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CA 2634898 A1 2007/06/28

(21) 2 634 898

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2006/12/19
(87) Date publication PCT/PCT Publication Date: 2007/06/28
(85) Entrée phase nationale/National Entry: 2008/06/23
(86) N° demande PCT/PCT Application No.: EP 2006/069944
(87) N° publication PCT/PCT Publication No.: 2007/071692
(30) Priorité/Priority: 2005/12/21 (GB0526038.5)

(51) Cl.Int./Int.Cl. *A61K 39/00* (2006.01), *A61P 31/04* (2006.01), *C07K 14/31* (2006.01), *C12N 15/31* (2006.01), *C12N 15/62* (2006.01)

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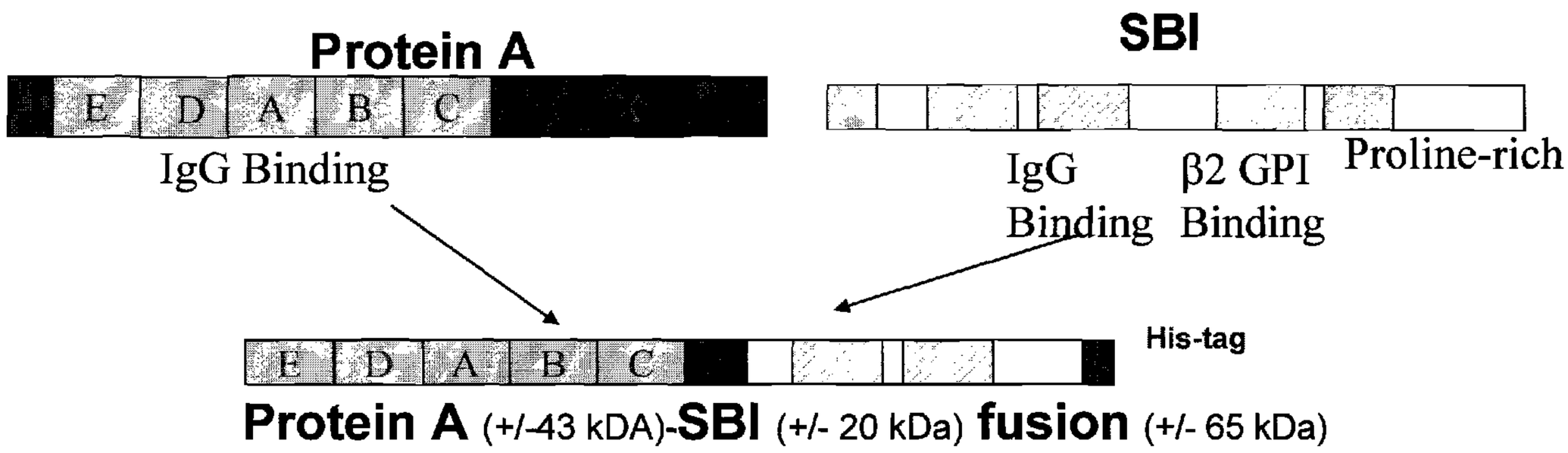
(54) Titre : COMPOSITION IMMUNOGENE
(54) Title: IMMUNOGENIC COMPOSITION



(57) Abrégé/Abstract:
The present invention discloses a polypeptide comprising: a protein A part including at least one IgG binding domain and an Sbi part including at least one IgG binding domain. In a further embodiment, the invention discloses an immunogenic composition comprising at least two different staphylococcal polypeptides, each comprising an IgG binding domain.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 June 2007 (28.06.2007)

PCT

(10) International Publication Number
WO 2007/071692 A3

(51) International Patent Classification:
A61K 39/00 (2006.01) *C12N 15/62* (2006.01)
C07K 14/31 (2006.01) *A61P 31/04* (2006.01)
C12N 15/31 (2006.01)

(21) International Application Number:
PCT/EP2006/069944

(22) International Filing Date:
19 December 2006 (19.12.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0526038.5 21 December 2005 (21.12.2005) GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

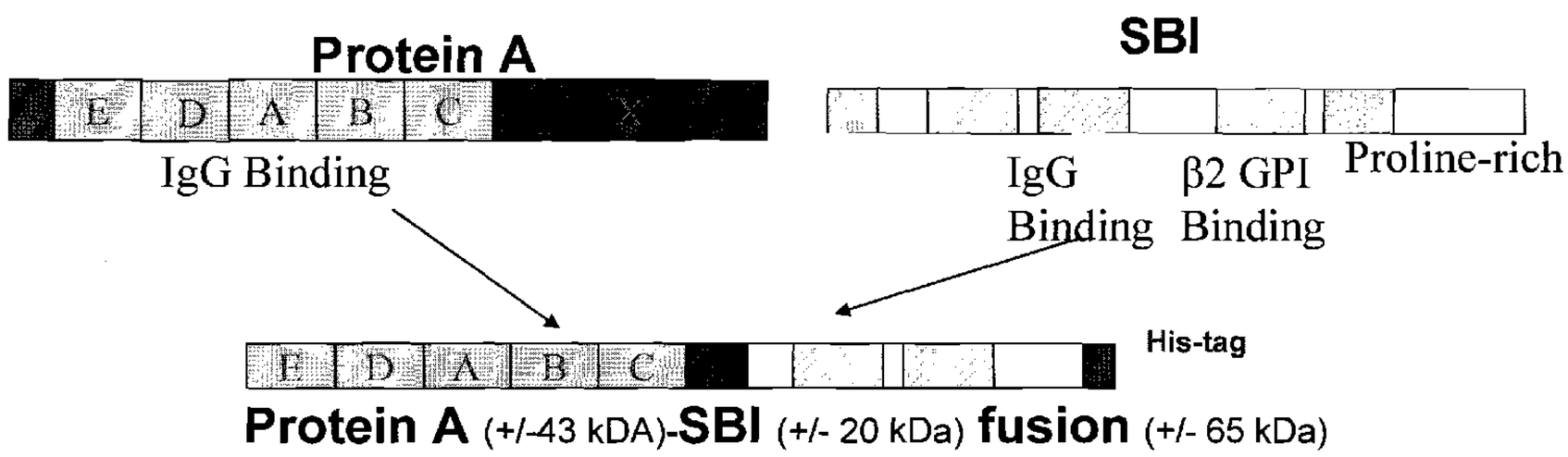
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
13 September 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IMMUNOGENIC COMPOSITION



(57) Abstract: The present invention discloses a polypeptide comprising: a protein A part including at least one IgG binding domain and an Sbi part including at least one IgG binding domain. In a further embodiment, the invention discloses an immunogenic composition comprising at least two different staphylococcal polypeptides, each comprising an IgG binding domain.

WO 2007/071692 A3

Immunogenic Composition

The present invention relates to the field of Staphylococcal immunogenic compositions and vaccines, their manufacture and the use of such compositions in medicine. More particularly, it relates to compositions comprising an immunoglobulin binding domain derived from *S. aureus* protein A and and immunoglobulin domain derived from the Sbi protein. Fusion proteins comprising an IgG binding domain from protein A and an IgG binding domain from Sbi are described as well as a polynucleotide encoding such a protein.

Protein A is a well studied cell wall associated protein in *S. aureus* which binds to the Fc and Fab regions of IgG from several species. Protein A consists of five consecutive domains, all with IgG binding activity, followed by a region anchoring the protein in the cell wall (Lofdahl et al 1983 Eur J Biochem 156; 637-643). Protein A has been investigated as a vaccine component for use in mastitis a vaccine (Carter and Kerr J. Diary Sci 2003 86; 1177-1186).

More recently Sbi was identified as a second IgG binding protein expressed by *S. aureus* (Zhang et al 1998, Microbiology 144; 985-991 and Zhang et al 1999, 145; 177-183). Sbi protein consists of about 436 amino acids and has an immunoglobulin binding specificity similar to protein A. Sbi contains two IgG binding domains towards the N-terminus of the protein and a further apolipoprotein H binding domain towards the C-terminus of Sbi.

S. aureus infections are treated with antibiotics, with penicillin being the drug of choice whereas vancomycin is used for methicillin resistant isolates. The percentage of staphylococcal strains exhibiting wide-spectrum resistance to antibiotics has become increasingly prevalent since the 1980's (Panlilo et al 1992, Infect.Control. Hosp. Epidemiol. 13; 582), posing a threat for effective antimicrobial therapy. In addition, the recent emergence of vancomycin resistant *S. aureus* strain has aroused fear that methicillin resistant *S.aureus* strains will emerge and spread for which no effective therapy is available.

An alternative approach of using antibodies against staphylococcal antigens in passive immunotherapy has been investigated. Therapy involving administration of polyclonal antisera are under development (WO 00/15238, WO 00/12132).

Attempts to protect against *S. aureus* bacteremia in an infant rat model using various doses of staphylococcal protein A antiserum were unsuccessful. Even the highest levels

of antibody did not significantly reduce mortality, bacteremia or metastatic infection to lungs or liver (Greenberg et al Infection and Immunity 57; 1113-1118 1989).

There remains a need to develop a vaccine which protects against staphylococcal disease. The provision of a method of blocking one of the staphylococcal defence mechanisms would improve the likelihood of producing an effective active or passive immunogenic composition.

Accordingly there is provided a polypeptide comprising: a protein A-like part having an amino acid sequence having at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 12-40, or an immunogenic fragment thereof optionally comprising at least one IgG binding domain and an Sbi-like part which has an amino acid sequence having at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 1-11, or an immunogenic fragment thereof, optionally comprising at least one IgG binding domain.

Such a polypeptide comprises IgG binding domains from each of the known *S. aureus* IgG binding proteins and is a convenient way of introducing the polypeptide sequences allowing an immune response to be raised against both of the IgG binding proteins.

In a further aspect of the invention there is provided, a polynucleotide comprising a Protein A encoding region having at least 85% identity to a polynucleotide sequence selected from the group consisting of SEQ ID NOs 54-72 or fragment thereof encoding at least one IgG binding domain and an Sbi-encoding region which has at least 85% identity to a polynucleotide sequence selelcted from the group consisting of SEQ ID NOs 43-53 or fragment thereof encoding at least one IgG binding domain.

In a further aspect of the invention there is provided, an immunogenic composition comprising two staphylococcal polypeptides, each comprising an IgG binding domain.

In a further aspect of the invention, there is provided a process for making the vaccine of the invention comprising the step of adding a pharmaceutically acceptable excipient to a composition comprising an IgG domain from protein A and an IgG domain from Sbi.

In a further aspect of the invention, there is provided an immunogenic composition of the invention for use in the treatment of prevention of staphylococcal disease.

In a further aspect of the invention, there is provided a use of the immunogenic composition of the invention in the preparation of a medicament for the treatment or prevention of staphylococcal disease.

In a further aspect of the invention, there is provided a method of treating or preventing staphylococcal disease comprising administering the immunogeninc composition of the invention to a patient in need thereof.

Description of Figures

Figure 1 – Schematic drawing of the ProteinA-Sbi fusion protein

Figure 2 – Coomassie blue stained 4-20% PAGE showing expressin of the ProteinA-Sbi fusion protein. Lanes 1 and 6 contain molecular weight markers, lane 2 contains the AR58 *E. coli* prior to induction, lane 3 contains AR58 *E.coli* after 4 hours induction, lane 4 contains AR58 *E. coli* transformed with ProteinA-Sbi plasmid prior to induction, lane 5 contains AR58 *E. coli* transformed with ProteinA-Sbi after 4 hours induction.

Figure 3 – Coomassie blue stained 4-20% PAGE showing purification of the Protein A-Sbi fusion protein. Lanes 1 and 7 contain molecular weight markers, lane 2 contains the soluble fraction prior to loading onto the Hi-Trap column, lane 3 contains the soluble fraction in an uninduced culture, lane 4 contains 1μg of the column purified protein, lane 5 contains 0.5μg of the column purified protein, lane 6 contains 0.25μg of the colurnn purified protein.

Figure 4 – Graph showing the mortality follow-up after a challenge with *S. aureus* 5 Reynolds (3 10E6) in CD1 mice. The line marked with triangle shows survival after immunisation with killed whole cells of *S. aureus* 5 Reynolds adjuvanted with AlPO4. The line marked with diamonds shows survival after immunisation with adjuvant alone. The line marked with crosses shows survival after inoculation with protein A adjuvanted with AlPO4. The line marked with squares shows survival after inoculation with ProteinA-Sbi fusion protein adjuvanted with AlPO4.

Figure 5 – Amino acid alignments of proteinA and Sbi proteins from different strains of *S. aureus.*

Detailed description

The present application discloses an immunogenic composition comprising at least or exactly two, three, four or five different staphylococcal polypeptides, each comprising an IgG binding domain. Such an immunogenic composition may further comprise further antigens which do not comprise an IgG binding domain.

A staphylococcal polypeptide is defined as a polypeptide which is expressed by a staphylococcal bacterium for instance *S. aureus* or *S. epidermidis*. The staphylococcal polypeptide is either derived from a staphylococcal strain or is expressed recombinantly in

a different expression system, for example in *E. coli*. The staphylococcal polypeptide may be a full length protein or a fragment of a full length protein that contains an IgG binding domain.

By different, it is meant that the polypeptides are encoded by separate genes. In an embodiment, the different polypeptides are present as noncovalently linked polypeptides (i.e. separate or free proteins). In an embodiment, the different polypeptides are present as a covalently cross-linked conjugate. In an embodiment, the different polypeptides are present as at least one fusion protein.

An IgG binding domain is a domain that is capable of binding to IgG. The interaction between an antigen and the CDR regions of the Fv domains which typically interact with the antigen to which the antibody is raised, is excluded from this definition. An IgG binding domain typically interacts with the Fc domain or with conserved regions of the Fab domains. The binding of individual IgG binding domains of protein A to Fc and $F(ab')_2$ doamins has been analysed (Jansson et al FEMS Immunology and Medical Microbiology 20; 69-78 1998). Optionally, an IgG binding domain is capable of binding to an antibody or fragment thereof (such as Fc or Fab) with an affinity of 0.1-1000, 1-500, 1-100 or 5-50 x 10-6 M-1 or approximately 10 x 10-6 M-1.

An IgG binding domain typically has an amino acid sequence which is at least 60%, 70%, 80%, 85%, 90%, 95% or 98% identical to that of SEQ ID NOs: 1, 2, or 12-21. Fragments of SEQ ID NOs 1, 2, or 12-21 which bind to IgG and/or are capable of generating an immune response against Protein A or Sbi, are also considered to be IgG binding proteins.

