

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 February 2007 (15.02.2007)

PCT

(10) International Publication Number
WO 2007/018482 A2

(51) International Patent Classification:
A61K 39/085 (2006.01)

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(21) International Application Number:
PCT/US2005/023511

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

(22) International Filing Date: 1 July 2005 (01.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/585,698 6 July 2004 (06.07.2004) US

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

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Published:

— without international search report and to be republished
upon receipt of that report

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

(54) Title: POLYPEPTIDES FOR INDUCING A PROTECTIVE IMMUNE RESPONSE AGAINST STAPHYLOCOCCUS AU-
REUS

NDTPKDETKSTESNTNQDTNTTKDVIALKDVKTSPEDAVKKAEEYKQGKLGISFENSNGEWAYKVTQQKSCEE
SEVLVADKNKKVINKKTEKEDTMNENDNFKYSDAIDYKKAIEGQKEFDGDIKEWSLEKDDGKLVYNIDLKKGKNG
KQEVTVDAKNGKVLKSEQDH

(57) Abstract: The present invention features polypeptides comprising an amino acid sequence structurally related to SEQ ID NO: 1 and uses of such polypeptides. SEQ ID NO: 1 is a truncated derivative of a full-length *S. aureus* polypeptide. The full-length naturally occurring polypeptide is referred to herein as full-length "ORF2331". A His-tagged derivative of SEQ ID NO: 1 was found to produce a protective immune response against *S. aureus*.



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TITLE OF THE INVENTION
POLYPEPTIDES FOR INDUCING A PROTECTIVE IMMUNE RESPONSE AGAINST
STAPHYLOCOCCUS AUREUS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Application No. 60/585,698, filed July 6, 2004 hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 The references cited throughout the present application are not admitted to be prior art to the claimed invention.

Staphylococcus aureus is a pathogen responsible for a wide range of diseases and conditions. Examples of diseases and conditions caused by *S. aureus* include bacteremia, infective endocarditis, folliculitis, furuncle, carbuncle, impetigo, bullous impetigo, cellulitis, botryomycosis, toxic
15 shock syndrome, scalded skin syndrome, central nervous system infections, infective and inflammatory eye disease, osteomyelitis and other infections of joints and bones, and respiratory tract infections. (*The Staphylococci in Human Disease*, Crossley and Archer (eds.), Churchill Livingstone Inc. 1997.)

Immunological based strategies can be employed to control *S. aureus* infections and the spread of *S. aureus*. Immunological based strategies include passive and active immunization. Passive
20 immunization employs immunoglobulins targeting *S. aureus*. Active immunization induces immune responses against *S. aureus*.

Potential *S. aureus* vaccines target *S. aureus* polysaccharides and polypeptides. Targeting can be achieved using suitable *S. aureus* polysaccharides or polypeptides as vaccine components. Examples of polysaccharides that may be employed as possible vaccine components
25 include *S. aureus* type 5 and type 8 capsular polysaccharides. (*Shinefield et al., N. Eng. J. Med.* 346:491-496, 2002.) Examples of polypeptides that may be employed as possible vaccine components include collagen adhesin, fibrinogen binding proteins, and clumping factor. (Mamo *et al., FEMS Immunology and Medical Microbiology* 10:47-54, 1994, Nilsson *et al., J. Clin. Invest.* 101:2640-2649, 1998, Josefsson *et al., The Journal of Infectious Diseases* 184:1572-1580, 2001.)

30 Information concerning *S. aureus* polypeptide sequences has been obtained from sequencing the *S. aureus* genome. (Kuroda *et al., Lancet* 357:1225-1240, 2001, Baba *et al., Lancet* 359:1819-1827, 2000, Kunsch *et al., European Patent Publication* EP 0 786 519, published July 30, 1997.) To some extent bioinformatics has been employed in efforts to characterize polypeptide sequences obtained from genome sequencing. (Kunsch *et al., European Patent Publication* EP 0 786 519,
35 published July 30, 1997.)

Techniques such as those involving display technology and sera from infected patients can be used in an effort to identify genes coding for potential antigens. (Foster *et al.*, International Publication Number WO 01/98499, published December 27, 2001, Meinke *et al.*, International Publication Number WO 02/059148, published August 1, 2002, Etz *et al.*, *PNAS* 99:6573-6578, 2002.)

