

**PURIFICATION OF *STAPHYLOCOCCUS AUREUS* TYPE 5 AND TYPE 8
CAPSULAR SACCHARIDES**

This application claims the benefit of U.S. Provisional Application Serial No. 61/256,905, filed on 30th October 2009, which is incorporated by reference herein in its entirety.

5 TECHNICAL FIELD

This invention is in the field of purifying bacterial capsular polysaccharides, particularly those of *Staphylococcus aureus* type 5 and type 8, and particularly for use in the preparation of vaccines.

BACKGROUND ART

10 The capsular saccharides of bacteria have been used for many years in vaccines against capsulated bacteria. As saccharides are T-independent antigens, however, they are poorly immunogenic. Conjugation to a carrier can convert T-independent antigens into T-dependent antigens, thereby enhancing memory responses and allowing protective immunity to develop. The most effective saccharide vaccines are therefore based on glycoconjugates, and the prototype conjugate vaccine was against *Haemophilus influenzae* type b ('Hib') [e.g. see chapter 14 of ref. 96].

15 Another bacterium for which conjugate vaccines have been described is *Staphylococcus aureus* (*S.aureus*). Various polysaccharides have been isolated from *S.aureus* for use in glycoconjugates. Two polysaccharides of particular interest are the type 5 and type 8 capsular polysaccharides. Approximately 60% of human *S.aureus* strains are type 8 and approximately 30% are type 5. Much of the work on type 5 and type 8 conjugates has been performed by Fattom *et al.*, and is described in documents such as
20 references 1 to 9.

The starting point for polysaccharide-based vaccines is the polysaccharide itself, and this is generally purified from the target bacterium. Fattom *et al.* have developed a complex process for purification of the type 5 and type 8 capsular polysaccharides that is described in detail in reference 1, and involves the following key steps after bacterial culture: suspension of bacterial cells in buffer, treatment with
25 lysostaphin, treatment with DNase and RNase, centrifugation, dialysis against buffer, treatment with protease, further dialysis, filtration, addition of ethanol to 25% with calcium chloride to precipitate contaminants; further addition of ethanol to 75% to precipitate the polysaccharide; collection and drying of the precipitate; anion exchange chromatography; dialysis; lyophilisation; size exclusion chromatography; dialysis and final lyophilisation.

30 The Fattom process involves the use of lysostaphin to lyse the bacterial cell walls and thereby release capsular polysaccharide. However, this step is time-consuming and makes the process difficult to scale-up to an industrial setting. It also increases the overall cost and complexity of the process. Other

researchers have attempted to omit this step and develop a simpler, more efficient method of purifying the polysaccharide. For example, reference [10] describes an alternative process that involves autoclaving *S.aureus* cells, ultrafiltration of the polysaccharide-containing supernatant, concentration, lyophilisation, treatment with sodium metaperiodate, further ultrafiltration, diafiltration, high performance size exclusion liquid chromatography, dialysis and freeze-drying. The authors suggest that this method provides a good yield and is suitable for large scale production of polysaccharide. In this method, the lysostaphin treatment is replaced by autoclaving to release capsular polysaccharide. The method was further developed in reference [11]. An important step in these alternative methods is the treatment with sodium metaperiodate. This step is carried out to remove teichoic acid contamination of the capsular polysaccharide. However, once again this step increases the duration, complexity and overall cost of the process. Reference [12] describes a similar process that again involves autoclaving to release capsular polysaccharide and treatment with sodium metaperiodate to remove teichoic acid. In contrast, most other groups use processes that retain lysostaphin treatment (see, for example, references 13, 14, 15, 16, 17 and 18), sometimes including treatment with sodium metaperiodate (e.g. in references 13 and 14).

The above methods are complex and may leave contamination in the resultant polysaccharide. There is thus a need for further and improved processes for purifying *S.aureus* type 5 and type 8 capsular polysaccharides, and particularly for less complex processes that result in less contamination.

DISCLOSURE OF THE INVENTION

The invention is based on a purification process in which the polysaccharide is initially released from the bacterial cells by treatment with an acid. This step removes the need for lysostaphin treatment and can be used as an alternative to autoclaving, as in the above processes. The inventors have found that the process results in a purified polysaccharide with low teichoic acid contamination. This means that it is not necessary to treat the polysaccharide with sodium metaperiodate. The purified polysaccharide also has low peptidoglycan contamination, making it particularly suitable for medical uses. The inventors' process can be quick and simple because laborious steps in previous processes are not necessary.

The invention provides a method for releasing capsular polysaccharide from *S.aureus* type 5 or type 8 cells, comprising the step of treating the cells with acid. The invention further provides a process for purifying capsular polysaccharide from *S.aureus* type 5 or type 8 cells comprising this method. Other processing steps may be included in the process, such as enzymatic treatment, e.g. to remove nucleic acid, protein and/or peptidoglycan contaminants; diafiltration, e.g. to remove low molecular weight contaminants; anion exchange chromatography, e.g. to remove residual protein; and concentration.

Accordingly, the invention provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, comprising the step of releasing the polysaccharide from *S.aureus* type 5 or type 8 cells by treating the cells with acid. Similarly, the invention provides, in a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, the improvement consisting of the use of acid treatment of *S.aureus* type 5 or type 8 cells to release the polysaccharide from the cells. Release by acid treatment removes the need for lysostaphin treatment or autoclaving to release the polysaccharide.

The invention also provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the process does not involve a step of lysostaphin treatment. Similarly, the invention provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the process does not involve a step of sodium metaperiodate treatment. Typically, the process does not involve one or both of these steps.

The invention also provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the process provides a composition comprising the polysaccharide and a level of peptidoglycan contamination that is less than 5% (e.g. $\leq 4\%$, $\leq 3\%$, $\leq 2\%$, $\leq 1\%$, etc.) by weight peptidoglycan relative to the total weight of the polysaccharide. Typically, the composition comprises less than 4%, particularly less than 3%, by weight peptidoglycan. The inventors have found that levels of about 2% or even about 1% can be obtained using the methods of the invention. The inventors have found that compositions with this level of peptidoglycan are useful in vaccine manufacture. In contrast, reference 17 teaches that levels above 5% should be used for this purpose. The level of peptidoglycan contamination may be measured using the methods described herein.

Similarly, the invention provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the process provides a composition comprising the polysaccharide and a level of protein contamination that is less than 5% (e.g. $\leq 4\%$, $\leq 3\%$, $\leq 2\%$, $\leq 1\%$, $\leq 0.5\%$, etc.) by weight protein relative to the total weight of the polysaccharide. Typically, the composition comprises less than 3%, particularly about 2.4%, by weight protein. The level of protein contamination may be measured using a MicroBCA assay (Pierce).

The invention also provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the process provides a composition comprising the polysaccharide and a level of nucleic acid contamination that is less than 1% (e.g. $\leq 0.75\%$, $\leq 0.50\%$, $\leq 0.25\%$, $\leq 0.10\%$, $\leq 0.01\%$, etc.) by weight nucleic acid relative to the total weight of the polysaccharide. Typically, the composition comprises less than 0.25%, particularly about 0.09%, by weight nucleic acid. The level of nucleic acid contamination may be measured by absorption at 260nm in a spectrophotometer.

The invention also provides a process for purifying *S.aureus* type 5 or type 8 capsular polysaccharide, wherein (a) the level of peptidoglycan acid contamination is less than 5% (as described above); (b) the level of protein contamination is less than 5% (as described above); (c) the level of nucleic acid contamination that is less than 1% (as described above).

- 5 The invention also provides a composition comprising a *S.aureus* type 5 or type 8 capsular polysaccharide, obtainable by any of the processes of the invention.

In particular, the invention provides a composition comprising *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the composition comprises a level of peptidoglycan contamination that is less than 5% (e.g. $\leq 4\%$, $\leq 3\%$, $\leq 2\%$, $\leq 1\%$, etc.) by weight peptidoglycan relative to the total weight of the polysaccharide. Typically, the composition comprises less than 3%, particularly less than 2%, by weight peptidoglycan. Compositions with levels of about 2% or even about 1% are specifically provided by the invention.

Similarly, the invention provides a composition comprising *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the composition comprises a level of protein contamination that is less than 5% (e.g. $\leq 4\%$, $\leq 3\%$, $\leq 2\%$, $\leq 1\%$, $\leq 0.5\%$, etc.) by weight protein relative to the total weight of the polysaccharide. Typically, the composition comprises less than 3%, particularly about 2.4%, by weight protein.

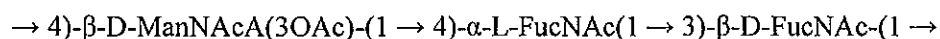
The invention also provides a composition comprising *S.aureus* type 5 or type 8 capsular polysaccharide, wherein the composition comprises a level of nucleic acid contamination that is less than 1% (e.g. $\leq 0.75\%$, $\leq 0.50\%$, $\leq 0.25\%$, $\leq 0.10\%$, $\leq 0.01\%$, etc.) by weight nucleic acid relative to the total weight of the polysaccharide. Typically, the composition comprises less than 0.25%, particularly about 0.09%, by weight nucleic acid.

The invention also provides a composition comprising *S.aureus* type 5 or type 8 capsular polysaccharide, wherein a) a level of peptidoglycan acid contamination is less than 5% (as described above); (b) the level of protein contamination is less than 5% (as described above); (c) the level of nucleic acid contamination that is less than 1% (as described above).

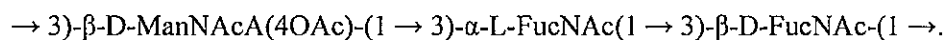
The capsular polysaccharide

The invention is based on the capsular polysaccharides of *S.aureus* type 5 and type 8. The structures of type 5 and type 8 capsular polysaccharides were described in references 19 and 20 as:

30 Type 5

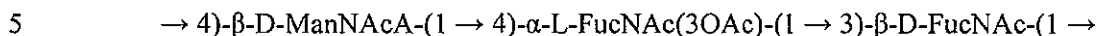


Type 8

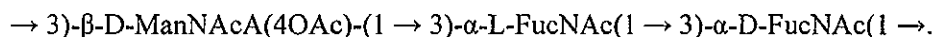


Recent NMR spectroscopy data [21] has led to a revision of these structures to:

Type 5



Type 8



After release from the *S.aureus* type 5 or type 8 cells, the polysaccharide may be chemically modified relative to the capsular polysaccharide as found in nature. For example, the polysaccharide may be de-O-acetylated (partially or fully), de-N-acetylated (partially or fully), N-propionated (partially or fully), *etc.* De-acetylation may occur before, during or after other processing steps, but typically occurs before any conjugation step. Depending on the particular polysaccharide, de-acetylation may or may not affect immunogenicity *e.g.* the NeisVac-C™ vaccine uses a de-O-acetylated polysaccharide, whereas Menjugate™ is acetylated, but both vaccines are effective. The effect of de-acetylation *etc.* can be assessed by routine assays. For example, the relevance of O-acetylation on *S.aureus* type 5 or type 8 capsular polysaccharides is discussed in reference 6. The native polysaccharides are said in this document to have 75% O-acetylation. These polysaccharides induced antibodies to both the polysaccharide backbone and O-acetyl groups. Polysaccharides with 0% O-acetylation still elicited antibodies to the polysaccharide backbone. Both types of antibody were opsonic against *S.aureus* strains that varied in their O-acetyl content. Accordingly, the type 5 or type 8 capsular polysaccharides used in the present invention may have between 0 and 100% O-acetylation. For example, the degree of O-acetylation of the type 5 capsular polysaccharide may be 10-100%, 10-100%, 20-100%, 30-100%, 40-100%, 50-100%, 60-100%, 70-100%, 80-100%, 90-100%, 50- 90%, 60-90%, 70-90% or 80-90%. Alternatively, 0% O-acetylated type 5 capsular polysaccharide may be used. Similarly, the degree of O-acetylation of the type 8 capsular polysaccharide may be 10-100%, 10-100%, 20-100%, 30-100%, 40-100%, 50-100%, 60-100%, 70-100%, 80-100%, 90-100%, 50- 90%, 60-90%, 70-90% or 80-90%. Alternatively, 0% O-acetylated type 8 capsular polysaccharide may be used. In one embodiment, the degree of O-acetylation of the type 5 and type 8 capsular polysaccharides may be 10-100%, 20-100%, 30-100%, 40-100%, 50-100%, 60-100%, 70-100%, 80-100%, 90-100%, 50-90%, 60-90%, 70-90% or 80-90%. In other embodiments, 0% O-acetylated type 5 and type 8 capsular polysaccharides are used. The degree of N-acetylation of the type 5 capsular polysaccharide used in the invention may be 0-100%, 50-100%, 75-100%, 80-100%, 90-100%, or 95-100%. Typically, the degree of N-acetylation of the type

5 capsular polysaccharide is 100%. Similarly, the degree of N-acetylation of the type 8 capsular polysaccharide used in the invention may be 0-100%, 50-100%, 75-100%, 80-100%, 90-100%, or 95-100%. Typically, the degree of N-acetylation of the type 8 capsular polysaccharide is 100%. In one embodiment, the degree of N-acetylation of the type 5 and type 8 capsular polysaccharides may be 0-100%, 50-100%, 75-100%, 80-100%, 90-100%, or 95-100%. Typically, the degree of N-acetylation of the type 5 and type 8 capsular polysaccharides are 100%.

The degree of O-acetylation of the polysaccharide can be determined by any method known in the art, for example, by proton NMR (*e.g.* as described in references 22, 23, 24 or 25). A further method is described in reference 26. Similar methods may be used to determine the degree of N-acetylation of the polysaccharide. O-acetyl groups may be removed by hydrolysis, for example by treatment with a base such as anhydrous hydrazine [27] or NaOH [6]. Similar methods may be used to remove N-acetyl groups. To maintain high levels of O-acetylation on type 5 and/or 8 capsular polysaccharides, treatments that lead to hydrolysis of the O-acetyl groups are minimised, *e.g.* treatments at extremes of pH.

Starting material

The process of the invention starts with *S.aureus* type 5 or type 8 cells. Typically, the cells are grown by fermentation prior to release of capsular polysaccharide. Suitable methods of cultivating *S.aureus* type 5 or type 8 cells are well known to the skilled person and are disclosed, for example, in references 1 to 21 and the references cited therein. After cell growth, the cells are usually deactivated. A suitable method for deactivation is treatment with phenol:ethanol, *e.g.* as described in reference 1.

The cells may be centrifuged prior to release of capsular polysaccharide. The process may therefore start with the cells in the form of a wet cell paste. Typically, however, the cells are resuspended in an aqueous medium that is suitable for the next step in the process, *e.g.* in a buffer or in distilled water. The cells may be washed with this medium prior to re-suspension. In another embodiment, the cells may be treated in suspension in their original culture medium. Alternatively, the cells are treated in a dried form.

Acid treatment

In the method of the invention, *S.aureus* type 5 or type 8 cells are treated with acid. This step results in release of capsular polysaccharide from the cells. In contrast, previous methods have used lysostaphin treatment or autoclaving to release the polysaccharide. The acid treatment of the invention is preferably carried out using a mild acid, *e.g.* acetic acid, to minimise damage to the polysaccharide. The skilled person would be capable of identifying suitable acids and conditions (*e.g.* of concentration, temperature and/or time) for release of the polysaccharide. For example, the inventors have found that treatment of

cells suspended at about 0.5mg/ml in distilled water with 1% acetic acid (v/v) at 100°C for 2 hours is suitable. Treatment with other acids, *e.g.* trifluoroacetic or other organic acids, may also be suitable.

The efficacy of different acid treatments may be tested using routine methods. For example, after acid treatment, the cells may be isolated and treated using known methods of *S.aureus* type 5 or type 8 capsular polysaccharide release (*e.g.* the lysostaphin-based method of reference 1) to see if additional capsular polysaccharide can be released. If additional capsular polysaccharide is released, then the acid treatment conditions may be altered so that a greater proportion of the capsular saccharide is released during acid treatment. In this way, it is possible to optimise the acid treatment conditions so that an optimal amount of capsular saccharide is released. For example, the inventors have found that after treatment of cells suspended at about 0.5mg/ml in distilled water with 1% acetic acid (v/v) at 100°C for 2 hours, very little additional capsular saccharide is releasable from the cells by subsequent lysostaphin treatment.

The inventors have found that after acid treatment, the degree of O-acetylation of the type 5 capsular polysaccharide may be between 60-100%. In particular, the degree of O-acetylation may be between the 65-95%, particularly 70-90%. Typically, the degree of O-acetylation is between 75-85%, *e.g.* about 80%. Similar values may be obtained for the type 8 capsular saccharide. If desired, the degree of O-acetylation of the capsular saccharide may then be altered by further processing steps as discussed above.

After acid treatment, the reaction mixture is typically neutralised. This may be achieved by the addition of a base, *e.g.* NaOH. The cells may be centrifuged and the polysaccharide-containing supernatant collected for storage and/or additional processing.

Enzymatic treatment

The polysaccharide obtained after acid treatment may be impure and contaminated with bacterial nucleic acids and proteins. These contaminants may be removed by enzymatic treatment. For example, RNA may be removed by treatment with RNase, DNA with DNase and protein with protease (*e.g.* pronase). The skilled person would be capable of identifying suitable enzymes and conditions for removal of the contaminants. For example, the inventors have found that treatment of polysaccharide-containing supernatant with 50µg/ml each of DNase and RNase at 37°C for 6-8 hours is suitable. Other suitable conditions are disclosed in the literature, *e.g.* in reference 1.

The polysaccharide obtained after acid treatment may also or alternatively be contaminated with peptidoglycan. This contaminant may also be removed by enzymatic treatment. The inventors have found that treatment with mutanolysin is effective at removing peptidoglycan contamination. The

skilled person would be capable of identifying suitable conditions for removal of the peptidoglycan with mutanolysin. For example, the inventors have found that treatment of polysaccharide-containing supernatant with 180U/ml each of mutanolysin at 37°C for 16 hours is suitable. After treatment, the suspension may be clarified by centrifugation and the polysaccharide-containing supernatant collected for storage and/or additional processing.

Diafiltration

The process of the invention may involve a step of diafiltration. This step is typically performed after the acid treatment and/or enzymatic treatment discussed above. The inventors have found that a diafiltration step, particularly by tangential flow filtration, is particularly effective for removing impurities from the polysaccharide. The impurities are typically low molecular weight contaminants like teichoic and/or peptidoglycan fragments. The tangential flow filtration is suitably carried out against 1M NaCl (*e.g.* against about 10 volumes) and then NaPi 10mM pH 7.2 buffer (*e.g.* against another 10 volumes). The filtration membrane should thus be one that allows passage of small molecular weight contaminants while retaining the capsular polysaccharide. A cut-off in the range 10kDa-30kDa is typical. The inventors have found that tangential flow filtration using a 30kDa cut-off membrane is particularly suitable for large-scale processes.

At least 5 cycles of tangential flow diafiltration are usually performed *e.g.* 6, 7, 8, 9, 10, 11 or more.

The polysaccharide-containing retentate from the diafiltration is collected for storage and/or additional processing.

Anion exchange chromatography

The polysaccharide may be further purified by a step of anion exchange chromatography. The inventors have found that anion exchange chromatography is particularly effective at removing residual protein and nucleic acid contamination, while maintaining a good yield of the polysaccharide.

The anion exchange chromatography step may be performed after the acid treatment, enzymatic treatment and/or diafiltration steps discussed above.

The anion exchange chromatography may be carried out using any suitable anionic exchange matrix. Commonly used anion exchange matrices are resins such as Q-resins (based on quaternary amines) and DEAE resins (based on diethylaminoethane). The inventors have found that DEAE-resins (*e.g.* a DEAE-Sephacrose™ Fast Flow resin (GE Healthcare)) are particularly suitable, although other resins may be used.

Appropriate starting buffers and mobile phase buffers for the anion exchange chromatography can also be determined by routine experiments without undue burden. Typical buffers for use in anion exchange chromatography include N-methyl piperazine, piperazine, L-histidine, bis-Tris, bis-Tris propane, triethanolamine, Tris, N-methyl-diethanolamine, diethanolamine, 1,3-diaminopropane, ethanolamine, piperidine, sodium chloride and phosphate buffers. The inventors have found that phosphate buffers, e.g. a sodium phosphate buffer, are suitable as the starting buffer for the anion exchange chromatography. The buffer may be at any suitable concentration. For example, 10 mM sodium phosphate has been found to be suitable. Material bound to the anionic exchange resin may be eluted with a suitable buffer. The inventors have found that a gradient of NaCl 1M is suitable.

- 10 Eluate fractions containing polysaccharide may be determined by measuring UV absorption at 215 nm. Fractions containing polysaccharide, usually combined together, are collected for storage and/or additional processing.

The anion exchange chromatography step may be repeated, e.g. 1, 2, 3, 4 or 5 times. Typically the anion exchange chromatography step is carried out once.

15 ***Gel filtration***

The process of the invention may involve one or more step(s) of gel filtration. This gel filtration is used to select polysaccharide molecules of a particular length and to further reduce contamination, particularly by proteins. However, the inventors have found that contrary to previous methods like those of references 1 to 9, a gel filtration step is not required to obtain polysaccharide of high purity.

- 20 Accordingly, this step may be omitted from the processes of the invention. The omission of this step is advantageous because it simplifies the process and reduces the overall cost.

When present, the gel filtration step(s) may be performed after the acid treatment, enzymatic treatment, diafiltration and/or anion exchange chromatography steps discussed above. Typically, any gel filtration step(s) are carried out after the anion exchange chromatography step discussed above.

