to another aspect, there is provided a process for producing polypeptides of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host cell and recovering the expressed polypeptide product. Alternatively, the polypeptides can be produced according to established synthetic chemical techniques i.e. solution phase or solid phase synthesis of oligopeptides which are ligated to produce the full polypeptide (block ligation).

General methods for obtention and evaluation of polynucleotides and polypeptides are described in the following references: Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons,

Inc. New York; PCR Cloning Protocols, from Molecular Cloning to Genetic Engineering, Edited by White B.A., Humana Press, Totowa, New Jersey, 1997, 490 pages; Protein Purification, Principles and Practices, Scopes R.K., Springer-Verlag, New York, 3rd Edition, 1993, 380 pages; Current Protocols in Immunology, Edited by Coligan J.E. et al., John Wiley & Sons Inc., New York.

For recombinant production, host cells are transfected with vectors which encode the polypeptide, and then cultured in a nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. Suitable vectors are those that are viable and replicable in the chosen host and include chromosomal, non-chromosomal and synthetic DNA sequences e.g. bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA. The polypeptide sequence may be incorporated in the vector at the appropriate site using restriction enzymes such that it is operably linked to an expression control region comprising a promoter, ribosome binding site (consensus region or Shine-Dalgarno sequence), and optionally an operator (control element). One can select individual components of the expression control region that are appropriate for a given host and vector according to established molecular biology principles (Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York). Suitable promoters include but are not limited to LTR or SV40 promoter, E.coli lac, tac or trp promoters and the phage lambda P_L promoter. Vectors will preferably incorporate an origin of replication as well as selection markers i.e. ampicilin resistance gene. Suitable bacterial vectors include pET, pQE70, pQE60, pQE-9, pD10 phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 and eukaryotic vectors pBlueBacIII, pWLNEO, pSV2CAT, pOG44, pXT1, pSG, pSVK3, pBPV, pMSG and pSVL. Host cells may be bacterial i.e. E.coli, Bacillus subtilis, Streptomyces; fungal i.e. Aspergillus niger,

Aspergillus nidulins; yeast i.e. Saccharomyces or eukaryotic i.e. CHO, COS.

Upon expression of the polypeptide in culture, cells are typically harvested by centrifugation then disrupted by physical or chemical means (if the expressed polypeptide is not secreted into the media) and the resulting crude extract retained to isolate the polypeptide of interest. Purification of the polypeptide from culture media or lysate may be achieved by established techniques depending on the properties of the polypeptide i.e. using ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography. Final purification may be achieved using HPLC.

According to a further aspect, the streptococcal polypeptides of the invention may be used in a diagnostic test for streptococcus infection, in particular Streptococcus pyogenes infection. Several diagnostic methods are possible, for example detecting streptococcus organism in a biological sample, the following procedure may be followed:

a) obtaining a biological sample from a host;

b) incubating an antibody or fragment thereof reactive with a streptococcus polypeptide of the invention with the biological sample to form a mixture; and

c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of streptococcus.

Alternatively, a method for the detection of antibody specific to a streptococcus antigen in a biological sample containing or suspected of containing said antibody may be performed as follows:

a) obtaining a biological sample from a host;

b) incubating one or more streptococcus polypeptides of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to streptococcus.

One of skill in the art will recognize that this diagnostic test may take several forms, including an immunological test such as an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay or a latex agglutination assay, essentially to determine whether antibodies specific for the protein are present in an organism.

The DNA sequences encoding polypeptides of the invention may also be used to design DNA probes for use in detecting the presence of streptococcus in a biological sample suspected of containing such bacteria. The detection method of this invention comprises:

a) obtaining the biological sample from a host;

b) incubating one or more DNA probes having a DNA sequence encoding a polypeptide of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound DNA probe in the mixture which indicates the presence of streptococcus bacteria.

The DNA probes of this invention may also be used for detecting circulating streptococcus i.e. Streptococcus pyogenes nucleic acids in a sample, for example using a polymerase chain reaction, as a method of diagnosing streptococcus infections. The probe may be synthesized using conventional techniques and may be immobilized on a solid phase, or may be labelled with a detectable label. A preferred DNA probe for this application is an oligomer having a sequence complementary to at least about 6 contiguous nucleotides of the Streptococcus pyogenes polypeptides of the invention.

Another diagnostic method for the detection of streptococcus in a host comprises:

a) labelling an antibody reactive with a polypeptide of the invention or fragment thereof with a detectable label;

b) administering the labelled antibody or labelled fragment to the host; and

c) detecting specifically bound labelled antibody or labelled fragment in the host which indicates the presence of streptococcus.

A further aspect of the invention is the use of the streptococcus polypeptides of the invention as immunogens for the production of specific antibodies for the diagnosis and in particular the treatment of streptococcus infection. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against streptococcus infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for a number of epitopes associated with the Streptococcus pyogenes polypeptides but is preferably specific for one.

A further aspect of the invention is the use of the antibodies directed to the polypeptides of the invention for passive immunization. One could use the antibodies described in the present application. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against streptococcal infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin

class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for a number of epitopes associated with the streptococcal polypeptides but is preferably specific for one.

According to one aspect, the present invention provides the use of an antibody for treatment and/or prophylaxis of streptococcal infections.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLE 1

This example illustrates the cloning and molecular characteristics of BVH-P2 gene and corresponding polypeptide

The coding region of S. pyogenes BVH-P2 gene (SEQ ID NO: 1) was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from genomic DNA of serotype M3 S. pyogenes strain ATCC12384 using the following oligonuceotide primers that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Xho*I (CTCGAG): DMAR124 and DMAR125, which are presented in Table 1. PCR products were purified from agarose gel using a QIAquick gel extraction kit

from QIAgen following the manufacturer's instructions (Chatsworth, CA), and digested with *Nde*I and *Xho*I (Pharmacia Canada Inc, Baie d'Urfé, Canada). The pET-21b(+) vector (Novagen, Madison, WI) was digested with *Nde*I and *Xho*I and purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA). The *Nde*I-*Xho*I PCR products were ligated to the *Nde*I-*Xho*I pET-21b(+) expression vector. The ligated products were transformed into E. coli strain DH5 [ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *hsd*R17(r_K-m_K+) *deo*R *thi*-1 *sup*E44 λ⁻*gyr*A96 *rel*A1] (Gibco BRL, Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). Recombinant pET-21b(+) plasmid (rpET21b(+)) containing BVH-P2 gene was purified using a QIAgen plasmid kit (Chatsworth, CA) and DNA insert was sequenced (Taq Dye Deoxy Terminator Cycle Sequencing kit, ABI, Foster City, CA).

Table 1. Oligonucleotide primers used for PCR amplifications of S. pyogenes genes

Genes	Primers I.D.	Restriction site	Vector	Sequence	SEQ ID No
BVH-P2	DMAR124	*NdeI*	pET21b	5'-CGGAGAGAACATATGAAAAAGACATTAAC-3'	17
BVH-P2	DMAR125	*XhoI*	pET21b	5'-GGGCTCGAGCTGAAACAGTCCCTTAAAG-3'	18
BVH-P2	DMAR507	*BamHI*	pCMV-GH	5'-GAGCGGATCCTGAACAAAGTAG-3'	19
BVH-P2	DMAR508	*SalI*	pCMV-GH	5'-GGGGTCGACCTGAAACAGTCCCTTAA	20

				AG-3'	
BVH-P3	DMAR188	*NdeI*	pET21b	5'-GATGGGAAAGCATATGAGCCTCATTTTG-3'	21
BVH-P3	DMAR189	*XhoI*	pET21b	5'-GGCTCGAGTTTTGCTAGACCTTCAG-3'	22
BVH-P4	DMAR192	*NdeI*	pET21b	5'-GGGTTCATACATATGAACAAGAAATTTATTGG-3'	23
BVH-P4	DMAR193	*XhoI*	pET21b	5'-GGCTCGAGTTTTTCAGGAACTTTAATG-3'	24
BVH-P4	DMAR509	*BamHI*	pCMV-GH	5'-GTTTGGATCCTTGTGGTAATCGTGG-3'	25
BVH-P4	DMAR510	*SalI*	pCMV-GH	5'-GGGTCGACTTTTTCAGGAACTTTAATG-3'	26
BVH-P5	DMAR200	*NdeI*	pET21b	5'-GGTTCATTTTCATATGAACAAAAAAGTAATG-3'	27
BVH-P5	DMAR201	*XhoI*	pET21b	5'-GGCTCGAGGTTTTCAGGAACTGTGATGG-3'	28
BVH-P5	DMAR511	*BamHI*	pCMV-GH	5'-GGGGATCCTACCAATAACTCCGCTAAACA-3'	29

BVH-P5	DMAR512	*SalI*	pCMV-GH	5'-CAGGTCGACTTTTCAGGAACTGTGATGGTTC-3'	30
BVH-P6	DMAR235	*NdeI*	pET21b	5'-GGATAGTTTTCATATGAATCAAGAGATTAG-3'	31
BVH-P6	DMAR236	*XhoI*	pET21b	5'-CCCTCGAGATTGGTCTGATTCCAACTATC-3'	32
BVH-P6	DMAR513	*BamHI*	pCMV-GH	5'-TTTGGATCCTAATCAAGAGATTAGATATTC-3'	33
BVH-P6	DMAR514	*SalI*	pCMV-GH	5'-CCGTCGACATTGGTCTGATTCCAACTATC-3'	34

It was determined that the open reading frame (ORF) which codes for BVH-P2 contains 633-bp and encodes a 210 amino acid residues polypeptide with a predicted pI of 6.40 and a predicted molecular mass of 24,611.78 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :2) using the Spscan software (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 22 amino acid residues signal peptide (MKKTLTLLLALFAIGVTSSVRA), which ends with a cleavage site situated between an alanine and a glutamic acid residues.