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SUMMARY OF THE INVENTION

The present invention features polypeptides comprising an amino acid sequence structurally related to SEQ ID NO: 1 and uses of such polypeptides. SEQ ID NO: 1 is a truncated derivative of a full-length *S. aureus* polypeptide. The full-length naturally occurring polypeptide is referred to herein as full-length "ORF2331". A His-tagged derivative of SEQ ID NO: 1 was found to produce a protective immune response against *S. aureus*.

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Reference to "protective" immunity or immune response indicates a detectable level of protection against *S. aureus* infection. The level of protection can be assessed using animal models such as those described herein.

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Thus, a first aspect of the present invention describes a polypeptide immunogen comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1, wherein the polypeptide does not contain an amino terminus provided by amino acids 1-20 of SEQ ID NO: 3 and the polypeptide provides protective immunity against *S. aureus*. SEQ ID NO: 3 provides a full length ORF2331 polypeptide, wherein amino acids 1-20 provide the amino terminus domain containing a signal sequence.

20

Reference to comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1 indicates that a SEQ ID NO: 1 related region is present and additional polypeptide regions may be present. Reference to the polypeptide not containing an amino terminus provided by amino acids 1-20 of SEQ ID NO: 3, indicates that if additional polypeptide regions are present the polypeptide does not have an amino terminus provided by amino acids 1-20 of SEQ ID NO: 3.

25

Percent identity (also referred to as percent identical) to a reference sequence is determined by aligning the polypeptide sequence with the reference sequence and determining the number of identical amino acids in the corresponding regions. This number is divided by the total number of amino acids in the reference sequence (*e.g.*, SEQ ID NO: 1) and then multiplied by 100 and rounded to the nearest whole number.

30

Another aspect of the present invention describes an immunogen comprising an amino acid sequence that provides protective immunity against *S. aureus* and one or more additional regions or moieties covalently joined to the amino acid sequence at the carboxyl terminus or amino terminus, wherein each region or moiety is independently selected from a region or moiety having at least one of the following properties: enhances the immune response, facilitates purification, or facilitates polypeptide stability.

35

Reference to “additional region or moiety” indicates a region or moiety different from a ORF2331 region. The additional region or moiety can be, for example, an additional polypeptide region or a non-peptide region.

5 Another aspect of the present invention describes a composition able to induce protective immunity against *S. aureus* in a patient. The composition comprises a pharmaceutically acceptable carrier and an immunologically effective amount of an immunogen that provides protective immunity against *S. aureus*.

10 An immunologically effective amount is an amount sufficient to provide protective immunity against *S. aureus* infection. The amount should be sufficient to significantly prevent the likelihood or severity of a *S. aureus* infection.

Another aspect of the present invention describes a nucleic acid comprising a recombinant gene encoding a polypeptide that provides protective immunity against *S. aureus*. A recombinant gene contains recombinant nucleic acid encoding a polypeptide along with regulatory elements for proper transcription and processing (which may include translational and post translational elements). The recombinant gene can exist independent of a host genome or can be part of a host genome.

20 A recombinant nucleic acid is nucleic acid that by virtue of its sequence and/or form does not occur in nature. Examples of recombinant nucleic acid include purified nucleic acid, two or more nucleic acid regions combined together that provides a different nucleic acid than found in nature, and the absence of one or more nucleic acid regions (*e.g.*, upstream or downstream regions) that are naturally associated with each other.

Another aspect of the present invention describes a recombinant cell. The cell comprises a recombinant gene encoding a polypeptide that provides protective immunity against *S. aureus*.

25 Another aspect of the present invention describes a method of making a polypeptide that provides protective immunity against *S. aureus*. The method involves growing a recombinant cell containing recombinant nucleic acid encoding the polypeptide and purifying the polypeptide.

Another aspect of the present invention describes a polypeptide that provides protective immunity against *S. aureus* made by a process comprising the steps of growing a recombinant cell containing recombinant nucleic acid encoding the polypeptide in a host and purifying the polypeptide.

30 Different host cells can be employed.

Another aspect of the present invention describes a method of inducing a protective immune response in a patient against *S. aureus*. The method comprises the step of administering to the patient an immunologically effective amount of an immunogen that provides protective immunity against *S. aureus*.

Unless particular terms are mutually exclusive, reference to “or” indicates either or both possibilities. Occasionally phrases such as “and/or” are used to highlight either or both possibilities.