In an embodiment, the immunogenic composition of the invention comprises a protein A polypeptide from *S. aureus* or a fragment thereof containing an IgG binding domain.

In an embodiment, the protein A polypeptide has a sequence which is at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 100% identical to that of SEQ ID NO 12-40 or fragment thereof comprising an IgG binding domain.

In an embodiment, the protein A polypeptide is encoded by a polynucleotide having a sequence which is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 100% identical to SEQ ID NO: 54-72 or fragment thereof comprising an IgG binding domain.

Protein A is a 58kDa protein containing about or exactly 524 amino acid (or about or exactly 509 amino acids in the mature protein). It comprises 5 IgG binding domains (E, D, A, B and C) which are located towards the N-terminus of the protein as shown in figure 1. All IgG domains of Protein A share 91-100% identity (computed with the ClustalW program).

In an embodiment, the protein A polypeptide or fragment thereof comprises 1, 2, 3, 4 or 5 IgG binding domains. For example, the immunogenic composition comprises IgG binding domain(s), E; D; A; B; C; E and D; D and A; A and B; B and C; E and A; E and B; E and C; D and B; D and C; A and C; E, D and A; D, A and B; A, B and C; E, D, A and B; D, A, B and C; or E, D, A, B and C. Combinations comprising E and 1, 2, 3 or 4 further IgG binding domains selected from A, B, C and D are present in an embodiment. These combinations of IgG binding domains are present in a single polypeptide or may be present in 2, 3, 4 or 5 separate polypeptides. In an embodiment, the IgG domains have an amino acid sequence comprising the sequence of SEQ ID NO: 12-21. Where multiple protein A IgG binding domains are present, the sequence may be that of SEQ ID NO: 22-32.

Alternatively, the sequence of the protein A polypeptide is the whole or a fragment of SEQ ID NO 33-40, or variants sharing at least 80%, 90%, 95%, 98% or 99% identity to the sequence of SEQ ID NO: 33-40 or the polypeptide encoded by SEQ ID NO 65-72 or variants sharing at least 80%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 65-72. Such a fragment comprises at least 1, 2, 3, 4 or 5 IgG binding domains. Such a fragment optionally contains at least 50, 60, 70, 80, 90, 100, 150, 200, 250 or 300 amino acids.

The sequence of the Protein A IgG binding domains are represented by SEQ ID NOs 12-21 and the protein A IgG binding domains have sequences which are at least 70%, 80%, 85%, 90%, 95%, 98% or 100% identical to SEQ ID NOs 12-21.

In an embodiment, the fragments of Protein A consist essentially of the amino acid sequence of SEQ ID NOs 12-32 but additionally comprise a further 1, 2, 3, 4, 5, 10, 20, 30, 40, 50 or 100 amino acids on either or both the N and C terminal side of the SEQ ID 12-32. In an embodiment, the additional sequence is that found in Protein A as set out in SEQ ID NOs 33-40 or Figure 4.

Specific variants of the polypeptide of the invention are envisaged. In particular, the third amino acid of protein A IgG binding domain D may be K or N, the 24th amino acid of protein A IgG binding domain D may be E or A, the 46th amino acid of protein A IgG binding domain A may be A or S, the 53rd amino acid of protein A IgG binding domain A may be D or E, the 23rd amino acid of protein A IgG binding domain B may be N or T, the 40th amino acid of protein A IgG binding domain B may be Q or V , the 42nd amino acid of protein A IgG binding domain B may be A or K, the 43rd amino acid of protein A IgG binding domain B may be N or E and/or the 44th amino acid of protein A IgG binding domain B may be I or L.

In an embodiment, an N-terminal M residue is added to the sequence of SEQ ID NO 1-32.

In an embodiment, the immunogenic composition of the invention comprises an Sbi polypeptide from *S. aureus* or a fragment thereof containing an IgG binding domain.

Sbi is a protein of about 48kDa, containing about 436 amino acids. Two IgG binding domains are present towards the N-terminus of the protein and Sbi further comprises a apolipoprotein H (β2-GPI) binding domain followed by a proline rich domain (see Figure C).

In an embodiment, the Sbi polypeptide has a sequence which is at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 100% identical to SEQ ID NO: 1-11 or fragment thereof containing an IgG binding domain (optionally containing 2 IgG binding domains). Such a fragment optionally contains at least 50, 60, 70, 80, 90, 100, 150, 200, 250 or 300 amino acids.

In an embodiment, the Sbi polypeptide is encoded by a polynucleotide having a sequence which is at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 100% identical to SEQ ID NO: 43-53 or fragment thereof encoding an IgG binding domain (optionally encoding 2 IgG binding domains).

For example, fragments of Sbi consist of or comprise the N-terminal IgG binding domain, the C-terminal IgG binding domain or both IgG binding domains. In an embodiment, the fragments have amino acid sequences which are at least 70%, 80%, 85%, 90%, 95%, 98% or 100% identical to SEQ ID NOs 1-4.

In an embodiment, the fragments of Sbi consist essentially of the amino acid sequence of SEQ ID NOs 1-4 but additionally comprise a further 1, 2, 3, 4, 5, 10, 20, 30, 40, 50 or 100 amino acids on either or both the N and C terminal side of the SEQ ID 1-4. In an embodiment, the sequence is that of the Sbi protein as set out in SEQ ID NOs 5-11 and Figure 4.

Where both IgG binding domains of Sbi are present in an immunogenic composition, they may be present on separate polypeptide chains or in the same polypeptide chain.

In an embodiment, both protein A (or a fragment thereof comprising an IgG binding domain) and Sbi (or a fragment thereof comprising an IgG binding domain) are present in the immunogenic composition. These antigens may be present as separate proteins within the immunogenic composition of they may be covalently linked together , for example as a fusion protein or using a crosslinking reagent to link the two polypeptides.

In an embodiment, the polypeptide comprises a protein A part and an Sbi part. For example, the Protein A part has an amino acid sequence having at least 85%, 90%, 95%, 98% or 100% identity to an amino acid sequence from the group consisting of SEQ ID

NOs 12-40 or an immunogenic fragment thereof comprising at least one IgG binding domain and an Sbi part which has an amino acid sequence having at least 85%, 90%, 95%, 98% or 100% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 1-11 or an immunogenic fragment thereof comprising at least one IgG binding domain.

In the context of a fusion protein or a crosslinked conjugate, any of the fragments or variants of protein A or Sbi as described above may be incorporated.

In an embodiment, the polypeptide is a fusion protein containing the protein A part N-terminal to the Sbi part. Alternatively the fusion protein may contain the Sbi part N-terminal to the protein A part.

The polypeptide of the invention optionally further comprises sequence from further staphylococcal protein(s) optionally selected from the group consisting of Ebh (WO 02/59148), Elastin binding protein (EbpS WO 98/38312), EFB (FIB) (WO 94/06830), ClfA (US6008341), ClfB (WO 99/27109), SdrC (WO 99/27109), SdrG (WO 97/48727), FnbA (including variants desribed in WO 05/116064), FbpA, IsaA/PisA (DE 199 17 098), IsdA (WO 02/59148, WO 06/59247), IsdB (WO 02/059148, including variants described in WO 05/09379 and WO 05/09378), IsdC (WO 06/59247), HarA (WO 05/09378), alpha toxin (Hla US4615884), alpha toxin H35R mutant, penicillin binding protein 4 (WO 06/33918), SsaA (WO 05/115113), Aap (WO 05/86663), RAP (WO 99/32133), AhpC and variants (WO 06/78680), SasA (WO 06/121664).

In an embodiment, the polypeptide has a polypeptide sequence which is at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 100% identical to the sequence of SEQ ID NO:41 or 42 or 76 or fragment thereof comprising an IgG binding domain, preferably from both protein A and Sbi.

In an embodiment, the polypeptide is a fusion protein encoded by a polynucleotide having a sequence which is at least 70%, 80%, 85%, 90%, 95%, 98% or 100% identical to SEQ ID NO: 73-75 or fragment thereof comprising an IgG binding domain , preferably from both protein A and Sbi.

SEQ group 1 contains SEQ ID NOs 43-75.

SEQ group 2 contains SEQ ID NOs 1-42 and 76.

The present invention further provides for:

(a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, most preferably at least 97, 98 or 99% or exact identity, to that of any sequence of SEQ Group 2;
(b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97, 98 or 99% or exact identity to any sequence of SEQ Group 1 over the entire length of the selected sequence of SEQ Group 1; or
(c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of any sequence of SEQ Group 2.

The invention also provides an immunogenic fragment of a polypeptide of the invention, that is, a contiguous portion of the Protein A-Sbi polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the corresponding amino acid sequence selected from SEQ Group 2 ; That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the ProteinA-Sbi polypeptide. Such an immunogenic fragment may include, for example, the Protein A-Sbi polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In an embodiment, the immunogenic fragment of Protein A-Sbi according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, most preferably at least 97, 98 or 99% identity, to that a sequence selected from SEQ Group 2 over the entire length of said sequence.

A fragment is a polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of any polypeptide of the invention. As with Protein A-Sbi polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

In an embodiment, fragments include, for example, truncation polypeptides having a portion of an amino acid sequence selected from SEQ Group 2 or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host

cell, are also preferred. Further preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

In an embodiment, fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence selected from SEQ Group 2 or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence selected from SEQ Group 2 .

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides

The invention discloses any polynucleotide encoding any one of the polypeptides of the invention as described above.

A further embodiment of the invention is a polynucleotide comprising a Protein A encoding region having at least 85%, 90%, 95%, 98%, 99% or 100% identity to a polynucleotide sequence selected from the group consisting of SEQ ID NOs 54-73 and an Sbi-encoding region which has at least 85%, 90%, 95%, 98%, 99% or 100% identity to a polynucleotide sequence selected from the group consisting of SEQ ID NOs 43-53.

In the case of SEQ ID NO: 47-53 and 65-72, the sequences are of the complete Sbi or protein A, respectively. Fragments of these sequences encoding at least 1, 2, 3, 4 or 5 IgG domains may be counted as the protein A or Sbi encoding part of the polynicelotides of the invention.

Specific variants of the polynucleotide of the invention are envisaged. For example, the fragment contains 1, 2, 3, 4 or 5 IgG binding domains as depicted in Figure X. In particular, the third codon of protein A IgG binding domain D may encode K or N, the 24th codon of protein A IgG binding domain D may encode E or A, the 46th codon of protein A IgG binding domain A may encode A or S, the 53rd codon of protein A IgG binding domain A may encode D or E, the 23rd codon of protein A IgG binding domain B may encode N or T, the 40th codon of protein A IgG binding domain B may encode Q or V , the 42nd codon of protein A IgG binding domain B may encode A or K, the 43rd codon of protein A IgG binding domain B may encode N or E and/or the 44th codon of protein A IgG binding domain B may encode I or L.

In an embodiment of the invention the polynucleotide encodes a fusion protein comprising a protein A like part having at least 85%, 90%, 95%, 98% or 100% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 54-72 or an immunogenic fragment thereof comrpsing at least 1, 2, 3, 4 or 5 IgG binding domains and an Sbi-like part which has at least 85%, 90%, 95%, 98% or 100% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 43-53, or an immunogenic fragment thereof comprising at least one IgG binding domain.

In an embodiment the polynuclotide of the invention has a polynucleotide sequence having at least 85%, 90%, 95%, 98% or 100% identity to SEQ ID NO 73 or 74.

In a preferred embodiment of the invention the polynucleotide comprises a region encoding Protein A-Sbi polypeptides comprising sequences set out in SEQ Group1 which include full length gene, or a variant or fragment thereof.

Polynucleotides of the invention do not encompass a complete genomic DNA from a staphylococcal species, e.g. *S. aureus* or *S. epidermidis*.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing Protein A-Sbi polypeptides and polynucleotides, particularly *S. aureus* or *S. epidermidis* Protein A-Sbi polypeptides and polynucleotides, including, for example, unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, clinically

or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions, preferably immunogenic compositions, comprising the same.

Another aspect of the invention relates to isolated polynucleotides, that encode Protein A-Sbi polypeptides having a deduced amino acid sequence of SEQ Group 2 and polynucleotides closely related thereto and variants thereof.

An embodiment of the invention relates to Protein A-Sbi polypeptides from *S. aureus* or *S. epidermidis* comprising or consisting of an amino acid sequence selected from SEQ Group 2 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ Group 1, a polynucleotide of the invention encoding Protein A-Sbi polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *S. aureus* as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ Group 1, typically a library of clones of chromosomal DNA of a different staphylococcal strain in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene sequence.

Moreover, each DNA sequence set out in SEQ Group 1 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ Group 2 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotides of SEQ Group 1, between the start codon and the stop codon, encode respectively the polypeptides of SEQ Group 2. The nucleotide number of start codon and first nucleotide of the stop codon are listed in table 3 for each polynucleotide of SEQ Group 1.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

(a) a polynucleotide sequence which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97, 98 or 99% or exact identity to any sequence from SEQ Group 1 over the entire length of the polunucleotide sequence from SEQ Group 1; or

(b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97, 98 or 99% or 100% exact, to any amino acid sequence selected from SEQ Group 2 , over the entire length of the amino acid sequence from SEQ Group 2.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *S. aureus*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions (for example, using a temperature in the range of 45 – 65°C and an SDS concentration from 0.1 – 1%) with a labeled or detectable probe consisting of or comprising any sequence selected from SEQ Group 1 or a fragment thereof; and isolating a full-length gene and/or genomic clones containing said polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) set out in SEQ Group 1. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame with another coding sequence, such as a sequence encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention may also contain at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence may also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is

a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA 86:* 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell 37:* 767 (1984), both of which may be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *S. aureus* having an amino acid sequence set out in any of the sequences of SEQ Group 2. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptides having a deduced amino acid sequence of any of the sequences of SEQ Group 2 . Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding Protein A-Sbi variants, that have the amino acid sequence of Protein A-Sbi polypeptides of any sequence from SEQ Group 2 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of Protein A-Sbi polypeptides.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to polynucleotides encoding Protein A-Sbi polypeptides having an amino acid sequence set out in any of the sequences of SEQ Group 2 , and polynucleotides that are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 90% identical over its entire length to polynucleotides encoding Protein A-Sbi polypeptides and polynucleotides complementary thereto. In this regard, polynucleotides at least 95% identical over their entire length to the same are particularly preferred. Furthermore, those with at least 97% are highly

preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain substantially the same biological function or activity as mature polypeptides encoded by a DNA sequences selected from SEQ Group 1.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to Protein A-Sbi polynucleotide sequences, such as those polynucleotides in SEQ Group 1.