To confirm the presence by PCR amplification of BVH-P2 (SEQ ID NO :1) gene, the following 4 serologically distinct S. pyogenes strains were used: the serotype M1 S. pyogenes strain ATCC 700294 and the serotype M3 S. pyogenes strain ATCC12384 were obtained from the American Type Culture Collection (Rockville, MD, USA); the serotype M6 S. pyogenes SPY67 clinical isolate was

provided by the Centre de recherche en infectiologie du Centre hospitalier de l'université Laval, Sainte-Foy; and S. pyogenes strain B514 which was initially isolated from a mouse was provided by Susan Hollingshead, from University of Alabama, Birmingham. The E. coli strain XL1-Blue MRF' was used in these experiments as negative control. Chromosomal DNA was isolated from each S. pyogenes strain as previously described (Jayarao BM et al. 1991. J. Clin. Microbiol. 29:2774-2778). BVH-P2 (SEQ ID NO: 1) gene was amplified by PCR(Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from the genomic DNA purified from the 4 S. pyogenes strains, and the control E. coli strain using the oligonucleotides primers DMAR124 and DMAR125 (Table 1). PCR was performed with 30 cycles of 45 sec at 95°C, 45 sec at 50°C and 1 min at 72°C and a final elongation period of 7 min at 72°C. The PCR products were size fractionated in 1% agarose gels and were visualized by ethidium bromide staining. The results of these PCR amplifications are presented in Table 2. The analysis of the amplification products revealed that BVH-P2 (SEQ ID NO: 1) gene was present in the genome of all of the 4 S. pyogenes strains tested. No such product was detected when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

Table 2. Identification of S. pyogenes genes by PCR amplification

Strain Identification	**Identification by PCR amplification of**				
	BVH-P2	BVH-P3	BVH-P4	BVH-P5	BVH-P6
ATCC700294 (M1)	+	+	+	+	+
ATCC12384 (M3)	+	+	+	+	+
SPY67 (M6)	+	+	+	+	+
B514*	+	+	+	+	+
E. coli XL1 Blue MRF'	-	-	-	-	-

*Mouse isolate

EXAMPLE 2

This example illustrates the cloning and molecular characteristics of BVH-P3 gene and corresponding polypeptide

The coding region of S. pyogenes BVH-P3 gene (SEQ ID NO: 3) was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using the following oligos that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Xho*I (CTCGAG): DMAR188 and DMAR189, which are presented in Table 1. The methods used for cloning BVH-P3 into an expression vector and sequencing are similar to the methods described in Example 1.

It was determined that the open reading frame (ORF) which codes for BVH-P3 contains 921-bp and encodes a 306 amino acid residues polypeptide with a predicted pI of 5.73 and a predicted molecular mass of 33,882.36 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :4) using the Spscan sofware (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 27 amino acid residues signal peptide (MSLILGAFLSVFLLVACSSTGTKTAKS), which ends with a cleavage site situated between a serine and an aspartic acid residues. The BVH-P3 gene was shown to be present after PCR amplification using the oligonucleotide primers DMAR188 and DMAR189 in the 4 serologically S. pyogenes strains tested (Table 2). The methods used for PCR amplification of the BVH-P3 gene were similar to the methods presented in Example 1. No such product was detected when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

EXAMPLE 3

This example illustrates the cloning and molecular characteristics of BVH-P4 gene and corresponding polypeptide

The coding region of S. pyogenes BVH-P4 gene (SEQ ID NO: 5) was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using the following oligos that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Xho*I (CTCGAG): DMAR192 and DMAR193, which are presented in Table 1. The methods used for cloning BVH-P4 into an expression vector and sequencing are similar to the methods described in Example 1.

It was determined that the open reading frame (ORF) which codes for BVH-P4 contains 1053-bp and encodes a 350 amino acid residues polypeptide with a predicted pI of 7.90 and a predicted molecular mass of 36,392.50 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :6) using the Spscan sofware (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 19 amino acid residues signal peptide (MNKKFIGLGLASVAVLSLA), which ends with a cleavage site situated between two alanine residues.

The BVH-P4 gene was shown to be present after PCR amplification using the oligonucleotide primers DMAR192 and DMAR193 in the 4 serologically S. pyogenes strains tested (Table 2). The methods used for PCR amplification of the BVH-P4 gene were similar to the methods presented in Example 1. No such product was detected when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

Sequencing of aditional BVH-P4 genes from other strains confirmed the high level of molecular conservation of this gene among S. pyogenes isolates. The respective coding region of S. pyogenes BVH-P4 gene from strains ATCC 12384 (SEQ ID NO : 11), SPY67 (SEQ ID NO: 13), and B514 (SEQ ID NO: 15) were amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from genomic DNA using the oligonucleotide primers DMAR192 and DMAR193 which are described in Table 1. PCR products were purified from agarose gel using a QIAquick gel

extraction kit from QIAgen following the manufacturer's instructions (Chatsworth, CA) and the DNA inserts were sequenced (Taq Dye Deoxy Terminator Cycle Sequencing kit, ABI, Foster City, CA). The predicted amino acid sequences from strains ATCC12384 (SEQ ID NO: 12), SPY67 (SEQ ID NO: 14), and B514 (SEQ ID NO: 16) were respectively presented in the following figures 12, 14, and 16. The figure 18 depicts the consensus predicted amino acid sequences established for S. pyogenes BVH-P4. Pairwise comparison of these BVH-P4 amino acid sequences indicated that the level of identity was higher than 99% clearly showing the high level of conservation of BVH-P4 among S. pyogenes isolates.

EXAMPLE 4

This example illustrates the cloning and molecular characteristics of BVH-P5 gene and corresponding polypeptide

The coding region of S. pyogenes BVH-P5 gene (SEQ ID NO: 7) was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using the following oligos that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Xho*I (CTCGAG): DMAR200 and DMAR201, which are presented in Table 1. The methods used for cloning BVH-P5 into an expression vector and sequencing are similar to the methods described in Example 1.

It was determined that the open reading frame (ORF) which codes for BVH-P5 contains 1044-bp and encodes a 347 amino acid residues polypeptide with a predicted pI of 5.65 and a predicted molecular mass of 36,808.91 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :8) using the Spscan sofware (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 17 amino acid residues signal peptide (MNKKVMSLGLVSTALFT), which ends with a cleavage site situated between a threonine and a leucine residues.

The BVH-P5 gene was shown to be present after PCR amplification using the oligonucleotide primers DMAR200 and DMAR201 in the 4 serologically S. pyogenes strains tested,(Table 2). The methods used for PCR amplification of the BVH-P5 gene were similar to the methods presented in example 1. No such product was detected when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

EXAMPLE 5

This example illustrates the cloning and molecular characteristics of BVH-P6 gene and corresponding polypeptide.

The coding region of S. pyogenes BVH-P6 gene (SEQ ID NO:9) was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using the following oligonucleotide primers that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Xho*I (CTCGAG): DMAR235 and DMAR236, which are presented in Table 1. The methods used for cloning BVH-P6 into an expression vector and sequencing are similar to the methods described in Example 1.

It was determined that the open reading frame (ORF) which codes for BVH-P6 contains 1020-bp and encodes a 339 amino acid residues polypeptide with a predicted pI of 6.66 and a predicted molecular mass of 38,017.78 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :10) using the Spscan sofware (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 33 amino acid residues signal peptide (MRKRCYSTSAAVLAAVTLFVLSVDRGVIADSFS), which ends with a cleavage site situated between a serine and an alanine residues. The BVH-P6 gene was shown to be present after PCR amplification using the oligonucleotide primers DMAR235 and DMAR236 in the 4 serologically S. pyogenes strains tested,(Table 2). The methods used for PCR amplification of the BVH-P6 gene were similar to the methods presented in example 1. No such product was detected

when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

EXAMPLE 6

This example illustrates the cloning of S. pyogenes genes in CMV plasmid pCMV-GH.

The DNA coding regions of S. pyogenes proteins were inserted in phase downstream of a human growth hormone (hGH) gene which was under the transcriptional control of the cytomegalovirus (CMV) promotor in the plasmid vector pCMV-GH (Tang et al., Nature, 1992, 356 :152). The CMV promotor is a non functional plasmid in E. coli cells but active upon administration of the plasmid in eukaryotic cells. The vector also incorporated the ampicillin resistance gene.