Reference to open-ended terms such as “comprises” allows for additional elements or steps. Occasionally phrases such as “one or more” are used with or without open-ended terms to highlight the possibility of additional elements or steps.

Unless explicitly stated reference to terms such as “a” or “an” is not limited to one. For example, “a cell” does not exclude “cells”. Occasionally phrases such as one or more are used to highlight the presence of a plurality.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the amino acid sequence of SEQ ID NO: 1.

Figure 2 illustrate a sequence comparison between SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5. SEQ ID NO: 2 is a His-tagged construct of SEQ ID NO: 1. The SEQ ID NO: 1 region is shown underlined. SEQ ID NOs: 3, 4 and 5 are different ORF2331 sequences. Some amino acid differences between the sequences are shown in bold.

Figure 3 illustrates a nucleic acid sequence SEQ ID NO: 6 encoding SEQ ID NO: 2. The region encoding SEQ ID NO: 1 is shown in bold.

Figure 4 illustrate survival data using a SEQ ID NO: 2 polypeptide in aluminum hydroxyphosphate adjuvant (AHP). The polypeptide is referred to as “SEQ 2”.

DETAILED DESCRIPTION OF THE INVENTION

The ability of SEQ ID NO: 1 related polypeptides to provide protective immunity is illustrated in the Examples provided below using SEQ ID NO: 2. SEQ ID NO: 2 is a His-Tag derivative of SEQ ID NO: 1. The His-Tag facilitates polypeptide purification and identification.

SEQ ID NO: 1 is a derivative of a full-length *S. aureus* polypeptide designated full-length ORF2331 (SEQ ID NO: 3). SEQ ID NO: 1 was obtained from ORF2331 by removing the first 20 amino acids of SEQ ID NO: 3. The first 20 amino acids contain the signal sequence.

Polypeptides structurally related to SEQ ID NO: 1 include polypeptides containing corresponding regions present in different *S. aureus* strains and derivatives of naturally occurring regions. The amino acid sequence of SEQ ID NO: 1 is illustrated in Figure 1.

ORF2331 Sequences

ORF2331 related sequences have been given different designations in different references. Examples of different designations are provided in Kuroda *et al.*, *Lancet* 357:1225-1240, 2001 (SAV0361 and SA0359); and Baba *et al.*, *Lancet* 359:1819-1827, 2002 (MW0227).

A polypeptide sequence corresponding to an ORF2331 related sequence appears to be provided in different patent publications. (Meinke *et al.*, International Publication Number WO 02/059148, published August 1, 2002, and Massignani *et al.*, International Publication Number WO 02/094868, published November 28, 2002.)

Other naturally occurring ORF2331 sequences can be identified based on the presence of a high degree of sequence similarity or contiguous amino acids compared to a known ORF2331 sequence. Contiguous amino acids provide characteristic tags. In different embodiments, a naturally occurring ORF2331 sequence is a sequence found in a *Staphylococcus*, preferably *S. aureus*, having at least 20, at least 30, or at least 50 contiguous amino acids as in SEQ ID NO: 1; and/or having at least 85% sequence similarity or identity with SEQ ID NO: 1.

Sequence similarity can be determined by different algorithms and techniques well known in the art. Generally, sequence similarity is determined by techniques aligning two sequences to obtain maximum amino acid identity, allowing for gaps, additions and substitutions in one of the sequences.

Sequence similarity can be determined, for example, using a local alignment tool utilizing the program lalign (developed by Huang and Miller, *Adv. Appl. Math.* 12:337-357, 1991, for the «sim» program). The options and environment variables are: -f # Penalty for the first residue a gap (-14 by default); -g # Penalty for each additional residue in a gap (-4 by default) -s str (SMATRIX) the filename of an alternative scoring matrix file. For protein sequences, PAM250 is used by default -w # (LINLEN) output line length for sequence alignments (60).

SEQ ID NO: 1 Related Polypeptides

SEQ ID NO: 1 related polypeptides contain an amino acid sequence at least 85% identical to SEQ ID NO: 1. Reference to "polypeptide" does not provide a minimum or maximum size limitation.