The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. As herein used, the terms "stringent conditions" and "stringent hybridization conditions" mean hybridization occurring only if there is at least 95% and preferably at least 97% identity between the sequences. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein. Solution hybridization may also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in any of the sequences of SEQ Group 1 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in the corresponding sequences of SEQ Group 1 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and

genomic DNA to isolate full-length cDNAs and genomic clones encoding Protein A-Sbi and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the Protein A-Sbi sequences. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of Protein A-Sbi genes may be isolated by screening using a DNA sequences provided in SEQ Group 1 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA 85:* 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ Group 1 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more

prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

In accordance with an aspect of the invention, there is provided the use of a polynucleotide of the invention for therapeutic or prophylactic purposes, in particular genetic immunization.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff *et al., Hum Mol Genet* (1992) 1: 363, Manthorpe *et al., Hum. Gene Ther.* (1983) 4: 419), delivery of DNA complexed with specific protein carriers (Wu *et al., J Biol Chem.* (1989) 264: 16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, *PNAS USA*, (1986) 83: 9551), encapsulation of DNA in various forms of liposomes (Kaneda *et al., Science* (1989) 243: 375), particle bombardment (Tang *et al., Nature* (1992) 356:152, Eisenbraun *et al., DNA Cell Biol* (1993) 12: 791) and *in vivo* infection using cloned retroviral vectors (Seeger *et al., PNAS USA* (1984) 81: 5849).

Vectors, Host Cells, Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention may be prepared by processes well known in those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems that comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al., BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al., MOLECULAR CLONING: A*

LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, conjugation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci, *E. coli*, streptomyces, cyanobacteria, *Bacillus subtilis*, *Neisseria meningitidis, Haemophilus influenzae* and *Moraxella catarrhalis*; fungal cells, such as cells of a yeast, *Kluveromyces*, *Saccharomyces*, *Pichia*, a basidiomycete, *Candida albicans* and *Aspergillus*; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses, retroviruses, and alphaviruses and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid

extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelian Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), *Listeria*, *Salmonella* , *Shigella*, BCG, streptococci. These viruses and bacteria can be virulent, or attenuated in various ways in order to obtain live vaccines. Such live vaccines also form part of the invention.

Immunogenic compositions comprising further antigens

The immunogenic compositions of the invention are optionally combined with further antigens. The neutralisation of staphylococcal IgG binding proteins may allow an improved immune response to be generated against further antigens.

In an embodiment, the immunogenic composition of the invention comprises a further staphylococcal antigen. In an embodiment, the further staphylococcal antigen is derived from *S. aureus* or *S. epidermidis*. Examples of further antigens include staphylococcal polysaccharide such as *S. aureus* type 5 capsular polysaccharide, *S. aureus* type 8 capsular polysaccharide, PNAG which is optionally less than 50%, 40%, 30%, 20% or 10% acetylated.

Most strains of *S.aureus* that cause infection in man contain either Type 5 or Type 8 polysaccharides. Approximately 60% of human strains are Type 8 and approximately 30% are Type 5. The structures of Type 5 and Type 8 capsular polysaccharide antigens are described in Moreau et al Carbohydrate Res. 201; 285 (1990) and Fournier et al Infect. Immun. 45; 87 (1984). Both have FucNAcp in their repeat unit as well as ManNAcA which can be used to introduce a sulfhydryl group. The structures were reported as :

Type 5

→4)-β-D-ManNAcA(3OAc)-(1 →4)-α-L-FucNAc(1 →3)-β-D-FucNAc-(1 →

Type 8

→3)-β-D-ManNAcA(4OAc)-(1 →3)-α-L-FucNAc(1 →3)-β-D-FucNAc-(1 →

Recently (Jones Carbohydrate Research 340, 1097-1106 (2005)) NMR spectroscopy revised to structures to :

Type 5

→4)-β-D-ManNAcA-(1 →4)-α-L-FucNAc(3OAc)-(1 →3)-β-D-FucNAc-(1 →

Type 8

→3)-β-D-ManNAcA(4OAc)-(1 →3)-α-L-FucNAc(1 →3)-α-D-FucNAc(1 →

Polysaccharides may be extracted from the appropriate strain of *S. aureus* using method well known to the skilled man, for instance as described in US6294177. For example, ATCC 12902 is a Type 5 *S. aureus* strain and ATCC 12605 is a Type 8 *S. aureus* strain.

In an embodiment, both Type 5 and Type 8 capsular polysaccharides are present in the immunogenic composition of the invention. The immunogenic composition of the invention alternatively contains either type 5 or type 8 polysaccharide.

Polysaccharides are of native size or alternatively may be sized, for instance by microfluidisation, ultrasonic irradiation or by chemical treatment. The invention also covers oligosaccharides derived from the type 5 and 8 polysaccharides from *S. aureus*.

The type 5 and 8 polysaccharides included in the immunogenic composition of the invention are preferably conjugated to a carrier protein as described below or are alternatively unconjugated.

The immunogenic compositions of the invention alternatively contains either type 5 or type 8 polysaccharide.

PNAG is a polysaccharide adhesin and is composed of a polymer of β-(1 →6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents. This polysaccharide is present in both *S.aureus* and *S. epidermidis* (Joyce et al 2003, Carbohydrate Research 338; 903; Maira-Litran et al 2002, Infect. Imun. 70; 4433).

PIA (or PNAG) may be of different sizes varying from over 400kDa to between 75 and 400kDa to between 10 and 75kDa to oligosaccharides composed of up to 30 repeat units (of β-(1 →6)-linked glucosamine substituted with N-acetyl and O-succinyl constituents). Any size of PIA polysaccharide or oligosaccharide may be use in an immunogenic composition of the invention, however a size of over 40kDa is preferred. Sizing may be achieved by any method known in the art, for instance by microfluidisation, ultrasonic irradiation or by chemical cleavage (WO 03/53462, EP497524, EP497525).

In an embodiment, the size range of PIA (PNAG) is 40-400kDa, 50-350kDa, 40-300kDa, 60-300kDa, 50-250kDa or 60-200kDa.

PIA (PNAG) can have different degree of acetylation due to substitution on the amino groups by acetate. PIA produced in vitro is almost fully substituted on amino groups (95-100%). Alternatively, a deacetylated PIA (PNAG) can be used having less than 60%, preferably less than 50%, 40%, 30%, 20%, 10% N-acetylation. Use of a deacetylated PIA (PNAG) is preferred since non-acetylated epitopes of PNAG are efficient at mediating opsonic killing of Gram positive bacteria, preferably *S. aureus* and/or *S. epidermidis*. In an embodiment, the PIA (PNAG) has a size between 40kDa and 300kDa and is deacetylated so that less than 60%, 50%, 40%, 30% or 20% of amino groups are acetylated.

The term deacetylated PNAG (dPNAG) refers to a PNAG polysaccharide or oligosaccharide in which less than 60%, 50%, 40%, 30%, 20% or 10% of the amino agroups are acetylated.

In an embodiment, PNAG is a deaceylated to form dPNAG by chemically treating the native polysaccharide. For example, the native PNAG is treated with a basic solution such that the pH rises to above 10. For instance the PNAG is treated with 0.1-5M, 0.2-4M, 0.3-3M, 0.5-2M, 0.75-1.5M or 1M NaOH , KOH or NH_4OH. Treatment is for at least 10 or 30 minutes, or 1, 2, 3, 4, 5, 10, 15 or 20 hours at a temperature of 20-100, 25-80, 30-60 or 30-50 or 35-45 °C. dPNAG may be prepared as described in WO 04/43405.

The polysaccharide(s) included in the immunogenic composition of the invention are preferably conjugated to a carrier protein as described below or alternatively unconjugated.

In an embodiment, the immunogenic composition fo the invention comprises Type 5 and 8 capsular polysaccharides and PNAG.

In an embodiment, the immunogenic composition of the invention comprises the *S. aureus* 336 antigen described in US6294177.

The 336 antigen comprises β-linked hexosamine, contains no O-acetyl groups and specifically binds to antibodies to *S. aureus* Type 336 deposited under ATCC 55804. It is further characterised in US 2006/0228368.

In an embodiment, the 336 antigen is a polysaccharide which is of native size or alternatively may be sized, for instance by microfluidisation, ultrasonic irradiation or by chemical treatment. The invention also covers oligosaccharides derived from the 336 antigen.

The 336 antigen, where included in the immunogenic composition of the invention is preferably conjugated to a carrier protein as described below or are alternatively unconjugated.

In an embodiment, the carrier protein is independently selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM197, protein D, alpha toxin, SdrG, ClfA, IsdA, IsdB, IsdH, protein A, Sbi and a proteinA-Sbi fusion protein or fragments thereof.

In an embodiment, the polysaccharides and PNAG utilised in the invention are linked to a protein carrier which provides bystander T –cell help. Examples of these carriers which are currently commonly used for the production of polysaccharide immunogens include the Diphtheria and Tetanus toxoids (DT, DT crm197 and TT respectively), Keyhole Limpet Haemocyanin (KLH), and the purified protein derivative of Tuberculin (PPD), protein D from *Haemophilus influenzae*, pneumolysin or fragments of any of the above. Fragments suitable for use include fragments encompassing T-helper epitopes. In particular protein D fragment will preferably contain the N-terminal 1/3 of the protein. Protein D is an IgD–binding protein from *Haemophilus influenzae* (EP 0 594 610 B1) and is a potential immunogen.

Despite the common use of these carriers and their success in the induction of anti polysaccharide antibody responses they are associated with several drawbacks. For example, it is known that antigen specific immune responses may be suppressed by the presence of pre-existing antibodies directed against the carrier, in this case Tetanus toxin (Di John *et al*; Lancet, December 16, 1989). In the population at large, a very high percentage of people will have pre-existing immunity to both DT and TT as people are routinely vaccinated with these antigens. In the UK for example 95% of children receive the DTP vaccine comprising both DT and TT. Other authors have described the problem

of epitope suppression to peptide vaccines in animal models (Sad *et al*, Immunology, 1991; 74:223-227; Schutze *et al*, J. Immunol. 135: 4, 1985; 2319-2322).

An alternative staphylococcal carrier protein is alpha toxoid. The native form may be conjugated to a polysaccharide since the process of conjugation reduces toxicity. Alternatively a genetically detoxified alpha toxin such as the His35Leu or His 35 Arg variants are used as carriers since residual toxicity is lower. Alternatively the alpha toxin is chemically detoxified by treatment with a cross-linking reagent, formaldehyde or glutaraldehyde. A genetically detoxified alpha toxin is optionally chemically detoxified, preferably by treatment with a cross-linking reagent, formaldehyde or glutaraldehyde to further reduce toxicity.

The polysaccharides may be linked to the carrier protein(s) by any known method (for example, by Likhite, U.S. Patent 4,372,945 by Armor et al., U.S. Patent 4,474,757, and Jennings et al., U.S. Patent 4,356,170). Preferably, CDAP conjugation chemistry is carried out (see WO95/08348).

In CDAP, the cyanylating reagent 1-cyano-dimethylaminopyridinium tetrafluoroborate (CDAP) is preferably used for the synthesis of polysaccharide-protein conjugates. The cyanilation reaction can be performed under relatively mild conditions, which avoids hydrolysis of the alkaline sensitive polysaccharides. This synthesis allows direct coupling to a carrier protein.

The polysaccharide is solubilized in water or a saline solution. CDAP is dissolved in acetonitrile and added immediately to the polysaccharide solution. The CDAP reacts with the hydroxyl groups of the polysaccharide to form a cyanate ester. After the activation step, the carrier protein is added. Amino groups of lysine react with the activated polysaccharide to form an isourea covalent link. After the coupling reaction, a large excess of glycine is then added to quench residual activated functional groups. The product is then passed through a gel permeation column to remove unreacted carrier protein and residual reagents.

In an embodiment, the immunogenic composition of the invention comprises a protein selected from the group consisting of Ebh (WO 02/59148), Elastin binding protein (EbpS WO 98/38312), EFB (FIB) (WO 94/06830), ClfA (US6008341), ClfB (WO 99/27109), SdrC (WO 99/27109), SdrG (WO 97/48727), FnbA (including variants desribed in WO

05/116064), FbpA, IsaA/PisA (DE 199 17 098), IsdA (WO 02/59148, WO 06/59247), IsdB (WO 02/059148, including variants described in WO 05/09379 and WO 05/09378), IsdC (WO 06/59247), HarA (WO 05/09378), alpha toxin (Hla US4615884), alpha toxin H35R mutant, penicillin binding protein 4 (WO 06/33918), SsaA (WO 05/115113), Aap (WO 05/86663), RAP (WO 99/32133), AhpC and variants (WO 06/78680), SasA (WO 06/121664).

In a preferred embodiment, immunogenic composition of the invention further comprises a number of proteins equal to or greater than 2, 3, 4, 5 or 6 selected from 2, 3 or 4 of the following groups:

- group a) - at least one staphylococcal extracellular component binding protein or fragment thereof selected from the group consisting of Ebh. Elastin binding protein (EbpS), EFB (FIB), ClfA, ClfB, SdrC, SdrG, FnbA,SsaA, SasA, Aap, AhpC, penicillin binding protein 4, IsaA/PisA;
- group b) - at least one staphylococcal transporter protein or fragment thereof selected from the group consisting of IsdA, IsdB, IsdC, HarA;
- group c) - at least one staphylococcal regulator of virulence, toxin or fragment thereof selected from the group consisting of alpha toxin (Hla), alpha toxin H35R mutant, RAP.

Vaccines

Another aspect of the invention is a vaccine comprising the immunogenic composition of the invention and a pharmaceutically acceptable carrier.

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with the polynucleotide and/or polypeptide or the invention, or a fragment or variant thereof, or a combination thereof as described above, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly staphylococcal infection including *S. aureus* and/or *S. epidermidis* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of polynucleotide and/or polypeptide or the invention, or a fragment or a variant thereof, for expressing polynucleotide and/or polypeptide of the invention, or a fragment or a variant thereof, or a combination thereof as described above, *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for

example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a polynucleotide and/or polypeptide of the invention encoded therefrom, or a combination thereof as described above, wherein the composition comprises a recombinant polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A polypeptide of the invention or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

In a vaccine composition according to the invention, a polypeptide and/or polynucleotide, or a fragment, or a mimotope, or a variant thereof , or a combination thereof as described above, may be present in a vector, such as the live recombinant vectors described above for example live bacterial vectors.