The coding regions of BVH-P2 (SEQ ID NO: 1), BVH-P4 (SEQ ID NO: 5), BVH-P5 (SEQ ID NO: 7), and BVH-P6 (SEQ ID NO: 9) genes without their leader peptide regions were amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, Ca) from from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using oligonucleotide primers that contained base extensions for the addition of restriction sites *BamHI* (GGATCC) and *SalI* (GTCGAC) which are described in Table 1. The PCR products were purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA), digested with restriction enzymes (Pharmacia Canada Inc, Baie d'Urfe, Canada). The pCMV-GH vector (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas) was digested with *BamHI* and *SalI* and purified from agarose gel using the QIAquick gel extraction kit from QIAgen (Chatsworth, CA). The *BamHI*-*SalI* DNA fragments were ligated to the *BamHI*-*SalI* pCMV-GH vector to create the hGH-BVH-P2, hGH-BVH-P4, hGH-BVH-P5, and hGH-BVH-P6 fusion proteins under the control of the CMV promoter. The ligated products were transformed into E. coli strain DH5α [ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *endA1* *recA1* *hsdR17*(r_K-m_K+) *deoR* *thi*-1 *sup*E44 λ^- *gyrA96* *relA1*] (Gibco BRL,

Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). The recombinant pCMV plasmids were purified using a QIAgen plasmid kit (Chatsworth, CA) and the nucleotide sequences of the DNA inserts were verified by DNA sequencing.

EXAMPLE 7

This example illustrates the use of DNA to elicit an immune response to S. pyogenes protein antigens.

Groups of 8 female BALB/c mice (Charles River, St-Constant, Québec, Canada) were immunized by intramuscular injection of 100 µl three times at two- or three-week intervals with 50 µg of recombinant pCMV-GH encoding BVH-P2 (SEQ ID NO: 1), BVH-P4 (SEQ ID NO: 5), BVH-P5 (SEQ ID NO: 7), and BVH-P6 (SEQ ID NO: 9) genes in presence of 50 µg of granulocyte-macrophage colony-stimulating factor (GM-CSF)- expressing plasmid pCMV-GH-GM-CSF (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas). As control, groups of mice were injected with 50 µg of pCMV-GH in presence of 50 µg of pCMV-GH-GM-CSF. Blood samples were collected from the orbital sinus prior to each immunization and seven days following the third injection and serum antibody responses were determined by ELISA using the corresponding His-tagged labeled S. pyogenes recombinant proteins as coating antigens. The production and purification of these His-tagged labeled S. pyogenes recombinant proteins are presented in Example 8.

EXAMPLE 8

This example illustrates the production and purification of S. pyogenes recombinant proteins.

The recombinant pET-21b(+)plasmids with BVH-P2 (SEQ ID NO: 1), BVH-P3 (SEQ ID NO: 3), BVH-P4 (SEQ ID NO: 5), BVH-P5 (SEQ ID NO: 7), and BVH-P6 (SEQ ID NO: 9) were used to transform by electroporation (Gene Pulser II apparatus, BIO-RAD Labs,

Mississauga, Canada) E. coli strain BL21(DE3) (F^-ompT $hsdS_B$ ($r^-_Bm^-_B$) gal dcm (DE3)) (Novagen, Madison, WI). In this strain of E. coli, the T7 promotor controlling expression of the recombinant protein is specifically recognized by the T7 RNA polymerase (present on the λDE3 prophage) whose gene is under the control of the lac promotor which is inducible by isopropyl-ß-d-thio-galactopyranoside (IPTG). The transformants BL21(DE3)/rpET were grown at 37°C with agitation at 250 rpm in LB broth (peptone 10g/L, yeast extract 5g/L, NaCl 10g/L) containing 100 µg of carbenicillin (Sigma-Aldrich Canada Ltd., Oakville, Canada) per ml until the A_{600} reached a value of 0.6. In order to induce the production of His-tagged S. pyogenes recombinant proteins, the cells were incubated for 3 additional hours in the presence of IPTG at a final concentration of 1 mM. Induced cells from a 500 ml culture were pelleted by centrifugation and frozen at -70°C.

The purification of the recombinant proteins from the soluble cytoplasmic fraction of IPTG-induced BL21(DE3)/rpET21b(+) was done by affinity chromatography based on the properties of the His•Tag sequence (6 consecutive histidine residues) to bind to divalent cations (Ni^{2+}) immobilized on the His•Bind metal chelation resin. Briefly, the pelleted cells obtained from a 500 mL culture induced with IPTG was resuspended in lysis buffer (20 mM Tris, 500 mM NaCl, 10 mM imidazole, pH 7.9) containing 1mM PMSF, sonicated and centrifuged at 12,000 X g for 20 min to remove debris. The supernatant was deposited on a Ni-NTA agarose column (Qiagen, Mississauga, Ontario, Canada). The His-tagged labeled S. pyogenes recombinant proteins were eluted with 250 mM imidazole-500mM NaCl-20 mM Tris pH 7.9. The removal of the salt and imidazole from the samples was done by dialysis against PBS at 4°C. The quantities of recombinant proteins obtained from the soluble fraction of E. coli was estimated by MicroBCA (Pierce, Rockford, Illinois).

EXAMPLE 9

This example illustrates the reactivity of the His-tagged S. pyogenes recombinant proteins with human sera and sera collected from mice after immunization with S. pyogenes antigenic preparations

As shown in Table 3, all purified recombinant proteins were recognized in immunoblots by the antibodies present in the pool of normal sera. It indicates that humans which are normally in contact with S. pyogenes do develop antibodies that are specific to these proteins. These particular human antibodies might be implicated in the protection against S. pyogenes infection. In addition, immunoblots also revealed that sera collected from mice immunized with S. pyogenes antigenic preparation enriched membrane proteins which protected mice against lethal challenge also developed antibodies that recognized BVH-P3, BVH-P4 and BVH-P5 His-tagged recombinant proteins. This result indicates that these proteins were present in S. pyogenes antigenic preparation that protected mice against infection and that they induced antibodies that reacted with the corresponding His-tagged recombinant protein.

Table 3. Reactivity in immunoblots of human sera and sera collected from mice after immunization with S. pyogenes antigenic preparations with S. pyogenes His-tagged fusion recombinant proteins.

Purified recombinant protein I.D.[1]	**Apparent molecular weight (kDa)**[2]	**Reactivity in immunoblots with**	
		Human sera[3]	Mouse sera[4]
BVH-P2	25	+	-
BVH-P3	34	+	+
BVH-P4	35	+	+
BVH-P5	34	+	+
BVH-P6	35	+	-

[1]His-tagged recombinant proteins produced and purified as described in Example 7 were used to perform the immunoblots.

[2]Molecular weight of the His-tagged recombinant protein were estimated after SDS-PAGE.

[3]Two sera collected from healthy human volunteers were pooled together and diluted 1/500 to perform the immunoblots.

[4]Mouse sera collected after immunization with S. pyogenes antigenic preparations enriched membrane proteins were pooled and diluted 1/500 to perform the immunoblots. These mice were protected against a lethal S. pyogenes challenge.

EXAMPLE 10

This example illustrates the accessibility to antibodies of the S. pyogenes BVH-P4 polypeptide at the surface of intact streptococcal cells.

Bacteria were grown in Tood Hewitt (TH) broth (Difco Laboratories, Detroit MI) with 0.5% Yeast extract (Difco Laboratories) and 0.5% peptone extract (Merck, Darmstadt, Germany) at 37°C in a 8% CO_2 atmosphere to give an OD_{490nm} of 0.600 (~10^8 CFU/ml). Dilutions of anti-BVH-P4 or control sera were then added and allowed to bind to the cells, which were incubated for 2 h at 4°C. Samples were washed 4 times in blocking buffer [phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA)], and then 1 ml of goat fluorescein (FITC)-conjugated anti-mouse IgG + IgM diluted in blocking buffer was added. After an additional incubation of 60 min at room temperature, samples were washed 4 times in blocking buffer and fixed with 0.25 % formaldehyde in PBS buffer for 18-24 h at 4°C. Cells were washed 2 times in PBS buffer and resuspended in 500 µl of PBS buffer. Cells were kept in the dark at 4°C until analyzed by flow cytometry (Epics® XL; Beckman Coulter, Inc.). Flow cytometric analysis revealed that BVH-P4-specific antibodies efficiently recognized their corresponding surface exposed epitopes on the heterologous (ATCC12384; serotype M3) S.

pyogenes strain tested. It was determined that more than 90 % of the 10,000 S. pyogenes cells analyzed were labeled with the antibodies present in the BVH-P4 specific anti-sera. It appears that the BVH-P4 polypeptide is accessible at the surface where it can be recognized by antibodies.

EXAMPLE 11

This example illustrates the protection against fatal S. pyogenes infection induced by passive immunization of mice with rabbit hyper-immune sera.

New Zealand rabbits (Charles River laboratories, St-Constant, Canada) are injected subcutaneously at multiple sites with 50 μg and 100 μg of the different His-tagged S. pyogenes recombinant proteins that were produced and purified as described in Example 8 and adsorbed to Alhydrogel adjuvant (Superfos Biosector a/s). Rabbits are immunized three times at three-week intervals with the different His-tagged S. pyogenes recombinant proteins. Blood samples are collected three weeks after the third injection. The antibodies present in the serum are purified by precipitation using 40% saturated ammonium sulfate. Groups of 10 female CD-1 mice (Charles River) are injected intravenously with 500 μl of purified serum collected from rabbits immunized with the different His-tagged S. pyogenes recombinant proteins, or rabbits immunized with an unrelated control recombinant protein. Eighteen hours later the mice are challenged with approximately $2x10^7$ CFU of the type 3 S. pyogenes strain ATCC12384. Samples of the S. pyogenes challenge inoculum are plated on blood agar plates to determine the CFU and to verify the challenge dose. Deaths are recorded for a period of 5 days.

EXAMPLE 12

This example illustrates the protection of mice against fatal S. pyogenes infection induced by immunization.