A polypeptide at least 85% identical to SEQ ID NO: 1 contains up to about 25 amino acid alterations from SEQ ID NO: 1. In different embodiments, the SEQ ID NO: 1 related polypeptide is at 90%, at least 94%, or at least 99% identical to SEQ ID NO: 1; differs from SEQ ID NO: 1 by 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid alterations; or consists essentially of SEQ ID NO: 1. Each amino acid alteration is independently either a deletion, substitution, or addition.

Reference to “consists essentially” of indicated amino acids indicates that the referred to amino acids are present and additional amino acids may be present. The additional amino acids can be at the carboxyl or amino terminus. In different embodiments 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 additional amino acids are present. A preferred additional amino acid is an amino terminus methionine.

Alterations can be made to SEQ ID NO: 1 to obtain derivatives that can induce protective immunity against *S. aureus*. Alterations can be performed, for example, to obtain a derivative retaining the ability to induce protective immunity against *S. aureus* or to obtain a derivative that in addition to providing protective immunity also has a region that can achieve a particular purpose.

The sequence comparison provided in Figure 2 can be used to guide the design of potential alterations to SEQ ID NO: 1. In addition, alterations can be made taking into account other ORF2331 sequences and known properties of amino acids.

Generally, in substituting different amino acids to retain activity it is preferable to exchange amino acids having similar properties. Factors that can be taken into account for an amino acid substitution include amino acid size, charge, polarity, and hydrophobicity. The effect of different amino acid R-groups on amino acid properties are well known in the art. (See, for example, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-2002, Appendix 1C.)

In exchanging amino acids to maintain activity, the replacement amino acid should have one or more similar properties such as approximately the same charge and/or size and/or polarity and/or hydrophobicity. For example, substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

Alterations to achieve a particular purpose include those designed to facilitate production or efficacy of the polypeptide; or cloning of the encoded nucleic acid. Polypeptide production can be facilitated through the use of an initiation codon (*e.g.*, coding for methionine) suitable for recombinant expression. The methionine may be later removed during cellular processing. Cloning can be facilitated by, for example, the introduction of restriction sites which can be accompanied by amino acid additions or changes.

Efficacy of a polypeptide to induce an immune response can be enhanced through epitope enhancement. Epitope enhancement can be performed using different techniques such as those involving alteration of anchor residues to improve peptide affinity for MHC molecules and those increasing affinity of the peptide-MHC complex for a T-cell receptor. (Berzofsky *et al.*, *Nature Review* 1:209-219, 2001.)

Preferably, the polypeptide is a purified polypeptide. A “purified polypeptide” is present in an environment lacking one or more other polypeptides with which it is naturally associated and/or is represented by at least about 10% of the total protein present. In different embodiments, the purified

polypeptide represents at least about 50%, at least about 75%, or at least about 95% of the total protein in a sample or preparation.

In an embodiment, the polypeptide is “substantially purified.” A substantially purified polypeptide is present in an environment lacking all, or most, other polypeptides with which the polypeptide is naturally associated. For example, a substantially purified *S. aureus* polypeptide is present in an environment lacking all, or most, other *S. aureus* polypeptides. An environment can be, for example, a sample or preparation.

Reference to “purified” or “substantially purified” does not require a polypeptide to undergo any purification and may include, for example, a chemically synthesized polypeptide that has not been purified.

Polypeptide stability can be enhanced by modifying the polypeptide carboxyl or amino terminus. Examples of possible modifications include amino terminus protecting groups such as acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or *t*-butyloxycarbonyl; and carboxyl terminus protecting groups such as amide, methylamide, and ethylamide.

In an embodiment of the present invention the polypeptide immunogen is part of an immunogen containing one or more additional regions or moieties covalently joined to the polypeptide at the carboxyl terminus or amino terminus, where each region or moiety is independently selected from a region or moiety having at least one of the following properties: enhances the immune response, facilitates purification, or facilitates polypeptide stability. Polypeptide stability can be enhanced, for example, using groups such as polyethylene glycol that may be present on the amino or carboxyl terminus.

Polypeptide purification can be enhanced by adding a group to the carboxyl or amino terminus to facilitate purification. Examples of groups that can be used to facilitate purification include polypeptides providing affinity tags. Examples of affinity tags include a six-histidine tag, trpE, glutathione and maltose-binding protein.

The ability of a polypeptide to produce an immune response can be enhanced using groups that generally enhance an immune response. Examples of groups that can be joined to a polypeptide to enhance an immune response against the polypeptide include cytokines such as IL-2. (Buchan *et al.*, 2000. *Molecular Immunology* 37:545-552.)