Also provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. *et al.* Science 273: 352 (1996).

In an embodiment, the immunogenic composition of the invention is mixed with a pharmaceutically acceptable excipient, and/or optionally with an adjuvant.

The vaccines of the present invention are optionally adjuvanted. Suitable adjuvants include an aluminum salt such as aluminum hydroxide gel (alum) or aluminium phosphate, but may also be a salt of calcium, magnesium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized polysaccharides, or polyphosphazenes.

Optionally the adjuvant is a preferential inducer of either a TH1 or a TH2 type of response. High levels of Th1-type cytokines tend to favor the induction of cell mediated immune responses to a given antigen, whilst high levels of Th2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

It is important to remember that the distinction of Th1 and Th2-type immune response is not absolute. In reality an individual will support an immune response which is described as being predominantly Th1 or predominantly Th2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173). Traditionally, Th1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of Th1-type immune responses are not produced by T-cells, such as IL-12. In contrast, Th2-type responses are associated with the secretion of Il-4, IL-5, IL-6, IL-10. Suitable adjuvant systems which promote a predominantly Th1 response include: Monophosphoryl lipid A or a derivative thereof, particularly 3-de-O-acylated monophosphoryl lipid A (3D-MPL) (for its preparation see GB 2220211 A); and a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with either an aluminium salt (for instance aluminium phosphate or aluminium hydroxide) or an oil-in-water emulsion. In such combinations, antigen and 3D-MPL are contained in the same particulate structures, allowing for more

efficient delivery of antigenic and immunostimulatory signals. Studies have shown that 3D-MPL is able to further enhance the immunogenicity of an alum-adsorbed antigen [Thoelen *et al.* Vaccine (1998) 16:708-14; EP 689454-B1].

An enhanced system involves the combination of a monophosphoryl lipid A and a saponin derivative, particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol as disclosed in WO 96/33739. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210, and is a preferred formulation. Preferably the vaccine additionally comprises a saponin, more preferably QS21. The formulation may also comprise an oil in water emulsion and tocopherol (WO 95/17210). The present invention also provides a method for producing a vaccine formulation comprising mixing a protein of the present invention together with a pharmaceutically acceptable excipient, such as 3D-MPL. Unmethylated CpG containing oligonucleotides (WO 96/02555) are also preferential inducers of a TH1 response and are suitable for use in the present invention.

The vaccine preparations of the present invention may be used to protect or treat a mammal susceptible to infection, by means of administering said vaccine via systemic or mucosal route. These administrations may include injection *via* the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or *via* mucosal administration to the oral/alimentary, respiratory, genitourinary tracts. Although the vaccine of the invention may be administered as a single dose, components thereof may also be co-administered together at the same time or at different times. For co-administration, the optional Th1 adjuvant may be present in any or all of the different administrations. In addition to a single route of administration, 2 different routes of administration may be used. In addition, the vaccines of the invention may be administered IM for priming doses and IN for booster doses.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccines. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that for polysaccharide or oligosaccharide antigens each dose will comprise 0.1-100 μg of saccharide, 0.1-50 μg for saccharide conjugates, 0.1-10 μg, 1-10 μg, or 1 to 5 μg is.

The content of protein antigens in the vaccine will typically be in the range 1-100μg, preferably 5-50μg, most typically in the range 5 - 25μg. Following an initial vaccination, subjects may receive one or several booster immunizations adequately spaced.

Vaccine preparation is generally described in Vaccine Design ("The subunit and adjuvant approach" (eds Powell M.F. & Newman M.J.) (1995) Plenum Press New York). Encapsulation within liposomes is described by Fullerton, US Patent 4,235,877.

The vaccines of the present invention may be stored in solution or lyophilized. Optionally the solution is lyophilized in the presence of a sugar such as sucrose, trehalose or lactose. Optionally they are lyophilized and extemporaneously reconstituted prior to use. Lyophilizing may result in a more stable composition (vaccine).

A further aspect of the invention relates to a process for making the vaccine of the invention comprising the step of adding a pharmaceutically acceptable excipient to the immunogenic composition of the invention.

A further aspect of the invention relates to the immunogenic composition, polypeptide or polynucleotide of the invention for use in the treatment of prevention of staphylococcal disease.

A further aspect of the invention relates to a use of the immunogenic composition, polypeptide or polynucleotide of the invention in the preparation of a medicament for the treatment or prevention of staphylococcal disease.

A further aspect of the invention relates to a method of treating or preventing staphylococcal disease comprising administering the immunogenic composition, vaccine, polypeptide or polynucleotide of the invention to a patient in need thereof.

The invention also encompasses method of treatment or staphylococcal infection, particularly hospital acquired nosocomial infections.

The immunogenic composition or vaccine of the invention is particularly advantageous to use in cases of elective surgery. Such patients will know the date of surgery in advance and could be inoculated in advance. Since it is not know whether the patient will be exposed to *S.aureus* or *S. epidermidis* infection, it is preferred to inoculate with a vaccine of the invention that protects against both, as described above. Preferably adults over 16

awaiting elective surgery are treated with the immunogenic compositions and vaccines of the invention.

It is also advantageous to inoculate health care workers with the vaccine of the invention.

The vaccine preparations of the present invention may be used to protect or treat a mammal susceptible to infection, by means of administering said vaccine via systemic or mucosal route. These administrations may include injection *via* the intramuscular, intraperitoneal, intradermal or subcutaneous routes; or *via* mucosal administration to the oral/alimentary, respiratory, genitourinary tracts.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccines. Such amount will vary depending upon which specific immunogen is employed and how it is presented. The protein content of the vaccine will typically be in the range1 -100μg, preferably 5-50μg, most typically in the range 10 - 25μg. Generally, it is expected that each dose will comprise 0.1-100 μg of polysaccharide where present, preferably 0.1-50 μg, preferably 0.1-10 μg, of which 1 to 5 μg is the most preferable range. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisations adequately spaced.

Although the vaccines of the present invention may be administered by any route, administration of the described vaccines into the skin (ID) forms one embodiment of the present invention. Human skin comprises an outer “horny” cuticle, called the stratum corneum, which overlays the epidermis. Underneath this epidermis is a layer called the dermis, which in turn overlays the subcutaneous tissue. Researchers have shown that injection of a vaccine into the skin, and in particular the dermis, stimulates an immune response, which may also be associated with a number of additional advantages. Intradermal vaccination with the vaccines described herein forms a preferred feature of the present invention.

The conventional technique of intradermal injection, the "mantoux procedure", comprises steps of cleaning the skin, and then stretching with one hand, and with the bevel of a narrow gauge needle (26-31 gauge) facing upwards the needle is inserted at an angle of between 10-15°. Once the bevel of the needle is inserted, the barrel of the needle is lowered and further advanced whilst providing a slight pressure to elevate it under the skin. The liquid is then injected very slowly thereby forming a bleb or bump on the skin surface, followed by slow withdrawal of the needle.

More recently, devices that are specifically designed to administer liquid agents into or across the skin have been described, for example the devices described in WO 99/34850 and EP 1092444, also the jet injection devices described for example in WO 01/13977; US 5,480,381, US 5,599,302, US 5,334,144, US 5,993,412, US 5,649,912, US 5,569,189, US 5,704,911, US 5,383,851, US 5,893,397, US 5,466,220, US 5,339,163, US 5,312,335, US 5,503,627, US 5,064,413, US 5,520, 639, US 4,596,556, US 4,790,824, US 4,941,880, US 4,940,460, WO 97/37705 and WO 97/13537. Alternative methods of intradermal administration of the vaccine preparations may include conventional syringes and needles, or devices designed for ballistic delivery of solid vaccines (WO 99/27961), or transdermal patches (WO 97/48440; WO 98/28037); or applied to the surface of the skin (transdermal or transcutaneous delivery WO 98/20734 ; WO 98/28037).

When the vaccines of the present invention are to be administered to the skin, or more specifically into the dermis, the vaccine is in a low liquid volume, particularly a volume of between about 0.05 ml and 0.2 ml.

The content of antigens in the skin or intradermal vaccines of the present invention may be similar to conventional doses as found in intramuscular vaccines (see above). However, it is a feature of skin or intradermal vaccines that the formulations may be "low dose". Accordingly the protein antigens in "low dose" vaccines are preferably present in as little as 0.1 to 10μg, preferably 0.1 to 5 μg per dose; and the polysaccharide (preferably conjugated) antigens may be present in the range of 0.01-1μg, and preferably between 0.01 to 0.5 μg of polysaccharide per dose.

As used herein, the term "intradermal delivery" means delivery of the vaccine to the region of the dermis in the skin. However, the vaccine will not necessarily be located exclusively in the dermis. The dermis is the layer in the skin located between about 1.0 and about 2.0

mm from the surface in human skin, but there is a certain amount of variation between individuals and in different parts of the body. In general, it can be expected to reach the dermis by going 1.5 mm below the surface of the skin. The dermis is located between the stratum corneum and the epidermis at the surface and the subcutaneous layer below. Depending on the mode of delivery, the vaccine may ultimately be located solely or primarily within the dermis, or it may ultimately be distributed within the epidermis and the dermis.

A further embodiment of the invention is a method of preventing or treating staphylococcal infection comprising the step of administering the vaccine of the invention to a patient in need thereof, for example a patient awaiting elective surgery.

The term 'staphylococcal infection' encompasses infection caused by *S.aureus* and/or *S. epidermidis* and other staphylococcal strains capable of causing infection in a mammalian, preferably human host.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

Examples

Example 1 Recombinant expression in E.coli and purification of Protein A-Sbi Fusion Protein

A 65kDa Protein A-Sbi fusion protein was made by fusing a fragment of protein A gene to a fragment of an gene Sbi, cloning in the expression vector TCMP14 and expressing the fusion protein in *E. coli*.

The *S. aureus* Protein A fragment, encoding a 43kDa fragment of Protein A included the five IgG-binding domains but not including the signal peptide or the C terminal wall anchor region, was amplified from NCTC8325 DNA strain (ATCC35556D) as a 1164 base pair fragment. The primers used were:-

ggaattc **catatg** GCGCAACACGATGAAGCTC (including an NdeI site in bold)
and
cgc **ggatcc** GCCGACATGTACTCCGTTACCATC (including a BamHI site in bold)

The Sbi fragment, encoding a 20kDa fragment of Sbi including the two IgG binding domains but excluding the β-2-glycoprotein 1 IgG binding domain, was amplified from NCTC8325 DNA strain (ATCC35556D) as a 522 base pair fragment. The primers used were:

cgc **ggatcc** AGTGAAAACACGCAACAAACTTC (including a BamH1 site in bold)
and
gc **tctaga tta actagt** TGCTTTTTCAATTGAAACTTTTTCTAC (including a Xba1 site and a stop codon, underlined in bold and a Spe1 site in bold)

The two fragments were cloned into a TCMP14 vector using NdeI, BamHI and SpeI cloning sites and were transformed in *E.coli* AR58.

Bacteria cultures (4x250 ml) were performed at 30°C in LBT + kanamycin 50 µg/ml. Protein expression was induced at the temperature of 42°C for four hours. The bacterial culture was centrifuged to form bacterial cell pellets and pellets were conserved at –20°C until extraction. Figure 2 shows a coomassie strained 4-20% PAGE showing that Protein A-Sbi expression is seen in transformed cells after 4 hours at the induction temperature. A new band of 65kDa appears in lane 5 containing bacteria incubated at the induction temperature for 4 hours.

The bacterial pellet (corresponding to 4 x 250 ml of bacterial culture) was resuspended in

binding buffer containing 20 mM phosphate (Na2HPO4/NaH2PO4), 500 mM NaCl, pH 7.4. As proteins are sensitive to proteases, protease inhibitor (Pefablock 1mM) was added to the suspension. Then bacteria were lysed by 4 passages at the French Press. The lysate was centrifuged for 20 minutes at 13,000rpm and the supernatent was used for the purification. A Hi-Trap chelating HP (5 ml) column (Amersham Biosciences) was used for the purification. The supernatant was loaded onto the column which was subsequently washed with 20 mM phosphate (Na2HPO4/NaH2PO4), 500 mM NaCl, pH 7.4. The bound fusion protein was then eluted with elution buffer containing: 20mM phosphate (Na2HPO4/NaH2PO4), 500 mM NaCl, 100 mM imidazole, pH 7.4. After purification, eluted fractions were pooled, dialysed against 50mM phosphate (Na2HPO4/NaH2PO4), 150mM NaCl, pH 7.4, filtrated (0.22µm) and quantified using kit BCA ™ Protein Assay (Pierce). The yield of the purification was 4.73 mg.

SDS-PAGE and Western blot analysis were performed on the purified protein A-SBI. Figure 3 shows a coomassie stained 4-20% PAGE. A pure band of 65kDa was present in the purified protein samples.

Example 2 Evaluation of the proteinA-SBI fusion protein in a mice mortality model induced by *S. aureus* intraperitoneal infection

Method

Groups of 25 4 week old female CD1 mice were immunized intramuscularly three times (days 0, 14 and 28) with 8 µg of proteinA (ProtA) or ProtA-SBI fusion protein, both adjuvanted in $AlPO_4$. Control mice were immunised with the equivalent amount of $AlPO_4$ adjuvant or killed whole cell *S. aureus* serotype 5 Reynolds (5 10^8 CFU) adjuvanted with $AlPO_4$. On day 42, mice were challenged intraperitoneally with 500 µl of *S. aureus* strain 5 Reynolds (3 10^6 CFU) supplemented with 5% of mucin. Mortality of mice was followed until 4 days after challenge.

Results

The results are shown in Figure 4. As expected, no protection was observed in mice immunized with AlPO4 alone. A very good protection was observed in positive control group immunized with the homologous killed whole cell. No protection was observed after immunization with the ProtA. Although not statistically significant, a increased survival rate of 24% was obtained in mice immunized with the ProtA-SBI construct.

Conclusion

Immunisation with the combination of protein A and Sbi IgG domains in the fusion protein was able to provide greater protection than immunisation with protein A alone. The protection against *S. aureus* infection induced by immunization with the proteinA-SBI fusion protein in this model suggests that this antigen may be used in combination with other candidates in order to obtain an effective *S. aureus* vaccine.