- 25 The gel filtration step(s) may be carried out using any suitable gel filtration matrix. Commonly used gel filtration matrices are based on dextran gels, agarose gels, polyacrylamide gels, polyacryloylmorpholine gels, and polystyrene gels *etc.* Cross-linked dextran gels and mixed polyacrylamide/agarose gels may also be used. The inventors have found that dextran gels (e.g. a Sephacryl™ S300 gel (GE Healthcare)) are particularly suitable, although other gels may be used.

- 30 Appropriate mobile phase buffers for the gel filtration can be determined by routine experiments without undue burden. Typical buffers for use in gel filtration include N-methyl piperazine, piperazine, L-histidine, bis-Tris, bis-Tris propane, triethanolamine, Tris, N-methyl-diethanolamine, diethanolamine,

1,3-diaminopropane, ethanolamine, piperidine, sodium chloride and phosphate buffers. For example, sodium chloride buffers may be suitable. The buffer may be at any suitable concentration. For example, 50 mM sodium chloride may be used for the mobile phase.

Eluate fractions containing polysaccharide may be determined by measuring UV absorption at 215 nm.

- 5 Fractions containing polysaccharide, usually combined together, are collected for storage and/or additional processing.

Concentration

- In addition to, or instead of, the one or more step(s) of gel filtration, the process of the invention may involve one or more steps of concentrating the polysaccharide. This concentration is useful for obtaining
10 a sample of the correct concentration for any subsequent conjugation of the polysaccharide to a carrier molecule, as described below. However, the inventors have found that this concentration step is not required to obtain polysaccharide of high purity. Accordingly, this step may be omitted from the processes of the invention.

- When present, the concentration step(s) may be performed after the acid treatment, enzymatic treatment,
15 diafiltration, anion exchange chromatography and/or gel filtration steps discussed above. Typically, any concentration step(s) are carried out after the anion exchange chromatography step discussed above. If used in addition to the gel filtration step(s) discussed above, the concentration step(s) may be carried out before or after the gel filtration step(s) discussed above. However, typically, concentration step(s) are used instead of gel filtration step(s).

- 20 The concentration step(s) may be carried out by any suitable method. For example, the inventors have found that the concentration step(s) may be diafiltration step(s) as described above, for example tangential flow filtration using a 30kDa cut-off membrane. For example, a Hydrosart™ (Sartorius) 30kDa cut-off membrane (with a 200 cm² membrane area) may be used.

The concentrated polysaccharide sample is collected for storage and/or additional processing.

25 *Further treatment of the capsular polysaccharide*

After purification, the polysaccharide may be further treated to remove contaminants. This is particularly important in situations where even minor contamination is not acceptable (e.g. for human vaccine production).

- The molecular mass of the purified *S.aureus* type 5 or type 8 capsular polysaccharide can be measured
30 by gel filtration relative to pullulan standards, such as those available from Polymer Standard Service [28]. Typically, the purified polysaccharide is a mixture of polysaccharides with masses within a range

of values. For the type 5 capsular polysaccharide, the molecular mass of the purified polysaccharide typically is between 2-3500 kDa, *e.g.* between 10-2000 kDa, particularly between 20-1000 kDa and more particularly between 100-600 kDa. Similarly, for the type 8 capsular polysaccharide, the molecular mass of the purified polysaccharide may be between 2-3500 kDa, *e.g.* between 10-2000 kDa, particularly
5 between 20-1000 kDa and more particularly between 100-600 kDa.

The purified polysaccharide may be depolymerised to form an oligosaccharide. Oligosaccharides may be preferred for use in vaccines. Depolymerisation to oligosaccharide may occur before or after any of the steps mentioned above. Typically, depolymerisation takes place after the anion exchange chromatography described above. If the polysaccharide is concentrated after this chromatography, then
10 depolymerisation typically takes place after this concentration. Where the composition of the invention includes a depolymerised polysaccharide, it is preferred that depolymerisation precedes any conjugation

Full-length polysaccharides may be depolymerised to give shorter fragments for use in the invention by various methods. Preferably, the method described in reference 29 is used. Alternatively, other methods for depolymerisation of the polysaccharide may be used. For example, the polysaccharide may be
15 heated or subjected to microfluidisation [30] or sonic radiation [3]. Alternatively, depolymerisation by oxidation-reduction [31] or ozonolysis [32] may be used.

Oligosaccharides can be identified by chromatography, *e.g.* size exclusion chromatography. The products may be sized in order to remove short-length oligosaccharides. This can be achieved in various ways, such as gel filtration. Specific molecular masses can be measured by gel filtration relative to
20 pullulan standards, such as those available from Polymer Standard Service [33].

If N-acetyl groups in the native capsular polysaccharide have been de-N-acetylated then the processes of the invention may include a step of re-N-acetylation. Controlled re-N-acetylation can conveniently be performed using a reagent such as acetic anhydride (CH_3CO)₂O *e.g.* in 5% ammonium bicarbonate [34].

Further rounds of filtration, *e.g.* sterile filtration, can also be performed.

25 These additional steps can generally be performed at room temperature.

Storage

The *S.aureus* type 5 or type 8 capsular polysaccharide preparation may be lyophilised, *e.g.* by freeze-drying under vacuum, or frozen in solution (*e.g.* as the eluate from the final concentration step, if included) for storage at any stage during the purification process. Accordingly, it is not necessary for the
30 preparation to be transferred immediately from one step of the process to another. For example, if the polysaccharide preparation is to be purified by diafiltration, then it may be lyophilised or frozen in

solution prior to this purification. Similarly, the polysaccharide may be lyophilised or frozen in solution prior to the anion exchange chromatography step. If the polysaccharide preparation is to be purified by gel filtration, then it may be lyophilised or frozen in solution prior to this step. Similarly, if the polysaccharide preparation is to be concentrated, then it may be lyophilised or frozen in solution prior to this step. The lyophilised preparation is reconstituted in an appropriate solution prior to further treatment. Similarly, the frozen solution is defrosted prior to further treatment.

The purified polysaccharide obtained by the process of the invention may be processed for storage in any suitable way. For example, the polysaccharide may be lyophilised as described above. Alternatively, the polysaccharide may be stored in aqueous solution, typically at low temperature, *e.g.* at -20°C. Conveniently, the polysaccharide may be stored as the eluate from the anion exchange chromatography, gel filtration or concentration steps.

Conjugation

The final purified capsular polysaccharide of the invention can be used as an antigen without further modification *e.g.* for use in *in vitro* diagnostic assays, for use in immunisation, *etc.*

For immunisation purposes, however, it is preferred to conjugate the polysaccharide to a carrier molecule, such as a protein. In general, covalent conjugation of polysaccharides to carriers enhances the immunogenicity of polysaccharides as it converts them from T-independent antigens to T-dependent antigens, thus allowing priming for immunological memory. Conjugation is particularly useful for paediatric vaccines [*e.g.* ref. 35] and is a well known technique [*e.g.* reviewed in refs. 36 to 44]. Thus the processes of the invention may include the further step of conjugating the purified polysaccharide to a carrier molecule.

Conjugation of *S.aureus* type 5 and type 8 capsular polysaccharides has been widely reported *e.g.* see references 1 to 9. The typical process used in the literature for conjugation involves thiolation of a purified polysaccharide using cystamine. The reaction relies on the presence of carboxylate groups in the capsular polysaccharide. These groups react with cystamine in the presence of a carbodiimide, *e.g.* EDAC. The derivatised polysaccharide is then conjugated to a carrier protein such as the *Pseudomonas aeruginosa* endotoxin A (ETA), typically via a linker [2]. Conjugate vaccines prepared in this manner have been shown to be safe and immunogenic in humans [5]. Other researchers have carried out conjugation of purified type 5 and type 8 capsular polysaccharides by reductive amination [45 and 12]; glutaraldehyde coupling [45]; or reaction of hydroxyl groups on the polysaccharides with cyanating agents like CDAP [46] or cyanuric trichloride [11]. Preferably, the process described in reference 29 is used.

Preferred carrier proteins are bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof. The inventors have found that the CRM197 diphtheria toxin mutant [47] is suitable. *Pseudomonas aeruginosa* exotoxin A (ETA) and its non-toxic mutant recombinant exoprotein A (rEPA) have been used as carrier proteins for *S.aureus* type 5 or type 8 capsular polysaccharides ([1] and [2]).
5 *S.aureus* α -haemolysin (α -toxin) ([45] and [48]), ovalbumin [11] and human serum albumin [12] have also been used. These carriers may be used in the present invention.

Other suitable carrier proteins include the *N.meningitidis* outer membrane protein complex [49], synthetic peptides [50,51], heat shock proteins [52,53], pertussis proteins [54,55], cytokines [56], lymphokines [56], hormones [56], growth factors [56], human serum albumin (typically recombinant),
10 artificial proteins comprising multiple human CD4⁺ T cell epitopes from various pathogen-derived antigens [57] such as N19 [58], protein D from *H.influenzae* [59-61], pneumococcal surface protein PspA [62], pneumolysin [63] or its non-toxic derivatives [64], iron-uptake proteins [65], toxin A or B from *C.difficile* [66], a GBS protein [67], a GAS protein [68] etc.

Other suitable carrier proteins include *S.aureus* protein antigens, for example the *S.aureus* protein
15 antigens set out below.

Attachment to the carrier is preferably via a -NH₂ group e.g. in the side chain of a lysine residue in a carrier protein, or of an arginine residue. Attachment may also be via a -SH group e.g. in the side chain of a cysteine residue.

It is possible to use more than one carrier protein e.g. to reduce the risk of carrier suppression. Thus
20 different carrier proteins can be used for the type 5 and type 8 capsular polysaccharides, e.g. type 5 polysaccharide might be conjugated to CRM197 while type 8 polysaccharide might be conjugated to rEPA. It is also possible to use more than one carrier protein for a particular polysaccharide antigen e.g. type 5 polysaccharide might be in two groups, with one group conjugated to CRM197 and the other conjugated to rEPA. Typically, however, the same carrier protein is used for all polysaccharides.

25 A single carrier protein might carry more than one polysaccharide antigen [69,70]. For example, a single carrier protein might have conjugated to it type 5 and type 8 capsular polysaccharides. To achieve this goal, different polysaccharides can be mixed prior to the conjugation process. Typically, however, there are separate conjugates for each polysaccharide, with the different polysaccharides being mixed after conjugation. The separate conjugates may be based on the same carrier.

30 Conjugates with a polysaccharide:protein ratio (w/w) of between 1:20 (i.e. excess protein) and 20:1 (i.e. excess polysaccharide) are typically used. Ratios of 1:10 to 1:1 are preferred, particularly ratios between 1:5 and 1:2 and, most preferably, about 1:3. In contrast, type 5 and type 8 capsular polysaccharide

conjugates used in the literature tend to have higher ratios, *e.g.* between 0.73 and 1.08 in references 1, 2 and 3. In particular embodiments of the invention, the polysaccharide:protein ratio (w/w) for type 5 capsular polysaccharide conjugate is between 1:10 and 1:2; and/or the polysaccharide:protein ratio (w/w) for type 8 capsular polysaccharide conjugate is between 1:5 and 7:10.

- 5 Conjugates may be used in conjunction with free carrier [71]. When a given carrier protein is present in both free and conjugated form in a composition of the invention, the unconjugated form is preferably no more than 5% of the total amount of the carrier protein in the composition as a whole, and more preferably present at less than 2% by weight.

10 After conjugation, free and conjugated polysaccharides can be separated. There are many suitable methods, including hydrophobic chromatography, tangential ultrafiltration, diafiltration *etc.* [see also refs. 72 & 73, *etc.*].

Combinations of conjugates and other antigens

Polysaccharides prepared by the methods of the invention (in particular after conjugation as described above) can be mixed *e.g.* with each other and/or with other antigens. Thus the processes of the invention
15 may include the further step of mixing the polysaccharide with one or more further antigens. The invention therefore provides a composition comprising a polysaccharide prepared by the method of the invention and one or more further antigens. The composition is typically an immunogenic composition.

The further antigen(s) may comprise further polysaccharides prepared by the method of the invention, and so the invention provides a composition comprising more than one polysaccharide of the invention.
20 In particular, the present invention provides a composition comprising a type 5 capsular polysaccharide of the invention and a type 8 capsular polysaccharide of the invention. Alternatively, the further antigen(s) may be type 5 or type 8 capsular polysaccharides prepared by methods other than those of the invention, *e.g.* the methods of references 1 to 18 above. Accordingly, the invention provides a composition comprising a type 5 capsular polysaccharide and a type 8 capsular polysaccharide, wherein
25 one of the polysaccharides (the type 5 polysaccharide or the type 8 polysaccharide) is a polysaccharide of the invention and the other polysaccharide is not a polysaccharide of the invention.

Where multiple different *S.aureus* conjugates are mixed then these may include different types of conjugate from the same *S.aureus* serotype and/or conjugates from different *S.aureus* serotypes. For example, the conjugates may be from *S.aureus* type 5 and type 8. The composition will be produced by
30 preparing separate conjugates (*e.g.* a different conjugate for each serotype) and then combining the conjugates.

The further antigen(s) may comprise other *S.aureus* antigens, including the saccharide and protein antigens set out below.

The further antigen(s) may comprise antigens from non-*S.aureus* pathogens. Thus the compositions of the invention may further comprise one or more non-*S.aureus* antigens, including additional bacterial,
5 viral or parasitic antigens. These may be selected from the following:

- a protein antigen from *N.meningitidis* serogroup B, such as those in refs. 74 to 80, with protein ‘287’ (see below) and derivatives (e.g. ‘ΔG287’) being particularly preferred.
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 81, 82, 83, 84 etc.
- 10 – a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 85 from serogroup C or the oligosaccharides of ref. 86.
- a saccharide antigen from *Streptococcus pneumoniae* [e.g. refs. 87-89; chapters 22 & 23 of ref. 96].
- an antigen from hepatitis A virus, such as inactivated virus [e.g. 90, 91; chapter 15 of ref. 96].
- 15 – an antigen from hepatitis B virus, such as the surface and/or core antigens [e.g. 91,92; chapter 16 of ref. 96].
- an antigen from hepatitis C virus [e.g. 93].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or
20 agglutinogens 2 and 3 [e.g. refs. 94 & 95; chapter 21 of ref. 96].
- a diphtheria antigen, such as a diphtheria toxoid [e.g. chapter 13 of ref. 96].
- a tetanus antigen, such as a tetanus toxoid [e.g. chapter 27 of ref. 96].
- a saccharide antigen from *Haemophilus influenzae* B [e.g. chapter 14 of ref. 96]
- an antigen from *N.gonorrhoeae* [e.g. 74, 75, 76].
- 25 – an antigen from *Chlamydia pneumoniae* [e.g. 97, 98, 99, 100, 101, 102, 103].
- an antigen from *Chlamydia trachomatis* [e.g. 104].
- an antigen from *Porphyromonas gingivalis* [e.g. 105].
- polio antigen(s) [e.g. 106, 107; chapter 24 of ref. 96] such as IPV.
- rabies antigen(s) [e.g. 108] such as lyophilised inactivated virus [e.g. 109, RabAvert™].
- 30 – measles, mumps and/or rubella antigens [e.g. chapters 19, 20 and 26 of ref. 96].
- influenza antigen(s) [e.g. chapters 17 & 18 of ref. 96], such as the haemagglutinin and/or neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [e.g. 110].
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [e.g. 111, 112, 113].

- an antigen from *Streptococcus agalactiae* (group B streptococcus) [e.g. 68, 114-116].
- an antigen from *S.epidermidis* [e.g. type I, II and/or III capsular polysaccharide obtainable from strains ATCC-31432, SE-360 and SE-10 as described in refs. 117, 118 and 119.

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier in order to enhance immunogenicity. Conjugation of *H.influenzae* B, meningococcal and pneumococcal saccharide antigens is well known.

Toxic protein antigens may be detoxified where necessary (e.g. detoxification of pertussis toxin by chemical and/or genetic means [95]).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens may be adsorbed to an aluminium salt.

One type of preferred composition includes further antigens that affect the immunocompromised, and so the *S.aureus* polysaccharides of the invention can be combined with one or more antigens from the following non-*S.aureus* pathogens: *Streptococcus agalactiae*, *Staphylococcus epidermis*, influenza virus, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Listeria monocytogenes*, *Neisseria meningitidis*, and parainfluenza virus.

Another type of preferred composition includes further antigens from bacteria associated with nosocomial infections, and so the *S.aureus* polysaccharides of the invention can be combined with one or more antigens from the following non-*S.aureus* pathogens: *Clostridium difficile*, *Pseudomonas aeruginosa*, *Candida albicans*, and extraintestinal pathogenic *Escherichia coli*.

Antigens in the composition will typically be present at a concentration of at least 1µg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using proteins antigens in the composition of the invention, nucleic acid encoding the antigen may be used [e.g. refs. 120 to 128]. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA e.g. in the form of a plasmid) that encodes the protein.

In practical terms, there may be an upper limit to the number of antigens included in compositions of the invention. The number of antigens (including *S.aureus* antigens) in a composition of the invention may

be less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. The number of *S.aureus* antigens in a composition of the invention may be less than 6, less than 5, or less than 4.

5 *Pharmaceutical compositions and methods*

The invention provides processes for preparing pharmaceutical compositions, comprising the steps of mixing (a) a polysaccharide of the invention (optionally in the form of a conjugate) with (b) a pharmaceutically acceptable carrier. Typical 'pharmaceutically acceptable carriers' include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable excipients is available in reference 129.

Compositions of the invention may be in aqueous form (*i.e.* solutions or suspensions) or in a dried form (*e.g.* lyophilised). If a dried vaccine is used then it will be reconstituted into a liquid medium prior to injection. Lyophilisation of conjugate vaccines is known in the art *e.g.* the Menjugate™ product is presented in lyophilised form, whereas NeisVac-C™ and Meningitec™ are presented in aqueous form. To stabilise conjugates during lyophilisation, it may be typical to include a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at between 1mg/ml and 30mg/ml (*e.g.* about 25 mg/ml) in the composition.

The pharmaceutical compositions may be packaged into vials or into syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses.

Aqueous compositions of polysaccharides of the invention are suitable for reconstituting other vaccines from a lyophilised form. Where a composition of the invention is to be used for such extemporaneous reconstitution, the invention provides a process for reconstituting such a lyophilised vaccine, comprising the step of mixing the lyophilised material with an aqueous composition of the invention. The reconstituted material can be used for injection.

S.aureus antigens

As mentioned above, one or more further *S.aureus* antigens can be included in compositions of the invention. The antigens may be protein or saccharide antigens. *S.aureus* protein antigens may be used as carrier proteins for conjugates of the invention, carrier proteins for other conjugates, or as unconjugated protein antigens. *S.aureus* saccharide antigens may be used as the saccharides for other conjugates or as unconjugated saccharide antigens.

Suitable *S.aureus* saccharide antigens include the exopolysaccharide of *S.aureus*, which is a poly-N-acetylglucosamine (PNAG). This polysaccharide is present in both *S.aureus* and *S.epidermidis* and can be isolated from either source [130,131]. For example, PNAG may be isolated from *S.aureus* strain MN8m [132]. The saccharide antigen may be a polysaccharide having the size that arises during purification of the exopolysaccharide from bacteria, or it may be a polysaccharide achieved by fragmentation of such a polysaccharide *e.g.* size can vary from over 400kDa to between 75 and 400kDa, or between 10 and 75kDa, or up to 30 repeat units. The saccharide antigen can have various degrees of N-acetylation and, as described in reference 133, the PNAG may be less than 40% N-acetylated (*e.g.* less than 35, 30, 20, 15, 10 or 5% N-acetylated; deacetylated PNAG is also known as dPNAG). Deacetylated epitopes of PNAG can elicit antibodies that are capable of mediating opsonic killing. The preparation of dPNAG is described in reference 134. The PNAG may or may not be O-succinylated *e.g.* it may be O-succinylated on fewer less than 25, 20, 15, 10, 5, 2, 1 or 0.1% of residues. The PNAG may be conjugated to a carrier molecule as described above or alternatively unconjugated.

Another suitable *S.aureus* saccharide antigen is the type 336 antigen, which is a β -linked hexosamine with no O-acetylation [135,136]. The type 336 antigen is cross-reactive with antibodies raised against the 336 strain (ATCC 55804). The type 336 antigen may be conjugated to a carrier molecule as described above or alternatively unconjugated.

Suitable *S.aureus* protein antigens include the following *S.aureus* antigens (or antigens comprising immunogenic fragment(s) thereof) [*e.g.* see references 137-144]: AhpC, AhpF, Autolysin amidase, Autolysin glucosaminidase, Collagen binding protein CAN, EbhB, GehD lipase, Heparin binding protein HBP (17kDa), Laminin receptor, MAP, MntC (also known as SitC), MRPII, Npase, ORF0594, ORF0657n, ORF0826, PBP4, RAP (RNA III activating protein), Sai-1, SasK, SBI, SdrG, SdrH, SSP-1, SSP-2 and Vitronectin-binding protein.

Further suitable *S.aureus* protein antigens include a *clfA* antigen; a *clfB* antigen; a *sdrE2* antigen; a *sdrC* antigen; a *sasF* antigen, a *emp* antigen; a *sdrD* antigen; a *spa* antigen; a *esaC* antigen; a *esxA* antigen; a *esxB* antigen; a *sta006* antigen; a *isdC* antigen; a *Hla* antigen; a *sta011* antigen; a *isdA* antigen; a *isdB* antigen; and a *sta073* antigen, as described below. One or more (*i.e.* 1, 2, 3, 4, 5, 6 or more) of these

antigens may be present in a composition of the invention. Of these antigens, the use of one or more (*i.e.* 1, 2, 3, 4, 5, 6 or more) of a esxA antigen; a esxB antigen; a sta006 antigen; a Hla antigen; a sta011 antigen; and/or a sta073 antigen is specifically envisaged.