Polypeptide Production

Polypeptides can be produced using standard techniques including those involving chemical synthesis and those involving purification from a cell producing the polypeptide. Techniques for chemical synthesis of polypeptides are well known in the art. (See *e.g.*, Vincent, *Peptide and Protein Drug Delivery*, New York, N.Y., Decker, 1990.) Techniques for recombinant polypeptide production

and purification are also well known in the art. (See for example, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-2002.)

Obtaining polypeptides from a cell is facilitated using recombinant nucleic acid techniques to produce the polypeptide. Recombinant nucleic acid techniques for producing a polypeptide involve introducing, or producing, a recombinant gene encoding the polypeptide in a cell and expressing the polypeptide.

A recombinant gene contains nucleic acid encoding a polypeptide along with regulatory elements for polypeptide expression. The recombinant gene can be present in a cellular genome or can be part of an expression vector.

The regulatory elements that may be present as part of a recombinant gene include those naturally associated with the polypeptide encoding sequence and exogenous regulatory elements not naturally associated with the polypeptide encoding sequence. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing a recombinant gene in a particular host or increasing the level of expression. Generally, the regulatory elements that are present in a recombinant gene include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. A preferred element for processing in eukaryotic cells is a polyadenylation signal.

Expression of a recombinant gene in a cell is facilitated through the use of an expression vector. Preferably, an expression vector in addition to a recombinant gene also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

Due to the degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be used to code for a particular polypeptide. The degeneracy of the genetic code arises because almost all amino acids are encoded by different combinations of nucleotide triplets or "codons". Amino acids are encoded by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU

C=Cys=Cysteine: codons UGC, UGU

D=Asp=Aspartic acid: codons GAC, GAU

E=Glu=Glutamic acid: codons GAA, GAG

F=Phe=Phenylalanine: codons UUC, UUU

G=Gly=Glycine: codons GGA, GGC, GGG, GGU

H=His=Histidine: codons CAC, CAU

I=Ile=Isoleucine: codons AUA, AUC, AUU

K=Lys=Lysine: codons AAA, AAG

L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU

M=Met=Methionine: codon AUG

N=Asn=Asparagine: codons AAC, AAU

P=Pro=Proline: codons CCA, CCC, CCG, CCU

Q=Gln=Glutamine: codons CAA, CAG

5 R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

10 Y=Tyr=Tyrosine: codons UAC, UAU

Suitable cells for recombinant nucleic acid expression of SEQ ID NO: 1 related polypeptides are prokaryotes and eukaryotes. Examples of prokaryotic cells include *E. coli*; members of the *Staphylococcus* genus, such as *S. aureus*; members of the *Lactobacillus* genus, such as *L. plantarum*; members of the *Lactococcus* genus, such as *L. lactis*; and members of the *Bacillus* genus, such as *B. subtilis*. Examples of eukaryotic cells include mammalian cells; insect cells; yeast cells such as members of the *Saccharomyces* genus (*e.g.*, *S. cerevisiae*), members of the *Pichia* genus (*e.g.*, *P. pastoris*), members of the *Hansenula* genus (*e.g.*, *H. polymorpha*), members of the *Kluyveromyces* genus (*e.g.*, *K. lactis* or *K. fragilis*) and members of the *Schizosaccharomyces* genus (*e.g.*, *S. pombe*).

Techniques for recombinant gene production, introduction into a cell, and recombinant gene expression are well known in the art. Examples of such techniques are provided in references such as Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-2002, and Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

If desired, expression in a particular host can be enhanced through codon optimization. Codon optimization includes use of more preferred codons. Techniques for codon optimization in different hosts are well known in the art.

SEQ ID NO: 1 related polypeptides may contain post translational modifications, for example, N-linked glycosylation, O-linked glycosylation, or acetylation. Reference to "polypeptide" or an "amino acid" sequence of a polypeptide includes polypeptides containing one or more amino acids having a structure of a post-translational modification from a host cell, such as a yeast host.