Example 3 Bioinformatic analysis of protein A and Sbi sequences

The sequences of Sbi from *S. aureus* strains Col, Mu50, NCTC8325, N315, Mw2, MRSA252 and MSSA476 were compared and the percentage identity was calculated using the ClustalW program. The alignment is shown in Figure 5 and the sequences were found to share 92-100% identity.

The IgG binding domains were identified as shown in Table 1.

Strain	Domain	Begin (aa)	End (aa)	Begin (nT)	End (nT)
NCTC8325	Dom1	43	94	127	282
	Dom2	95	148	283	444
Mu50	Dom1	45	96	133	288
	Dom2	97	150	289	450
N315	Dom1	43	94	127	282
	Dom2	95	148	283	444
Mw2	Dom1	43	94	127	282
	Dom2	95	148	283	444
Col	Dom1	43	94	127	282
	Dom2	95	148	283	444
MRSA252	Dom1	43	94	127	282
	Dom2	95	148	283	444
MSSA476	Dom1	43	94	127	282
	Dom2	95	148	283	444

The sequences of Protein A from *S. aureus* strains Col, Mu50, NCTC8325, N315, Mw2, MRSA252 and MSSA476 were compared and the percentage identity was calculated using the ClustalW program. The alignment is shown in Figure 4 and the sequences were found to share 91-100% identity. The IgG binding domains are identified in Table 2.

Strain	Domain	Begin (aa)	End (aa)	Begin (nT)	End (nT)
NCTC8325	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
	Dom5	270	323	808	969

Mu50	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
N315	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
Mw2	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
	Dom5	270	323	808	969
COL	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
	Dom5	270	323	808	969
MRSA252	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
	Dom5	270	323	808	969
MSSA476	Dom1	35	88	103	264
	Dom2	96	149	286	447
	Dom3	154	207	460	621
	Dom4	212	265	634	795
	Dom5	270	323	808	969
V8	Dom1	47	100	139	300
	Dom2	108	161	336	483
	Dom3	166	219	496	657
	Dom4	224	277	670	831

Example 4 Construction of a fusion protein containing ProtA-SdrG-Sbi (+/- 150 kDa)

The sdrG gene was cloned in the Protein A – Sbi construct described in Example 1, by inserting sdrG into the *BamHI* site between gene ProtA and gene Sbi to form a ProtA-SdrG-Sbi construct.

SdrG was amplified by PCR from genomic DNA of the strain *S. epidermidis* 12228 using the following primers:

Primer 1 : fus SdrG28 - 01 **(BamHI).**

CGCGGATCCGAGGAGAATTCAGTACAAGAC

Primer 2 : fus SdrG28 - 02 **(BamHI).**

CGCGGATCCTTCGTCATCATAGTATCCGTTATC

The resultant construct had the sequence of SEQ ID NO: 75. This construct was used to express ProteinA-SdrG-Sbi fusion protein using the protocol described in example 1. The expression resulted in a 150kDa protein protein A-SdrG-Sbi protein which was visualized by running on a 4-20% polyacrylamide gel and staining with commassie blue or by western blotting using an anti-His tag antibody.

Sequences

SEQ ID NO 1 Sbi domain 1

TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS

SEQ ID NO 2 Sbi domain 2

KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA

SEQ ID NO 3 SBI domains 1 and 2

TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKN
PDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA

SEQ ID NO 4 Sbi part of fusion protein

SENTQQTSTKHQTTQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSE
SLKDSKNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKAK
ERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKA

SEQ ID NO 5 Sbi Col

MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH
LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE
KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN
SKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKA
PMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKVESPKVEVPQIQSPKVE
VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDQTVLTVL
GSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQET
ASSIKNTLSNLLSFWK

SEQ ID NO 6 Sbi Mu50

MHMKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQV
LHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTE
QEKNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYE
ANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVN
KAPMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQSKLLG
YYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDQTVLTVLGSGSKSYI
QPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQETASSIKNTL
SNLLSFWK

SEQ ID NO 7 sbi MRSA252

MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH
LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE
KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN
SKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKA
PMDVQKHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPKVE
VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNKYYQYKGLIDKTVLTTI
GSGYGSYIKPLEVSKESGNLAKSYAQVRNYVTESINTGKVLYAFYQKPELVKTAIKAQET

ATTFKNALTGIFKSFWK

SEQ ID NO 8 sbi MSSA476

MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH
LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE
KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN
SKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNVKDSIENRRLAQREVNKA
PMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPKVE
VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDKAVLTLL
GDGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQET
ASSIKNTITGLFNSFWK

SEQ ID NO 9 sbi MW2

MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH
LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE
KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN
SKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNVKDSIENRRLAQREVNKA
PMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPKVE
VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDKAVLTLL
GDGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQET
ASSIKNTITGLFNSFWK

SEQ ID NO 10 sbi N315

MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH
LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE
KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN
SKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKA
PMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPKVE
VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDQTVLTVL
GSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQET
ASSIKNTLSNLLSFWK

SEQ ID NO 11 sbi NCTC8325

MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYVTDQQKAFYQVLH
LKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQE
KNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEAN
SKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKA
PMDVKEHLQKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKVESPKVEVPQIQSPKVE
VPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDQTVLTVL
GSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQET
ASSIKNTLSNLLSFWK

SEQ ID NO 12 SPA domain 1

NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS

SEQ ID NO 13 SPA domain 2 (V8)

QQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNES

SEQ ID NO 14 domain 2 (Mu50)

QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES

SEQ ID NO 15 domain 3 (MRSA)

ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNES

SEQ ID NO 16 domain 3 (V8)

ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA

SEQ ID NO 17 domain 3 (MU50)

ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES

SEQ ID NO 18 domain 4 (MRSA)

ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA

SEQ ID NO 19 domain 4 (V8)

ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA

SEQ ID NO 20 domain 4 (MU50)

ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA

SEQ ID NO 21 domain 5

ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA

SEQ ID NO 22 domains 1+2

MAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQ
APKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNES

SEQ ID NO 23 domains 2 -3

QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNES

SEQ ID NO 24 domains 3-4

ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ

SANLLAEAKKLNDA

SEQ ID NO 25 domains 4-5

ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKD
DPSVSKEILAEAKKLNDAQA

SEQ ID NO 26 domains 1-3

MAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQ
APKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNES

SEQ ID NO 27 domains 2-4

QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDA

SEQ ID NO 28 domains 3-5

QAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKD
DPSVSKEILAEAKKLNDAQA

SEQ ID NO 29 domains 1-4

AQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQ
APKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDA

SEQ ID NO 30 domains 2-5

QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKD
DPSVSKEILAEAKKLNDAQA

SEQ ID NO 31 domains 1-5

AQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQ

APKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKD
DPSVSKEILAEAKKLNDAQA

SEQ ID NO 32 protein A part of fusion protein

MAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQ
APKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEA
KKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANL
LSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQ
SANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKD
DPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPG
KEDNNKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDGNK
PGKEDGNGVHVG

SEQ ID NO 33 SPA NCTC8425

MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNFNKDQQSAFYEILNMPNLNEA
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFI
QSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGK
EDNNKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGN
GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQ
ALPETGEENPFIGTTVFGGLSLALGAALLAGRRREL

SEQ ID NO 34 SPA Mu50

MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNFNKDQQSAFYEILNMPNLNEA
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNKKPGKEDGNKPGKEDGNKPGKEDNKKP
GKEDGNKPGKEDNNKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNGVHVVK
PGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQALPETG
EENPFIGTTVFGGLSLALGAALLAGRRREL

SEQ ID NO 35 SPA N315

MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNFNKDQQSAFYEILNMPNLNEA
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNKKPGKEDGNKPGKEDGNKPGKEDNKKP
GKEDGNKPGKEDNNKPGKEDGNKPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGNGVHVVK
PGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQALPETG
EENPFIGTTVFGGLSLALGAALLAGRRREL

SEQ ID NO 36 SPA MW2

MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNFNKDQQSAFYEILNMPNLNEA
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFI
QSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDGNKPGKEDGNKPGKEDNNKPGK
EDGNKPGKEDNKKPGKEDGNKPGKEDNNKPGKEDGNGVHVVKPGDTVNDIAKANGTTADK
IAADNKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLAL
GAALLAGRRREL

SEQ ID NO 37 SPA Col

LKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNFNKDQQSAFYEILNMPNLNEA
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFI
QSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGK
EDGNKPGKEDNKKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGNGVHVVKPG
DTVNDIAKANGTTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEE
NPFIGTTVFGGLSLALGAALLAGRRREL

SEQ ID NO 38 MRSA252

LKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNKFNKDQQSAFYEILNMPNLNEE
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLAEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFI
QSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDNNKPGKEDGNKPGKEDNKKPGK
EDGNKPGKEDNKKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGN
GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQ
ALPETGEENPFIGTTVFGGLSLALGAALLAGRRREL

SEQ ID NO 39 SPA MSSA476

LKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFYQVLNMPNLNADQ
RNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNNFNKDQQSAFYEILNMPNLNEA
QRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQR
NGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQRNG
FIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQRNGFI
QSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDGNKPGKEDGNKPGKEDNNKPGK
EDGNKPGKEDNKKPGKEDGNKPGKEDNNKPGKEDGNGVHVVKPGDTVNDIAKANGTTADK
IAADNKLADKNMIKPGQELVVDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLAL
GAALLAGRRREL

SEQ ID NO 40 SPA V8

MMTLQIHTGGINLKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQHDEAQQNAFY
QVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADAQQNKFNKDQQSAF
YEILNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYE
ILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEIL
HLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKEDNNKPGKED

GNKPGKEDNKKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNGV
HVVKPGDTVNDIAKANGTTADKIAVDNKLADKNMIKPGQELVVDKKQPANHADANKAQAL
PETGEENPFIGTTVFGGLSLALGAALLAGRRREL

SEQ ID NO 41 fusion protein

MAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADA
QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKAD
NNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNK
FNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFN
KEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPG
KEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDN
KKPGKEDGNKPGKEDGNKPGKEDGNGVHVGGSSENTQQTSTKHQTTQNNYVTDQQKAFYQ
VLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLT
EQEKNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAY
EANSKLPKDLRDKNNRFVEKVSIEKA

SEQ ID NO 42 fusion protein full length

MAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSQAPKADA
QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNESQAPKAD
NNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNK
FNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFN
KEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPG
KEDNNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDN
KKPGKEDGNKPGKEDGNKPGKEDGNGVHVGGSSENTQQTSTKHQTTQNNYVTDQQKAFYQ
VLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLT
EQEKNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAY
EANSKLPKDLRDKNNRFVEKVSIEKATSGHHHHHH

SEQ ID NO 43 Sbi domain 1

ACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACATCTAAAAG
GTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCAGAACGTGC
ACAAGAAGTATTCTCTGAATCACTTAAAGACAGC

SEQ ID NO 44 Sbi domain 2

AAGAACCCAGACCGACGTGTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATA
ACTTAACTGAACAAGAAAAAAATAATTACATTGCACAAATTAAAGAAAACCCTGATAGAAG
CCAACAAGTTTGGGTAGAATCAGTACAATCTTCTAAAGCT

SEQ ID NO 45 Sbi domains 1 and 2

ACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACATCTAAAAG
GTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCAGAACGTGC
ACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGACCGACGTGTTGCACAA
CAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAAAAAAATAATT
ACATTGCACAAATTAAAGAAAACCCTGATAGAAGCCAACAAGTTTGGGTAGAATCAGTACA

ATCTTCTAAAGCT

SEQ ID NO 46 sbi part of fusion protein

AGTGAAAACACGCAACAAACTTCAACTAAGCACCAAACAACTCAAAACAACTACGTAACAG
ATCAACAAAAAGCTTTTTATCAAGTATTACATCTAAAAGGTATCACAGAAGAACAACGTAA
CCAATACATCAAAACATTACGCGAACACCCAGAACGTGCACAAGAAGTATTCTCTGAATCA
CTTAAAGACAGCAAGAACCCAGACCGACGTGTTGCACAACAAAACGCTTTTTACAATGTTC
TTAAAAATGATAACTTAACTGAACAAGAAAAAAATAATTACATTGCACAAATTAAAGAAAA
CCCTGATAGAAGCCAACAAGTTTGGGTAGAATCAGTACAATCTTCTAAAGCTAAAGAACGT
CAAAATATTGAAAATGCGGATAAAGCAATTAAAGATTTCCAAGATAACAAAGCACCACACG
ATAAATCAGCAGCATATGAAGCTAACTCAAAATTACCTAAAGATTTACGTGATAAAAACAA
CCGCTTTGTAGAAAAAGTTTCAATTGAAAAAGCA

SEQ ID NO 47 Sbi Col

ATGAAAAATAAATATATCTCGAAGTTGCTAGTTGGGGCAGCAACAATTACGTTAGCTACA
ATGATTTCAAATGGGGAAGCAAAAGCGAGTGAAAACACGCAACAAACTTCAACTAAGCAC
CAAACAACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACAT
CTAAAAGGTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCA
GAACGTGCACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGACCGACGT
GTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAA
AAAAATAATTACATTGCACAAATTAAAGAAAACCCTGATAGAAGCCAACAAGTTTGGGTA
GAATCAGTACAATCTTCTAAAGCTAAAGAACGTCAAAATATTGAAAATGCGGATAAAGCA
ATTAAAGATTTCCAAGATAACAAAGCACCACACGATAAATCAGCAGCATATGAAGCTAAC
TCAAAATTACCTAAAGATTTACGTGATAAAAACAACCGCTTTGTAGAAAAAGTTTCAATT
GAAAAAGCAATCGTTCGTCATGATGAGCGTGTGAAATCAGCAAATGATGCAATCTCAAAA
TTAAATGAAAAAGATTCAATTGAAAACAGACGTTTAGCACAACGTGAAGTTAACAAAGCA
CCTATGGATGTAAAAGAGCATTTACAGAAACAATTAGACGCATTAGTTGCTCAAAAAGAT
GCTGAAAAGAAAGTGGCGCCAAAAGTTGAGGCTCCTCAAATTCAATCACCACAAATTGAA
AAACCTAAAGTAGAATCACCAAAAGTTGAAGTCCCTCAAATTCAATCACCAAAAGTTGAG
GTTCCTCAATCTAAATTATTAGGTTACTACCAATCATTAAAAGATTCATTTAACTATGGT
TACAAGTATTTAACAGATACTTATAAAAGCTATAAAGAAAAATATGATACAGCAAAGTAC
TACTATAATACGTACTATAAATACAAAGGTGCGATTGATCAAACAGTATTAACAGTACTA
GGTAGTGGTTCTAAATCTTACATCCAACCATTGAAAGTTGATGATAAAAACGGCTACTTA
GCTAAATCATATGCACAAGTAAGAAACTATGTAACTGAGTCAATCAATACTGGTAAAGTA
TTATATACTTTCTACCAAAACCCAACATTAGTAAAAACAGCTATTAAAGCTCAAGAAACT
GCATCATCAATCAAAAATACATTAAGTAATTTATTATCATTCTGGAAATAA