For example, a composition of the invention may comprise one of the following combinations of
5 *S.aureus* protein antigens:

- (1) A esxA antigen, a esxB antigen, a sta006 antigen and a Hla antigen. The esxA and esxB antigens can usefully be combined as a hybrid polypeptide, as discussed below, *e.g.* a EsxAB hybrid with a esxB antigen downstream of a esxA antigen. The Hla antigen may be a detoxified mutant *e.g.* including a H35L mutation.
- 10 (2) A esxA antigen, a esxB antigen, a sta006 antigen and a sta011 antigen. The esxA and esxB antigens may be combined as a hybrid polypeptide, as discussed below, *e.g.* an EsxAB hybrid.
- (3) A esxA antigen, a esxB antigen and a sta011 antigen. The esxA and esxB antigens can usefully be combined as a hybrid polypeptide, as discussed below, *e.g.* a EsxAB
15 hybrid.
- (4) A esxA antigen, a esxB antigen, a Hla antigen, a sta006 antigen and a sta011 antigen. The esxA and esxB antigens may be combined as a hybrid polypeptide, as discussed below, *e.g.* an EsxAB hybrid. The Hla antigen may be a detoxified mutant *e.g.* including a H35L mutation.
- 20 (5) A esxA antigen, a esxB antigen and a Hla antigen. The esxA and esxB antigens can usefully be combined as a hybrid polypeptide, as discussed below, *e.g.* a EsxAB hybrid. The Hla antigen may be a detoxified mutant *e.g.* including a H35L mutation.
- (6) A Hla antigen, a sta006 antigen and a sta011 antigen. The Hla antigen may be a detoxified mutant *e.g.* including a H35L mutation.
- 25 (7) A esxA antigen and a esxB antigen. The esxA and esxB antigens can usefully be combined as a hybrid polypeptide, as discussed below, *e.g.* an EsxAB hybrid.
- (8) A esxA antigen, a esxB antigen and a sta006 antigen. The esxA and esxB antigens can usefully be combined as a hybrid polypeptide, as discussed below, *e.g.* a EsxAB hybrid.
- (9) A esxA antigen, a esxB antigen, a sta011 antigen and a sta073 antigen. The esxA and
30 esxB antigens may be combined as a hybrid polypeptide, as discussed below, *e.g.* an EsxAB hybrid.
- (10) A sta006 antigen and a sta011 antigen.

Further *Staphylococcus aureus* antigens are disclosed in reference 145.

clfA

The 'clfA' antigen is annotated as 'clumping factor A'. In the NCTC 8325 strain clfA is SAOUHSC_00812 and has amino acid sequence SEQ ID NO: 1 (GI:88194572). In the Newman strain it is nwmn_0756 (GI:151220968).

- 5 Useful clfA antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 1 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 1; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 1, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These clfA proteins include variants of SEQ ID NO: 1. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 1 while retaining at least one epitope of SEQ ID NO: 1. The final 368 C-terminal amino acids of SEQ ID NO: 1 can usefully be omitted. The first 39 N-terminal amino acids of SEQ ID NO: 1 can usefully be omitted. Other fragments omit one or more protein domains.

SEQ ID NO: 2 is a useful fragment of SEQ ID NO: 1 ('ClfA₄₀₋₅₅₉'). This fragment omits the long repetitive region towards the C-terminal of SEQ ID NO: 1.

clfB

- 20 The 'clfB' antigen is annotated as 'clumping factor B'. In the NCTC 8325 strain clfB is SAOUHSC_02963 and has amino acid sequence SEQ ID NO: 3 (GI:88196585). In the Newman strain it is nwmn_2529 (GI:151222741).

- Useful clfB antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 3 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 3, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These clfB proteins include variants of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 3 while retaining at least one epitope of SEQ ID NO: 3. The final 40 C-terminal amino acids of SEQ ID NO: 3 can usefully be omitted. The first 44 N-terminal amino acids of SEQ ID NO: 3 can usefully be omitted. Other

fragments omit one or more protein domains. ClfB is naturally a long protein and so the use of fragments is helpful *e.g.* for purification, handling, fusion, expression, *etc.*

SEQ ID NO: 4 is a useful fragment of SEQ ID NO: 3 ('ClfB₄₅₋₅₅₂'). This fragment includes the most exposed domain of ClfB and is more easily used at an industrial scale. It also reduces the antigen's similarity with human proteins. Other useful fragments, based on a 3-domain model of ClfB, include: ClfB₄₅₋₃₆₀ (also known as CLfB-N12; SEQ ID NO: 5); ClfB₂₁₂₋₅₄₂ (also known as CLfB-N23; SEQ ID NO: 6); and ClfB₃₆₀₋₅₄₂ (also known as CLfB-N3; SEQ ID NO: 7).

sdrE2

The 'sdrE2' antigen is annotated as 'Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrE'. In the Newman strain sdrE2 is NWMN_0525 and has amino acid sequence SEQ ID NO: 8 (GI:151220737).

Useful sdrE2 antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 8 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 8; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 8, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sdrE2 proteins include variants of SEQ ID NO: 8. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 8. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 8 while retaining at least one epitope of SEQ ID NO: 8. The final 38 C-terminal amino acids of SEQ ID NO: 8 can usefully be omitted. The first 52 N-terminal amino acids of SEQ ID NO: 8 can usefully be omitted. Other fragments omit one or more protein domains. SdrE2 is naturally a long protein and so the use of fragments is very helpful *e.g.* for purification, handling, fusion, expression, *etc.*

SEQ ID NO: 9 is a useful fragment of SEQ ID NO: 8 ('SdrE₅₃₋₆₃₂'). This fragment includes the most exposed domain of SdrE2 and is more easily used at an industrial scale. It also reduces the antigen's similarity with human proteins.

sdrC

The 'sdrC' antigen is annotated as 'sdrC protein'. In the NCTC 8325 strain sdrC is SAOUHSC_00544 and has amino acid sequence SEQ ID NO: 10 (GI:88194324).

Useful sdrC antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 10 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ

ID NO: 10; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 10, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sdrC proteins include variants of SEQ ID NO: 10. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 10. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 10 while retaining at least one epitope of SEQ ID NO: 10. The final 38 C-terminal amino acids of SEQ ID NO: 10 can usefully be omitted. The first 50 N-terminal amino acids of SEQ ID NO: 10 can usefully be omitted. Other fragments omit one or more protein domains. SdrC is naturally a long protein and so the use of fragments is helpful *e.g.* for purification, handling, fusion, expression, *etc.*

SEQ ID NO: 11 is a useful fragment of SEQ ID NO: 10 ('SdrC₅₁₋₅₁₈'). This fragment includes the most exposed domain of SdrC and is more easily used at an industrial scale. It also reduces the antigen's similarity with human proteins.

sasF

The 'sasF' antigen is annotated as 'sasF protein'. In the NCTC 8325 strain sasF is SAOUHSC_02982 and has amino acid sequence SEQ ID NO: 12 (GI:88196601).

Useful sasF antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 12 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 12; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 12, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sasF proteins include variants of SEQ ID NO: 12. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 12. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 12 while retaining at least one epitope of SEQ ID NO: 12. The final 39 C-terminal amino acids of SEQ ID NO: 12 can usefully be omitted. The first 37 N-terminal amino acids of SEQ ID NO: 12 can usefully be omitted. Other fragments omit one or more protein domains.

emp

The 'emp' antigen is annotated as 'extracellular matrix and plasma binding protein'. In the NCTC 8325 strain emp is SAOUHSC_00816 and has amino acid sequence SEQ ID NO: 13 (GI:88194575). In the Newman strain it is nwmn_0758 (GI:151220970).

Useful emp antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 13 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 13, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These emp proteins include variants of SEQ ID NO: 13. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 13 while retaining at least one epitope of SEQ ID NO: 13. The first 26 N-terminal amino acids of SEQ ID NO: 13 can usefully be omitted. Other fragments omit one or more protein domains.

SEQ ID NOs: 14, 15, 16 and 17 are useful fragments of SEQ ID NO: 13 ('Emp₃₅₋₃₄₀', 'Emp₂₇₋₃₃₄', 'Emp₃₅₋₃₃₄' and 'Emp₂₇₋₁₄₇', respectively).

sdrD

The 'sdrD' antigen is annotated as 'sdrD protein'. In the NCTC 8325 strain sdrD is SAOUHSC_00545 and has amino acid sequence SEQ ID NO: 18 (GI:88194325).

Useful sdrD antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 18 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 18; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 18, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sdrD proteins include variants of SEQ ID NO: 18. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 18. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 18 while retaining at least one epitope of SEQ ID NO: 18. The final 38 C-terminal amino acids of SEQ ID NO: 18 can usefully be omitted. The first 52 N-terminal amino acids of SEQ ID NO: 18 can usefully be omitted. Other fragments omit one or more protein domains. SdrD is naturally a long protein and so the use of fragments is very helpful *e.g.* for purification, handling, fusion, expression, *etc.*

SEQ ID NO: 19 is a useful fragment of SEQ ID NO: 18 ('SdrD₅₃₋₅₉₂'). This fragment includes the most exposed domain of SdrD and is more easily used at an industrial scale. It also reduces the antigen's similarity with human proteins. A nother useful fragment, with the same C-terminus residue, is SdrD₃₉₄₋₅₉₂ (also known as SdrD-N3; SEQ ID NO: 20).

spa

The 'spa' antigen is annotated as 'protein A' or 'SpA'. In the NCTC 8325 strain spa is SAOUHSC_00069 and has amino acid sequence SEQ ID NO: 21 (GI:88193885). In the Newman strain it is nwmn_0055 (GI:151220267). All *S.aureus* strains express the structural gene for spa, a well characterized virulence factor whose cell wall-anchored surface protein product has five highly homologous immunoglobulin binding domains designated E, D, A, B, and C [146]. These domains display ~80% identity at the amino acid level, are 56 to 61 residues in length, and are organized as tandem repeats [147]. SpA is synthesized as a precursor protein with an N-terminal signal peptide and a C-terminal sorting signal [148,149]. Cell wall-anchored spa is displayed in great abundance on the staphylococcal surface [150,151]. Each of its immunoglobulin binding domains is composed of anti-parallel α -helices that assemble into a three helix bundle and can bind the Fc domain of immunoglobulin G (IgG) [152,153], the VH3 heavy chain (Fab) of IgM (*i.e.* the B cell receptor) [154], the von Willebrand factor at its A1 domain [155] and/or the TNF- α receptor I (TNFRI) [156], which is displayed on surfaces of airway epithelia.

Useful spa antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 21 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 21; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 21, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These spa proteins include variants of SEQ ID NO: 21. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 21. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 21 while retaining at least one epitope of SEQ ID NO: 21. The final 35 C-terminal amino acids of SEQ ID NO: 21 can usefully be omitted. The first 36 N-terminal amino acids of SEQ ID NO: 21 can usefully be omitted. Other fragments omit one or more protein domains. Reference 157 suggests that individual IgG-binding domains might be useful immunogens, alone or in combination.

SEQ ID NO: 22 is a useful fragment of SEQ ID NO: 21 ('Spa₃₇₋₃₂₅'). This fragment contains all the five SpA Ig-binding domains and includes the most exposed domain of SpA. It also reduces the antigen's similarity with human proteins. Other useful fragments may omit 1, 2, 3 or 4 of the natural A, B, C, D and/or E domains. As reported in reference 157, other useful fragments may include only 1, 2, 3 or 4 of the natural A, B, C, D and/or E domains *e.g.* comprise only the SpA(A) domain but not B to E, or comprise only the SpA(D) domain but not A, B, C or E, *etc.* Thus a spa antigen useful with the invention

may include 1, 2, 3, 4 or 5 IgG-binding domains, but ideally has 4 or fewer. If an antigen includes only one type of spa domain (*e.g.* only the Spa(A) or Spa(D) domain), it may include more than one copy of this domain *e.g.* multiple Spa(D) domains in a single polypeptide chain. An individual domain within the antigen may be mutated at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids relative to SEQ ID NO: 21 (*e.g.* see ref. 157, disclosing mutations at residues 3 and/or 24 of domain D, at residue 46 and/or 53 of domain A, *etc.*). Such mutants should not remove the antigen's ability to elicit an antibody that recognises SEQ ID NO: 21, but may remove the antigen's binding to IgG. In certain aspects a spa antigen includes a substitution at (a) one or more amino acid substitution in an IgG Fc binding sub-domain of Spa domain A, B, C, D and/or E that disrupts or decreases binding to IgG Fc, and (b) one or more amino acid substitution in a V_H3 binding sub-domain of Spa domain A, B, C, D, and/or E that disrupts or decreases binding to V_H3. In certain embodiments, a variant Spa comprises at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more variant Spa domain D peptides.

esaC

The 'esaC' antigen is annotated as 'esaC'. In the NCTC 8325 strain esaC is SAOUHSC_00264 and has amino acid sequence SEQ ID NO: 23 (GI:88194069).

Useful esaC antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 23 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 23; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 23, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These esaC proteins include variants of SEQ ID NO: 23. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 23. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 23 while retaining at least one epitope of SEQ ID NO: 23. Other fragments omit one or more protein domains.

esxA

The 'esxA' antigen is annotated as 'protein'. In the NCTC 8325 strain esxA is SAOUHSC_00257 and has amino acid sequence SEQ ID NO: 24 (GI:88194063).

Useful esxA antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 24 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 24; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 24, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 or more). These

esxA proteins include variants of SEQ ID NO: 24. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 24. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 24 while retaining at least one epitope of SEQ ID NO: 24. Other fragments omit one or more protein domains.

esxB

The 'esxB' antigen is annotated as 'esxB'. In the NCTC 8325 strain esxB is SAOUHSC_00265 and has amino acid sequence SEQ ID NO: 25 (GI:88194070).

Useful esxB antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 25 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 25; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 25, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These esxB proteins include variants of SEQ ID NO: 25. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 25. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 25 while retaining at least one epitope of SEQ ID NO: 25. Other fragments omit one or more protein domains.

sta006

The 'sta006' antigen is annotated as 'ferrichrome-binding protein', and has also been referred to as 'FhuD2' in the literature [158]. In the NCTC 8325 strain sta006 is SAOUHSC_02554 and has amino acid sequence SEQ ID NO: 26 (GI:88196199). In the Newman strain it is nwmn_2185 (GI:151222397).

Useful sta006 antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 26 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 26; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 26, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sta006 proteins include variants of SEQ ID NO: 26. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 26. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 26 while retaining at least one epitope of SEQ ID NO: 26. The first 17 N-terminal amino acids of SEQ ID NO: 26

can usefully be omitted. Other fragments omit one or more protein domains. Mutant forms of sta006 are reported in reference 159. A sta006 antigen may be lipidated *e.g.* with an acylated N-terminus cysteine.

isdC

The 'isdC' antigen is annotated as 'protein'. In the NCTC 8325 strain isdC is SAOUHSC_01082 and has amino acid sequence SEQ ID NO: 27 (GI:88194830).

Useful isdC antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 27 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 27; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 27, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These isdC proteins include variants of SEQ ID NO: 27. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 27. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 27 while retaining at least one epitope of SEQ ID NO: 27. The final 39 C-terminal amino acids of SEQ ID NO: 27 can usefully be omitted. The first 28 N-terminal amino acids of SEQ ID NO: 27 can usefully be omitted. Other fragments omit one or more protein domains. Useful fragments of IsdB are disclosed in reference 165.

Reference 160 discloses antigens which usefully include epitopes from both IsdB and IsdH.

Hla

The 'Hla' antigen is the 'alpha-hemolysin precursor' also known as 'alpha toxin' or simply 'hemolysin'. In the NCTC 8325 strain Hla is SAOUHSC_01121 and has amino acid sequence SEQ ID NO: 28 (GI:88194865). In the Newman strain it is nwmn_1073 (GI:151221285). Hla is an important virulence determinant produced by most strains of *S.aureus*, having pore-forming and haemolytic activity. Anti-Hla antibodies can neutralise the detrimental effects of the toxin in animal models, and Hla is particularly useful for protecting against pneumonia.

Useful Hla antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 28 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 28; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 28, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hla proteins include variants of SEQ ID NO: 28. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 28. Other preferred fragments lack one or more amino acids (*e.g.*

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 28 while retaining at least one epitope of SEQ ID NO: 28. The first 26 N-terminal amino acids of SEQ ID NO: 28 can usefully be omitted. Truncation at the C-terminus can also be used *e.g.* leaving only 50 amino acids
 5 (residues 27-76 of SEQ ID NO: 28) [161]. Other fragments omit one or more protein domains.

Hla's toxicity can be avoided in compositions of the invention by chemical inactivation (*e.g.* using formaldehyde, glutaraldehyde or other cross-linking reagents). Instead, however, it is preferred to use mutant forms of Hla which remove its toxic activity while retaining its immunogenicity. Such detoxified mutants are already known in the art. One useful Hla antigen has a mutation at residue 61 of SEQ ID
 10 NO: 28, which is residue 35 of the mature antigen (*i.e.* after omitting the first 26 N-terminal amino acids). Thus residue 61 may not be histidine, and may instead be *e.g.* Ile, Val or preferably Leu. A His-Arg mutation at this position can also be used. For example, SEQ ID NO: 29 is the mature mutant Hla-H35L sequence and a useful Hla antigen comprises SEQ ID NO: 29. Another useful mutation replaces a long loop with a short sequence *e.g.* to replace the 39mer at residues 136-174 of SEQ ID NO:
 15 28 with a tetramer such as PSGS (SEQ ID NO: 30), as in SEQ ID NO: 31 (which also includes the H35L mutation) and SEQ ID NO: 32 (which does not include the H35L mutation).

Further useful Hla antigens are disclosed in references 162 and 163.

SEQ ID NOs: 33, 34 & 35 are three useful fragments of SEQ ID NO: 28 ('Hla₂₇₋₇₆', 'Hla₂₇₋₈₉' and 'Hla₂₇₋₇₉', respectively). SEQ ID NOs: 36, 37 and 38 are the corresponding fragments from SEQ ID NO: 29.

20 sta011

The 'sta011' antigen is annotated as 'lipoprotein'. In the NCTC 8325 strain sta011 is SAOUHSC_00052 and has amino acid sequence SEQ ID NO: 39 (GI:88193872).

Useful sta011 antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 39 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%,
 25 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 39, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sta011 proteins include variants of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino
 30 acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39 while retaining at least one epitope of SEQ ID NO: 39. The first 23 N-terminal amino acids of SEQ ID NO: 39

can usefully be omitted. Other fragments omit one or more protein domains. A sta006 antigen may be lipidated *e.g.* with an acylated N-terminus cysteine.

Variant forms of SEQ ID NO: 39 which may be used for preparing sta011 antigens include, but are not limited to, SEQ ID NOs: 40, 41 and 42 with various Ile/Val/Leu substitutions.

5 isdA

The 'isdA' antigen is annotated as 'IsdA protein'. In the NCTC 8325 strain isdA is SAOUHSC_01081 and has amino acid sequence SEQ ID NO: 43 (GI:88194829). In the Newman strain it is nwmn_1041 (GI:151221253).

- Useful isdA antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 43 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 43; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 43, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These isdA proteins include variants of SEQ ID NO: 43. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 43. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 43 while retaining at least one epitope of SEQ ID NO: 43. The final 38 C-terminal amino acids of SEQ ID NO: 43 can usefully be omitted. The first 46 N-terminal amino acids of SEQ ID NO: 43 can usefully be omitted. Truncation to exclude the C-terminal 38mer of SEQ ID NO: 43 (beginning with the LPKTG motif) is also useful. Other fragments omit one or more protein domains.

- SEQ ID NO: 44 is a useful fragment of SEQ ID NO: 43 (amino acids 40-184 of SEQ ID NO: 43; 'IsdA₄₀₋₁₈₄') which includes the natural protein's heme binding site and includes the antigen's most exposed domain. It also reduces the antigen's similarity with human proteins. Other useful fragments are disclosed in references 164 and 165.

IsdA does not adsorb well to aluminium hydroxide adjuvants, so IsdA present in a composition may be unadsorbed or may be adsorbed to an alternative adjuvant *e.g.* to an aluminium phosphate.

isdB

- The 'isdB' antigen is annotated as 'neurofilament protein isdB'. In the NCTC 8325 strain isdB is SAOUHSC_01079 and has amino acid sequence SEQ ID NO: 45 (GI:88194828). IsdB has been proposed for use as a vaccine antigen on its own [166], but this may not prevent pneumonia.

Useful isdB antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 45 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 45; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 45, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These isdB proteins include variants of SEQ ID NO: 45. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 45. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 45 while retaining at least one epitope of SEQ ID NO: 45. The final 36 C-terminal amino acids of SEQ ID NO: 45 can usefully be omitted. The first 40 N-terminal amino acids of SEQ ID NO: 45 can usefully be omitted. Other fragments omit one or more protein domains. Useful fragments of IsdB are disclosed in references 165 and 167 *e.g.* lacking 37 internal amino acids of SEQ ID NO: 45.

In some embodiments, compositions of the invention do not include an isdB antigen.

sta073

The 'sta073' antigen is annotated as 'bifunctional autolysin precursor'. In the NCTC 8325 strain sta073 is SAOUHSC_00994 and has amino acid sequence SEQ ID NO: 46 (GI:88194750). In the Newman strain it is nwmn_0922 (GI:151221134). Proteomic analysis has revealed that this protein is secreted or surface-exposed.

Useful sta073 antigens can elicit an antibody (*e.g.* when administered to a human) that recognises SEQ ID NO: 46 and/or may comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 46; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 46, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These sta073 proteins include variants of SEQ ID NO: 46. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 46. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 46 while retaining at least one epitope of SEQ ID NO: 46. The first 24 N-terminal amino acids of SEQ ID NO: 46 can usefully be omitted. Other fragments omit one or more protein domains.