Groups of 8 female CD-1 mice (Charles River) are immunized subcutaneously three times at three-week intervals with 20 μg of affinity purified His-tagged *S. pyogenes* recombinant proteins in presence of 10 μg of QuilA adjuvant (Cedarlane Laboratories Ltd, Hornby, Canada) or, as control, with QuilA adjuvant alone in PBS. Blood samples are collected from the orbital sinus on day 1, 22 and 43 prior to each immunization and seven days (day 50) following the third injection. Two weeks later the mice are challenged with approximately $2x10^7$ CFU of the type 3 *S. pyogenes* strain ATCC12384. Samples of the *S. pyogenes* challenge inoculum are plated on blood agar plates to determine the CFU and to verify the challenge dose. Deaths are recorded for a period of 14 days.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

The claims defining the invention are as follows:

1. An isolated polynucleotide comprising:

(a) a polynucleotide encoding a polypeptide that comprises an amino acid sequence at least 70% identical to the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(b) a polynucleotide encoding a polypeptide that comprises an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(c) a polynucleotide encoding a polypeptide that comprises an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(d) a polynucleotide encoding a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(e) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 10; or

(f) a polynucleotide encoding a polypeptide comprising an immunogenic fragment consisting of at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*.

2. An isolated polynucleotide that is complementary to the polynucleotide of claim 1.

3. The polynucleotide of claim 1, wherein said polynucleotide is DNA.

4. The polynucleotide of claim 1, wherein said polynucleotide is RNA.

5. An isolated polynucleotide that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 10.

6. A vector comprising the polynucleotide of any one of claims 1 to 5, wherein the polynucleotide is operably linked to an expression control region.

7. A host cell transfected with the vector of claim 6.

8. A process for producing the polypeptide encoded by the polynucleotide of claim 1, said process comprising culturing the host cell according to claim 7 under conditions suitable for expression of the polypeptide.

9. An isolated polypeptide comprising:

(a) a polypeptide comprising an amino acid sequence at least 70% identical to the amino acid sequence set forth in

SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(b) a polypeptide comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(c) a polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(d) a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(e) a polypeptide capable of generating antibodies having binding specificity for a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO: 10;

(f) a polypeptide comprising an immunogenic fragment consisting of at least 10 contiguous amino acids of the amino acid sequence set forth in SEQ ID NO: 10, wherein the polypeptide can elicit an immune response to *Streptococcus pyogenes*;

(g) the polypeptide of (a), (b), (c), or (d) wherein the N-terminal Met residue is deleted; or

(h) the polypeptide of (a), (b), (c), or (d) wherein the secretory amino acid sequence is deleted.

10. A chimeric polypeptide comprising two or more immunogenic polypeptide fragments, wherein each of the two or more immunogenic polypeptide fragments comprise at least 10 contiguous amino acid residues from a polypeptide consisting of SEQ ID NO: 10, provided that the two or more

2002216863 12 Feb 2008

immunogenic polypeptide fragments are linked to form a chimeric polypeptide.

11. A pharmaceutical composition comprising the polypeptide according to claim 9 and a pharmaceutically acceptable carrier or diluent.

12. A pharmaceutical composition comprising the chimeric polypeptide according to claim 10 and a pharmaceutically acceptable carrier or diluent.

13. The pharmaceutical composition of claim 11 or 12 further comprising a pharmaceutically acceptable adjuvant.

14. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to the polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:10.

15. The antibody, or antigen-binding fragment thereof, according to claim 14 wherein the antibody is a polyclonal antibody or antigen-binding fragment thereof, or monoclonal antibody or antigen-binding fragment thereof.

16. The antibody, or antigen-binding fragment thereof, according to claim 14, wherein the antibody, or antigen-binding fragment thereof, is murine, rat, or human.

17. A method for therapeutic or prophylactic treatment of pharyngitis, erysipelas, impetigo, scarlet fever, bacteremia, necrotizing fasciitis, or toxic shock in a host susceptible to pharyngitis, erysipelas, impetigo, scarlet

fever, bacteremia, necrotizing fasciitis, or toxic shock, said method comprising administering to the host a therapeutic or prophylactic amount of the pharmaceutical composition according to any one of claims 11-13.

18. A method for therapeutic or prophylactic treatment of *Streptococcus pyogenes* bacterial infection in a host susceptible to *Streptococcus pyogenes* infection, said method comprising administering to the host a therapeutic or prophylactic amount of the pharmaceutical composition according to any one of claims 11-13.

19. The method according to claim 18 wherein the host is a human or non-human animal.

20. A method for diagnosing a streptococcal infection in a host susceptible to streptococcal infection comprising:

(a) obtaining a biological sample from the host;

(b) incubating an antibody, or antigen-binding fragment thereof, that specifically binds to the polypeptide according to claim 9 with the biological sample to form a mixture; and

(c) detecting specifically bound antibody or bound antigen-binding fragment in the mixture which indicates the presence of a streptococcal infection.

21. A method for detecting an antibody that specifically binds to *Streptococcus pyogenes* in a host susceptible to streptococcal infection comprising:

(a) obtaining a biological sample from a host;

(b) incubating one or more polypeptides according to claim 9 with the biological sample to form a mixture; and

(c) detecting specifically bound polypeptide in the mixture which indicates the presence of an antibody specific to that specifically binds to *Streptococcus pyogenes*.

22. Use of the pharmaceutical composition according to any one of claims 11 to 13 for the preparation of a medicament for the prophylactic or therapeutic treatment of pharyngitis, erysipelas, impetigo, scarlet fever, bacteremia, necrotizing fasciitis, or toxic shock in a host susceptible to pharyngitis, erysipelas, impetigo, scarlet fever, bacteremia, necrotizing fasciitis, or toxic shock.

23. Use of the pharmaceutical composition according to any one of claims 11-13 for preparation of a medicament for the prophylactic or therapeutic treatment of a streptococcal infection in a host susceptible to the streptococcal infection, wherein the streptococcal infection is a *Streptococcus pyogenes* infection.

24. Use according to claim 23 wherein the host is a human or non-human animal.

25. Kit comprising the isolated polypeptide according to claim 9 for detection or diagnosis of a streptococcal infection.

26. An isolated polynucleotide according to any one of claims 1 to 5, or polypeptide encoded thereby, substantially as herein described with reference to Example 5.

Figure 1; SEQ ID NO: 1.

```
   1 ATGAAAAAGA CATTAACTTT GCTACTGGCA CTCTTTGCCA TCGGGGTAAC TAGTAGCGTC
  61 AGAGCGGAGG ATGAACAAAG TAGTACACAA AAGCCAGTAA AATTTGATTT GGATGGACCT
 121 CAACAAAAAA TTAAAGATTA TAGTGGCAAC ACAATCACTC TAGAAGACTT ATATGTTGGT
 181 AGTAAAGTAG TAAAAATATA TATCCCTCAA GGATGGTGGG TATATCTTTA CAGACAATGT
 241 GATCATAACA GTAAAGAACG AGGAATTTTA GCTAGTCCTA TTCTCGAAAA AAATATAACA
 301 AAAACAGATC CTTATCGTCA ATATTATACA GGAGTACCTT ATATTCTTAA CTTAGGAGAA
 361 GATCCTTTGA AGAAAGGAGA AAAATTAACT TTCTCATTTA AAGGAGAAGA CGGATTTTAT
 421 GTCGGTAGCT ATATCTATAG AGACTCTGAT ACTATAAAAA AAGAAAAAGA AGCTGAAGAA
 481 GCACTTCAAA AAAAGGAAGA GGAAAAGCAA CAAAAACAGC TAGAAGAAAG CATGCTAAAG
 541 CAGATAAGAG AAGAAGACCA TAAACCTTGG CATCAGCGGT TAAGTGAGAG CATCCAAGAT
 601 CAGTGGTGGA ACTTTAAGGG ACTGTTTCAG TGA
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Figure 2; SEQ ID NO: 2

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   1 MKKTLTLLLA LFAIGVTSSV RAEDEQSSTQ KPVKFDLDGP QQKIKDYSGN TITLEDLYVG
  61 SKVVKIYIPQ GWWVYLYRQC DHNSKERGIL ASPILEKNIT KTDPYRQYYT GVPYILNLGE
 121 DPLKKGEKLT FSFKGEDGFY VGSYIYRDSD TIKKEKEAEE ALQKKEEEKQ QKQLEESMLK
 181 QIREEDHKPW HQRLSESIQD QWWNFKGLFQ
```

Figure 3; SEQ ID NO: 3.