SEQ ID NO 48 Sbi Mu50

ATACACATGAAAAATAAATATATCTCGAAGTTGCTAGTTGGGGCAGCAACAATTACTTTA
GCTACAATGATTTCAAATGGGGAAGCAAAAGCGAGTGAAAACACGCAACAAACTTCAACT
AAGCACCAAACAACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTA
TTACATCTAAAAGGTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAA
CACCCAGAACGTGCACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGAC
CGACGTGTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAA
CAAGAAAAAAATAATTACATTGCACAAATTAAAGAAAACCCTGATAGAAGCCAACAAGTT
TGGGTAGAATCAGTACAATCTTCTAAAGCTAAAGAACGTCAAAATATTGAAAATGCGGAT
AAAGCAATTAAAGATTTCCAAGATAACAAAGCACCACACGATAAATCAGCAGCATATGAA
GCTAACTCAAAATTACCTAAAGATTTACGCGATAAAAATAACCGCTTTGTAGAAAAAGTT
TCAATTGAAAAAGCAATCGTTCGTCATGATGAGCGTGTGAAATCAGCAAATGATGCAATC
TCAAAATTAAATGAAAAAGATTCAATTGAAAACAGACGTTTAGCACAACGTGAAGTTAAC
AAAGCACCTATGGATGTAAAAGAGCATTTACAGAAACAATTAGACGCATTAGTAGCTCAA
AAAGATGCTGAAAAGAAAGTGGCGCCAAAAGTTGAGGCTCCTCAAATTCAATCACCACAA

ATTGAAAAACCTAAAGCAGAATCACCAAAAGTTGAAGTCCCTCAATCTAAATTATTAGGT
TACTACCAATCATTAAAAGATTCATTTAACTATGGTTACAAGTATTTAACAGATACTTAT
AAAAGCTATAAAGAAAAATATGATACAGCAAAGTACTACTATAATACGTACTATAAATAC
AAAGGTGCGATTGATCAAACAGTATTAACAGTACTAGGTAGTGGTTCTAAATCTTACATC
CAACCATTGAAAGTTGATGATAAAAACGGCTACTTAGCTAAATCATATGCACAAGTAAGA
AACTATGTAACTGAGTCAATCAATACTGGTAAAGTATTATATACTTTCTACCAAAACCCA
ACATTAGTAAAAACAGCTATTAAAGCTCAAGAAACTGCATCATCAATCAAAAATACATTA
AGTAATTTATTATCATTCTGGAAATAA

SEQ ID NO 49 Sbi MRSA252

ATGAAAAATAAATATATCTCGAAGTTGCTAGTTGGGGCAGCAACAATTACTTTAGCTACA
ATGATTTCAAATGGGGAAGCAAAAGCGAGTGAAAACACGCAACAAACTTCAACTAAGCAC
CAAACAACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACAT
CTAAAAGGTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCA
GAACGTGCACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGACCGACGT
GTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAA
AAAAATAATTACATTGCACAAATTAAAGAAAACCCTGATAGAAGCCAACAAGTTTGGGTA
GAATCAGTACAATCTTCTAAAGCTAAAGAACGTCAAAATATTGAAAATGCGGATAAAGCA
ATTAAAGATTTCCAAGATAACAAAGCACCACACGATAAATCAGCAGCATATGAAGCTAAC
TCAAAATTACCTAAAGATTTACGTGATAAAAATAACCGCTTTGTAGAAAAAGTTTCAATT
GAAAAAGCAATCGTTCGTCATGATGAGCGTGTGAAATCAGCAAATGATGCAATCTCAAAA
TTAAATGAAAAAGATTCAATTGAAAACAGACGTTTAGCACAACGTGAAGTTAATAAAGCA
CCTATGGATGTACAAAAGCATTTACAGAAACAATTAGACGCATTAGTAGCTCAAAAAGAT
GCTGAAAAGAAAGTGGCGCCAAAAGTTGAGGCTCCTCAAATTCAATCACCACAAATTGAA
AAACCTAAAGCAGAATCACCAAAAGTTGAAGTCCCTCAAATTCAATCACCAAAAGTTGAG
GTTCCTCAATCTAAATTATTAGGTTACTACCAATCATTAAAAGATTCATTTAACTATGGT
TACAAGTATTTAACAGATACTTATAAAAGCTATAAAGAAAAATATGATACAGCAAAGTAC
TACTATAATAAATATTACCAATATAAAGGTTTGATTGATAAAACAGTTTTAACAACTATC
GGTAGTGGCTATGGTTCATACATTAAACCTCTTGAAGTAAGCAAAGAAAGCGGGAACTTA
GCTAAATCATATGCACAAGTAAGAAACTATGTAACTGAGTCAATCAACACTGGTAAAGTG
TTATACGCATTCTACCAAAAACCAGAATTAGTAAAAACAGCTATTAAAGCTCAAGAAACA
GCAACAACTTTCAAAAACGCTTTAACAGGCATATTCAAATCATTCTGGAAATAA

SEQ ID NO 50 Sbi MSSA476

ATGAAAAATAAATATATCTCGAAGTTGCTAGTTGGGGCAGCAACAATTACTTTAGCTACA
ATGATTTCAAATGGGGAAGCAAAAGCGAGTGAAAACACGCAACAAACTTCAACTAAGCAC
CAAACAACTCAAAACAACTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACAT
CTAAAAGGTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCA
GAACGTGCACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGACCGACGT
GTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAA
AAAAATAATTACATTGCACAAATTAAAGAAAACCCTGATAGAAGCCAACAAGTTTGGGTA
GAATCAGTACAATCTTCTAAAGCTAAAGAACGTCAAAATATTGAAAATGCGGATAAAGCA
ATTAAAGATTTCCAAGATAACAAAGCACCACACGATAAATCAGCAGCATATGAAGCTAAC
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GCTAAATCATATGCACAAGTAAGAAACTATGTAACTGAGTCAATCAATACTGGTAAAGTA
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SEQ ID NO 51 Sbi MW2

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GTTGCACAACAAAACGCTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAA
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SEQ ID NO 52 Sbi N315

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GCTAAATCATATGCACAAGTAAGAAACTATGTAACTGAGTCAATCAATACTGGTAAAGTA
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SEQ ID NO 53 Sbi NCTC8325

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SEQ ID NO 54 SPA domain 1

AATGCTGCGCAACACGATGAAGCTCAACAAAATGCTTTTTATCAAGTCTTAAATATGCCT
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AGTGCTAACGTTTTAGGTGAAGCTCAAAAACTTAATGACTCT

SEQ ID NO 55 SPA domain 2 (V8)

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AGGTGAAGCTAAAAAATTAAACGAATCT

SEQ ID NO 56 domain 2 (Mu50)

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SEQ ID NO 57 domain 3 (MRSA)

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SEQ ID NO 58 domain 3 (V8)

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SEQ ID NO 59 domain 3 (MU50)

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SEQ ID NO 60 domain 4 (MRSA)

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SEQ ID NO 61 domain 4 (V8)

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AGCAAAGAAATTTTAGCAGAAGCTAAAAAGCTAAACGATGCT

SEQ ID NO 62 domain 4 (MU50)

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AGCAAAGAAATTTTAGCAGAAGCTAAAAAGCTAAACGATGCT

SEQ ID NO 63 domain 5

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AACTTAACTGAAGAACAACGTAACGGCTTCATCCAAAGCCTTAAAGACGATCCTTCAGTG
AGCAAAGAAATTTTAGCAGAAGCTAAAAAGCTAAACGATGCT

SEQ ID NO 64 protein A part of fusion protein

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TTAAATGCTGATCAACGCAATGGTTTTATCCAAAGCCTTAAAGATGATCCAAGCCAAAGT
GCTAACGTTTTAGGTGAAGCTCAAAAACTTAATGACTCTCAAGCTCCAAAAGCTGATGCG
CAACAAAATAACTTCAACAAAGATCAACAAAGCGCCTTCTATGAAATCTTGAACATGCCT
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SEQ ID NO 65 SPA NCTC8325

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SEQ ID NO 66 SPA Mu50

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SEQ ID NO 67 SPA N315

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SEQ ID NO 68 SPA MW2

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SEQ ID NO 69 PSA COL

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SEQ ID NO 70 SPA MRSA252

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SEQ ID NO 71 SPA MSSA476

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SEQ ID NO 72 SPA V8

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SEQ ID NO 73 fusion protein

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SEQ ID NO 74 fusion protein with His tag

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Sequence ID NO 75 – protein A-SdrG-Sbi fusion protein

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Sequence ID No 76 Protein A-SdrG-Sbi fusion protein

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103

AMENDED CLAIMS

1. An immunogenic composition comprising two different isolated staphylococcal polypeptides, each comprising an IgG binding domain.

2. The immunogenic composition of claim 1 comprising an isolated protein A polypeptide from *S. aureus* or a fragment thereof containing an IgG binding domain.

3. The immunogenic composition of claim 2 wherein the isolated protein A polypeptide has a sequence which is at least 80% identical to SEQ ID NO 12-40 or fragment thereof comprising an IgG binding domain.

4. The immunogenic composition of claim 2 wherein the isolated protein A polypeptide is encoded by a polynucleotide having a sequence which is at least 80% identical to SEQ ID NO: 54-72 or fragment thereof comprising an IgG binding domain.

5. The immunogenic composition of any one of claims 2-4 wherein the isolated protein A polypeptide or fragment thereof comprises 1, 2, 3, 4 or 5 IgG binding domains.

6. The immunogenic composition of any one of claims 1-5 comprising an isolated Sbi polypeptide from *S. aureus* or a fragment thereof containing an IgG binding domain.

7. The immunogenic composition of claim 6 wherein the isolated Sbi polypeptide has a sequence which is at least 80% identical to SEQ ID NO:1-11 or fragment thereof containing an IgG binding domain.

8. The immunogenic composition of claim 6 or 7 wherein the isolated Sbi polypeptide is encoded by a polynucleotide having a sequence which is at least 80% identical to SEQ ID NO: 43-53 or fragment thereof comprising an IgG binding domain.

9. The immunogenic composition of any one of claims 6-8 wherein the isolated Sbi polypeptide or fragment thereof comprises two IgG binding domains.

10. The immunogenic composition of any preceding claim wherein the isolated protein A polypeptide or fragment thereof is covalently bonded to the isolated Sbi polypeptide or fragment thereof to form a fusion protein.

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11. The immunogenic composition of claim 10 wherein the fusion protein has a polypeptide sequence which is at least 80% identical to the sequence of SEQ ID NO:41-42 or 76 or fragment thereof comprising an IgG binding domain from both Protein A and Sbi.

12. The immunogenic composition of claim 10 wherein the fusion protein is encoded by a polynucleotide having a sequence which is at least 80% identical to SEQ ID NO: 73-75 or fragment thereof encoding an IgG binding domain from both Protein A and Sbi.

13. The immunogenic composition of any one of claims 1-12 comprising a further staphylococcal antigen.

14. The immunongenic composition of claim 13 wherein the further staphylococcal antigen is derived from *S. aureus*.

15. The immunogenic composition of claims 14 wherein the further staphylococcal antigen comprises *S. aureus* type 5 capsular polysaccharide.

16. The immunogenic composition of any one of claims 13-15 wherein the further staphylococcal antigen comprises *S. aureus* type 8 capsular polysaccharide.

17. The immunogenic composition of any one of claims 13-16 wherein the further staphylococcal antigen comprises PNAG.

18. The immunogenic composition of claim 17 wherein the PNAG is less than 50%, 40%, 30%, 20% or 10% N-acetylated.

19. The immunogenic composition of any one of claims 15-18 wherein the *S. aureus* type 5 capsular polysaccharide is conjugated to a carrier protein.

20. The immunogenic composition of any one of claims 16-19 wherein the *S. aureus* type 8 capsular polysaccharide is conjugated to a carrier protein.

21. The immunogeninc composition of any one of claims 15-20 wherein the PNAG is conjugated to a carrier protein.

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22. The immunogenic composition of any one of clams 15-21 wherein the carrier protein is independently selected from the group consisting of tetanus toxoid, diphtheria toxoid, CRM197, protein D, alpha toxin, SdrG, ClfA, IsdA, IsdB, IsdH, protein A, Sbi and a proteinA-Sbi fusion protein or fragments thereof.

23. The immunogenic composition of any one of claims 18-22 wherein the further staphylococcal antigen comprises a protein selected from the group consisting of Ebh, Elastin binding protein (EbpS), EFB (FIB), ClfA, ClfB, SdrC, SdrG, FnbA, FbpA, IsaA/PisA, Penicillin binding protein 4, AhpC, SsaA, Aap, SasA, IsdA, IsdB, IsdC, HarA (IsdH), alpha toxin (Hla), alpha toxin H35R mutant and RAP.

24. A polypeptide comprising: a) a protein A part having an amino acid sequence having at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 12-40, or an immunogenic fragment thereof comprising at least one IgG binding domain and b) an Sbi part which has an amino acid sequence having at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 1-11, or an immunogenic fragment thereof, comprising at least one IgG binding domain.

25. The polypeptide of claim 24 having an amino acid sequence which is at least 85% identical to SEQ ID NO 41 or 42 or 76.

26. The polypeptide of claim 24 or 25, conjugated to at least one further antigen.

27. The polypeptide of claim 26 wherein the at least one further antigen comprises *S. aureus* type 5 polysaccharide or oligosaccharide, *S. aureus* type 8 polysaccharide or oligosaccharide or PNAG (optionally less than 50%, 40%, 30%, 20% or 10% N-acetylated).