Post translational modifications can be produced chemically or by making use of suitable hosts. For example, in *S. cerevisiae* the nature of the penultimate amino acid appears to determine whether the N-terminal methionine is removed. Furthermore, the nature of the penultimate amino acid also determines whether the N-terminal amino acid is N^α-acetylated (Huang *et al.*, *Biochemistry* 26: 8242-8246, 1987). Another example includes a polypeptide targeted for secretion due to the presence of

a secretory leader (e.g., signal peptide), where the protein is modified by N-linked or O-linked glycosylation. (Kukuruzinska *et al.*, *Ann. Rev. Biochem.* 56:915-944, 1987.)

Adjuvants

Adjuvants are substances that can assist an immunogen in producing an immune response. Adjuvants can function by different mechanisms such as one or more of the following: increasing the antigen biologic or immunologic half-life; improving antigen delivery to antigen-presenting cells; improving antigen processing and presentation by antigen-presenting cells; and inducing production of immunomodulatory cytokines. (Vogel, *Clinical Infectious Diseases* 30(suppl. 3):S266-270, 2000.)

A variety of different types of adjuvants can be employed to assist in the production of an immune response. Examples of particular adjuvants include aluminum hydroxide, aluminum phosphate, or other salts of aluminum, calcium phosphate, DNA CpG motifs, monophosphoryl lipid A, cholera toxin, *E. coli* heat-labile toxin, pertussis toxin, muramyl dipeptide, Freund's incomplete adjuvant, MF59, SAF, immunostimulatory complexes, liposomes, biodegradable microspheres, saponins, nonionic block copolymers, muramyl peptide analogues, polyphosphazene, synthetic polynucleotides, IFN- γ , IL-2 and IL-12. (Vogel *Clinical Infectious Diseases* 30(suppl 3):S266-270, 2000, Klein *et al.*, *Journal of Pharmaceutical Sciences* 89:311-321, 2000.)

Patients For Inducing Protective Immunity

A "patient" refers to a mammal capable of being infected with *S. aureus*. A patient can be treated prophylactically or therapeutically. Prophylactic treatment provides sufficient protective immunity to reduce the likelihood, or severity, of a *S. aureus* infection. Therapeutic treatment can be performed to reduce the severity of a *S. aureus* infection.

Prophylactic treatment can be performed using a vaccine containing an immunogen described herein. Such treatment is preferably performed on a human. Vaccines can be administered to the general population or to those persons at an increased risk of *S. aureus* infection.

Persons with an increased risk of *S. aureus* infection include health care workers; hospital patients; patients with a weakened immune system; patients undergoing surgery; patients receiving foreign body implants, such a catheter or a vascular device; patients facing therapy leading to a weakened immunity; and persons in professions having an increased risk of burn or wound injury. (*The Staphylococci in Human Disease*, Crossley and Archer (ed.), Churchill Livingstone Inc. 1997.)

Non-human patients that can be infected with *S. aureus* include cows, pigs, sheep, goats, rabbits, horses, dogs, cats and mice. Treatment of non-human patients is useful in protecting pets and livestock, and in evaluating the efficacy of a particular treatment.

Combination Vaccines

SEQ ID NO: 1 related polypeptides can be used alone, or in combination with other immunogens, to induce an immune response. Additional immunogens that may be present include: one or more additional *S. aureus* immunogens, such as those referenced in the Background of the Invention *supra*; one or more immunogens targeting one or more other *Staphylococcus* organisms such as *S. epidermidis*, *S. haemolyticus*, *S. warneri*, or *S. lugunensis*; and one or more immunogens targeting other infections organisms.

Animal Model System

An animal model system was used to evaluate the efficacy of an immunogen to produce a protective immune response against *S. aureus*. The animal model was a slow kinetics lethality model involving *S. aureus* prepared from cells in stationary phase, appropriately titrated, and intravenously administered. This slow kinetics of death provides sufficient time for the specific immune defense to fight off the bacterial infection (*e.g.*, 10 days rather 24 hours).

S. aureus cells in stationary phase can be obtained from cells grown on solid medium. They can also be obtained from liquid, however the results with cells grown on solid media were more reproducible. Cells can conveniently be grown overnight on solid medium. For example, *S. aureus* can be grown from about 18 to about 24 hours under conditions where the doubling time is about 20-30 minutes.

S. aureus can be isolated from solid or liquid medium using standard techniques to maintain *S. aureus* potency. Isolated *S. aureus* can be stored, for example, at -70°C as a washed high density suspension ($> 10^9$ colony forming units (CFU)/mL) in phosphate buffered saline containing glycerol.