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   1 ATGAGCCTCA TTTTGGGTGC TTTTTTATCT GTTTTTCTTT TAGTAGCTTG TTCGTCAACT
  61 GGCACTAAAA CTGCTAAGAG TGATAAATTA AAAGTCGTGG CAACCAATTC AATTATTGCC
 121 GACATGACAA AAGCTATTGC TGGTGATAAA ATCGATCTGC ACAGCATTGT GCCAATCGGT
 181 CAAGACCCTC ATGAGTACGA ACCATTACCA GAAGATGTTG AAAAAACAAG TAATGCTGAT
 241 GTGATTTTCT ATAATGGTAT CAATCTAGAA GATGGCGGGC AAGCTTGGTT CACCAAACTA
 301 GTGAAAAATG CTCAAAAAAC GAAAAACAAA GATTACTTTG CCGTGTCTGA TGGCATTGAT
 361 GTGATTTACT TGGAAGGTGC AAGCGAAAAA GGAAAAGAAG ATCCACATGC TTGGTTAAAT
 421 CTCGAAAACG GAATCATTTA TTCAAAAAAC ATTGCCAAAC AATTGATTGC AAAGGATCCT
 481 AAAAACAAAG AAACTTATGA AAAGAACCTA AAAGCTTATG TGGCTAAATT GGAAAAACTA
 541 GACAAAGAAG CCAAATCAAA ATTTGATGCT ATTGCAGAAA ATAAAAAATT GATTGTGACT
 601 AGTGAAGGCT GCTTCAAGTA CTTTTCAAAA GCTTACGGTG TCCCATCTGC TTATATCTGG
 661 GAAATTAACA CCGAAGAAGA AGGAACACCA GATCAAATTT CATCATTGAT TGAAAAACTA
 721 AAAGTCATCA AGCCATCTGC GCTTTTTGTA GAGTCAAGTG TCGATAGACG CCCTATGGAA
 781 ACTGTTTCTA AAGATAGTGG TATTCCTATT TATTCTGAGA TCTTTACAGA TTCAATTGCT
 841 AAAAAAGGTA AACCTGGCGA TAGTTATTAT GCTATGATGA AATGGAACCT TGACAAAATT
 901 TCTGAAGGTC TAGCAAAATA A
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Figure 4; SEQ ID NO: 4

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   1 MSLILGAFLS VFLLVACSST GTKTAKSDKL KVVATNSIIA DMTKAIAGDK IDLHSIVPIG
  61 QDPHEYEPLP EDVEKTSNAD VIFYNGINLE DGGQAWFTKL VKNAQKTKNK DYFAVSDGID
 121 VIYLEGASEK GKEDPHAWLN LENGIIYSKN IAKQLIAKDP KNKETYEKNL KAYVAKLEKL
 181 DKEAKSKFDA IAENKKLIVT SEGCFKYFSK AYGVPSAYIW EINTEEEGTP DQISSLIEKL
 241 KVIKPSALFV ESSVDRRPME TVSKDSGIPI YSEIFTDSIA KKGKPGDSYY AMMKWNLDKI
 301 SEGLAK
```

Figure 5; SEQ ID NO: 5.

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   1 ATGAACAAGA AATTTATTGG TCTTGGTTTA GCGTCAGTGG CTGTGCTGAG TTTAGCTGCT
  61 TGTGGTAATC GTGGTGCTTC TAAAGGTGGG GCATCAGGAA AAACTGATTT AAAAGTTGCA
 121 ATGGTTACCG ATACTGGTGG TGTAGATGAC AAATCATTCA ACCAATCAGC ATGGGAAGGC
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 181 CTGCAATCTT GGGGTAAAGA AATGGGCCTT CAAAAAGGAA CAGGTTTCGA TTATTTTCAA
 241 TCTACAAGTG AATCTGAGTA TGCAACTAAT CTCGATACAG CAGTTTCAGG AGGGTATCAA
 301 CTGATTTATG GTATCGGCTT TGCATTGAAA GATGCTATTG CTAAAGCAGC TGGAGATAAT
 361 GAAGGAGTTA AGTTTGTTAT TATCGATGAT ATTATCGAAG GAAAAGATAA TGTAGCCAGT
 421 GTTACCTTTG CCGACCATGA AGCTGCTTAT CTTGCAGGAA TTGCAGCTGC AAAAACAACA
 481 AAAACAAAAA CAGTTGGTTT CGTGGGCGGT ATGGAAGGAA CTGTCATAAC TCGATTTGAA
 541 AAAGGTTTTG AAGCAGGAGT TAAGTCTGTT GACGATACAA TCCAAGTTAA AGTTGATTAT
 601 GCTGGATCAT TTGGTGACGC TGCAAAAGGA AAAACAATCG CAGCAGCTCA GTATGCAGCA
 661 GGTGCTGATG TTATTTACCA GGCAGCAGGA GGCACTGGAG CAGGTGTATT TAATGAAGCA
 721 AAAGCTATTA ATGAAAAACG TAGTGAAGCT GATAAAGTTT GGGTTATTGG TGTTGACCGT
 781 GATCAAAAAG ACGAAGGAAA ATACACTTCT AAAGATGGCA AAGAAGCAAA CTTTGTACTT
 841 GCATCATCAA TCAAAGAAGT CGGTAAAGCT GTTCAGTTAA TCAACAAGCA AGTAGCAGAT
 901 AAAAAATTCC CTGGAGGAAA AACAACTGTC TATGGTCTAA AAGATGGCGG TGTTGAAATC
 961 GCAACTACAA ATGTTTCAAA AGAAGCTGTT AAAGCTATTA AAGAAGCGAA AGCAAAAATT
1021 AAATCTGGTG ACATTAAAGT TCCTGAAAAA TAG
```

Figure 6; SEQ ID NO: 6

```
   1 MNKKFIGLGL ASVAVLSLAA CGNRGASKGG ASGKTDLKVA MVTDTGGVDD KSFNQSAWEG
  61 LQSWGKEMGL QKGTGFDYFQ STSESEYATN LDTAVSGGYQ LIYGIGFALK DAIAKAAGDN
 121 EGVKFVIIDD IIEGKDNVAS VTFADHEAAY LAGIAAAKTT KTKTVGFVGG MEGTVITRFE
 181 KGFEAGVKSV DDTIQVKVDY AGSFGDAAKG KTIAAAQYAA GADVIYQAAG GTGAGVFNEA
 241 KAINEKRSEA DKVWVIGVDR DQKDEGKYTS KDGKEANFVL ASSIKEVGKA VQLINKQVAD
 301 KKFPGGKTTV YGLKDGGVEI ATTNVSKEAV KAIKEAKAKI KSGDIKVPEK
```

Figure 7; SEQ ID NO: 7.

```
   1 ATGAACAAAA AAGTAATGTC ACTTGGTCTT GTTTCGACTG CCCTATTCAC ATTAGGAGGC
  61 TGTACCAATA ACTCCGCTAA ACAAACAACT GACAATTCAT TAAAAATCGC TATGATTACT
 121 AATCAGACGG GTATTGATGA CAAGTCATTT AACCAGTCAG CCTGGGAAGG CTTACAAGCT
 181 TGGGGAAAAG AAAATAAACT TGAAAAAGGA AAAGGCTATG ATTATTTCCA ATCAGCCAAT
 241 GAATCAGAGT TTACCACAAA CCTTGAGTCA GCAGTAACCA ATGGTTATAA TCTTGTTTTT
 301 GGGATTGGAT TTCCATTACA TGACGCTGTA GAAAAAGTAG CCGCAAACAA TCCTGACAAC
 361 CATTTTGCAA TTGTGGATGA TGTGATTAAA GGTCAAAAAA ATGTTGCAAG TATCACCTTT
 421 TCAGACCATG AAGCGGCATA CCTAGCCGGT GTTGCAGCAG CTAAAACGAC AAAAACCAAG
 481 CAAGTTGGTT TTGTAGGTGG TATGGAAGGA GATGTTGTCA AGCGCTTTGA AAAAGGTTTT
 541 GAAGCTGGTG TGAAATCAGT AGATGATACC ATCAAAGTAA GAGTTGCTTA TGCAGGCTCT
 601 TTTGCAGATG CTGCCAAAGG CAAGACGATT GCAGCTGCTC AATACGCTGA AGGCGCAGAT
 661 GTTATTTATC ATGCAGCAGG AGGCACAGGG GCGGGTGTCT TTAGCGAAGC TAAGTCTATC
 721 AACGAAAAAC GTAAAGAAGA AGATAAGGTT TGGGTTATTG GTGTTGACCG TGACCAAAGT
 781 GAAGATGGAA AATACACTAC AAAAGATGGC AAGTCAGCTA ATTTTGTTTT GACCTCAAGT
 841 ATCAAGGAAG TCGGAAAAGC TTTAGTAAAA GTAGCCGTAA AAACCTCAGA AGACCAATTC
 901 CCAGGTGGTC AAATAACCAC TTTTGGTTTA AAAGAAGGTG GTGTTAGCCT TACAACGGAT
 961 GCTCTGACAC AAGACACTAA AAAAGCTATT GAGGCTGCTA AAAAAGCGAT TATCGAAGGA
1021 ACCATCACAG TTCCTGAAAA CTAA
```

Figure 8; SEQ ID NO: 8

```
   1 MNKKVMSLGL VSTALFTLGG CTNNSAKQTT DNSLKIAMIT NQTGIDDKSF NQSAWEGLQA
  61 WGKENKLEKG KGYDYFQSAN ESEFTTNLES AVTNGYNLVF GIGFPLHDAV EKVAANNPDN
 121 HFAIVDDVIK GQKNVASITF SDHEAAYLAG VAAAKTTKTK QVGFVGGMEG DVVKRFEKGF
 181 EAGVKSVDDT IKVRVAYAGS FADAAKGKTI AAAQYAEGAD VIYHAAGGTG AGVFSEAKSI
 241 NEKRKEEDKV WVIGVDRDQS EDGKYTTKDG KSANFVLTSS IKEVGKALVK VAVKTSEDQF
 301 PGGQITTFGL KEGGVSLTTD ALTQDTKKAI EAAKKAIIEG TITVPEN
```