Sta073 does not adsorb well to aluminium hydroxide adjuvants, so Sta073 present in a composition may be unadsorbed or may be adsorbed to an alternative adjuvant *e.g.* to an aluminium phosphate.

Hybrid polypeptides

S.aureus protein antigens used in the invention may be present in the composition as individual separate polypeptides. Where more than one antigen is used, however, they do not have to be present as separate polypeptides. Instead, at least two (e.g. 2, 3, 4, 5, or more) antigens can be expressed as a single polypeptide chain (a 'hybrid' polypeptide). Hybrid polypeptides offer two main advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

- 10 The hybrid polypeptide may comprise two or more polypeptide sequences from each of the antigens listed above, or two or more variants of the same antigen in the cases in which the sequence has partial variability across strains.

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten antigens are useful. In particular, hybrids consisting of amino acid sequences from two, three, four, or five antigens are preferred, such as two or three antigens.

Different hybrid polypeptides may be mixed together in a single formulation. Hybrids may be combined with non-hybrid antigens selected from the first, second or third antigen groups. Within such combinations, an antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Hybrid polypeptides can be represented by the formula $\text{NH}_2\text{-A-}\{-\text{X-L-}\}_n\text{-B-COOH}$, wherein: X is an amino acid sequence of a *S.aureus* antigen, as described above; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; n is an integer of 2 or more (e.g. 2, 3, 4, 5, 6, etc.). Usually n is 2 or 3.

- 25 If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of $X_2 \dots X_n$ will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

- 30 For each n instances of $\{-\text{X-L-}\}$, linker amino acid sequence -L- may be present or absent. For instance, when $n=2$ the hybrid may be $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-L}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-X}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-X}_2\text{-L}_2\text{-COOH}$, etc. Linker amino acid sequence(s) -L- will typically be short (e.g. 20 or fewer

amino acids *i.e.* 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_{*n*} where *n* = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_{*n*} where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is
 5 GSGGGG (SEQ ID NO: 47) or GSGSGGGG (SEQ ID NO: 48), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the (Gly)₄ tetrapeptide being a typical poly-glycine linker. Other suitable linkers, particularly for use as the final L_{*n*} are ASGGGS (SEQ ID NO: 49 *e.g.* encoded by SEQ ID NO: 50) or a Leu-Glu dipeptide.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino
 10 acids *i.e.* 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_{*n*} where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X₁ lacks its own N-terminus methionine, -A- is preferably an
 15 oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine *e.g.* Met-Ala-Ser, or a single Met residue.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino
 acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short
 20 peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_{*n*} where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more, such as SEQ ID NO: 51), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

One hybrid polypeptide of the invention may include both EsxA and EsxB antigens. These may be in either order, N- to C- terminus. SEQ ID NOs: 52 ('EsxAB'; encoded by SEQ ID NO: 53) and 54
 25 ('EsxBA') are examples of such hybrids, both having hexapeptide linkers ASGGGS (SEQ ID NO: 49).

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references 168-175, *etc.*

30 "GI" numbering is used above. A GI number, or "GenInfo Identifier", is a series of digits assigned consecutively to each sequence record processed by NCBI when sequences are added to its databases. The GI number bears no resemblance to the accession number of the sequence record. When a sequence

is updated (*e.g.* for correction, or to add more annotation or information) then it receives a new GI number. Thus the sequence associated with a given GI number is never changed.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. 176. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. 177.

Where the invention concerns an “epitope”, this epitope may be a B-cell epitope and/or a T-cell epitope. Such epitopes can be identified empirically (*e.g.* using PEPSCAN [178,179] or similar methods), or they can be predicted (*e.g.* using the Jameson-Wolf antigenic index [180], matrix-based approaches [181], MAPITOPE [182], TEPITOPE [183,184], neural networks [185], OptiMer & EpiMer [186, 187], ADEPT [188], Tsites [189], hydrophilicity [190], antigenic index [191] or the methods disclosed in references 192-196, *etc.*). Epitopes are the parts of an antigen that are recognised by and bind to the antigen binding sites of antibodies or T-cell receptors, and they may also be referred to as “antigenic determinants”.

Where an antigen “domain” is omitted, this may involve omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, of an extracellular domain, *etc.*

The term “comprising” encompasses “including” as well as “consisting” *e.g.* a composition “comprising” X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term “about” in relation to a numerical value *x* means, for example, $x \pm 10\%$.

The word “substantially” does not exclude “completely” *e.g.* a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

Where the invention provides a process involving multiple sequential steps, the invention can also provide a process involving less than the total number of steps. The different steps can be performed at very different times by different people in different places (*e.g.* in different countries).

It will be appreciated that sugar rings can exist in open and closed form and that, whilst closed forms are shown in structural formulae herein, open forms are also encompassed by the invention. Similarly, it will be appreciated that sugars can exist in pyranose and furanose forms and that, whilst pyranose forms are

shown in structural formulae herein, furanose forms are also encompassed. Different anomeric forms of sugars are also encompassed.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 illustrates a process for purifying *S.aureus* type 5 and type 8 capsular polysaccharides based on the method of reference 13.

Figure 2 shows a DEAE Sepharose chromatogram of capsular polysaccharide and a ¹H NMR spectrum of capsular polysaccharide-containing fractions (fractions 68-80) prepared according to the method of Figure 1.

Figure 3 shows a S300 Sephacryl chromatogram of capsular polysaccharide and a ¹H NMR spectrum of capsular polysaccharide-containing fractions (fractions 22-44) prepared according to the method of Figure 1.

Figure 4 illustrates an exemplary process of the invention for purifying *S.aureus* type 5 and type 8 capsular polysaccharides.

Figure 5 shows a DEAE Sepharose chromatogram of capsular polysaccharide prepared according to a method of the invention.

Figure 6 shows a ¹H NMR spectrum for purified *S.aureus* type 5 capsular polysaccharide.

Figure 7 shows the chemical structure of the peptidoglycan of *S.aureus* based on references 197, 198, 199 and 200. The repeat unit is highlighted.

MODES FOR CARRYING OUT THE INVENTION

A. Purification of *S.aureus* type 5 capsular polysaccharide (comparative example)

S.aureus type 5 capsular polysaccharide was purified according to the scheme illustrated in Figure 1, based on the method of reference 13. The conditions and rationale for the various steps of this method are described in Table 1:

Step	Conditions	Rationale
Bacterial growth on plates		
Bacterial pellet centrifugation		Harvest of cells
Reaction with Lysostaphin	100 µg/ml of Lysostaphin overnight at 37°C	Cell wall lysis and release of capsular polysaccharide
Reaction with DNase/RNase	50 µg/ml of DNase and RNase at 37°C for 6-8hrs	Nucleic acid hydrolysis
Reaction with NaIO ₄	0.05M NaIO ₄ for 5hrs at RT in the dark	Teichoic acid hydrolysis
Diafiltration 30kDa	Washing with NaCl 1M and H ₂ O	Low molecular weight species removal
Anion exchange chromatography (DEAE SepharoseFF resin)	NaCl 1M gradient	Separation according to charge (protein removal)
Gel filtration (Sephacryl S300)	NaPi 10 mM pH 7.2 and NaCl 10 mM	Separation according to molecular weight

Table 1

Bacterial pellet centrifugation and enzymatic reactions (Lysostaphin and RNase/DNase)

5 *S.aureus* was grown in solid medium to provide a bacterial suspension of 600-800 ml. The wet cell pellet, harvested by centrifugation at 8000 rpm, had a mass of around 30-50 g. The harvested pellet was washed three times with 50mM Tris-2mM MgSO₄ pH7.5 and then suspended at 0.25-0.5g per ml in 50mM Tris-2mM MgSO₄ pH7.5 and treated with 0.1-0.13mg/ml of lysostaphin (Sigma-Aldrich). The reaction mixture was incubated at 37°C for 16hrs (ON) with mild stirring. 0.05 mg/ml of DNase/RNase (Sigma-Aldrich) was added to the suspension and incubated for 5-7hrs at 37°C. The suspension was then
10 clarified by centrifugation.

Reaction with NaIO₄

The material was incubated with 50mM NaIO₄ (Sigma-Aldrich) in the dark for 5-7hrs. NaIO₄ was then removed by the addition of excess glycerol for 30 minutes with stirring in the light.

30 kDa tangential flow filtration

15 Tangential flow filtration was carried out as indicated in Table 2:

Membrane type	Sartorius Hydrosart™ 30kDa
Surface area	0.1m ²
P _{in} /P _{out}	0.4/0.0 bar
Permeate flow rate	80 ml/min
Diafiltration volumes	10 volumes of NaCl 1M followed by 10 volumes of distilled water
Product recovery	Retentate volume + two washings with distilled water equal to the dead volume of the system (with completely open retentate and closed permeate)

Table 2

The tangential flow filtration was performed in a Sartorius™ holder for 0.1m² cassettes using a WatsonMarlon™ peristaltic pump. Afterwards, the membrane was washed with NaOH 1M and stored in NaOH 0.1M at +2-8°C.

DEAE Sepharose Fast Flow chromatography

Residual protein, nucleic acid and other impurities were removed by anion exchange chromatography carried out in accordance with Table 3:

Resin	DEAE Sepharose™ Fast Flow resin (G&E Healthcare)
Column dimension	Ø = 5cm; h = 7.5cm; V = 150ml
Equilibration	10mM NaPi buffer pH7.2 q.b. to reach 1.8-2.0mS/cm eluate conductivity
Load	Retentate from 30K UF buffered to 10mM NaPi buffer pH7.2
Elution	20 column volumes of 10mM NaPi buffer pH7.2
Stripping	20 column volumes of NaCl 1M

Table 3

- 10 The chromatography was performed using an Akta™ system (G&E Healthcare) and the capsular polysaccharide was detected by measuring UV absorption at 215nm. The capsular polysaccharide solution was first added to 100mM NaPi buffer pH7.2 to obtain a final buffer concentration of 10mM NaPi pH7.2. The DEAE resin was pre-equilibrated with 100mM NaPi buffer pH7.2 to pH7.2 and then equilibrated with 10mM NaPi buffer pH7.2 to achieve the indicated conductivity (10mM NaPi buffer
- 15 pH7.2 conductivity). The resultant fractions were analyzed by NMR and those containing capsular polysaccharide pooled together (Figure 2).

S300 Sephacryl chromatography

The polysaccharide was further purified by gel-filtration chromatography carried out in accordance with Table 4:

Resin	S300 Sephacryl™ resin (G&E Healthcare)
Column dimension	Ø = 2.6cm; h = 95cm; V = 500ml
Equilibration	50mMNaCl buffer q.b. to reach 6.3-6.5mS/cm eluate conductivity
Load	12-14ml
Elution	50mMNaCl buffer

Table 4

The chromatography was performed on an Akta™ system (G&E Healthcare) and the capsular polysaccharide was detected by measuring UV absorption at 215nm. The resultant fractions were analyzed by NMR and those containing capsular polysaccharide pooled together (Figure 3).

5 B. Purification of *S.aureus* type 5 and type 8 capsular polysaccharides (example)

S.aureus type 5 and type 8 capsular polysaccharides were purified according to the scheme illustrated in Figure 4. The conditions and rationale for the various steps of this method are described in Table 5:

Step	Conditions	Rationale
Bacterial growth on plates		
Bacterial pellet centrifugation		Harvest of cells
Reaction with AcOH1%	2hrs at 100°C	Cell wall lysis and release of capsular polysaccharide
Reaction with mutanolysin	180U/ml of mutanolysin at 37°C over-night	Further removal of peptidoglycan
Reaction with DNase/RNase	50 µg/ml of DNase and RNase at 37°C for 6-8hrs	Nucleic acid hydrolysis
Diafiltration 30kDa	Washing with NaCl 1M and H ₂ O	Low molecular weight species removal
Anion exchange chromatography (DEAE SepharoseFF resin)	NaCl 1M gradient	Separation according to charge (protein removal)

Table 5

Bacterial pellet centrifugation and acid and enzymatic reactions (acetic acid, RNase/DNase and mutanolysin)

S.aureus was grown in solid medium to provide a bacterial suspension of 600-800 ml. The wet cell pellet, harvested by centrifugation at 8000 rpm, had a mass of around 30-50 g. The harvested pellet was washed three times with 50mM Tris-2mM MgSO₄ pH7.5 and then suspended at 0.5-0.6g per ml in distilled water and stirred vigorously while the temperature was raised to 100°C. Acetic acid was then added to a final concentration of 1% and the mixture kept at 100°C for 2hrs. The mixture was neutralised with NaOH 1M and centrifuged at 8000 rpm.

The supernatant was decanted from the pellet and combined with 0.05 mg/ml of DNase/RNase (Sigma-Aldrich). The mixture was then incubated for 5-7hrs at 37°C and afterwards clarified by centrifugation.

180U/ml of mutanolysin (Sigma-Aldrich) was then added to the suspension and the mixture incubated over-night (for 16hrs) at 37°C with mild stirring. The suspension was then clarified again by centrifugation

30 kDa tangential flow filtration

- 5 Tangential flow filtration was carried out as indicated in Table 6:

Membrane type	Sartorius Hydrosart™ 30kDa
Surface area	0.2m ²
P _{in} /P _{out}	0.7/0.0 bar
Permeate flow rate	11 ml/min
Diafiltration volumes	10 volumes of NaCl 1M followed by 10 volumes of NaPi 10mM pH 7.2 buffer
Product recovery	Retentate volume + two washings with distilled water equal to the dead volume of the system (with completely open retentate and closed permeate)

Table 6

- The tangential flow filtration was performed in a Sartorius™ holder for 0.2m² cassettes using a WatsonMarlon™ peristaltic pump. Afterwards, the membrane was washed with NaOH 1M and stored in NaOH 0.1M at +2-8°C.
- 10

DEAE Sepharose Fast Flow chromatography

Residual protein, nucleic acid and other impurities were removed by anion exchange chromatography carried out in accordance with Table 7:

Resin	DEAE Sepharose™ Fast Flow resin (G&E Healthcare)
Column dimension	Ø = 5cm; h = 7.5cm; V = 150ml
Equilibration	10mM NaPi buffer pH7.2 q.b. to reach 1.8-2.0mS/cm eluate conductivity
Load	Retentate from 30K UF
Elution	20 column volumes of 10mM NaPi buffer pH7.2
Stripping	20 column volumes of NaCl 1M

Table 7

- 15 The chromatography was performed using an Akta™ system (G&E Healthcare) and the capsular polysaccharide was detected by measuring UV absorption at 215nm. The capsular polysaccharide solution was first added to 100mM NaPi buffer pH7.2 to obtain a final buffer concentration of 10mM NaPi pH7.2. The DEAE resin was pre-equilibrated with 100mM NaPi buffer pH7.2 to pH7.2 and then equilibrated with 10mM NaPi buffer pH7.2 to achieve the indicated conductivity (10mM NaPi buffer

pH7.2 conductivity). The resultant fractions were analyzed by NMR and those containing capsular polysaccharide pooled together (Figure 5).

30 kDa tangential flow filtration

Tangential flow filtration was carried out to remove NaCl left over from the anion exchange chromatography and to concentrate the purified polysaccharides. The filtration was carried out as indicated in Table 8:

Membrane type	Sartorius Hydrosart™ 30kDa
Surface area	0.2m ²
P _{in} /P _{out}	0.7/0.0 bar
Permeate flow rate	11 ml/min
Diafiltration volumes	10 volumes of distilled water
Product recovery	Retentate volume + two washings with distilled water equal to the dead volume of the system (with completely open retentate and closed permeate)

Table 8

The tangential flow filtration was performed in a Sartorius™ holder for 0.2m² cassettes using a WatsonMarlon™ peristaltic pump. Afterwards, the membrane was washed with NaOH 1M and stored in NaOH 0.1M at +2-8°C. The purified polysaccharide was analysed by NMR (e.g. Figure 6 for the type 5 capsular polysaccharide).

C. Determination of peptidoglycan contamination in purified polysaccharide

The peptidoglycan (Figure 7) content of purified type 5 polysaccharide obtained according to the methods in sections A and B above was determined by amino acid analysis using HPAEC-PAD according to the Dionex AAA-Direct™ system (AminoPac™ PA10 AAA-Direct™, Dionex) in accordance with the manufacturer's instructions. Briefly, 20μL of 100μM norleucine was added to 200μL of polysaccharide at 250μg/mL in water in a 400°C treated glass tube and dried using a Speedvac system. The norleucine serves as an internal standard. Samples were hydrolyzed *in vacuo* using the vapor of boiling hydrochloric acid/phenol in order to yield free amino acids from residual protein and peptidoglycan contamination. Separation of free amino acids was performed on an AminoPac™ PA10 column (2x250mm) equipped with an AminoPac™ PA10 guard column (2x50mm) using a gradient condition for amino acids and carbohydrates according to the manufacturer's recommendations. These gradient conditions are summarized in Table 9:

Time (min)	%E1	%E2	%E3	Curve	Comments
Initiation	84	16	0		Autosampler fills the sample loop
0.0	84	16	0		Valve from Load to Inject
2.0	84	16	0		Begin hydroxide gradient
12.1	68	32	0	8	
16.0	68	32	0		Begin acetate gradient
24.0	36	24	40	8	
40.0	36	24	40		
40.1	20	80	0	5	Column wash with hydroxide
42.1	20	80	0		
42.2	84	16	0	5	Equilibrate to starting conditions
65.0	84	16	0		

Eluent E1: Deionized Water; Eluent E2: 0.250 M Sodium Hydroxide; Eluent E3: 1.0 M Sodium Acetate and Flow=0.25mL/min

Table 9

Detection was performed using a AAA-Direct waveform potential (Table 10).

Time (sec)	Potential (V) vs. Ag/AgCl	Potential (V) vs. pH	Integration
0.000	-0.20	+0.13	
0.040	-0.20	+0.13	
0.050	0.00	+0.33	
0.210	0.00	+0.33	Begin
0.220	+0.22	+0.55	
0.460	+0.22	+0.55	
0.470	0.00	+0.33	
0.560	0.00	+0.33	End
0.570	-0.20	-1.67	
0.580	-0.20	-1.67	
0.590	+0.60	+0.93	
0.600	-0.20	+0.13	

Table 10

- 5 The quantification was performed using a non-hydrolyzed 17 amino acid standard solution (Fluka P/N 09428) in the range 2.5-50 μ M. Standard samples were analyzed with and without norleucine, at the same sample concentration. The ratio of the norleucine peak area in the sample divided by the average norleucine peak area in the standards was used as a correction factor for possible amino acid loss in the hydrolysis step. A BSA sample was used as control sample.

10 *Peptidoglycan content estimation*

Peptidoglycan content was estimated using two different methods. The first method (method 1) was based on the method used in reference 17, which involves a summation of the lysine, alanine, glycine and glutamate content. In the second method (method 2), a conversion factor is calculated for each amino acid according to the following formula:

$$\frac{(\text{molecular mass of amino acid}) \times (\text{number of residues in the peptidoglycan structure})}{(\text{molecular mass of the repeating unit of peptidoglycan})}$$

The molecular mass of the repeating unit of peptidoglycan is 1233.27 Da (Figure 7). The peptidoglycan content was then calculated as the average peptidoglycan concentration obtained by calculating the ratio of the amino acid concentration and the conversion factor.

The peptidoglycan content of the purified type 5 capsular polysaccharide after anionic exchange chromatography is given in Table 11:

Measurement method	Details of calculation	% Peptidoglycan	
		Measurement 1	Measurement 2
1	Calculated according to reference 17 as sum of Lys-Ala-Gly-Glx concentration	2.04	0.74
1	Calculated according to reference 17 as sum of all amino acids detectable except for Lys-Ala-Gly-Glx	0.48	0.85
2	Calculated using Ala and Gly concentration divided by PG conversion factor (Ala=0.2167, Gly=0.3043)	0.88	0.81

Table 11

The method of the invention provides a very low content of peptidoglycan in the purified polysaccharide.

D. Conjugation and immunogenicity of purified polysaccharides

Purified type 5 polysaccharides obtained from the methods in sections A and B above were conjugated to CRM197 according to the method of reference 29. Total saccharide in the conjugate was determined by HPAEC-PAD analysis and protein content by MicroBCA assay (Table 12).

Purification method	Lot	Protein (µg/ml)	Saccharide (µg/ml)	Saccharide/protein (w/w)
A	1	51.52	1.72	0.03
A	2	161.80	17.10	0.11
A	3	34.42	4.22	0.12
B	4	444.0	139.0	0.31
B	5	40.56	12.70	0.31

TABLE 12

The conjugates prepared using polysaccharides purified by the method of the invention (lots 4 and 5) had higher polysaccharide:protein ratios.

The immunogenicity of lot 5 was tested in a mouse lethal model of *S.aureus* infection. Briefly, CD1 mice were immunised by intraperitoneal injection with a 2 µg dose of antigen in an injection volume of 200 µl. Immunisations were carried out in groups of twelve mice according to the following scheme, prior to challenge by intraperitoneal injection of a bacterial suspension of 5×10^8 CFU type 5 *S.aureus*. Cultures of *S.aureus* were centrifuged, washed twice and diluted in PBS before challenge. Further dilutions were needed for the desired inoculum, which was experimentally verified by agar plating and colony formation. Animals were monitored for 14 days and lethal disease recorded.