The *S. aureus* challenge should have a potency providing about 80 to 90% death in an animal model over a period of about 7 to 10 days starting on the first or second day. Titration experiments can be performed using animal models to monitor the potency of the stored *Staphylococcus* inoculum. The titration experiments can be performed about one to two weeks prior to an inoculation experiment.

Initial potency for titration experiments can be based on previous experiments. For *S. aureus* and the animal model strain Becker a suitable potency was generally found in the range of 5×10^8 to 8×10^8 CFU/ml.

Administration

Immunogens can be formulated and administered to a patient using the guidance provided herein along with techniques well known in the art. Guidelines for pharmaceutical

administration in general are provided in, for example, *Vaccines* Eds. Plotkin and Orenstein, W.B. Sanders Company, 1999; *Remington's Pharmaceutical Sciences 20th Edition*, Ed. Gennaro, Mack Publishing, 2000; and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990, each of which are hereby incorporated by reference herein.

5 Pharmaceutically acceptable carriers facilitate storage and administration of an immunogen to a patient. Pharmaceutically acceptable carriers may contain different components such as a buffer, sterile water for injection, normal saline or phosphate buffered saline, sucrose, histidine, salts and polysorbate.

10 Immunogens can be administered by different routes such as subcutaneous, intramuscular, or mucosal. Subcutaneous and intramuscular administration can be performed using, for example, needles or jet-injectors.

Suitable dosing regimens are preferably determined taking into account factors well known in the art including age, weight, sex and medical condition of the patient; the route of administration; the desired effect; and the particular compound employed. The immunogen can be used
15 in multi-dose vaccine formats. It is expected that a dose would consist of the range of 1.0 µg to 1.0 mg total polypeptide. In different embodiments of the present invention the range is 0.01 mg to 1.0 mg and 0.1 mg to 1.0 mg.

The timing of doses depends upon factors well known in the art. After the initial administration one or more booster doses may subsequently be administered to maintain or boost
20 antibody titers. An example of a dosing regime would be day 1, 1 month, a third dose at either 4, 6 or 12 months, and additional booster doses at distant times as needed.

Generation of Antibodies

A SEQ ID NO: 1 related polypeptide can be used to generate antibodies and antibody
25 fragments that bind to the polypeptide or to *S. aureus*. Such antibodies and antibody fragments have different uses including use in polypeptide purification, *S. aureus* identification, or in therapeutic or prophylactic treatment against *S. aureus* infection.

Antibodies can be polyclonal or monoclonal. Techniques for producing and using antibodies are well known in the art. Examples of such techniques are described in Ausubel, *Current*
30 *Protocols in Molecular Biology*, John Wiley, 1987-2002, Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, and Kohler *et al.*, *Nature* 256:495-497, 1975.

EXAMPLES

Examples are provided below further illustrating different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1: Protective Immunity

This example illustrates the ability of SEQ ID NO: 1 related polypeptides to provide protective immunity in an animal model. SEQ ID NO: 2, a His-tagged derivative of SEQ ID NO: 1, was used to provide protective immunity.

SEQ ID NO: 2 Cloning and Expression

The protein was designed to be expressed from the pET16 vector with the His-Tag residues encoded by the vector. The vector provides the initiating Met codon followed by a Gly codon, the 10 His-Tag, and an additional 12 codons for Ser Ser Gly His Ile Glu Gly Arg His Met Leu Glu. (Amino acids 1-24 of SEQ ID NO: 2.) The designed DNA sequence encodes a 194 amino acid altered form of mature ORF2331.

The ORF2331 DNA sequence was translated using Vector NTI software and the resulting amino acid sequence (SEQ ID NO: 1) was analyzed. PCR primers were designed to amplify the gene starting at the 21st residue (Asn) to remove a signal sequence and membrane binding site and ending prior to the stop codon at the terminal His residue. The forward PCR primers contained an XhoI restriction site to facilitate cloning into the expression vector. The reverse PCR primer included a BlnI restriction site to facilitate cloning into the expression vector and a stop codon.

PCR amplified sequences were digested with XhoI and BlnI then ligated into the pET16b vector (Novagen) digested with XhoI and BlnI. The ligation reaction was transformed into *E. coli* strain NovaBlue and colonies selected by growth in LB with 100 µg/mL ampicillin. DNA minipreps were made (Qiagen), and insert integrity determined by restriction digestion and sequence confirmation. A clone was selected containing no DNA changes from the desired sequence.