Figure 9; SEQ ID NO: 9.

```
   1 ATGAGAAAAA GATGCTATTC AACTTCAGCT GCAGTATTGG CAGCAGTGAC TTTATTTGTT
  61 CTATCGGTAG ATCGTGGTGT TATAGCAGAT AGTTTTTCTG CTAATCAAGA GATTAGATAT
 121 TCGGAAGTAA CACCTTATCA CGTTACTTCC GTTTGGACCA AAGGAGTTAC TCCTCCAGCA
 181 AACTTCACTC AAGGTGAAGA TGTTTTTCAC GCTCCTTATG TTGCTAACCA AGGATGGTAT
 241 GATATTACCA AAACATTCAA TGGAAAAGAC GATCTTCTTT GCGGGGCTGC CACAGCAGGG
 301 AATATGCTTC ACTGGTGGTT CGATCAAAAC AAAGACCAAA TTAAACGTTA TTTGGAAGAG
 361 CATCCAGAAA AGCAAAAAAT AAACTTCAAT GGCGAACAGA TGTTTGACGT AAAAGAAGCT
 421 ATCGACACTA AAAACCACCA GCTAGATAGT AAATTATTTG AATATTTTAA AGAAAAAGCT
 481 TTCCCTTATC TATCTACTAA ACACCTAGGA GTTTTCCCTG ATCATGTAAT TGATATGTTC
 541 ATTAACGGCT ACCGCCTTAG TCTAACTAAC CACGGTCCAA CGCCAGTAAA AGAAGGTAGT
 601 AAAGATCCCC GAGGTGGTAT TTTTGACGCC GTATTTACAA GAGGTGATCA AAGTAAGCTA
 661 TTGACAAGTC GTCATGATTT TAAAGAAAAA AATCTCAAAG AAATCAGTGA TCTCATTAAG
 721 AAAGAGTTAA CCGAAGGCAA GGCTCTAGGC CTATCACACA CCTACGCTAA CGTACGCATC
 781 AACCATGTTA TAAACCTGTG GGGAGCTGAC TTTGATTCTA ACGGGAACCT TAAAGCTATT
 841 TATGTAACAG ACTCTGATAG TAATGCATCT ATTGGTATGA AGAAATACTT TGTTGGTGTT
 901 AATTCCGCTG GAAAAGTAGC TATTTCTGCT AAAGAAATAA AAGAAGATAA TATTGGTGCT
 961 CAAGTACTAG GGTTATTTAC ACTTTCAACA GGGCAAGATA GTTGGAATCA GACCAATTAA
```

Figure 10; SEQ ID NO: 10

```
   1 MRKRCYSTSA AVLAAVTLFV LSVDRGVIAD SFSANQEIRY SEVTPYHVTS VWTKGVTPPA
  61 NFTQGEDVFH APYVANQGWY DITKTFNGKD DLLCGAATAG NMLHWWFDQN KDQIKRYLEE
 121 HPEKQKINFN GEQMFDVKEA IDTKNHQLDS KLFEYFKEKA FPYLSTKHLG VFPDHVIDMF
 181 INGYRLSLTN HGPTPVKEGS KDPRGGIFDA VFTRGDQSKL LTSRHDFKEK NLKEISDLIK
 241 KELTEGKALG LSHTYANVRI NHVINLWGAD FDSNGNLKAI YVTDSDSNAS IGMKKYFVGV
 301 NSAGKVAISA KEIKEDNIGA QVLGLFTLST GQDSWNQTN
```

Figure 11; SEQ ID NO: 11.

```
   1 TCTTGGTTTA GCGTCAGTGG CTGTGCTGAG TTTAGCTGCT TGTGGTAATC GTGGTGCTTC
  61 TAAAGGTGGG GCATCAGGAA AAACTGATTT AAAAGTTGCA ATGGTTACCG ATACTGGTGG
 121 TGTAGATGAC AAATCATTCA ACCAATCAGC ATGGGAAGGC CTGCAATCTT GGGGTAAAGA
 181 AATGGGCCTT CAAAAAGGAA CAGGTTTCGA TTATTTTCAA TCTACAAGTG AATCTGAGTA
 241 TGCAACTAAT CTTGATACAG CAGTTTCAGG AGGGTATCAA CTGATTTATG GTATCGGCTT
 301 TGCATTGAAA GATGCTATTG CTAAAGCAGC TGGAGATAAT GAAGGAGTTA AGTTTGTTAT
 361 TATCGATGAT ATTATCGAAG GAAAAGATAA TGTAGCCAGT GTTACCTTTG CTGACCATGA
 421 AGCTGCTTAT CTTGCAGGAA TTGCAGCTGC AAAAACAACA AAAACAAAAA CAGTTGGTTT
 481 CGTGGGCGGT ATGGAAGGAA CTGTCATAAC TCGATTTGAA AAAGGTTTTG AAGCAGGAGT
 541 TAAGTCTGTT GACGATACAA TCCAAGTTAA AGTTGATTAT GCTGGATCAT TTGGTGACGC
 601 TGCAAAAGGA AAAACAATCG CAGCAGCTCA GTATGCAGCA GGTGCTGATG TTATTTACCA
 661 GGCAGCAGGA GGCACTGGAG CAGGTGTATT TAATGAAGCA AAAGCTATTA ATGAAAAACG
 721 TAGTGAAGCT GATAAAGTTT GGGTTATTGG TGTTGACCGT GATCAAAAAG ACGAAGGAAA
 781 ATACACTTCT AAAGATGGCA AAGAAGCAAA CTTTGTACTT GCATCATCAA TCAAAGAAGT
 841 TGGTAAAGCT GTTCAGTTAA TCAACAAACA AGTAGCAGAT AAAAAATTCC CTGGAGGAAA
 901 AACAACTGTC TATGGTCTAA AAGATGGCGG TGTTGAAATC GCAACTACAA ATGTTTCAAA
 961 AGAAGCTGTT AAAGCTATTA AAGAAGCGAA AGC
```

Figure 12; SEQ ID NO: 12.

```
   1 LGLASVAVLS LAACGNRGAS KGGASGKTDL KVAMVTDTGG VDDKSFNQSA WEGLQSWGKE
  61 MGLQKGTGFD YFQSTSESEY ATNLDTAVSG GYQLIYGIGF ALKDAIAKAA GDNEGVKFVI
 121 IDDIIEGKDN VASVTFADHE AAYLAGIAAA KTTKTKTVGF VGGMEGTVIT RFEKGFEAGV
 181 KSVDDTIQVK VDYAGSFGDA AKGKTIAAAQ YAAGADVIYQ AAGGTGAGVF NEAKAINEKR
 241 SEADKVWVIG VDRDQKDEGK YTSKDGKEAN FVLASSIKEV GKAVQLINKQ VADKKFPGGK
```

301 TTVYGLKDGG VEIATTNVSK EAVKAIKEAK

Figure 13; SEQ ID NO: 13.

```
   1 TCTTGGTTTA GCGTCACTGG CTGTGCTGAG TTTAGCTGCT TGTGGTAATC GTGGTGCTTC
  61 TAAAGGTGGG GCATCAGGAA AAACTGATTT AAAAGTTGCA ATGGTTACCG ATACTGGTGG
 121 TGTAGATGAC AAATCATTCA ACCAATCAGC ATGGGAAGGC CTGCAATCTT GGGGTAAAGA
 181 AATGGGCCTT CAAAAAGGAA CAGGTTTCGA TTATTTTCAA TCTACAAGTG AATCTGAGTA
 241 TGCAACTAAT CTCGATACAG CAGTTTCAGG AGGATATCAA CTGATTTATG GTATCGGCTT
 301 TGCATTGAAA GATGCTATTG CTAAAGCAGC TGGAGATAAT GAAGGAGTTA AGTTTGTTAT
 361 TATCGATGAT ATTATCGAAG GAAAAGATAA TGTAGCCAGT GTTACCTTTG CCGACCATGA
 421 AGCTGCTTAT CTTGCAGGAA TTGCGGCTGC AAAAACAACA AAAACAAAAA CAGTTGGTTT
 481 CGTGGGCGGT ATGGAAGGAA CTGTCATAAC TCGATTTGAA AAAGGTTTTG AAGCAGGAGT
 541 TAAGTCTGTT GACGATACAA TCCAAGTTAA AGTTGATTAT GCTGGATCAT TTGGTGACGC
 601 TGCAAAAGGA AAAACAATCG CAGCAGCTCA GTATGCAGCA GGTGCTGATG TTATTTACCA
 661 GGCAGCAGGA GGCACTGGAG CAGGTGTATT TAATGAAGCA AAAGCTATTA ATGAAAAACG
 721 TAGTGAAGCT GATAAAGTTT GGGTTATTGG TGTTGACCGT GATCAAAAAG ACGAAGGAAA
 781 ATACACTTCT AAAGATGGCA AAGAAGCAAA CTTTGTACTT GCATCATCAA TCAAAGAAGT
 841 TGGTAAAGCT GTTCAGTTAA TCAACAAACA AGTAGCAGAT AAAAAATTCC CTGGAGGAAA
 901 AACAACTGTC TATGGTTTAA AAGATGGCGG TGTTGAAATC GCAACTACAA ATGTTTCAAA
 961 AGAAGCTGTT AAAGCTATTA AAGAAGCGAA AGC
```