28. A polynucleotide comprising: a) a Protein A encoding region having at least 85% identity to a polynucleotide sequence selected from the group consisting of SEQ ID NOs 54-72 or fragment thereof encoding at least one IgG binding domain and b) an Sbi-encoding region which has at least 85% identity to a polynucleotide sequence selelcted from the group consisting of SEQ ID NOs 43-53 or fragment thereof encoding at least one IgG binding domain.

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29. The polynuclotide of claim 28 having a polynucleotide sequence having at least 85% identity to SEQ ID NO 73-75 or fragment therof encoding at least one protein A IgG binding domain and at least one Sbi binding domain.

30. A polynucleotide encoding a fusion protein comprising: a) a protein A like part having at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 12-40 or an immunogenic fragment thereof optionally comprising at least one IgG binding domain and b) an Sbi-like part which has at least 85% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 1-11, or an immunogenic fragment thereof optionally comprising at least one IgG binding domain.

31. A vaccine comprising the immunogenic composition of any one of claims 1-23 or the polypeptide of claims 24-27 and a pharmaceutically acceptable carrier.

32. A process for making the vaccine of claim 31 comprising the step of adding a pharmaceutically acceptable excipient to the immunogenic composition of any one of claims 1-23 or the polypeptide of any one of claims 24-27.

33. An immunogenic composition according to any one of claims 1-23 for use in the treatment of prevention of staphylococcal disease.

34. A use of the immunogenic composition of any one of claims 1-23 in the preparation of a medicament for the treatment or prevention of staphylococcal disease.

35. A method of treating or preventing staphylococcal disease comprising administering the immunogeninc composition of any one of claims 1-23 to a patient in need thereof.

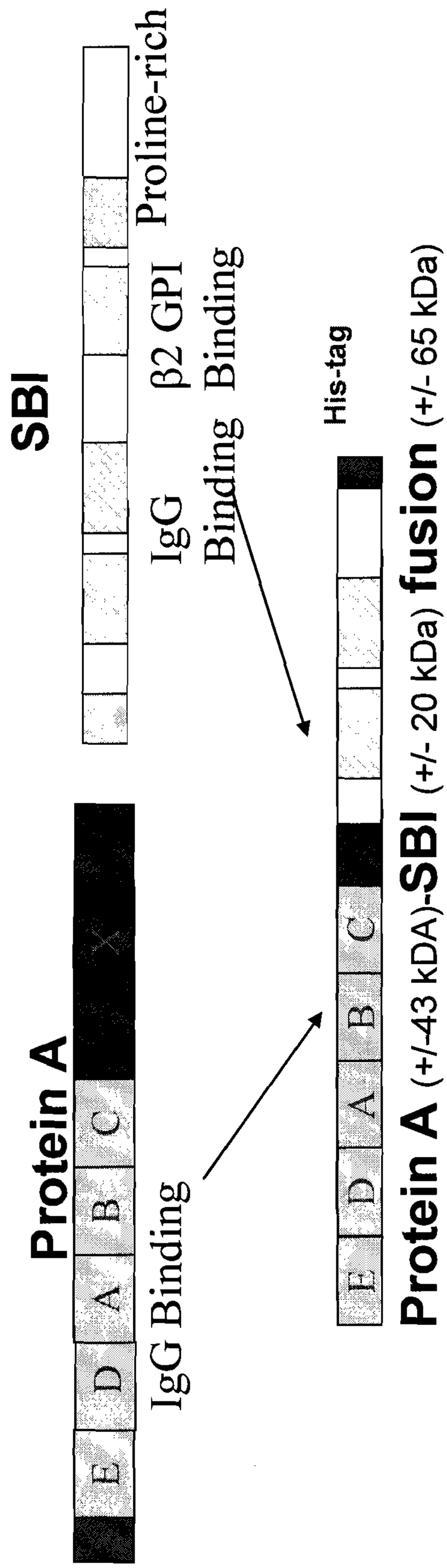
SBI
Proline-rich
β2 GPI Binding
IgG Binding
His-tag
Protein A
IgG Binding
E
D
A
B
C
Protein A (+/-43 kDA)-SBI (+/- 20 kDa) fusion (+/- 65 kDa)

Figure 1

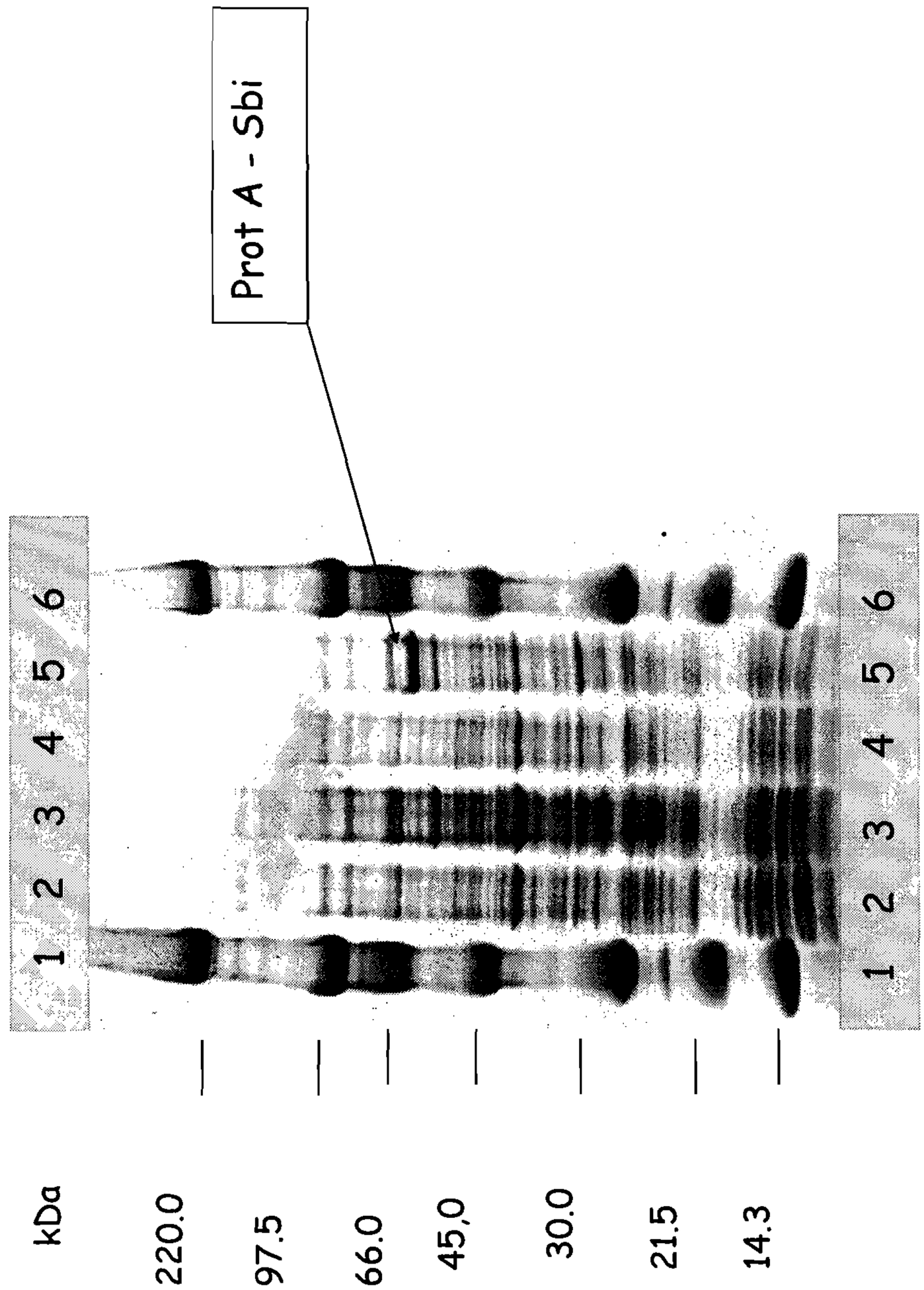

Figure 2

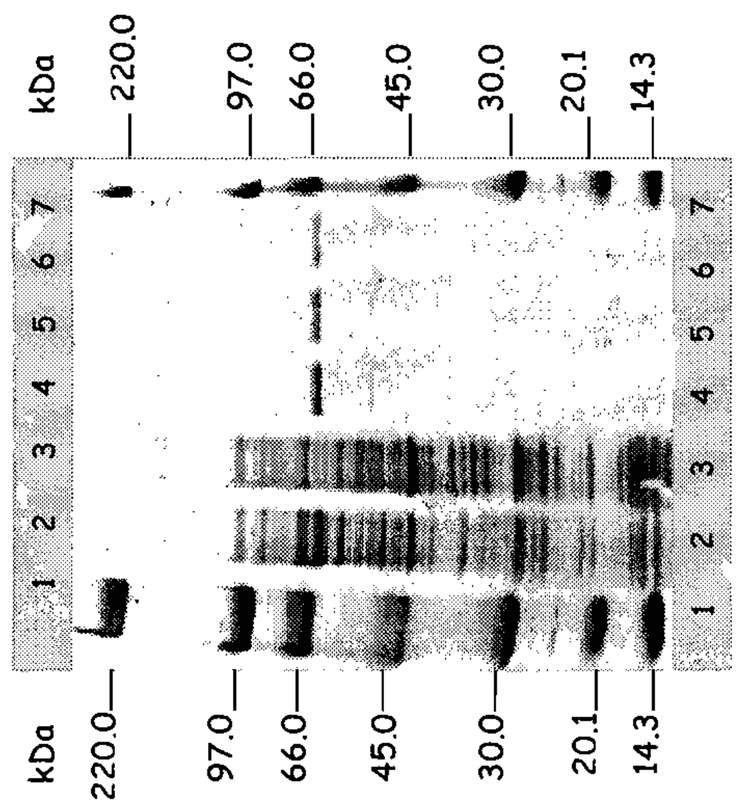

Figure 3

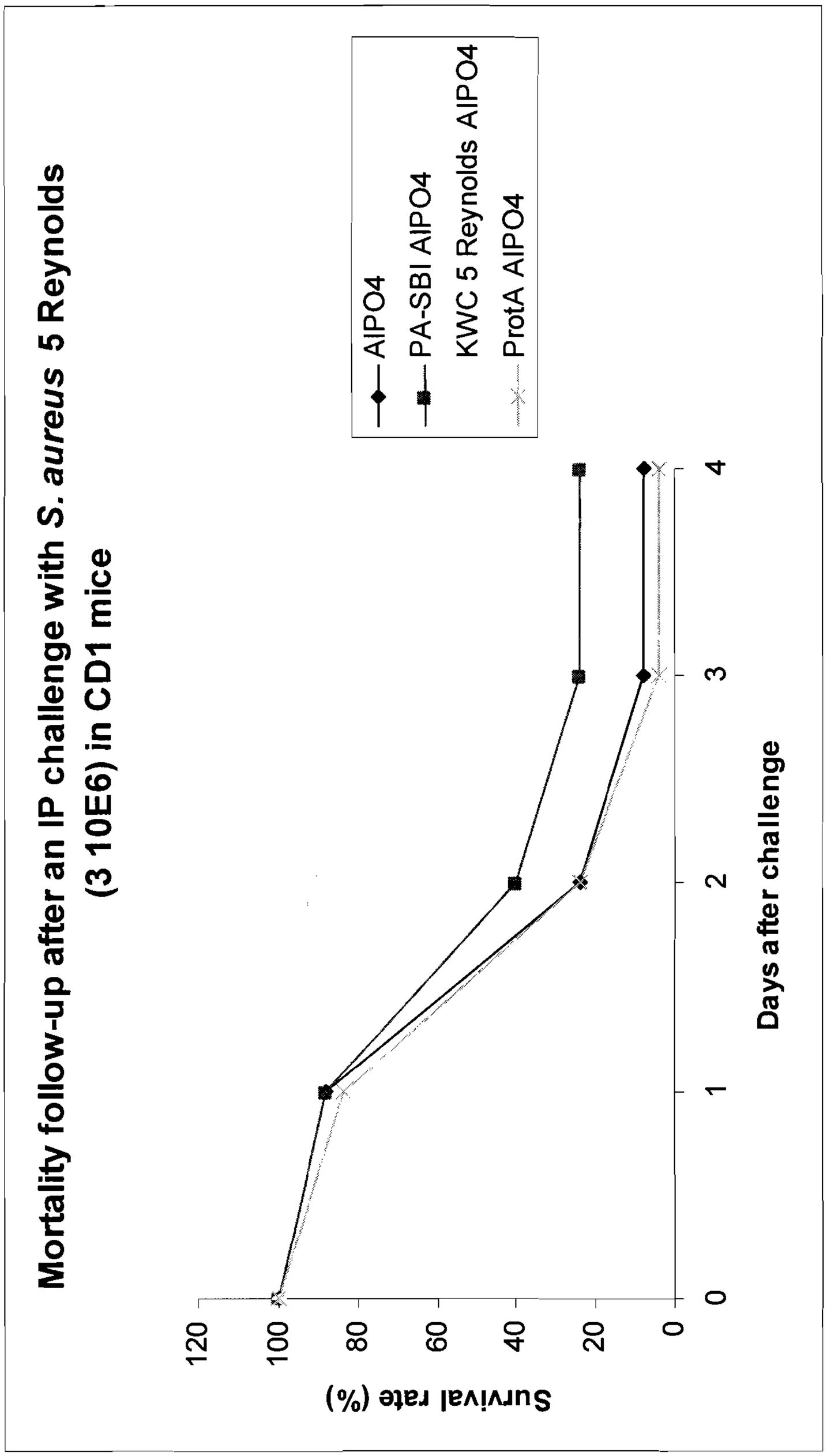
Mortality follow-up after an IP challenge with S. aureus 5 Reynolds
(3 10E6) in CD1 mice
AlPO4
PA-SBI AlPO4
KWC 5 Reynolds AlPO4
ProtA AlPO4
Survival rate (%)
120
100
80
60
40
20
0
0
1
2
3
4
Days after challenge

Figure 4

Figure 5 : SBI comparisons

Full length protein sequences alignment.