Group 1 – PBS plus alum

Group 2 – Type 5 capsular polysaccharide-CRM conjugate (Lot 5) plus alum

Group 4 – Type 5 capsular polysaccharide-CRM conjugate (Lot 5) plus EsxAB, Sta006 and Sta011 proteins and alum

Group 5 – Type 5 capsular polysaccharide-CRM conjugate (Lot 5) plus HlaH35L, Sta006 and Sta011 proteins and alum

Survival data is presented in Table 13:

Group	Time (days)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	100	25	17	17	17	17	17	17	17	17	8	0	0	0
2	100	50	50	50	50	50	50	50	50	42	42	42	42	42
4	100	67	67	67	67	67	67	67	67	67	67	67	67	67
5	100	100	100	100	100	100	83	83	75	75	75	75	75	75

TABLE 13

The conjugates prepared using polysaccharides purified by the method of the invention gave a high level of survival. Survival was enhanced by addition of *S.aureus* protein antigens.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A method for releasing capsular polysaccharide from *S.aureus* type 5 or type 8 cells, comprising the step of treating the cells with acid.
2. The method according to claim 1, wherein the cells are *S.aureus* type 5 cells.
- 5 3. The method according to claim 1, wherein the cells are *S.aureus* type 8 cells.
4. The method according to any one of claims 1-3, wherein the cells are in the form of a wet cell paste or are suspended in an aqueous medium.
5. The method according to any one of claims 1-4, wherein the acid treatment is carried out using acetic acid.
- 10 6. The method according to any one of claims 1-5, wherein the acid treatment results in the capsular polysaccharide having a degree of O-acetylation between 60-100%.
7. The method according to any one of claims 1-6, wherein the method further comprises a step of neutralisation.
8. The method according to any one of claims 1-7, wherein the method further comprises a step of
15 centrifugation of the cells and collection of the polysaccharide-containing supernatant.
9. A process for purifying capsular polysaccharide from *S.aureus* type 5 or type 8 cells comprising the method according to any one of claims 1-8.
10. The process according to claim 9, wherein the process further comprises a step of treatment of the capsular polysaccharide with DNase and/or RNase.
- 20 11. The process according to claim 9 or claim 10, wherein the process further comprises a step of treatment of the capsular polysaccharide with mutanolysin.
12. The process according to any one of claims 9-11, wherein the process further comprises a step of diafiltration.
13. The process according to claim 12, wherein the diafiltration is tangential flow filtration.
- 25 14. The process according to any one of claims 9-13, wherein the process further comprises a step of anion exchange chromatography.
15. The process according to any one of claims 9-14, wherein the process further comprises a step of gel filtration.

16. The process according to any one of claims 9-15, wherein the process further comprises a step of concentration of the polysaccharide.
17. The process according to any one of claims 9-16, wherein the molecular mass of the purified polysaccharide is between 2-3500 kDa.
- 5 18. The process according to any one of claims 9-17, wherein the process further comprises a step of depolymerisation of the purified polysaccharide to form an oligosaccharide.
19. The process according to any one of claims 9-18, wherein the process further comprises a step of sterile filtration.
- 10 20. The process according to any one of claims 9-19, wherein the process provides a composition comprising the polysaccharide and a level of peptidoglycan contamination that is less than 5% by weight peptidoglycan relative to the total weight of the polysaccharide.
21. The process according to claim 20, wherein the level of peptidoglycan contamination is about 2%.
22. The process according to any one of claims 9-21, wherein the process provides a composition comprising the polysaccharide and a level of protein contamination that is less than 5% by weight
15 protein relative to the total weight of the polysaccharide.
23. The process according to any one of claims 9-22, wherein the process provides a composition comprising the polysaccharide and a level of nucleic acid contamination that is less than 1% by weight nucleic acid relative to the total weight of the polysaccharide.
- 20 24. The process according to any one of claims 9-23, wherein the process further comprises a step of conjugation to a carrier molecule.
25. A composition comprising a *S.aureus* type 5 or type 8 capsular polysaccharide obtainable by the process according to any one of claims 9-23 or a conjugate obtainable by the process of claim 24.
26. The composition of claim 25 further comprising one or more *S.aureus* protein antigen(s) selected from the group consisting of a clfA antigen; a clfB antigen; a sdrE2 antigen; a sdrC antigen; a sasF
25 antigen; a emp antigen; a sdrD antigen; a spa antigen; a esaC antigen; a esxA antigen; a esxB antigen; a sta006 antigen; a isdC antigen; a Hla antigen; a sta011 antigen; a isdA antigen; a isdB antigen; and a sta073 antigen.
27. The composition of claim 26, wherein the one or more *S.aureus* protein antigen(s) are selected from the group consisting of a esxA antigen; a esxB antigen; a sta006 antigen; a Hla antigen; a sta011
30 antigen; and a sta073 antigen.

28. The composition of claim 27, wherein the composition comprises *S.aureus* protein antigens according to one of combinations (1) to (10) below:

- (1) a csxA antigen, a csxB antigen, a sta006 antigen and a Hla antigen;
- (2) a csxA antigen, a csxB antigen, a sta006 antigen and a sta011 antigen;
- 5 (3) a csxA antigen, a csxB antigen and a sta011 antigen;
- (4) a csxA antigen, a csxB antigen, a Hla antigen, a sta006 antigen and a sta011 antigen;
- (5) a csxA antigen, a csxB antigen and a Hla antigen;
- (6) a Hla antigen, a sta006 antigen and a sta011 antigen;
- (7) a csxA antigen and a csxB antigen;
- 10 (8) a csxA antigen, a csxB antigen and a sta006 antigen;
- (9) a csxA antigen, a csxB antigen, a sta011 antigen and a sta073 antigen; and
- (10) a sta006 antigen and a sta011 antigen.

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pctib2010054934-seq1

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Asn Gly Asp Val Val Ala Lys Ala Thr Tyr Asp Ile Leu Thr Lys Thr
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Tyr Thr Phe Val Phe Thr Asp Tyr Val Asn Asn Lys Glu Asn Ile Asn
305 310 315 320
Gly Gln Phe Ser Leu Pro Leu Phe Thr Asp Arg Ala Lys Ala Pro Lys
325 330 335
Ser Gly Thr Tyr Asp Ala Asn Ile Asn Ile Ala Asp Glu Met Phe Asn
340 345 350
Asn Lys Ile Thr Tyr Asn Tyr Ser Ser Pro Ile Ala Gly Ile Asp Lys
355 360 365
Pro Asn Gly Ala Asn Ile Ser Ser Gln Ile Ile Gly Val Asp Thr Ala
370 375 380
Ser Gly Gln Asn Thr Tyr Lys Gln Thr Val Phe Val Asn Pro Lys Gln
385 390 395 400
Arg Val Leu Gly Asn Thr Trp Val Tyr Ile Lys Gly Tyr Gln Asp Lys
405 410 415
Ile Glu Glu Ser Ser Gly Lys Val Ser Ala Thr Asp Thr Lys Leu Arg
420 425 430
Ile Phe Glu Val Asn Asp Thr Ser Lys Leu Ser Asp Ser Tyr Tyr Ala
435 440 445
Asp Pro Asn Asp Ser Asn Leu Lys Glu Val Thr Asp Gln Phe Lys Asn
450 455 460
Arg Ile Tyr Tyr Glu His Pro Asn Val Ala Ser Ile Lys Phe Gly Asp
465 470 475 480
Ile Thr Lys Thr Tyr Val Val Leu Val Glu Gly His Tyr Asp Asn Thr
485 490 495
Gly Lys Asn Leu Lys Thr Gln Val Ile Gln Glu Asn Val Asp Pro Val
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Thr Asn Arg Asp Tyr Ser Ile Phe Gly Trp Asn Asn Glu Asn Val Val
515 520 525

pctib2010054934-seq1

Arg Tyr Gly Gly Gly Ser Ala Asp Gly Asp Ser Ala Val Asn Pro Lys
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 Asp Pro Thr Pro Gly Pro Pro Val Asp Pro Glu Pro Ser Pro Asp Pro
 545 550 555 560
 Glu Pro Glu Pro Thr Pro Asp Pro Glu Pro Ser Pro Asp Pro Glu Pro
 565 570 575
 Glu Pro Ser Pro Asp Pro Asp Pro Asp Ser Asp Ser Asp Ser Asp Ser
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 595 600 605
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 675 680 685
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 740 745 750
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 755 760 765
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 770 775 780
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 785 790 795 800
 Asp Ser Asp Ser Arg Val Thr Pro Pro Asn Asn Glu Gln Lys Ala Pro
 805 810 815
 Ser Asn Pro Lys Gly Glu Val Asn His Ser Asn Lys Val Ser Lys Gln
 820 825 830
 His Lys Thr Asp Ala Leu Pro Glu Thr Gly Asp Lys Ser Glu Asn Thr
 835 840 845
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pctib2010054934-seq1

<211> 508

<212> PRT

<213> Staphylococcus aureus

<400> 4

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Thr Ala Asn Asp Thr Ser Asp Ile Ser Ala Asn Thr Asn Ser Ala Asn
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Val Asp Ser Thr Thr Lys Pro Met Ser Thr Gln Thr Ser Asn Thr Thr
50     55     60
Thr Thr Glu Pro Ala Ser Thr Asn Glu Thr Pro Gln Pro Thr Ala Ile
65     70     75     80
Lys Asn Gln Ala Thr Ala Ala Lys Met Gln Asp Gln Thr Val Pro Gln
85     90     95
Glu Ala Asn Ser Gln Val Asp Asn Lys Thr Thr Asn Asp Ala Asn Ser
100    105    110
Ile Ala Thr Asn Ser Glu Leu Lys Asn Ser Gln Thr Leu Asp Leu Pro
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Gln Ser Ser Pro Gln Thr Ile Ser Asn Ala Gln Gly Thr Ser Lys Pro
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Ser Val Arg Thr Arg Ala Val Arg Ser Leu Ala Val Ala Glu Pro Val
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Val Asn Ala Ala Asp Ala Lys Gly Thr Asn Val Asn Asp Lys Val Thr
165    170    175
Ala Ser Asn Phe Lys Leu Glu Lys Thr Thr Phe Asp Pro Asn Gln Ser
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Gly Asn Thr Phe Met Ala Ala Asn Phe Thr Val Thr Asp Lys Val Lys
195    200    205
Ser Gly Asp Tyr Phe Thr Ala Lys Leu Pro Asp Ser Leu Thr Gly Asn
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Gly Asp Val Asp Tyr Ser Asn Ser Asn Asn Thr Met Pro Ile Ala Asp
225    230    235    240
Ile Lys Ser Thr Asn Gly Asp Val Val Ala Lys Ala Thr Tyr Asp Ile
245    250    255
Leu Thr Lys Thr Tyr Thr Phe Val Phe Thr Asp Tyr Val Asn Asn Lys
260    265    270
Glu Asn Ile Asn Gly Gln Phe Ser Leu Pro Leu Phe Thr Asp Arg Ala
275    280    285
Lys Ala Pro Lys Ser Gly Thr Tyr Asp Ala Asn Ile Asn Ile Ala Asp
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Glu Met Phe Asn Asn Lys Ile Thr Tyr Asn Tyr Ser Ser Pro Ile Ala
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Gly Ile Asp Lys Pro Asn Gly Ala Asn Ile Ser Ser Gln Ile Ile Gly
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pctib2010054934-seq1

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 Asn Pro Lys Gln Arg Val Leu Gly Asn Thr Trp Val Tyr Ile Lys Gly
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 Tyr Gln Asp Lys Ile Glu Glu Ser Ser Gly Lys Val Ser Ala Thr Asp
 370 375 380
 Thr Lys Leu Arg Ile Phe Glu Val Asn Asp Thr Ser Lys Leu Ser Asp
 385 390 395 400
 Ser Tyr Tyr Ala Asp Pro Asn Asp Ser Asn Leu Lys Glu Val Thr Asp
 405 410 415
 Gln Phe Lys Asn Arg Ile Tyr Tyr Glu His Pro Asn Val Ala Ser Ile
 420 425 430
 Lys Phe Gly Asp Ile Thr Lys Thr Tyr Val Val Leu Val Glu Gly His
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 Tyr Asp Asn Thr Gly Lys Asn Leu Lys Thr Gln Val Ile Gln Glu Asn
 450 455 460
 Val Asp Pro Val Thr Asn Arg Asp Tyr Ser Ile Phe Gly Trp Asn Asn
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 <213> Staphylococcus aureus

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 35 40 45
 Val Asp Ser Thr Thr Lys Pro Met Ser Thr Gln Thr Ser Asn Thr Thr
 50 55 60
 Thr Thr Glu Pro Ala Ser Thr Asn Glu Thr Pro Gln Pro Thr Ala Ile
 65 70 75 80
 Lys Asn Gln Ala Thr Ala Ala Lys Met Gln Asp Gln Thr Val Pro Gln
 85 90 95
 Glu Ala Asn Ser Gln Val Asp Asn Lys Thr Thr Asn Asp Ala Asn Ser
 100 105 110
 Ile Ala Thr Asn Ser Glu Leu Lys Asn Ser Gln Thr Leu Asp Leu Pro
 115 120 125
 Gln Ser Ser Pro Gln Thr Ile Ser Asn Ala Gln Gly Thr Ser Lys Pro
 130 135 140
 Ser Val Arg Thr Arg Ala Val Arg Ser Leu Ala Val Ala Glu Pro Val
 145 150 155 160

pctib2010054934-seq1

Val Asn Ala Ala Asp 165 Ala Lys Gly Thr Asn 170 Val Asn Asp Lys Val 175 Thr
 Ala Ser Asn Phe 180 Lys Leu Glu Lys Thr 185 Thr Phe Asp Pro Asn 190 Gln Ser
 Gly Asn Thr 195 Phe Met Ala Ala Asn 200 Phe Thr Val Thr Asp 205 Lys Val Lys
 Ser Gly 210 Asp Tyr Phe Thr Ala 215 Lys Leu Pro Asp Ser 220 Leu Thr Gly Asn
 Gly 225 Asp Val Asp Tyr Ser 230 Asn Ser Asn Asn Thr 235 Met Pro Ile Ala Asp 240
 Ile Lys Ser Thr Asn 245 Gly Asp Val Val Ala 250 Lys Ala Thr Tyr Asp 255 Ile
 Leu Thr Lys Thr 260 Tyr Thr Phe Val Phe 265 Thr Asp Tyr Val Asn 270 Asn Lys
 Glu Asn Ile 275 Asn Gly Gln Phe Ser 280 Leu Pro Leu Phe Thr 285 Asp Arg Ala
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 Lys Leu 50 Pro Asp Ser Leu Thr 55 Gly Asn Gly Asp Val 60 Asp Tyr Ser Asn
 Ser 65 Asn Asn Thr Met 70 Pro Ile Ala Asp Ile Lys 75 Ser Thr Asn Gly Asp 80
 Val Val Ala Lys 85 Ala Thr Tyr Asp Ile Leu Thr 90 Lys Thr Tyr Thr Phe 95
 Val Phe Thr Asp 100 Tyr Val Asn Asn Lys 105 Glu Asn Ile Asn Gly Gln Phe 110
 Ser Leu 115 Pro Leu Phe Thr Asp Arg Ala Lys Ala Pro Lys 125 Ser Gly Thr
 Tyr Asp 130 Ala Asn Ile Asn Ile 135 Ala Asp Glu Met Phe 140 Asn Asn Lys Ile
 Thr 145 Tyr Asn Tyr Ser Ser 150 Pro Ile Ala Gly Ile Asp Lys Pro Asn Gly 160
 Ala Asn Ile Ser Ser Gln Ile Ile Gly Val Asp Thr Ala Ser Gly Gln

pctib2010054934-seq1

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 195 200 205
 Ser Ser Gly Lys Val Ser Ala Thr Asp Thr Lys Leu Arg Ile Phe Glu
 210 215 220
 Val Asn Asp Thr Ser Lys Leu Ser Asp Ser Tyr Tyr Ala Asp Pro Asn
 225 230 235 240
 Asp Ser Asn Leu Lys Glu Val Thr Asp Gln Phe Lys Asn Arg Ile Tyr
 245 250 255
 Tyr Glu His Pro Asn Val Ala Ser Ile Lys Phe Gly Asp Ile Thr Lys
 260 265 270
 Thr Tyr Val Val Leu Val Glu Gly His Tyr Asp Asn Thr Gly Lys Asn
 275 280 285
 Leu Lys Thr Gln Val Ile Gln Glu Asn Val Asp Pro Val Thr Asn Arg
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 35 40 45
 Val Tyr Ile Lys Gly Tyr Gln Asp Lys Ile Glu Glu Ser Ser Gly Lys
 50 55 60
 Val Ser Ala Thr Asp Thr Lys Leu Arg Ile Phe Glu Val Asn Asp Thr
 65 70 75 80
 Ser Lys Leu Ser Asp Ser Tyr Tyr Ala Asp Pro Asn Asp Ser Asn Leu
 85 90 95
 Lys Glu Val Thr Asp Gln Phe Lys Asn Arg Ile Tyr Tyr Glu His Pro
 100 105 110
 Asn Val Ala Ser Ile Lys Phe Gly Asp Ile Thr Lys Thr Tyr Val Val
 115 120 125
 Leu Val Glu Gly His Tyr Asp Asn Thr Gly Lys Asn Leu Lys Thr Gln
 130 135 140
 Val Ile Gln Glu Asn Val Asp Pro Val Thr Asn Arg Asp Tyr Ser Ile
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Asp Gly Asp Ser Ala Val Asn
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Glu Ala Lys Ala Ala Glu Asn Thr Ser Thr Glu Asn Ala Lys Gln Asp
 50 55 60

Asp Ala Thr Thr Ser Asp Asn Lys Glu Val Val Ser Glu Thr Glu Asn
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Asn Ser Thr Thr Glu Asn Asn Ser Thr Asn Pro Ile Lys Lys Glu Thr
 85 90 95

Asn Thr Asp Ser Gln Pro Glu Ala Lys Lys Glu Ser Thr Ser Ser Ser
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Thr Gln Lys Gln Gln Asn Asn Val Thr Ala Thr Thr Glu Thr Lys Pro
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Gln Asn Ile Glu Lys Glu Asn Val Lys Pro Ser Thr Asp Lys Thr Ala
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Thr Glu Asp Thr Ser Val Ile Leu Glu Glu Lys Lys Ala Pro Asn Asn
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Thr Asn Asn Asp Val Thr Thr Lys Pro Ser Thr Ser Glu Pro Ser Thr
 165 170 175

Ser Glu Ile Gln Thr Lys Pro Thr Thr Pro Gln Glu Ser Thr Asn Ile
 180 185 190

Glu Asn Ser Gln Pro Gln Pro Thr Pro Ser Lys Val Asp Asn Gln Val
 195 200 205

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

Leu Lys Asn Asn Pro Glu Lys Leu Lys Glu Leu Val Arg Asn Asp Ser
 225 230 235 240

Asn Thr Asp His Ser Thr Lys Pro Val Ala Thr Ala Pro Thr Ser Val
 245 250 255

Ala Pro Lys Arg Val Asn Ala Lys Met Arg Phe Ala Val Ala Gln Pro
 260 265 270

Ala Ala Val Ala Ser Asn Asn Val Asn Asp Leu Ile Lys Val Thr Lys
 275 280 285

Gln Thr Ile Lys Val Gly Asp Gly Lys Asp Asn Val Ala Ala Ala His
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pctib2010054934-seq1