Figure 14; SEQ ID NO: 14.

```
   1 LGLASVAVLS LAACGNRGAS KGGASGKTDL KVAMVTDTGG VDDKSFNQSA WEGLQSWGKE
  61 MGLQKGTGFD YFQSTSESEY ATNLDTAVSG GYQLIYGIGF ALKDAIAKAA GDNEGVKFVI
 121 IDDIIEGKDN VASVTFADHE AAYLAGIAAA KTTKTKTVGF VGGMEGTVIT RFEKGFEAGV
 181 KSVDDTIQVK VDYAGSFGDA AKGKTIAAAQ YAAGADVIYQ AAGGTGAGVF NEAKAINEKR
 241 SEADKVWVIG VDRDQKDEGK YTSKDGKEAN FVLASSIKEV GKAVQLINKQ VADKKFPGGK
 301 TTVYGLKDGG VEIATTNVSK EAVKAIKEAK
```

Figure 15; SEQ ID NO: 15.

```
   1 TCTTGGTTTA GCGTCAGTGG CTGTGCTGAG TTTAGCTGCT TGTGGTAATC GTGGTGCTTC
  61 TAAAGGTGGG GCAGCAGGAA AAACTGATTT AAAAGTTGCA ATGGTTACCG ATACTGGTGG
 121 TGTAGATGAT AAATCATTCA ACCAATCAGC ATGGGAAGGC CTGCAATCTT GGGGTAAAGA
 181 AATGGGCCTT CAAAAAGGAA CAGGTTTCGA TTATTTTCAA TCTACAAGTG AATCTGAGTA
 241 TGCAACTAAT CTCGATACAG CAGTTTCAGG AGGGTATCAA CTGATTTATG GTATCGGCTT
 301 TGCATTGAAA GATGCTATTG CTAAAGCAGC TGGAGATAAT GAAGGAGTTA AGTTTGTTAT
 361 TATCGATGAT ATTATCGAAG GAAAAGATAA TGTAGCCAGT GTTACCTTTG CCGACCATGA
 421 AGCTGCTTAT CTTGCAGGAA TTGCAGCTGC AAAAACAACA AAAACAAAAA CAGTTGGTTT
 481 CGTGGGCGGT ATGGAAGGAA CTGTCATAAC TCGATTTGAA AAAGGTTTTG AAGCAGGAGT
 541 TAAGTCTGTT GACGATACAA TCCAAGTTAA AGTTGATTAT GCTGGATCAT TTGGTGACGC
 601 TGCAAAAGGA AAAACAATCG CAGCAGCTCA GTATGCAGCA GGTGCTGATG TTATTTACCA
 661 GGCAGCAGGA GGCACTGGAG CAGGTGTATT TAATGAAGCA AAAGCTATTA ATGAAAAACG
 721 TAGTGAAGCT GATAAAGTTT GGGTTATTGG TGTTGACCGT GATCAAAAAG ACGAAGGAAA
 781 ATACACTTCT AAAGATGGCA AAGAAGCAAA CTTTGTACTT GCATCATCAA TCAAAGAAGT
 841 TGGTAAAGCT GTTCAGTTAA TCAACAAGCA AGTAGCAGAT AAAAAATTCC CTGGAGGAAA
 901 AACAACTGTC TATGGTCTAA AAGATGGCGG TGTTGAAATC GCAACTACAA ATGTTTCAAA
 961 AGAAGCTGTT AAAGCTATTA AAGAAGCGAA AGC
```

Figure 16; SEQ ID NO: 16.

```
   1 LGLASVAVLS LAACGNRGAS KGGAAGKTDL KVAMVTDTGG VDDKSFNQSA WEGLQSWGKE
  61 MGLQKGTGFD YFQSTSESEY ATNLDTAVSG GYQLIYGIGF ALKDAIAKAA GDNEGVKFVI
```

```
121 IDDIIEGKDN VASVTFADHE AAYLAGIAAA KTTKTKTVGF VGGMEGTVIT RFEKGFEAGV
181 KSVDDTIQVK VDYAGSFGDA AKGKTIAAAQ YAAGADVIYQ AAGGTGAGVF NEAKAINEKR
241 SEADKVWVIG VDRDQKDEGK YTSKDGKEAN FVLASSIKEV GKAVQLINKQ VADKKFPGGK
301 TTVYGLKDGG VEIATTNVSK EAVKAIKEAK
```