2 IgG-binding domains : from amino acid 42-93 (light grey) and 94-147 (medium grey) for COL, NCTC, N315, MSSA, MW2 and MRSA.

```
                        *        20         *        40         *
SBI_COL   : --MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  48
SBI_NCTC  : --MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  48
SBI_MU50  : MHMKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  50
SBI_N315  : --MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  48
SBI_MSSA  : --MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  48
SBI_MW2   : --MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  48
SBI_MRSA  : --MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKHQTTQNNYV :  48

                    60         *        80         *       100
SBI_COL   : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD :  98
SBI_NCTC  : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD :  98
SBI_MU50  : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD : 100
SBI_N315  : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD :  98
SBI_MSSA  : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD :  98
SBI_MW2   : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD :  98
SBI_MRSA  : TDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPD :  98

                        *       120         *       140         *
SBI_COL   : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 148
SBI_NCTC  : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 148
SBI_MU50  : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 150
SBI_N315  : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 148
SBI_MSSA  : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 148
SBI_MW2   : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 148
SBI_MRSA  : RRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 148

                    160         *       180         *       200
SBI_COL   : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 198
SBI_NCTC  : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 198
SBI_MU50  : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 200
SBI_N315  : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 198
SBI_MSSA  : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 198
SBI_MW2   : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 198
SBI_MRSA  : KERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKV : 198

                        *       220         *       240         *
SBI_COL   : SIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHL : 248
SBI_NCTC  : SIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHL : 248
SBI_MU50  : SIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHL : 250
SBI_N315  : SIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHL : 248
SBI_MSSA  : SIEKAIVRHDERVKSANDAISKLNVKDSIENRRLAQREVNKAPMDVKEHL : 248
SBI_MW2   : SIEKAIVRHDERVKSANDAISKLNVKDSIENRRLAQREVNKAPMDVKEHL : 248
SBI_MRSA  : SIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVQKHL : 248
```

```
                    260         *         280         *         300
SBI_COL   : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKVESPKVEVPQIQSPK : 298
SBI_NCTC  : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKVESPKVEVPQIQSPK : 298
SBI_MU50  : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESP----------K : 290
SBI_N315  : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPK : 298
SBI_MSSA  : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPK : 298
SBI_MW2   : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPK : 298
SBI_MRSA  : QKQLDALVAQKDAEKKVAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPK : 298

                    *         320         *         340         *
SBI_COL   : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKY : 348
SBI_NCTC  : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKY : 348
SBI_MU50  : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKY : 340
SBI_N315  : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKY : 348
SBI_MSSA  : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKY : 348
SBI_MW2   : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNTYYKY : 348
SBI_MRSA  : VEVPQSKLLGYYQSLKDSFNYGYKYLTDTYKSYKEKYDTAKYYYNKYYQY : 348

                    360         *         380         *         400
SBI_COL   : KGAIDQTVLTVLGSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTG : 398
SBI_NCTC  : KGAIDQTVLTVLGSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTG : 398
SBI_MU50  : KGAIDQTVLTVLGSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTG : 390
SBI_N315  : KGAIDQTVLTVLGSGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTG : 398
SBI_MSSA  : KGAIDKAVLTLLGDGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTG : 398
SBI_MW2   : KGAIDKAVLTLLGDGSKSYIQPLKVDDKNGYLAKSYAQVRNYVTESINTG : 398
SBI_MRSA  : KGLIDKTVLTTIGSGYGSYIKPLEVSKESGNLAKSYAQVRNYVTESINTG : 398

                    *         420         *
SBI_COL   : KVLYTFYQNPTLVKTAIKAQETASSIKNTLSNLL-SFWK : 436
SBI_NCTC  : KVLYTFYQNPTLVKTAIKAQETASSIKNTLSNLL-SFWK : 436
SBI_MU50  : KVLYTFYQNPTLVKTAIKAQETASSIKNTLSNLL-SFWK : 428
SBI_N315  : KVLYTFYQNPTLVKTAIKAQETASSIKNTLSNLL-SFWK : 436
SBI_MSSA  : KVLYTFYQNPTLVKTAIKAQETASSIKNTITGLFNSFWK : 437
SBI_MW2   : KVLYTFYQNPTLVKTAIKAQETASSIKNTITGLFNSFWK : 437
SBI_MRSA  : KVLYAFYQKPELVKTAIKAQETATTFKNALTGIFKSFWK : 437
```

Figure 5 : Protein A (Spa) comparisons

Full length protein sequences alignment.

Identical and similar (same physico-chemical properties) amino acid in one column are highlighted in black.

4 or 5 IgG-binding domains are highlighted by underlining,

```
                        *        20         *        40         *
SPA_MRSA  : ------------LKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38
SPA_V8    : MMTLQIHTGGINLKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  50
SPA_MU50  : ------------MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38
SPA_N315  : ------------MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38
SPA_MW2   : ------------MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38
SPA_MSSA  : ------------LKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38
SPA_NCTC  : ------------MKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38
SPA_COL   : ------------LKKKNIYSIRKLGVGIASVTLGTLLISGGVTPAANAAQ :  38

                      60         *        80         *       100
SPA_MRSA  : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88
SPA_V8    : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 100
SPA_MU50  : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88
SPA_N315  : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88
SPA_MW2   : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88
SPA_MSSA  : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88
SPA_NCTC  : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88
SPA_COL   : HDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS :  88

                        *       120         *       140         *
SPA_MRSA  : QAPKADAQQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTN : 138
SPA_V8    : QAPKADAQQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTN : 150
SPA_MU50  : QAPKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTN : 138
SPA_N315  : QAPKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTN : 138
SPA_MW2   : QAPKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTN : 138
SPA_MSSA  : QAPKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTN : 138
SPA_NCTC  : QAPKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTN : 138
SPA_COL   : QAPKADAQQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTN : 138

                      160         *       180         *       200
SPA_MRSA  : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188
SPA_V8    : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 200
SPA_MU50  : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188
SPA_N315  : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188
SPA_MW2   : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188
SPA_MSSA  : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188
SPA_NCTC  : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188
SPA_COL   : VLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLK : 188

                        *       220         *       240         *
SPA_MRSA  : DDPSQSANLLAEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238
```

```
SPA_V8   : DDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHLPNLTEEQR : 250
SPA_MU50 : DDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238
SPA_N315 : DDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238
SPA_MW2  : DDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238
SPA_MSSA : DDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238
SPA_NCTC : DDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238
SPA_COL  : DDPSQSANLLSEAKKLNESQAPKADNKFNKEQQNAFYEILHLPNLNEEQR : 238

                    260         *         280         *         300
SPA_MRSA : NGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHL : 288
SPA_V8   : NGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKE--------- : 291
SPA_MU50 : NGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNKKPGKE--------- : 279
SPA_N315 : NGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNKKPGKE--------- : 279
SPA_MW2  : NGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHL : 288
SPA_MSSA : NGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHL : 288
SPA_NCTC : NGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHL : 288
SPA_COL  : NGFIQSLKDDPSQSANLLAEAKKLNDAQAPKADNKFNKEQQNAFYEILHL : 288

                  *         320         *         340         *
SPA_MRSA : PNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKED : 338
SPA_V8   : ---------------------------------DNNKPGKEDGNKPGKED : 308
SPA_MU50 : ---------------------------------DGNKPGKEDGNKPGKED : 296
SPA_N315 : ---------------------------------DGNKPGKEDGNKPGKED : 296
SPA_MW2  : PNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKED : 338
SPA_MSSA : PNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKED : 338
SPA_NCTC : PNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKED : 338
SPA_COL  : PNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDAQAPKEEDNNKPGKED : 338

                    360         *         380         *         400
SPA_MRSA : NNKPGKED-------------------------GNKPGKEDNKKPGKEDGN : 364
SPA_V8   : NKKPGKED-------------------------GNKPGKEDNKKPGKEDGN : 334
SPA_MU50 : NKKPGKED-------------------------GNKPGKEDNNKPGKEDGN : 322
SPA_N315 : NKKPGKED-------------------------GNKPGKEDNNKPGKEDGN : 322
SPA_MW2  : GNKPGKED-------------------------GNKPGKEDNNKPGKEDGN : 364
SPA_MSSA : GNKPGKED-------------------------GNKPGKEDNNKPGKEDGN : 364
SPA_NCTC : NNKPGKEDNNKPGKEDNNKPGKEDNNKPGKEDGNKPGKEDNKKPGKEDGN : 388
SPA_COL  : NNKPGKEDNNKPGKEDNNKPGK--------EDGNKPGKEDNKKPGKEDGN : 380

                  *         420         *         440         *
SPA_MRSA : KPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKPGKEDGNKP : 414
SPA_V8   : KPGKEDGNKPGKEDGNKPGKEDGN---------------------------- : 358
SPA_MU50 : KPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGN------------------- : 354
SPA_N315 : KPGKEDNNKPGKEDGNKPGKEDGNKPGKEDGN------------------- : 354
SPA_MW2  : KPGKEDNKKPGKEDGNKPGKEDNNKPGKEDGN------------------- : 396
SPA_MSSA : KPGKEDNKKPGKEDGNKPGKEDNNKPGKEDGN------------------- : 396
SPA_NCTC : KPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGN------------------- : 420
SPA_COL  : KPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGN------------------- : 412

                    460         *         480         *         500
SPA_MRSA : GKEDGNGVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 464
SPA_V8   : ------GVHVVKPGDTVNDIAKANGTTADKIAVDNKLADKNMIKPGQELV : 402
SPA_MU50 : ------GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 398
SPA_N315 : ------GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 398
```

```
SPA_MW2  : ------GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 440
SPA_MSSA : ------GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 440
SPA_NCTC : ------GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 464
SPA_COL  : ------GVHVVKPGDTVNDIAKANGTTADKIAADNKLADKNMIKPGQELV : 456

                    *        520         *        540         *
SPA_MRSA : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 514
SPA_V8   : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 452
SPA_MU50 : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 448
SPA_N315 : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 448
SPA_MW2  : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 490
SPA_MSSA : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 490
SPA_NCTC : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 514
SPA_COL  : VDKKQPANHADANKAQALPETGEENPFIGTTVFGGLSLALGAALLAGRRR : 506

                    560
SPA_MRSA : EL-------- : 516
SPA_V8   : EL-------- : 454
SPA_MU50 : EL-------- : 450
SPA_N315 : EL-------- : 450
SPA_MW2  : EL-------- : 492
SPA_MSSA : EL-------- : 492
SPA_NCTC : EL-------- : 516
SPA_COL  : EL-------- : 508
```

Figure 5 : SBI and Spa IgG-binding domains comparison

Identical and similar (same physico-chemical properties) amino acid in one column are highlighted in black.

```
                        *         20         *         40         *
SPA_MRSA_5  : ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_MSSA_5  : ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_COL_5   : ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_MW2_5   : ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_NCTC_5  : ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_V8_4    : ADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_MU50_4  : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_N315_4  : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSVSKEILAEAKKLNDA : 54
SPA_NCTC_4  : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA : 54
SPA_MW2_4   : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA : 54
SPA_COL_4   : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA : 54
SPA_MRSA_4  : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA : 54
SPA_MSSA_4  : ADNKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA : 54
SPA_V8_3    : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDA : 54
SPA_NCTC_3  : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES : 54
SPA_MU50_3  : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES : 54
SPA_N315_3  : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES : 54
SPA_MW2_3   : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES : 54
SPA_COL_3   : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES : 54
SPA_MSSA_3  : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAKKLNES : 54
SPA_MRSA_3  : ADNNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNES : 54
SPA_NCTC_2  : QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_MU50_2  : QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_N315_2  : QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_MW2_2   : QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_COL_2   : QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_MSSA_2  : QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_MRSA_2  : QQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_V8_2    : QQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDPSQSTNVLGEAKKLNES : 54
SPA_NCTC_1  : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_MU50_1  : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_N315_1  : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_MW2_1   : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_COL_1   : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_MRSA_1  : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_MSSA_1  : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SPA_V8_1    : NAAQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDS : 54
SBI_COL_1   : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_MU50_1  : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_MRSA_1  : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_MSSA_1  : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_MW2_1   : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_N315_1  : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_NCTC_1  : TQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDS-- : 52
SBI_COL_2   : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
SBI_MU50_2  : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
SBI_MRSA_2  : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
SBI_MSSA_2  : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
SBI_MW2_2   : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
SBI_N315_2  : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
SBI_NCTC_2  : KNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKA : 54
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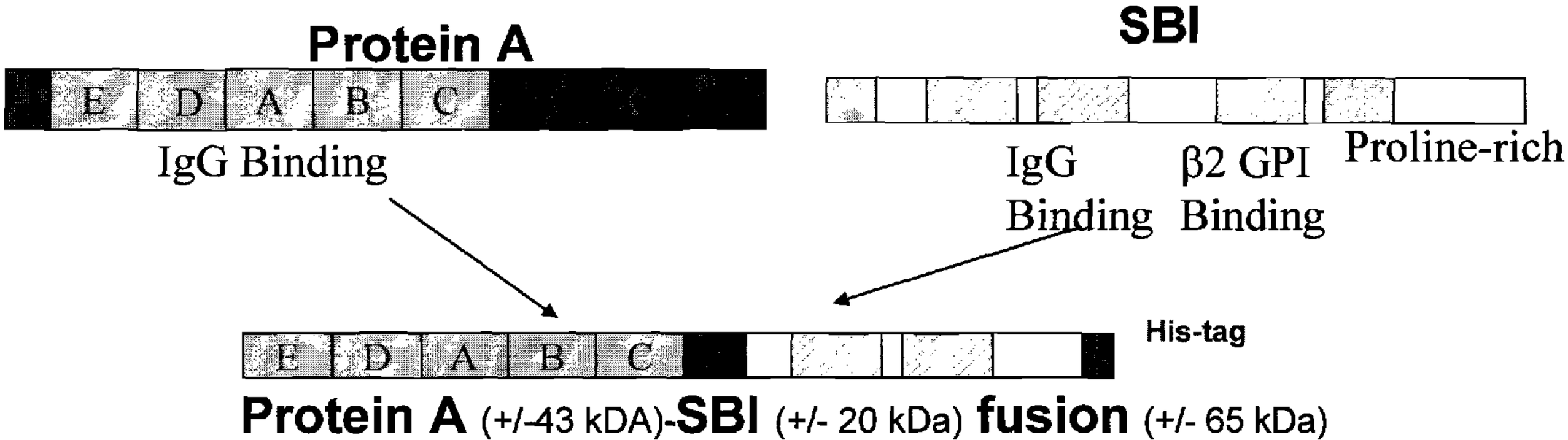
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B
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SBI
IgG Binding
β2 GPI Binding
Proline-rich
E
D
A
B
C
His-tag
Protein A (+/-43 kDA)-SBI (+/- 20 kDa) fusion (+/- 65 kDa)