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 Val Lys Lys Gly Asp Thr Met Thr Ile Asn Tyr Asp Lys Asn Val Ile
 325 330 335
 Pro Ser Asp Leu Thr Asp Lys Asn Asp Pro Ile Asp Ile Thr Asp Pro
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 Ser Gly Glu Val Ile Ala Lys Gly Thr Phe Asp Lys Ala Thr Lys Gln
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 Ile Thr Tyr Thr Phe Thr Asp Tyr Val Asp Lys Tyr Glu Asp Ile Lys
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 Glu Thr Ser Leu Asn Leu Thr Phe Ala Thr Ala Gly Lys Glu Thr Ser
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 Gln Asn Val Thr Val Asp Tyr Gln Asp Pro Met Val His Gly Asp Ser
 420 425 430
 Asn Ile Gln Ser Ile Phe Thr Lys Leu Asp Glu Asp Lys Gln Thr Ile
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 Glu Gln Gln Ile Tyr Val Asn Pro Leu Lys Lys Ser Ala Thr Asn Thr
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 Lys Val Asp Ile Ala Gly Ser Gln Val Asp Asp Tyr Gly Asn Ile Lys
 465 470 475 480
 Leu Gly Asn Gly Ser Thr Ile Ile Asp Gln Asn Thr Glu Ile Lys Val
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 Tyr Lys Val Asn Ser Asp Gln Gln Leu Pro Gln Ser Asn Arg Ile Tyr
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 Asp Phe Ser Gln Tyr Glu Asp Val Thr Ser Gln Phe Asp Asn Lys Lys
 515 520 525
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 Ala Tyr Ile Ile Lys Val Val Ser Lys Tyr Thr Pro Thr Ser Asp Gly
 545 550 555 560
 Glu Leu Asp Ile Ala Gln Gly Thr Ser Met Arg Thr Thr Asp Lys Tyr
 565 570 575
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 580 585 590
 Asp Thr Gly Gly Gly Asp Gly Thr Val Lys Pro Glu Glu Lys Leu Tyr
 595 600 605
 Lys Ile Gly Asp Tyr Val Trp Glu Asp Val Asp Lys Asp Gly Val Gln
 610 615 620
 Gly Thr Asp Ser Lys Glu Lys Pro Met Ala Asn Val Leu Val Thr Leu
 625 630 635 640
 Thr Tyr Pro Asp Gly Thr Thr Lys Ser Val Arg Thr Asp Ala Asn Gly
 645 650 655
 His Tyr Glu Phe Gly Gly Leu Lys Asp Gly Glu Thr Tyr Thr Val Lys

pctib2010054934-seq1

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Gly Lys Asp Asp Met Ser	Leu Asp Thr Gly Phe Tyr	Lys Glu Pro Lys
705	710	715
Tyr Asn Leu Gly Asp Tyr Val	Trp Glu Asp Thr Asn Lys	Asp Gly Ile
725	730	735
Gln Asp Ala Asn Glu Pro Gly	Ile Lys Asp Val Lys Val	Thr Leu Lys
740	745	750
Asp Ser Thr Gly Lys Val Ile	Gly Thr Thr Thr Thr	Asp Ala Ser Gly
755	760	765
Lys Tyr Lys Phe Thr Asp	Leu Asp Asn Gly Asn Tyr	Thr Val Glu Phe
770	775	780
Glu Thr Pro Ala Gly Tyr Thr	Pro Thr Val Lys Asn Thr	Thr Ala Asp
785	790	795
Asp Lys Asp Ser Asn Gly	Leu Thr Thr Thr Gly Val	Ile Lys Asp Ala
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Leu Gly Asp Tyr Val Trp Tyr	Asp Ser Asn Lys Asp Gly	Lys Gln Asp
835	840	845
Ser Thr Glu Lys Gly Ile Lys	Asp Val Thr Val Thr	Leu Gln Asn Glu
850	855	860
Lys Gly Glu Val Ile Gly Thr	Thr Lys Thr Asp Glu	Asn Gly Lys Tyr
865	870	875
Arg Phe Asp Asn Leu Asp	Ser Gly Lys Tyr Lys Val	Ile Phe Glu Lys
885	890	895
Pro Ala Gly Leu Thr Gln Thr	Val Thr Asn Thr Thr Glu	Asp Asp Lys
900	905	910
Asp Ala Asp Gly Gly Glu Val	Asp Val Thr Ile Thr Asp	His Asp Asp
915	920	925
Phe Thr Leu Asp Asn Gly Tyr	Phe Glu Glu Asp Thr Ser	Asp Ser Asp
930	935	940
Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp
945	950	955
Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp
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980	985	990
Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp
995	1000	1005
Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp Ser Asp	Ser Asp Ser Asp
1010	1015	1020

pctib2010054934-seq1

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 1075 1080 1085
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 1090 1095 1100
 Ser Asp Ala Gly Lys His Thr Pro Val Lys Pro Met Ser Thr Thr Lys
 1105 1110 1115 1120
 Asp His His Asn Lys Ala Lys Ala Leu Pro Glu Thr Gly Ser Glu Asn
 1125 1130 1135
 Asn Gly Ser Asn Asn Ala Thr Leu Phe Gly Gly Leu Phe Ala Ala Leu
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 35 40 45
 Gln Pro Glu Ala Lys Lys Glu Ser Thr Ser Ser Ser Thr Gln Lys Gln
 50 55 60
 Gln Asn Asn Val Thr Ala Thr Thr Glu Thr Lys Pro Gln Asn Ile Glu
 65 70 75 80
 Lys Glu Asn Val Lys Pro Ser Thr Asp Lys Thr Ala Thr Glu Asp Thr
 85 90 95
 Ser Val Ile Leu Glu Glu Lys Lys Ala Pro Asn Asn Thr Asn Asn Asp
 100 105 110
 Val Thr Thr Lys Pro Ser Thr Ser Glu Pro Ser Thr Ser Glu Ile Gln
 115 120 125
 Thr Lys Pro Thr Thr Pro Gln Glu Ser Thr Asn Ile Glu Asn Ser Gln
 130 135 140
 Pro Gln Pro Thr Pro Ser Lys Val Asp Asn Gln Val Thr Asp Ala Thr
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 Asn Pro Lys Glu Pro Val Asn Val Ser Lys Glu Glu Leu Lys Asn Asn
 165 170 175
 Pro Glu Lys Leu Lys Glu Leu Val Arg Asn Asp Ser Asn Thr Asp His
 180 185 190

pctib2010054934-seq1

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Val Asn Ala Lys Met Arg Phe Ala Val Ala Gln Pro Ala Ala Val Ala
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Ser Asn Asn Val Asn Asp Leu Ile Lys Val Thr Lys Gln Thr Ile Lys
225 230 235 240
Val Gly Asp Gly Lys Asp Asn Val Ala Ala Ala His Asp Gly Lys Asp
245 250 255
Ile Glu Tyr Asp Thr Glu Phe Thr Ile Asp Asn Lys Val Lys Lys Gly
260 265 270
Asp Thr Met Thr Ile Asn Tyr Asp Lys Asn Val Ile Pro Ser Asp Leu
275 280 285
Thr Asp Lys Asn Asp Pro Ile Asp Ile Thr Asp Pro Ser Gly Glu Val
290 295 300
Ile Ala Lys Gly Thr Phe Asp Lys Ala Thr Lys Gln Ile Thr Tyr Thr
305 310 315 320
Phe Thr Asp Tyr Val Asp Lys Tyr Glu Asp Ile Lys Ser Arg Leu Thr
325 330 335
Leu Tyr Ser Tyr Ile Asp Lys Lys Thr Val Pro Asn Glu Thr Ser Leu
340 345 350
Asn Leu Thr Phe Ala Thr Ala Gly Lys Glu Thr Ser Gln Asn Val Thr
355 360 365
Val Asp Tyr Gln Asp Pro Met Val His Gly Asp Ser Asn Ile Gln Ser
370 375 380
Ile Phe Thr Lys Leu Asp Glu Asp Lys Gln Thr Ile Glu Gln Gln Ile
385 390 395 400
Tyr Val Asn Pro Leu Lys Lys Ser Ala Thr Asn Thr Lys Val Asp Ile
405 410 415
Ala Gly Ser Gln Val Asp Asp Tyr Gly Asn Ile Lys Leu Gly Asn Gly
420 425 430
Ser Thr Ile Ile Asp Gln Asn Thr Glu Ile Lys Val Tyr Lys Val Asn
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Tyr Glu Asp Val Thr Ser Gln Phe Asp Asn Lys Lys Ser Phe Ser Asn
465 470 475 480
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485 490 495
Lys Val Val Ser Lys Tyr Thr Pro Thr Ser Asp Gly Glu Leu Asp Ile
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Ala Gln Gly Thr Ser Met Arg Thr Thr Asp Lys Tyr Gly Tyr Tyr Asn
515 520 525
Tyr Ala Gly Tyr Ser Asn Phe Ile Val Thr Ser Asn Asp Thr Gly Gly
530 535 540
Gly Asp Gly Thr Val Lys Pro Glu Glu Lys Leu Tyr Lys Ile Gly Asp

pctib2010054934-seq1

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Lys	Ala 50	Ala	Glu	His	Thr	Asn 55	Gly	Glu	Leu	Asn	Gln 60	Ser	Lys	Asn	Glu
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Gln	Leu	Lys	Asp	Asn 85	Thr	Gln	Thr	Ala	Thr 90	Ala	Asp	Gln	Pro	Lys 95	Val
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Asn	Ile	Asp 195	Ile	Ala	Ile	Asp	Lys 200	Gly	His	Val	Asn	Gln 205	Thr	Thr	Gly
Lys	Thr 210	Glu	Phe	Trp	Ala	Thr 215	Ser	Ser	Asp	Val	Leu 220	Lys	Leu	Lys	Ala
Asn 225	Tyr	Thr	Ile	Asp	Asp 230	Ser	Val	Lys	Glu	Gly 235	Asp	Thr	Phe	Thr	Phe 240
Lys	Tyr	Gly	Gln	Tyr 245	Phe	Arg	Pro	Gly	Ser 250	Val	Arg	Leu	Pro	Ser 255	Gln
Thr	Gln	Asn	Leu 260	Tyr	Asn	Ala	Gln	Gly 265	Asn	Ile	Ile	Ala	Lys 270	Gly	Ile
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pctib2010054934-seq1

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 Lys Arg Lys Asn Ala Thr Thr Asp Lys Thr Ala Tyr Lys Met Glu Val
 305 310 315 320
 Thr Leu Gly Asn Asp Thr Tyr Ser Glu Glu Ile Ile Val Asp Tyr Gly
 325 330 335
 Asn Lys Lys Ala Gln Pro Leu Ile Ser Ser Thr Asn Tyr Ile Asn Asn
 340 345 350
 Glu Asp Leu Ser Arg Asn Met Thr Ala Tyr Val Asn Gln Pro Lys Asn
 355 360 365
 Thr Tyr Thr Lys Gln Thr Phe Val Thr Asn Leu Thr Gly Tyr Lys Phe
 370 375 380
 Asn Pro Asn Ala Lys Asn Phe Lys Ile Tyr Glu Val Thr Asp Gln Asn
 385 390 395 400
 Gln Phe Val Asp Ser Phe Thr Pro Asp Thr Ser Lys Leu Lys Asp Val
 405 410 415
 Thr Asp Gln Phe Asp Val Ile Tyr Ser Asn Asp Asn Lys Thr Ala Thr
 420 425 430
 Val Asp Leu Met Lys Gly Gln Thr Ser Ser Asn Lys Gln Tyr Ile Ile
 435 440 445
 Gln Gln Val Ala Tyr Pro Asp Asn Ser Ser Thr Asp Asn Gly Lys Ile
 450 455 460
 Asp Tyr Thr Leu Asp Thr Asp Lys Thr Lys Tyr Ser Trp Ser Asn Ser
 465 470 475 480
 Tyr Ser Asn Val Asn Gly Ser Ser Thr Ala Asn Gly Asp Gln Lys Lys
 485 490 495
 Tyr Asn Leu Gly Asp Tyr Val Trp Glu Asp Thr Asn Lys Asp Gly Lys
 500 505 510
 Gln Asp Ala Asn Glu Lys Gly Ile Lys Gly Val Tyr Val Ile Leu Lys
 515 520 525
 Asp Ser Asn Gly Lys Glu Leu Asp Arg Thr Thr Thr Asp Glu Asn Gly
 530 535 540
 Lys Tyr Gln Phe Thr Gly Leu Ser Asn Gly Thr Tyr Ser Val Glu Phe
 545 550 555 560
 Ser Thr Pro Ala Gly Tyr Thr Pro Thr Thr Ala Asn Val Gly Thr Asp
 565 570 575
 Asp Ala Val Asp Ser Asp Gly Leu Thr Thr Thr Gly Val Ile Lys Asp
 580 585 590
 Ala Asp Asn Met Thr Leu Asp Ser Gly Phe Tyr Lys Thr Pro Lys Tyr
 595 600 605
 Ser Leu Gly Asp Tyr Val Trp Tyr Asp Ser Asn Lys Asp Gly Lys Gln
 610 615 620
 Asp Ser Thr Glu Lys Gly Ile Lys Gly Val Lys Val Thr Leu Gln Asn
 625 630 635 640
 Glu Lys Gly Glu Val Ile Gly Thr Thr Glu Thr Asp Glu Asn Gly Lys
 645 650 655

pctib2010054934-seq1

Tyr Arg Phe Asp Asn Leu Asp Ser Gly Lys Tyr Lys Val Ile Phe Glu
 660 665 670
 Lys Pro Ala Gly Leu Thr Gln Thr Gly Thr Asn Thr Thr Glu Asp Asp
 675 680 685
 Lys Asp Ala Asp Gly Gly Glu Val Asp Val Thr Ile Thr Asp His Asp
 690 695 700
 Asp Phe Thr Leu Asp Asn Gly Tyr Tyr Glu Glu Glu Thr Ser Asp Ser
 705 710 715 720
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 725 730 735
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 740 745 750
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 755 760 765
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 770 775 780
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 785 790 795 800
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 805 810 815
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 820 825 830
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 835 840 845
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 850 855 860
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 865 870 875 880
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 885 890 895
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 900 905 910
 Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 915 920 925
 Asp Ser Asp Ser Asp Ser Asp Ala Gly Lys His Thr Pro Ala Lys Pro
 930 935 940
 Met Ser Thr Val Lys Asp Gln His Lys Thr Ala Lys Ala Leu Pro Glu
 945 950 955 960
 Thr Gly Ser Glu Asn Asn Asn Ser Asn Asn Gly Thr Leu Phe Gly Gly
 965 970 975
 Leu Phe Ala Ala Leu Gly Ser Leu Leu Leu Phe Gly Arg Arg Lys Lys
 980 985 990
 Gln Asn Lys
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<210> 11

pctib2010054934-seq1

<211> 468

<212> PRT

<213> Staphylococcus aureus

<400> 11

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

pctib2010054934-seq1

Asn Ala Lys Asn Phe Lys Ile Tyr Glu Val Thr Asp Gln Asn Gln Phe
 340 345 350
 Val Asp Ser Phe Thr Pro Asp Thr Ser Lys Leu Lys Asp Val Thr Asp
 355 360 365
 Gln Phe Asp Val Ile Tyr Ser Asn Asp Asn Lys Thr Ala Thr Val Asp
 370 375 380
 Leu Met Lys Gly Gln Thr Ser Ser Asn Lys Gln Tyr Ile Ile Gln Gln
 385 390 395 400
 Val Ala Tyr Pro Asp Asn Ser Ser Thr Asp Asn Gly Lys Ile Asp Tyr
 405 410 415
 Thr Leu Asp Thr Asp Lys Thr Lys Tyr Ser Trp Ser Asn Ser Tyr Ser
 420 425 430
 Asn Val Asn Gly Ser Ser Thr Ala Asn Gly Asp Gln Lys Lys Tyr Asn
 435 440 445
 Leu Gly Asp Tyr Val Trp Glu Asp Thr Asn Lys Asp Gly Lys Gln Asp
 450 455 460

Ala Asn Glu Lys
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<210> 12
 <211> 635
 <212> PRT
 <213> Staphylococcus aureus

<400> 12
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 1 5 10 15
 Cys Ser Thr Met Met Ala Thr Ser Ile Ile Leu Thr Asn Ile Leu Pro
 20 25 30
 Tyr Asp Ala Gln Ala Ala Ser Glu Lys Asp Thr Glu Ile Thr Lys Glu
 35 40 45
 Ile Leu Ser Lys Gln Asp Leu Leu Asp Lys Val Asp Lys Ala Ile Arg
 50 55 60
 Gln Ile Glu Gln Leu Lys Gln Leu Ser Ala Ser Ser Lys Glu His Tyr
 65 70 75 80
 Lys Ala Gln Leu Asn Glu Ala Lys Thr Ala Ser Gln Ile Asp Glu Ile
 85 90 95
 Ile Lys Arg Ala Asn Glu Leu Asp Ser Lys Asp Asn Lys Ser Ser His
 100 105 110
 Thr Glu Met Asn Gly Gln Ser Asp Ile Asp Ser Lys Leu Asp Gln Leu
 115 120 125
 Leu Lys Asp Leu Asn Glu Val Ser Ser Asn Val Asp Arg Gly Gln Gln
 130 135 140
 Ser Gly Glu Asp Asp Leu Asn Ala Met Lys Asn Asp Met Ser Gln Thr
 145 150 155 160
 Ala Thr Thr Lys His Gly Glu Lys Asp Asp Lys Asn Asp Glu Ala Met
 165 170 175
 Val Asn Lys Ala Leu Glu Asp Leu Asp His Leu Asn Gln Gln Ile His
 180 185 190

pctib2010054934-seq1

Lys Ser Lys Asp Ala Ser Lys Asp Thr Ser Glu Asp Pro Ala Val Ser
195 200 205
Thr Thr Asp Asn Asn His Glu Val Ala Lys Thr Pro Asn Asn Asp Gly
210 215 220
Ser Gly His Val Val Leu Asn Lys Phe Leu Ser Asn Glu Glu Asn Gln
225 230 235 240
Ser His Ser Asn Arg Leu Thr Asp Lys Leu Gln Gly Ser Asp Lys Ile
245 250 255
Asn His Ala Met Ile Glu Lys Leu Ala Lys Ser Asn Ala Ser Thr Gln
260 265 270
His Tyr Thr Tyr His Lys Leu Asn Thr Leu Gln Ser Leu Asp Gln Arg
275 280 285
Ile Ala Asn Thr Gln Leu Pro Lys Asn Gln Lys Ser Asp Leu Met Ser
290 295 300
Glu Val Asn Lys Thr Lys Glu Arg Ile Lys Ser Gln Arg Asn Ile Ile
305 310 315 320
Leu Glu Glu Leu Ala Arg Thr Asp Asp Lys Lys Tyr Ala Thr Gln Ser
325 330 335
Ile Leu Glu Ser Ile Phe Asn Lys Asp Glu Ala Val Lys Ile Leu Lys
340 345 350
Asp Ile Arg Val Asp Gly Lys Thr Asp Gln Gln Ile Ala Asp Gln Ile
355 360 365
Thr Arg His Ile Asp Gln Leu Ser Leu Thr Thr Ser Asp Asp Leu Leu
370 375 380
Thr Ser Leu Ile Asp Gln Ser Gln Asp Lys Ser Leu Leu Ile Ser Gln
385 390 395 400
Ile Leu Gln Thr Lys Leu Gly Lys Ala Glu Ala Asp Lys Leu Ala Lys
405 410 415
Asp Trp Thr Asn Lys Gly Leu Ser Asn Arg Gln Ile Val Asp Gln Leu
420 425 430
Lys Lys His Phe Ala Ser Thr Gly Asp Thr Ser Ser Asp Asp Ile Leu
435 440 445
Lys Ala Ile Leu Asn Asn Ala Lys Asp Lys Lys Gln Ala Ile Glu Thr
450 455 460
Ile Leu Ala Thr Arg Ile Glu Arg Gln Lys Ala Lys Leu Leu Ala Asp
465 470 475 480
Leu Ile Thr Lys Ile Glu Thr Asp Gln Asn Lys Ile Phe Asn Leu Val
485 490 495
Lys Ser Ala Leu Asn Gly Lys Ala Asp Asp Leu Leu Asn Leu Gln Lys
500 505 510
Arg Leu Asn Gln Thr Lys Lys Asp Ile Asp Tyr Ile Leu Ser Pro Ile
515 520 525
Val Asn Arg Pro Ser Leu Leu Asp Arg Leu Asn Lys Asn Gly Lys Thr
530 535 540
Thr Asp Leu Asn Lys Leu Ala Asn Leu Met Asn Gln Gly Ser Asp Leu
545

pctib2010054934-seq1

545 550 555 560
 Leu Asp Ser Ile Pro Asp Ile Pro Thr Pro Lys Pro Glu Lys Thr Leu
 565 570 575
 Thr Leu Gly Lys Gly Asn Gly Leu Leu Ser Gly Leu Leu Asn Ala Asp
 580 585 590
 Gly Asn Val Ser Leu Pro Lys Ala Gly Glu Thr Ile Lys Glu His Trp
 595 600 605
 Leu Pro Ile Ser Val Ile Val Gly Ala Met Gly Val Leu Met Ile Trp
 610 615 620
 Leu Ser Arg Arg Asn Lys Leu Lys Asn Lys Ala
 625 630 635

<210> 13
 <211> 340
 <212> PRT
 <213> Staphylococcus aureus

<400> 13
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 Gln Ile Met Asn Ser Asn His Ala Lys Ala Ser Val Thr Glu Ser Val
 20 25 30
 Asp Lys Lys Phe Val Val Pro Glu Ser Gly Ile Asn Lys Ile Ile Pro
 35 40 45
 Ala Tyr Asp Glu Phe Lys Asn Ser Pro Lys Val Asn Val Ser Asn Leu
 50 55 60
 Thr Asp Asn Lys Asn Phe Val Ala Ser Glu Asp Lys Leu Asn Lys Ile
 65 70 75 80
 Ala Asp Ser Ser Ala Ala Ser Lys Ile Val Asp Lys Asn Phe Val Val
 85 90 95
 Pro Glu Ser Lys Leu Gly Asn Ile Val Pro Glu Tyr Lys Glu Ile Asn
 100 105 110
 Asn Arg Val Asn Val Ala Thr Asn Asn Pro Ala Ser Gln Gln Val Asp
 115 120 125
 Lys His Phe Val Ala Lys Gly Pro Glu Val Asn Arg Phe Ile Thr Gln
 130 135 140
 Asn Lys Val Asn His His Phe Ile Thr Thr Gln Thr His Tyr Lys Lys
 145 150 155 160
 Val Ile Thr Ser Tyr Lys Ser Thr His Val His Lys His Val Asn His
 165 170 175
 Ala Lys Asp Ser Ile Asn Lys His Phe Ile Val Lys Pro Ser Glu Ser
 180 185 190
 Pro Arg Tyr Thr His Pro Ser Gln Ser Leu Ile Ile Lys His His Phe
 195 200 205
 Ala Val Pro Gly Tyr His Ala His Lys Phe Val Thr Pro Gly His Ala
 210 215 220
 Ser Ile Lys Ile Asn His Phe Cys Val Val Pro Gln Ile Asn Ser Phe
 225 230 235 240

pctib2010054934-seq1

Lys Val Ile Pro Pro Tyr Gly His Asn Ser His Arg Met His Val Pro
245 250 255
Ser Phe Gln Asn Asn Thr Thr Ala Thr His Gln Asn Ala Lys Val Asn
260 265 270
Lys Ala Tyr Asp Tyr Lys Tyr Phe Tyr Ser Tyr Lys Val Val Lys Gly
275 280 285
Val Lys Lys Tyr Phe Ser Phe Ser Gln Ser Asn Gly Tyr Lys Ile Gly
290 295 300
Lys Pro Ser Leu Asn Ile Lys Asn Val Asn Tyr Gln Tyr Ala Val Pro
305 310 315 320
Ser Tyr Ser Pro Thr His Tyr Val Pro Glu Phe Lys Gly Ser Leu Pro
325 330 335
Ala Pro Arg Val
340