Figure 17

```
700294     1 TCTTGGTTTAGCGTCAGTGGCTGTGCTGAGTTTAGCTGCTTGTGGTAATC  50
12384      1 TCTTGGTTTAGCGTCAGTGGCTGTGCTGAGTTTAGCTGCTTGTGGTAATC  50
SPY67      1 TCTTGGTTTAGCGTCAGTGGCTGTGCTGAGTTTAGCTGCTTGTGGTAATC  50
B514       1 TCTTGGTTTAGCGTCAGTGGCTGTGCTGAGTTTAGCTGCTTGTGGTAATC  50
             **************************************************

700294    51 GTGGTGCTTCTAAAGGTGGGGCATCAGGAAAAACTGATTTAAAAGTTGCA 100
12384     51 GTGGTGCTTCTAAAGGTGGGGCATCAGGAAAAACTGATTTAAAAGTTGCA 100
SPY67     51 GTGGTGCTTCTAAAGGTGGGGCATCAGGAAAAACTGATTTAAAAGTTGCA 100
B514      51 GTGGTGCTTCTAAAGGTGGGGCAGCAGGAAAAACTGATTTAAAAGTTGCA 100
             ********************** ************************

700294   101 ATGGTTACCGATACTGGTGGTGTAGATGACAAATCATTCAACCAATCAGC 150
12384    101 ATGGTTACCGATACTGGTGGTGTAGATGACAAATCATTCAACCAATCAGC 150
SPY67    101 ATGGTTACCGATACTGGTGGTGTAGATGACAAATCATTCAACCAATCAGC 150
B514     101 ATGGTTACCGATACTGGTGGTGTAGATGATAAATCATTCAACCAATCAGC 150
             ***************************** ********************

700294   151 ATGGGAAGGCCTGCAATCTTGGGGTAAAGAAATGGGCCTTCAAAAAGGAA 200
12384    151 ATGGGAAGGCCTGCAATCTTGGGGTAAAGAAATGGGCCTTCAAAAAGGAA 200
SPY67    151 ATGGGAAGGCCTGCAATCTTGGGGTAAAGAAATGGGCCTTCAAAAAGGAA 200
B514     151 ATGGGAAGGCCTGCAATCTTGGGGTAAAGAAATGGGCCTTCAAAAAGGAA 200
             **************************************************

700294   201 CAGGTTTCGATTATTTTCAATCTACAAGTGAATCTGAGTATGCAACTAAT 250
12384    201 CAGGTTTCGATTATTTTCAATCTACAAGTGAATCTGAGTATGCAACTAAT 250
SPY67    201 CAGGTTTCGATTATTTTCAATCTACAAGTGAATCTGAGTATGCAACTAAT 250
B514     201 CAGGTTTCGATTATTTTCAATCTACAAGTGAATCTGAGTATGCAACTAAT 250
             **************************************************

700294   251 CTCGATACAGCAGTTTCAGGAGGGTATCAACTGATTTATGGTATCGGCTT 300
12384    251 CTTGATACAGCAGTTTCAGGAGGGTATCAACTGATTTATGGTATCGGCTT 300
SPY67    251 CTCGATACAGCAGTTTCAGGAGGATATCAACTGATTTATGGTATCGGCTT 300
B514     251 CTCGATACAGCAGTTTCAGGAGGGTATCAACTGATTTATGGTATCGGCTT 300
             ** ******************* *************************

700294   301 TGCATTGAAAGATGCTATTGCTAAAGCAGCTGGAGATAATGAAGGAGTTA 350
12384    301 TGCATTGAAAGATGCTATTGCTAAAGCAGCTGGAGATAATGAAGGAGTTA 350
SPY67    301 TGCATTGAAAGATGCTATTGCTAAAGCAGCTGGAGATAATGAAGGAGTTA 350
B514     301 TGCATTGAAAGATGCTATTGCTAAAGCAGCTGGAGATAATGAAGGAGTTA 350
             **************************************************

700294   351 AGTTTGTTATTATCGATGATATTATCGAAGGAAAAGATAATGTAGCCAGT 400
12384    351 AGTTTGTTATTATCGATGATATTATCGAAGGAAAAGATAATGTAGCCAGT 400
SPY67    351 AGTTTGTTATTATCGATGATATTATCGAAGGAAAAGATAATGTAGCCAGT 400
B514     351 AGTTTGTTATTATCGATGATATTATCGAAGGAAAAGATAATGTAGCCAGT 400
             **************************************************

700294   401 GTTACCTTTGCCGACCATGAAGCTGCTTATCTTGCAGGAATTGCAGCTGC 450
12384    401 GTTACCTTTGCTGACCATGAAGCTGCTTATCTTGCAGGAATTGCAGCTGC 450
SPY67    401 GTTACCTTTGCCGACCATGAAGCTGCTTATCTTGCAGGAATTGCGGCTGC 450
B514     401 GTTACCTTTGCCGACCATGAAGCTGCTTATCTTGCAGGAATTGCAGCTGC 450
             *********** ********************************* *****

700294   451 AAAAACAACAAAAACAAAAACAGTTGGTTTCGTGGGCGGTATGGAAGGAA 500
12384    451 AAAAACAACAAAAACAAAAACAGTTGGTTTCGTGGGCGGTATGGAAGGAA 500
SPY67    451 AAAAACAACAAAAACAAAAACAGTTGGTTTCGTGGGCGGTATGGAAGGAA 500
B514     451 AAAAACAACAAAAACAAAAACAGTTGGTTTCGTGGGCGGTATGGAAGGAA 500
             **************************************************
```

```
700294   501 CTGTCATAACTCGATTTGAAAAAGGTTTTGAAGCAGGAGTTAAGTCTGTT 550
12384    501 CTGTCATAACTCGATTTGAAAAAGGTTTTGAAGCAGGAGTTAAGTCTGTT 550
SPY67    501 CTGTCATAACTCGATTTGAAAAAGGTTTTGAAGCAGGAGTTAAGTCTGTT 550
B514     501 CTGTCATAACTCGATTTGAAAAAGGTTTTGAAGCAGGAGTTAAGTCTGTT 550
             **************************************************

700294   551 GACGATACAATCCAAGTTAAAGTTGATTATGCTGGATCATTTGGTGACGC 600
12384    551 GACGATACAATCCAAGTTAAAGTTGATTATGCTGGATCATTTGGTGACGC 600
SPY67    551 GACGATACAATCCAAGTTAAAGTTGATTATGCTGGATCATTTGGTGACGC 600
B514     551 GACGATACAATCCAAGTTAAAGTTGATTATGCTGGATCATTTGGTGACGC 600
             **************************************************

700294   601 TGCAAAAGGAAAAACAATCGCAGCAGCTCAGTATGCAGCAGGTGCTGATG 650
12384    601 TGCAAAAGGAAAAACAATCGCAGCAGCTCAGTATGCAGCAGGTGCTGATG 650
SPY67    601 TGCAAAAGGAAAAACAATCGCAGCAGCTCAGTATGCAGCAGGTGCTGATG 650
B514     601 TGCAAAAGGAAAAACAATCGCAGCAGCTCAGTATGCAGCAGGTGCTGATG 650
             **************************************************

700294   651 TTATTTACCAGGCAGCAGGAGGCACTGGAGCAGGTGTATTTAATGAAGCA 700
12384    651 TTATTTACCAGGCAGCAGGAGGCACTGGAGCAGGTGTATTTAATGAAGCA 700
SPY67    651 TTATTTACCAGGCAGCAGGAGGCACTGGAGCAGGTGTATTTAATGAAGCA 700
B514     651 TTATTTACCAGGCAGCAGGAGGCACTGGAGCAGGTGTATTTAATGAAGCA 700
             **************************************************

700294   701 AAAGCTATTAATGAAAAACGTAGTGAAGCTGATAAAGTTTGGGTTATTGG 750
12384    701 AAAGCTATTAATGAAAAACGTAGTGAAGCTGATAAAGTTTGGGTTATTGG 750
SPY67    701 AAAGCTATTAATGAAAAACGTAGTGAAGCTGATAAAGTTTGGGTTATTGG 750
B514     701 AAAGCTATTAATGAAAAACGTAGTGAAGCTGATAAAGTTTGGGTTATTGG 750
             **************************************************

700294   751 TGTTGACCGTGATCAAAAAGACGAAGGAAAATACACTTCTAAAGATGGCA 800
12384    751 TGTTGACCGTGATCAAAAAGACGAAGGAAAATACACTTCTAAAGATGGCA 800
SPY67    751 TGTTGACCGTGATCAAAAAGACGAAGGAAAATACACTTCTAAAGATGGCA 800
B514     751 TGTTGACCGTGATCAAAAAGACGAAGGAAAATACACTTCTAAAGATGGCA 800
             **************************************************


700294   801 AAGAAGCAAACTTTGTACTTGCATCATCAATCAAAGAAGTCGGTAAAGCT 850
12384    801 AAGAAGCAAACTTTGTACTTGCATCATCAATCAAAGAAGTTGGTAAAGCT 850
SPY67    801 AAGAAGCAAACTTTGTACTTGCATCATCAATCAAAGAAGTTGGTAAAGCT 850
B514     801 AAGAAGCAAACTTTGTACTTGCATCATCAATCAAAGAAGTTGGTAAAGCT 850
             ************************************** *********

700294   851 GTTCAGTTAATCAACAAGCAAGTAGCAGATAAAAAATTCCCTGGAGGAAA 900
12384    851 GTTCAGTTAATCAACAAACAAGTAGCAGATAAAAAATTCCCTGGAGGAAA 900
SPY67    851 GTTCAGTTAATCAACAAACAAGTAGCAGATAAAAAATTCCCTGGAGGAAA 900
B514     851 GTTCAGTTAATCAACAAGCAAGTAGCAGATAAAAAATTCCCTGGAGGAAA 900
             ***************** ********************************

700294   901 AACAACTGTCTATGGTCTAAAAGATGGCGGTGTTGAAATCGCAACTACAA 950
12384    901 AACAACTGTCTATGGTCTAAAAGATGGCGGTGTTGAAATCGCAACTACAA 950
SPY67    901 AACAACTGTCTATGGTTTAAAAGATGGCGGTGTTGAAATCGCAACTACAA 950
B514     901 AACAACTGTCTATGGTCTAAAAGATGGCGGTGTTGAAATCGCAACTACAA 950
             ***************** ********************************

700294   951 ATGTTTCAAAAGAAGCTGTTAAAGCTATTAAAGAAGCGAAAGC 993
12384    951 ATGTTTCAAAAGAAGCTGTTAAAGCTATTAAAGAAGCGAAAGC 993
SPY67    951 ATGTTTCAAAAGAAGCTGTTAAAGCTATTAAAGAAGCGAAAGC 993
B514     951 ATGTTTCAAAAGAAGCTGTTAAAGCTATTAAAGAAGCGAAAGC 993
             *******************************************
```

Figure 18

```
700294     1 LGLASVAVLSLAACGNRGASKGGASGKTDLKVAMVTDTGGVDDKSFNQSA  50
12384      1 LGLASVAVLSLAACGNRGASKGGASGKTDLKVAMVTDTGGVDDKSFNQSA  50
```

```
SPY67      1 LGLASVAVLSLAACGNRGASKGGASGKTDLKVAMVTDTGGVDDKSFNQSA  50
B514       1 LGLASVAVLSLAACGNRGASKGGAAGKTDLKVAMVTDTGGVDDKSFNQSA  50
             ************************.************************

700294    51 WEGLQSWGKEMGLQKGTGFDYFQSTSESEYATNLDTAVSGGYQLIYGIGF 100
12384     51 WEGLQSWGKEMGLQKGTGFDYFQSTSESEYATNLDTAVSGGYQLIYGIGF 100
SPY67     51 WEGLQSWGKEMGLQKGTGFDYFQSTSESEYATNLDTAVSGGYQLIYGIGF 100
B514      51 WEGLQSWGKEMGLQKGTGFDYFQSTSESEYATNLDTAVSGGYQLIYGIGF 100
             **************************************************

700294   101 ALKDAIAKAAGDNEGVKFVIIDDIIEGKDNVASVTFADHEAAYLAGIAAA 150
12384    101 ALKDAIAKAAGDNEGVKFVIIDDIIEGKDNVASVTFADHEAAYLAGIAAA 150
SPY67    101 ALKDAIAKAAGDNEGVKFVIIDDIIEGKDNVASVTFADHEAAYLAGIAAA 150
B514     101 ALKDAIAKAAGDNEGVKFVIIDDIIEGKDNVASVTFADHEAAYLAGIAAA 150
             **************************************************

700294   151 KTTKTKTVGFVGGMEGTVITRFEKGFEAGVKSVDDTIQVKVDYAGSFGDA 200
12384    151 KTTKTKTVGFVGGMEGTVITRFEKGFEAGVKSVDDTIQVKVDYAGSFGDA 200
SPY67    151 KTTKTKTVGFVGGMEGTVITRFEKGFEAGVKSVDDTIQVKVDYAGSFGDA 200
B514     151 KTTKTKTVGFVGGMEGTVITRFEKGFEAGVKSVDDTIQVKVDYAGSFGDA 200
             **************************************************

700294   201 AKGKTIAAAQYAAGADVIYQAAGGTGAGVFNEAKAINEKRSEADKVWVIG 250
12384    201 AKGKTIAAAQYAAGADVIYQAAGGTGAGVFNEAKAINEKRSEADKVWVIG 250
SPY67    201 AKGKTIAAAQYAAGADVIYQAAGGTGAGVFNEAKAINEKRSEADKVWVIG 250
B514     201 AKGKTIAAAQYAAGADVIYQAAGGTGAGVFNEAKAINEKRSEADKVWVIG 250
             **************************************************

700294   251 VDRDQKDEGKYTSKDGKEANFVLASSIKEVGKAVQLINKQVADKKFPGGK 300
12384    251 VDRDQKDEGKYTSKDGKEANFVLASSIKEVGKAVQLINKQVADKKFPGGK 300
SPY67    251 VDRDQKDEGKYTSKDGKEANFVLASSIKEVGKAVQLINKQVADKKFPGGK 300
B514     251 VDRDQKDEGKYTSKDGKEANFVLASSIKEVGKAVQLINKQVADKKFPGGK 300
             **************************************************

700294   301 TTVYGLKDGGVEIATTNVSKEAVKAIKEAK 330
12384    301 TTVYGLKDGGVEIATTNVSKEAVKAIKEAK 330
SPY67    301 TTVYGLKDGGVEIATTNVSKEAVKAIKEAK 330
B514     301 TTVYGLKDGGVEIATTNVSKEAVKAIKEAK 330
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