<210> 14
<211> 306
<212> PRT
<213> Staphylococcus aureus

<400> 14
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Asp Glu Phe Lys Asn Ser Pro Lys Val Asn Val Ser Asn Leu Thr Asp
20 25 30
Asn Lys Asn Phe Val Ala Ser Glu Asp Lys Leu Asn Lys Ile Ala Asp
35 40 45
Ser Ser Ala Ala Ser Lys Ile Val Asp Lys Asn Phe Val Val Pro Glu
50 55 60
Ser Lys Leu Gly Asn Ile Val Pro Glu Tyr Lys Glu Ile Asn Asn Arg
65 70 75 80
Val Asn Val Ala Thr Asn Asn Pro Ala Ser Gln Gln Val Asp Lys His
85 90 95
Phe Val Ala Lys Gly Pro Glu Val Asn Arg Phe Ile Thr Gln Asn Lys
100 105 110
Val Asn His His Phe Ile Thr Thr Gln Thr His Tyr Lys Lys Val Ile
115 120 125
Thr Ser Tyr Lys Ser Thr His Val His Lys His Val Asn His Ala Lys
130 135 140
Asp Ser Ile Asn Lys His Phe Ile Val Lys Pro Ser Glu Ser Pro Arg
145 150 155 160
Tyr Thr His Pro Ser Gln Ser Leu Ile Ile Lys His His Phe Ala Val
165 170 175
Pro Gly Tyr His Ala His Lys Phe Val Thr Pro Gly His Ala Ser Ile
180 185 190
Lys Ile Asn His Phe Cys Val Val Pro Gln Ile Asn Ser Phe Lys Val
195 200 205
Ile Pro Pro Tyr Gly His Asn Ser His Arg Met His Val Pro Ser Phe
210 215 220

pctib2010054934-seq1

Gln Asn Asn Thr Thr Ala Thr His Gln Asn Ala Lys Val Asn Lys Ala
 225 230 235 240
 Tyr Asp Tyr Lys Tyr Phe Tyr Ser Tyr Lys Val Val Lys Gly Val Lys
 245 250 255
 Lys Tyr Phe Ser Phe Ser Gln Ser Asn Gly Tyr Lys Ile Gly Lys Pro
 260 265 270
 Ser Leu Asn Ile Lys Asn Val Asn Tyr Gln Tyr Ala Val Pro Ser Tyr
 275 280 285
 Ser Pro Thr His Tyr Val Pro Glu Phe Lys Gly Ser Leu Pro Ala Pro
 290 295 300

Arg Val
 305

<210> 15
 <211> 308
 <212> PRT
 <213> Staphylococcus aureus

<400> 15
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 Ile Asn Lys Ile Ile Pro Ala Tyr Asp Glu Phe Lys Asn Ser Pro Lys
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 Val Asn Val Ser Asn Leu Thr Asp Asn Lys Asn Phe Val Ala Ser Glu
 35 40 45
 Asp Lys Leu Asn Lys Ile Ala Asp Ser Ser Ala Ala Ser Lys Ile Val
 50 55 60
 Asp Lys Asn Phe Val Val Pro Glu Ser Lys Leu Gly Asn Ile Val Pro
 65 70 75 80
 Glu Tyr Lys Glu Ile Asn Asn Arg Val Asn Val Ala Thr Asn Asn Pro
 85 90 95
 Ala Ser Gln Gln Val Asp Lys His Phe Val Ala Lys Gly Pro Glu Val
 100 105 110
 Asn Arg Phe Ile Thr Gln Asn Lys Val Asn His His Phe Ile Thr Thr
 115 120 125
 Gln Thr His Tyr Lys Lys Val Ile Thr Ser Tyr Lys Ser Thr His Val
 130 135 140
 His Lys His Val Asn His Ala Lys Asp Ser Ile Asn Lys His Phe Ile
 145 150 155 160
 Val Lys Pro Ser Glu Ser Pro Arg Tyr Thr His Pro Ser Gln Ser Leu
 165 170 175
 Ile Ile Lys His His Phe Ala Val Pro Gly Tyr His Ala His Lys Phe
 180 185 190
 Val Thr Pro Gly His Ala Ser Ile Lys Ile Asn His Phe Cys Val Val
 195 200 205
 Pro Gln Ile Asn Ser Phe Lys Val Ile Pro Pro Tyr Gly His Asn Ser
 210 215 220
 His Arg Met His Val Pro Ser Phe Gln Asn Asn Thr Thr Ala Thr His

pctib2010054934-seq1

225					230					235						240
Gln	Asn	Ala	Lys	Val 245	Asn	Lys	Ala	Tyr	Asp 250	Tyr	Lys	Tyr	Phe	Tyr 255	Ser	
Tyr	Lys	Val	Val 260	Lys	Gly	Val	Lys	Lys 265	Tyr	Phe	Ser	Phe	Ser 270	Gln	Ser	
Asn	Gly	Tyr 275	Lys	Ile	Gly	Lys	Pro 280	Ser	Leu	Asn	Ile	Lys 285	Asn	Val	Asn	
Tyr	Gln 290	Tyr	Ala	Val	Pro	Ser 295	Tyr	Ser	Pro	Thr	His 300	Tyr	Val	Pro	Glu	
Phe 305	Lys	Gly	Ser													
<210>	16															
<211>	300															
<212>	PRT															
<213>	Staphylococcus aureus															
<400>	16															
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Asp	Glu	Phe	Lys 20	Asn	Ser	Pro	Lys	Val 25	Asn	Val	Ser	Asn	Leu 30	Thr	Asp	
Asn	Lys	Asn 35	Phe	Val	Ala	Ser	Glu 40	Asp	Lys	Leu	Asn	Lys 45	Ile	Ala	Asp	
Ser	Ser 50	Ala	Ala	Ser	Lys	Ile 55	Val	Asp	Lys	Asn	Phe 60	Val	Val	Pro	Glu	
Ser 65	Lys	Leu	Gly	Asn	Ile 70	Val	Pro	Glu	Tyr	Lys 75	Glu	Ile	Asn	Asn	Arg 80	
Val	Asn	Val	Ala	Thr 85	Asn	Asn	Pro	Ala	Ser 90	Gln	Gln	Val	Asp	Lys 95	His	
Phe	Val	Ala	Lys 100	Gly	Pro	Glu	Val	Asn 105	Arg	Phe	Ile	Thr	Gln 110	Asn	Lys	
Val	Asn	His 115	His	Phe	Ile	Thr	Thr 120	Gln	Thr	His	Tyr	Lys 125	Lys	Val	Ile	
Thr	Ser 130	Tyr	Lys	Ser	Thr	His 135	Val	His	Lys	His	Val 140	Asn	His	Ala	Lys	
Asp 145	Ser	Ile	Asn	Lys	His 150	Phe	Ile	Val	Lys	Pro 155	Ser	Glu	Ser	Pro	Arg 160	
Tyr	Thr	His	Pro	Ser 165	Gln	Ser	Leu	Ile	Ile 170	Lys	His	His	Phe	Ala 175	Val	
Pro	Gly	Tyr	His 180	Ala	His	Lys	Phe	Val 185	Thr	Pro	Gly	His	Ala 190	Ser	Ile	
Lys	Ile	Asn 195	His	Phe	Cys	Val	Val 200	Pro	Gln	Ile	Asn	Ser 205	Phe	Lys	Val	
Ile	Pro 210	Pro	Tyr	Gly	His	Asn 215	Ser	His	Arg	Met	His 220	Val	Pro	Ser	Phe	
Gln 225	Asn	Asn	Thr	Thr	Ala 230	Thr	His	Gln	Asn	Ala 235	Lys	Val	Asn	Lys	Ala 240	

pctib2010054934-seq1
Tyr Asp Tyr Lys Tyr Phe Tyr Ser Tyr Lys Val Val Lys Gly Val Lys
245 250 255
Lys Tyr Phe Ser Phe Ser Gln Ser Asn Gly Tyr Lys Ile Gly Lys Pro
260 265 270
Ser Leu Asn Ile Lys Asn Val Asn Tyr Gln Tyr Ala Val Pro Ser Tyr
275 280 285
Ser Pro Thr His Tyr Val Pro Glu Phe Lys Gly Ser
290 295 300

<210> 17
<211> 121
<212> PRT
<213> Staphylococcus aureus

<400> 17
Ser Val Thr Glu Ser Val Asp Lys Lys Phe Val Val Pro Glu Ser Gly
1 5 10 15
Ile Asn Lys Ile Ile Pro Ala Tyr Asp Glu Phe Lys Asn Ser Pro Lys
20 25 30
Val Asn Val Ser Asn Leu Thr Asp Asn Lys Asn Phe Val Ala Ser Glu
35 40 45
Asp Lys Leu Asn Lys Ile Ala Asp Ser Ser Ala Ala Ser Lys Ile Val
50 55 60
Asp Lys Asn Phe Val Val Pro Glu Ser Lys Leu Gly Asn Ile Val Pro
65 70 75 80
Glu Tyr Lys Glu Ile Asn Asn Arg Val Asn Val Ala Thr Asn Asn Pro
85 90 95
Ala Ser Gln Gln Val Asp Lys His Phe Val Ala Lys Gly Pro Glu Val
100 105 110
Asn Arg Phe Ile Thr Gln Asn Lys Val
115 120

<210> 18
<211> 1349
<212> PRT
<213> Staphylococcus aureus

<400> 18
Met Leu Asn Arg Glu Asn Lys Thr Ala Ile Thr Arg Lys Gly Met Val
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Ser Asn Arg Leu Asn Lys Phe Ser Ile Arg Lys Tyr Thr Val Gly Thr
20 25 30
Ala Ser Ile Leu Val Gly Thr Thr Leu Ile Phe Gly Leu Gly Asn Gln
35 40 45
Glu Ala Lys Ala Ala Glu Ser Thr Asn Lys Glu Leu Asn Glu Ala Thr
50 55 60
Thr Ser Ala Ser Asp Asn Gln Ser Ser Asp Lys Val Asp Met Gln Gln
65 70 75 80
Leu Asn Gln Glu Asp Asn Thr Lys Asn Asp Asn Gln Lys Glu Met Val
85 90 95
Ser Ser Gln Gly Asn Glu Thr Thr Ser Asn Gly Asn Lys Leu Ile Glu
100 105 110

pctib2010054934-seq1

Lys Glu Ser Val Gln Ser Thr Thr Gly Asn Lys Val Glu Val Ser Thr
 115 120 125
 Ala Lys Ser Asp Glu Gln Ala Ser Pro Lys Ser Thr Asn Glu Asp Leu
 130 135 140
 Asn Thr Lys Gln Thr Ile Ser Asn Gln Glu Ala Leu Gln Pro Asp Leu
 145 150 155 160
 Gln Glu Asn Lys Ser Val Val Asn Val Gln Pro Thr Asn Glu Glu Asn
 165 170 175
 Lys Lys Val Asp Ala Lys Thr Glu Ser Thr Thr Leu Asn Val Lys Ser
 180 185 190
 Asp Ala Ile Lys Ser Asn Asp Glu Thr Leu Val Asp Asn Asn Ser Asn
 195 200 205
 Ser Asn Asn Glu Asn Asn Ala Asp Ile Ile Leu Pro Lys Ser Thr Ala
 210 215 220
 Pro Lys Arg Leu Asn Thr Arg Met Arg Ile Ala Ala Val Gln Pro Ser
 225 230 235 240
 Ser Thr Glu Ala Lys Asn Val Asn Asp Leu Ile Thr Ser Asn Thr Thr
 245 250 255
 Leu Thr Val Val Asp Ala Asp Lys Asn Asn Lys Ile Val Pro Ala Gln
 260 265 270
 Asp Tyr Leu Ser Leu Lys Ser Gln Ile Thr Val Asp Asp Lys Val Lys
 275 280 285
 Ser Gly Asp Tyr Phe Thr Ile Lys Tyr Ser Asp Thr Val Gln Val Tyr
 290 295 300
 Gly Leu Asn Pro Glu Asp Ile Lys Asn Ile Gly Asp Ile Lys Asp Pro
 305 310 315 320
 Asn Asn Gly Glu Thr Ile Ala Thr Ala Lys His Asp Thr Ala Asn Asn
 325 330 335
 Leu Ile Thr Tyr Thr Phe Thr Asp Tyr Val Asp Arg Phe Asn Ser Val
 340 345 350
 Gln Met Gly Ile Asn Tyr Ser Ile Tyr Met Asp Ala Asp Thr Ile Pro
 355 360 365
 Val Ser Lys Asn Asp Val Glu Phe Asn Val Thr Ile Gly Asn Thr Thr
 370 375 380
 Thr Lys Thr Thr Ala Asn Ile Gln Tyr Pro Asp Tyr Val Val Asn Glu
 385 390 395 400
 Lys Asn Ser Ile Gly Ser Ala Phe Thr Glu Thr Val Ser His Val Gly
 405 410 415
 Asn Lys Glu Asn Pro Gly Tyr Tyr Lys Gln Thr Ile Tyr Val Asn Pro
 420 425 430
 Ser Glu Asn Ser Leu Thr Asn Ala Lys Leu Lys Val Gln Ala Tyr His
 435 440 445
 Ser Ser Tyr Pro Asn Asn Ile Gly Gln Ile Asn Lys Asp Val Thr Asp
 450 455 460
 Ile Lys Ile Tyr Gln Val Pro Lys Gly Tyr Thr Leu Asn Lys Gly Tyr

pctib2010054934-seq1

465		470		475		480
Asp Val Asn Thr	Lys 485	Glu Leu Thr Asp Val	Thr Asn Gln Tyr	Leu 495	Gln	
Lys Ile Thr Tyr	500	Gly Asp Asn Asn Ser	Ala Val Ile Asp Phe	Gly 510	Asn	
Ala Asp Ser	515	Ala Tyr Val Val	Met 520	Val Asn Thr Lys	Phe 525	Gln Tyr Thr
Asn Ser	530	Glu Ser Pro Thr	Leu 535	Val Gln Met Ala	Thr 540	Leu Ser Ser Thr
Gly 545	Asn Lys Ser Val	Ser 550	Thr Gly Asn Ala	Leu 555	Gly Phe Thr	Asn 560
Gln Ser Gly Gly	Ala 565	Gly Gln Glu Val	Tyr 570	Lys Ile Gly	Asn 575	Tyr Val
Trp Glu Asp	Thr 580	Asn Lys Asn Gly	Val 585	Gln Glu Leu Gly	Glu 590	Lys Gly
Val Gly	Asn 595	Val Thr Val Thr	Val 600	Phe Asp Asn Asn	Thr 605	Asn Thr Lys
Val Gly	Glu 610	Ala Val Thr	Lys 615	Glu Asp Gly Ser	Tyr 620	Leu Ile Pro Asn
Leu 625	Pro Asn Gly Asp	Tyr 630	Arg Val Glu Phe	Ser 635	Asn Leu Pro Lys	Gly 640
Tyr Glu Val	Thr 645	Pro Ser Lys Gln	Gly 650	Asn Asn Glu Glu	Leu 655	Asp Ser
Asn Gly Leu	Ser 660	Ser Val Ile Thr	Val 665	Asn Gly Lys Asp	Asn 670	Leu Ser
Ala Asp	Leu 675	Gly Ile Tyr Lys	Pro 680	Lys Tyr Asn Leu	Gly 685	Asp Tyr Val
Trp Glu	Asp 690	Thr Asn Lys	Asn 695	Gly Ile Gln Asp	Gln 700	Asp Glu Lys Gly
Ile 705	Ser Gly Val Thr	Val 710	Thr Leu Lys Asp	Glu 715	Asn Gly Asn Val	Leu 720
Lys Thr Val	Thr 725	Thr Asp Ala Asp	Gly 730	Lys Tyr Lys Phe	Thr 735	Asp Leu
Asp Asn Gly	Asn 740	Tyr Lys Val Glu	Phe 745	Thr Thr Pro Glu	Gly 750	Tyr Thr
Pro Thr	Thr 755	Val Thr Ser Gly	Ser 760	Asp Ile Glu Lys	Asp 765	Ser Asn Gly
Leu Thr	Thr 770	Thr Thr Gly Val	Ile 775	Asn Gly Ala Asp	Asn 780	Met Thr Leu Asp
Ser 785	Gly Phe Tyr Lys	Thr 790	Pro Lys Tyr Asn	Leu 795	Gly Asn Tyr Val	Trp 800
Glu Asp Thr	Asn 805	Lys Asp Gly Lys	Gln 810	Asp Ser Thr Glu	Lys 815	Gly Ile
Ser Gly Val	Thr 820	Val Thr Leu Lys	Asn 825	Glu Asn Gly Glu	Val 830	Leu Gln

pctib2010054934-seq1

Thr Thr Lys Thr Asp Lys Asp Gly Lys Tyr Gln Phe Thr Gly Leu Glu
835 840 845

Asn Gly Thr Tyr Lys Val Glu Phe Glu Thr Pro Ser Gly Tyr Thr Pro
850 855 860

Thr Gln Val Gly Ser Gly Thr Asp Glu Gly Ile Asp Ser Asn Gly Thr
865 870 875 880

Ser Thr Thr Gly Val Ile Lys Asp Lys Asp Asn Asp Thr Ile Asp Ser
885 890 895

Gly Phe Tyr Lys Pro Thr Tyr Asn Leu Gly Asp Tyr Val Trp Glu Asp
900 905 910

Thr Asn Lys Asn Gly Val Gln Asp Lys Asp Glu Lys Gly Ile Ser Gly
915 920 925

Val Thr Val Thr Leu Lys Asp Glu Asn Asp Lys Val Leu Lys Thr Val
930 935 940

Thr Thr Asp Glu Asn Gly Lys Tyr Gln Phe Thr Asp Leu Asn Asn Gly
945 950 955 960

Thr Tyr Lys Val Glu Phe Glu Thr Pro Ser Gly Tyr Thr Pro Thr Ser
965 970 975

Val Thr Ser Gly Asn Asp Thr Glu Lys Asp Ser Asn Gly Leu Thr Thr
980 985 990

Thr Gly Val Ile Lys Asp Ala Asp Asn Met Thr Leu Asp Ser Gly Phe
995 1000 1005

Tyr Lys Thr Pro Lys Tyr Ser Leu Gly Asp Tyr Val Trp Tyr Asp Ser
1010 1015 1020

Asn Lys Asp Gly Lys Gln Asp Ser Thr Glu Lys Gly Ile Lys Asp Val
1025 1030 1035 1040

Lys Val Thr Leu Leu Asn Glu Lys Gly Glu Val Ile Gly Thr Thr Lys
1045 1050 1055

Thr Asp Glu Asn Gly Lys Tyr Cys Phe Asp Asn Leu Asp Ser Gly Lys
1060 1065 1070

Tyr Lys Val Ile Phe Glu Lys Pro Ala Gly Leu Thr Gln Thr Val Thr
1075 1080 1085

Asn Thr Thr Glu Asp Asp Lys Asp Ala Asp Gly Gly Glu Val Asp Val
1090 1095 1100

Thr Ile Thr Asp His Asp Asp Phe Thr Leu Asp Asn Gly Tyr Phe Glu
1105 1110 1115 1120

Glu Asp Thr Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1125 1130 1135

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1140 1145 1150

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1155 1160 1165

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1170 1175 1180

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1185 1190 1195 1200

pctib2010054934-seq1

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1205 1210 1215
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1220 1225 1230
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1235 1240 1245
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1250 1255 1260
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
1265 1270 1275 1280
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Gly Lys His Thr Pro Val
1285 1290 1295
Lys Pro Met Ser Thr Thr Lys Asp His His Asn Lys Ala Lys Ala Leu
1300 1305 1310
Pro Glu Thr Gly Ser Glu Asn Asn Gly Ser Asn Asn Ala Thr Leu Phe
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Lys Lys Gln Asn Lys
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35 40 45
Asn Glu Thr Thr Ser Asn Gly Asn Lys Leu Ile Glu Lys Glu Ser Val
50 55 60
Gln Ser Thr Thr Gly Asn Lys Val Glu Val Ser Thr Ala Lys Ser Asp
65 70 75 80
Glu Gln Ala Ser Pro Lys Ser Thr Asn Glu Asp Leu Asn Thr Lys Gln
85 90 95
Thr Ile Ser Asn Gln Glu Ala Leu Gln Pro Asp Leu Gln Glu Asn Lys
100 105 110
Ser Val Val Asn Val Gln Pro Thr Asn Glu Glu Asn Lys Lys Val Asp
115 120 125
Ala Lys Thr Glu Ser Thr Thr Leu Asn Val Lys Ser Asp Ala Ile Lys
130 135 140
Ser Asn Asp Glu Thr Leu Val Asp Asn Asn Ser Asn Ser Asn Asn Glu
145 150 155 160
Asn Asn Ala Asp Ile Ile Leu Pro Lys Ser Thr Ala Pro Lys Arg Leu

pctib2010054934-seq1

pct1b2010054954-Seq1															
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Asp	Ala 210	Asp	Lys	Asn	Asn	Lys 215	Ile	Val	Pro	Ala	Gln 220	Asp	Tyr	Leu	Ser
Leu 225	Lys	Ser	Gln	Ile	Thr 230	Val	Asp	Asp	Lys	Val 235	Lys	Ser	Gly	Asp	Tyr 240
Phe	Thr	Ile	Lys	Tyr 245	Ser	Asp	Thr	Val	Gln 250	Val	Tyr	Gly	Leu	Asn 255	Pro
Glu	Asp	Ile	Lys 260	Asn	Ile	Gly	Asp	Ile 265	Lys	Asp	Pro	Asn	Asn 270	Gly	Glu
Thr	Ile	Ala 275	Thr	Ala	Lys	His	Asp 280	Thr	Ala	Asn	Asn	Leu 285	Ile	Thr	Tyr
Thr	Phe 290	Thr	Asp	Tyr	Val	Asp 295	Arg	Phe	Asn	Ser	Val 300	Gln	Met	Gly	Ile
Asn 305	Tyr	Ser	Ile	Tyr	Met 310	Asp	Ala	Asp	Thr	Ile 315	Pro	Val	Ser	Lys	Asn 320
Asp	Val	Glu	Phe	Asn 325	Val	Thr	Ile	Gly	Asn 330	Thr	Thr	Thr	Lys	Thr 335	Thr
Ala	Asn	Ile	Gln 340	Tyr	Pro	Asp	Tyr	Val 345	Val	Asn	Glu	Lys	Asn 350	Ser	Ile
Gly	Ser	Ala 355	Phe	Thr	Glu	Thr	Val 360	Ser	His	Val	Gly	Asn 365	Lys	Glu	Asn
Pro	Gly 370	Tyr	Tyr	Lys	Gln	Thr 375	Ile	Tyr	Val	Asn	Pro 380	Ser	Glu	Asn	Ser
Leu 385	Thr	Asn	Ala	Lys	Leu 390	Lys	Val	Gln	Ala	Tyr 395	His	Ser	Ser	Tyr	Pro 400
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Gln	Val	Pro	Lys 420	Gly	Tyr	Thr	Leu	Asn 425	Lys	Gly	Tyr	Asp	Val 430	Asn	Thr
Lys	Glu	Leu 435	Thr	Asp	Val	Thr	Asn 440	Gln	Tyr	Leu	Gln	Lys 445	Ile	Thr	Tyr
Gly	Asp 450	Asn	Asn	Ser	Ala	Val 455	Ile	Asp	Phe	Gly	Asn 460	Ala	Asp	Ser	Ala
Tyr 465	Val	Val	Met	Val	Asn 470	Thr	Lys	Phe	Gln	Tyr 475	Thr	Asn	Ser	Glu	Ser 480
Pro	Thr	Leu	Val	Gln 485	Met	Ala	Thr	Leu	Ser 490	Ser	Thr	Gly	Asn	Lys 495	Ser
Val	Ser	Thr	Gly 500	Asn	Ala	Leu	Gly	Phe 505	Thr	Asn	Asn	Gln	Ser 510	Gly	Gly
Ala	Gly	Gln 515	Glu	Val	Tyr	Lys	Ile 520	Gly	Asn	Tyr	Val	Trp 525	Glu	Asp	Thr

pctib2010054934-seq1
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 35 40 45
 Leu Lys Val Gln Ala Tyr His Ser Ser Tyr Pro Asn Asn Ile Gly Gln
 50 55 60
 Ile Asn Lys Asp Val Thr Asp Ile Lys Ile Tyr Gln Val Pro Lys Gly
 65 70 75 80
 Tyr Thr Leu Asn Lys Gly Tyr Asp Val Asn Thr Lys Glu Leu Thr Asp
 85 90 95
 Val Thr Asn Gln Tyr Leu Gln Lys Ile Thr Tyr Gly Asp Asn Asn Ser
 100 105 110
 Ala Val Ile Asp Phe Gly Asn Ala Asp Ser Ala Tyr Val Val Met Val
 115 120 125
 Asn Thr Lys Phe Gln Tyr Thr Asn Ser Glu Ser Pro Thr Leu Val Gln
 130 135 140
 Met Ala Thr Leu Ser Ser Thr Gly Asn Lys Ser Val Ser Thr Gly Asn
 145 150 155 160
 Ala Leu Gly Phe Thr Asn Asn Gln Ser Gly Gly Ala Gly Gln Glu Val
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<400> 21
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 35 40 45
 Gln Val Leu Asn Met Pro Asn Leu Asn Ala Asp Gln Arg Asn Gly Phe
 50 55 60
 Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu Gly
 65 70 75 80

pctib2010054934-seq1

Glu Ala Gln Lys Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala Gln
 85 90 95
 Gln Asn Asn Phe Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile Leu
 100 105 110
 Asn Met Pro Asn Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser
 115 120 125
 Leu Lys Asp Asp Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys
 130 135 140
 Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys
 145 150 155 160
 Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn
 165 170 175
 Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser
 180 185 190
 Gln Ser Ala Asn Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser Gln
 195 200 205
 Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe
 210 215 220
 Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly
 225 230 235 240
 Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu
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 Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Ala Asp Asn
 260 265 270
 Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His Leu
 275 280 285
 Pro Asn Leu Thr Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys
 290 295 300
 Asp Asp Pro Ser Val Ser Lys Glu Ile Leu Ala Glu Ala Lys Lys Leu
 305 310 315 320
 Asn Asp Ala Gln Ala Pro Lys Glu Glu Asp Asn Asn Lys Pro Gly Lys
 325 330 335
 Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys
 340 345 350
 Glu Asp Asn Asn Lys Pro Gly Lys Glu Asp Asn Asn Lys Pro Gly Lys
 355 360 365
 Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp Asn Lys Lys Pro Gly Lys
 370 375 380
 Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp Asn Lys Lys Pro Gly Lys
 385 390 395 400
 Glu Asp Gly Asn Lys Pro Gly Lys Glu Asp Gly Asn Lys Pro Gly Lys
 405 410 415
 Glu Asp Gly Asn Gly Val His Val Val Lys Pro Gly Asp Thr Val Asn
 420 425 430
 Asp Ile Ala Lys Ala Asn Gly Thr Thr Ala Asp Lys Ile Ala Ala Asp
 Page 34

pctib2010054934-seq1

435 440 445
 Asn Lys Leu Ala Asp Lys Asn Met Ile Lys Pro Gly Gln Glu Leu Val
 450 455 460
 Val Asp Lys Lys Gln Pro Ala Asn His Ala Asp Ala Asn Lys Ala Gln
 465 470 475 480
 Ala Leu Pro Glu Thr Gly Glu Glu Asn Pro Phe Ile Gly Thr Thr Val
 485 490 495
 Phe Gly Gly Leu Ser Leu Ala Leu Gly Ala Ala Leu Leu Ala Gly Arg
 500 505 510
 Arg Arg Glu Leu
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 Lys Asp Asp Pro Ser Gln Ser Ala Asn Val Leu Gly Glu Ala Gln Lys
 35 40 45
 Leu Asn Asp Ser Gln Ala Pro Lys Ala Asp Ala Gln Gln Asn Asn Phe
 50 55 60
 Asn Lys Asp Gln Gln Ser Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn
 65 70 75 80
 Leu Asn Glu Ala Gln Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp
 85 90 95
 Pro Ser Gln Ser Thr Asn Val Leu Gly Glu Ala Lys Lys Leu Asn Glu
 100 105 110
 Ser Gln Ala Pro Lys Ala Asp Asn Asn Phe Asn Lys Glu Gln Gln Asn
 115 120 125
 Ala Phe Tyr Glu Ile Leu Asn Met Pro Asn Leu Asn Glu Glu Gln Arg
 130 135 140
 Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn
 145 150 155 160
 Leu Leu Ser Glu Ala Lys Lys Leu Asn Glu Ser Gln Ala Pro Lys Ala
 165 170 175
 Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu
 180 185 190
 His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly Phe Ile Gln Ser
 195 200 205
 Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala Lys
 210 215 220
 Lys Leu Asn Asp Ala Gln Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys
 225 230 235 240

pctib2010054934-seq1

Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His Leu Pro Asn Leu Thr
245 250 255

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<210> 23
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<212> PRT
<213> Staphylococcus aureus

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35 40 45

Ser Phe Ala Leu Asp Asp Ile Glu Ser Thr Ala Lys Asp Val Gln Thr
50 55 60

Ala Lys Ser Ser Ala Asn Lys Asp Ser Val Thr Val Lys Gly Lys Ala
65 70 75 80

Pro Asn Thr Leu Tyr Ile Glu Lys Arg Asn Leu Met Lys Gln Lys Leu
85 90 95

Glu Met Leu Gly Glu Asp Ile Asp Lys Asn Lys Glu Ser Leu Gln Lys
100 105 110

Ala Lys Glu Ile Ala Gly Glu Lys Ala Ser Glu Tyr Phe Asn Lys Ala
115 120 125

Met Asn
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<212> PRT
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35 40 45

Ser Arg Phe Glu Glu Gln Phe Gln Gln Leu Ser Pro Lys Val Glu Lys
50 55 60

Phe Ala Gln Leu Leu Glu Glu Ile Lys Gln Gln Leu Asn Ser Thr Ala
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Asp Ala Val Gln Glu Gln Asp Gln Gln Leu Ser Asn Asn Phe Gly Leu
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pctib2010054934-seq1

Gln

<210> 25
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 <212> PRT
 <213> Staphylococcus aureus

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 Lys Gln Thr Gln Gln Leu Ala Glu Tyr Ile Glu Gly Ser Asp Trp Glu
 35 40 45
 Gly Gln Phe Ala Asn Lys Val Lys Asp Val Leu Leu Ile Met Ala Lys
 50 55 60
 Phe Gln Glu Glu Leu Val Gln Pro Met Ala Asp His Gln Lys Ala Ile
 65 70 75 80
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 85 90 95
 Gln Gly Leu Asp Arg Val Asn Pro
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 <211> 302
 <212> PRT
 <213> Staphylococcus aureus

<400> 26
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 Tyr Lys Met Asp Asp Gly Lys Thr Val Asp Ile Pro Lys Asp Pro Lys
 35 40 45
 Arg Ile Ala Val Val Ala Pro Thr Tyr Ala Gly Gly Leu Lys Lys Leu
 50 55 60
 Gly Ala Asn Ile Val Ala Val Asn Gln Gln Val Asp Gln Ser Lys Val
 65 70 75 80
 Leu Lys Asp Lys Phe Lys Gly Val Thr Lys Ile Gly Asp Gly Asp Val
 85 90 95
 Glu Lys Val Ala Lys Glu Lys Pro Asp Leu Ile Ile Val Tyr Ser Thr
 100 105 110
 Asp Lys Asp Ile Lys Lys Tyr Gln Lys Val Ala Pro Thr Val Val Val
 115 120 125
 Asp Tyr Asn Lys His Lys Tyr Leu Glu Gln Gln Glu Met Leu Gly Lys
 130 135 140
 Ile Val Gly Lys Glu Asp Lys Val Lys Ala Trp Lys Lys Asp Trp Glu
 145 150 155 160
 Glu Thr Thr Ala Lys Asp Gly Lys Glu Ile Lys Lys Ala Ile Gly Gln
 Page 37

pctib2010054934-seq1

	165		170		175
Asp	Ala	Thr	Val	Ser	Leu
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Tyr	Gly	Asp	Asn	Trp	Gly
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Gly	Leu	Lys	Met	Gln	Pro
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Trp	Ala	Glu	Val	Lys	Gln
	225				
Ile	Val	Ser	Thr	Ser	Glu
				245	
Asn	Met	Trp	Lys	Asn	Leu
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 <212> PRT
 <213> Staphylococcus aureus

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Thr	Leu
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Lys	Leu
Met	Ser
Lys	Asp
Ile	Asp
Pro	Phe
Pro	Thr
Ser	Ala
Ser	Glu

pctib2010054934-seq1

Asn Ala Gly Thr Pro Ala Tyr Ile Tyr Ala Ile Pro Val Ala Ser Leu
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Asn Val Glu
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<211> 319
<212> PRT
<213> Staphylococcus aureus

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35 40 45

Gly Asp Leu Val Thr Tyr Asp Lys Glu Asn Gly Met His Lys Lys Val
50 55 60

Phe Tyr Ser Phe Ile Asp Asp Lys Asn His Asn Lys Lys Leu Leu Val
65 70 75 80

Ile Arg Thr Lys Gly Thr Ile Ala Gly Gln Tyr Arg Val Tyr Ser Glu
85 90 95

Glu Gly Ala Asn Lys Ser Gly Leu Ala Trp Pro Ser Ala Phe Lys Val
100 105 110

Gln Leu Gln Leu Pro Asp Asn Glu Val Ala Gln Ile Ser Asp Tyr Tyr
115 120 125

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

Gly Phe Asn Gly Asn Val Thr Gly Asp Asp Thr Gly Lys Ile Gly Gly
145 150 155 160

Leu Ile Gly Ala Asn Val Ser Ile Gly His Thr Leu Lys Tyr Val Gln
165 170 175

Pro Asp Phe Lys Thr Ile Leu Glu Ser Pro Thr Asp Lys Lys Val Gly
180 185 190

Trp Lys Val Ile Phe Asn Asn Met Val Asn Gln Asn Trp Gly Pro Tyr
195 200 205

Asp Arg Asp Ser Trp Asn Pro Val Tyr Gly Asn Gln Leu Phe Met Lys
210 215 220

Thr Arg Asn Gly Ser Met Lys Ala Ala Asp Asn Phe Leu Asp Pro Asn
225 230 235 240

Lys Ala Ser Ser Leu Leu Ser Ser Gly Phe Ser Pro Asp Phe Ala Thr
245 250 255

Val Ile Thr Met Asp Arg Lys Ala Ser Lys Gln Gln Thr Asn Ile Asp
260 265 270

Val Ile Tyr Glu Arg Val Arg Asp Asp Tyr Gln Leu His Trp Thr Ser
275 280 285

pctib2010054934-seq1

Thr Asn Trp Lys Gly Thr Asn Thr Lys Asp Lys Trp Ile Asp Arg Ser
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Ser Glu Arg Tyr Lys Ile Asp Trp Glu Lys Glu Glu Met Thr Asn
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<210> 29
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 <212> PRT
 <213> Staphylococcus aureus

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 35 40 45

Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly Gln
 50 55 60

Tyr Arg Val Tyr Ser Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala Trp
 65 70 75 80

Pro Ser Ala Phe Lys Val Gln Leu Gln Leu Pro Asp Asn Glu Val Ala
 85 90 95

Gln Ile Ser Asp Tyr Tyr Pro Arg Asn Ser Ile Asp Thr Lys Glu Tyr
 100 105 110

Met Ser Thr Leu Thr Tyr Gly Phe Asn Gly Asn Val Thr Gly Asp Asp
 115 120 125

Thr Gly Lys Ile Gly Gly Leu Ile Gly Ala Asn Val Ser Ile Gly His
 130 135 140

Thr Leu Lys Tyr Val Gln Pro Asp Phe Lys Thr Ile Leu Glu Ser Pro
 145 150 155 160

Thr Asp Lys Lys Val Gly Trp Lys Val Ile Phe Asn Asn Met Val Asn
 165 170 175

Gln Asn Trp Gly Pro Tyr Asp Arg Asp Ser Trp Asn Pro Val Tyr Gly
 180 185 190

Asn Gln Leu Phe Met Lys Thr Arg Asn Gly Ser Met Lys Ala Ala Asp
 195 200 205

Asn Phe Leu Asp Pro Asn Lys Ala Ser Ser Leu Leu Ser Ser Gly Phe
 210 215 220

Ser Pro Asp Phe Ala Thr Val Ile Thr Met Asp Arg Lys Ala Ser Lys
 225 230 235 240

Gln Gln Thr Asn Ile Asp Val Ile Tyr Glu Arg Val Arg Asp Asp Tyr
 245 250 255

Gln Leu His Trp Thr Ser Thr Asn Trp Lys Gly Thr Asn Thr Lys Asp
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 50 55 60
 Tyr Arg Val Tyr Ser Glu Glu Gly Ala Asn Lys Ser Gly Leu Ala Trp
 65 70 75 80
 Pro Ser Ala Phe Lys Val Gln Leu Gln Leu Pro Asp Asn Glu Val Ala
 85 90 95
 Gln Ile Ser Asp Tyr Tyr Pro Arg Asn Ser Ile Asp Thr Pro Ser Gly
 100 105 110
 Ser Val Gln Pro Asp Phe Lys Thr Ile Leu Glu Ser Pro Thr Asp Lys
 115 120 125
 Lys Val Gly Trp Lys Val Ile Phe Asn Asn Met Val Asn Gln Asn Trp
 130 135 140
 Gly Pro Tyr Asp Arg Asp Ser Trp Asn Pro Val Tyr Gly Asn Gln Leu
 145 150 155 160
 Phe Met Lys Thr Arg Asn Gly Ser Met Lys Ala Ala Asp Asn Phe Leu
 165 170 175
 Asp Pro Asn Lys Ala Ser Ser Leu Leu Ser Ser Gly Phe Ser Pro Asp
 180 185 190
 Phe Ala Thr Val Ile Thr Met Asp Arg Lys Ala Ser Lys Gln Gln Thr
 195 200 205
 Asn Ile Asp Val Ile Tyr Glu Arg Val Arg Asp Asp Tyr Gln Leu His
 210 215 220
 Trp Thr Ser Thr Asn Trp Lys Gly Thr Asn Thr Lys Asp Lys Trp Ile
 225 230 235 240
 Asp Arg Ser Ser Glu Arg Tyr Lys Ile Asp Trp Glu Lys Glu Glu Met
 245 250 255

Thr Asn

<210> 32
 <211> 258
 <212> PRT
 <213> Staphylococcus aureus

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

<210> 33
 <211> 50
 <212> PRT
 <213> Staphylococcus aureus

<400> 33
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pctib2010054934-seq1

Asn Thr Thr Val Lys Thr Gly Asp Leu Val Thr Tyr Asp Lys Glu Asn
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Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
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Asn Lys
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<211> 63

<212> PRT

<213> Staphylococcus aureus

<400> 34

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Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
35 40 45

Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly
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<210> 35

<211> 53

<212> PRT

<213> Staphylococcus aureus

<400> 35

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Gly Met His Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
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Asn Lys Lys Leu Leu
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<210> 36

<211> 50

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<213> Staphylococcus aureus

<400> 36

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20 25 30

Gly Met Leu Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
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Asn Lys
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<211> 63

<212> PRT

<213> Staphylococcus aureus

pctib2010054934-seq1

<400> 37
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 Asn Thr Thr Val Lys Thr Gly Asp Leu Val Thr Tyr Asp Lys Glu Asn
 20 25 30
 Gly Met Leu Lys Lys Val Phe Tyr Ser Phe Ile Asp Asp Lys Asn His
 35 40 45
 Asn Lys Lys Leu Leu Val Ile Arg Thr Lys Gly Thr Ile Ala Gly
 50 55 60

<210> 38
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 <212> PRT
 <213> Staphylococcus aureus

<400> 38
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 35 40 45
 Asn Lys Lys Leu Leu
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 <212> PRT
 <213> Staphylococcus aureus

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 Val Lys Lys Ser Phe Glu Lys Thr Leu Ser Met Tyr Pro Ile Lys Asn
 35 40 45
 Leu Glu Asp Leu Tyr Asp Lys Glu Gly Tyr Arg Asp Asp Gln Phe Asp
 50 55 60
 Lys Asn Asp Lys Gly Thr Trp Ile Ile Asn Ser Glu Met Val Ile Gln
 65 70 75 80
 Pro Asn Asn Glu Asp Met Val Ala Lys Gly Met Val Leu Tyr Met Asn
 85 90 95
 Arg Asn Thr Lys Thr Thr Asn Gly Tyr Tyr Tyr Val Asp Val Thr Lys
 100 105 110
 Asp Glu Asp Glu Gly Lys Pro His Asp Asn Glu Lys Arg Tyr Pro Val
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 Lys Met Val Asp Asn Lys Ile Ile Pro Thr Lys Glu Ile Lys Asp Glu
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pctib2010054934-seq1

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Ala Pro Lys Leu Leu Leu Lys Gly Ser Gly Asn Leu Lys Gly Ser Ser
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115 120 125
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180 185 190
Tyr Asn Val Lys Gln Leu Arg Lys Arg Tyr Asp Ile Pro Thr Ser Lys
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Ala Pro Lys Leu Leu Leu Lys Gly Ser Gly Asn Leu Lys Gly Ser Ser
210 215 220
Val Gly Tyr Lys Asp Ile Glu Phe Thr Phe Val Glu Lys Lys Glu Glu
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pctib2010054934-seq1

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Page 46

pctib2010054934-seq1

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pctib2010054934-seq1

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275 280 285
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Lys Gln Ala Ser Lys Ala Lys Glu Leu Pro Lys Thr Gly Leu Thr Ser
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Gln Thr Val Leu Asn Asn Ala Ser Phe Trp Lys Glu Tyr Lys Phe Tyr
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pctib2010054934-seq1

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65 70 75 80
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Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
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180 185 190
Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
195 200 205
Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
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Phe Ser Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
225 230 235 240
His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
Page 49

pctib2010054934-seq1

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pctib2010054934-seq1

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Lys Ala Thr Thr Glu Gln Ala Lys Ala Glu Val Lys Asn Pro Thr Gln
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100 105 110

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Glu Asp Asp Asn Ser Glu Asn Gln Ile Asn Pro Glu Leu Ile Lys Ser
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Lys Ala Ala Thr Thr Ser Ala Pro Lys Ala Lys Thr Glu Ala Thr Pro
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Gly Arg Pro Glu Gly Ile Val Val His Asp Thr Ala Asn Asp Arg Ser
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pctib2010054934-seq1

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645 pcc1b2010034934-seq1 655

Page 53

pctib2010054934-seq1

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pctib2010054934-seq1

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 Gln Gly Leu Asp Arg Val Asn Pro Ala Ser Gly Gly Gly Ser Met Ala
 100 105 110
 Met Ile Lys Met Ser Pro Glu Glu Ile Arg Ala Lys Ser Gln Ser Tyr
 115 120 125
 Gly Gln Gly Ser Asp Gln Ile Arg Gln Ile Leu Ser Asp Leu Thr Arg
 130 135 140
 Ala Gln Gly Glu Ile Ala Ala Asn Trp Glu Gly Gln Ala Phe Ser Arg

pctib2010054934-seq1