E. coli BLR(DE3) cells (Novagen) were transformed and grown on LB plates containing ampicillin (100 µg/ml). Liquid LB (ampicillin) cultures were set up by inoculating with single colonies from the LB (ampicillin) plates and incubated at 37°C, 250 rpm overnight. The overnight culture was diluted back into fresh LB (ampicillin), grown until the A₆₀₀ was between 0.6 and 1.0, an un-induced cell pellet harvested and the rest of the culture induced by the addition of IPTG to final concentrations of 1 mM and allowed to express for three hours. Cell pellets were harvested from 1 ml culture by centrifugation at 5000 x g for 3 minutes at room temperature. Cell pellets were resuspended in 200 µl lysis buffer (Bugbuster, with protease inhibitors, Novagen), then soluble and insoluble fractions of the

induced samples were obtained. An equal volume of loading buffer (supplemented with β -mecapto ethanol to 5% final volume) was added prior to heating the samples at 90°C for 3 minutes. Extracts were run on Novex 4-20% Tris-Glycine gels and assayed for protein (Coomassie Blue stained) and blotted onto nitrocellulose and probed with anti-HIS6 antibodies (Zymed).

5

SEQ ID NO: 2 Purification

Frozen recombinant *E. coli* cell paste (48 grams) was thawed and resuspended in four volumes of Lysis Buffer (50 mM sodium phosphate, pH 8.0, 0.15 M NaCl, 2 mM magnesium chloride, 10 mM imidazole, 0.1% Tween-80, and 0.02% sodium azide. Benzonase (EM #1.01697.0002) was added to the cell suspension at 250 Units/mL), and protease inhibitor cocktail was added to the cell suspension at one tablet per 15 ml (Complete™, EDTA-Free, Roche # 1873580).

A lysate was prepared with a microfluidizer. The Lysate was stirred overnight at 4°C, and was clarified by centrifugation at 10,800 x g for 30 minutes at 4°C. The supernatant was filtered through a glass-fiber pre-filter (Millipore) and NaCl was added to a final concentration of 0.5 M from a 5 M stock solution. The Filtered Supernatant was added to Ni-NTA agarose chromatography resin (Qiagen #30250) and the slurry was mixed overnight at 4°C.

The slurry of chromatography resin was poured into a chromatography column and the non-bound fraction was collected by gravity from the column outlet. The column was washed with five column volumes of Wash Buffer (50 mM sodium phosphate, pH 8.0, 0.3 M NaCl, 0.1% Tween-80, and 20 mM imidazole). The column was eluted with Elution Buffer (50 mM sodium phosphate, pH 8.0, 0.3 M NaCl, 0.1% Tween-80, and 0.3 M imidazole).

Fractions containing protein were identified by dot blot on nitrocellulose membrane with Ponceau-S staining, and fractions containing the highest protein concentrations were pooled to make the Ni-IMAC Product. The Ni-IMAC Product was fractionated by SEC. SEC fractions containing the product protein were identified by SDS/PAGE with Coomassie staining. Product-containing SEC fractions were pooled to make the SEC Product. The SEC Product was filtered with a CUNO Zeta-Plus BioCap filter to remove endotoxin. The Filtrate was sterile-filtered and adsorbed on aluminum hydroxyphosphate adjuvant at a final concentration of 0.2 mg/ml.

Preparation of *S. aureus* Challenge

S. aureus was grown on TSA plates at 37°C overnight. The bacteria were washed from the TSA plates by adding 5 ml of PBS onto a plate and gently resuspending the bacteria with a sterile spreader. The bacterial suspension was spun at 6000 rpm for 20 minutes using a Sorvall RC-5B centrifuge (DuPont Instruments). The pellet was resuspended in 16% glycerol and aliquots were stored frozen at -70°C.

Prior to use, inocula were thawed, appropriately diluted and used for infection. Each stock was titrated at least 3 times to determine the appropriate dose inducing slow kinetics of death in naive mice. The potency of the bacterial inoculum (80 to 90% lethality) was constantly monitored to assure reproducibility of the model. Ten days before each challenge experiment, a group of 10 control
5 animals (immunized with adjuvant alone) were challenged and monitored.

Protection Studies for a SEQ ID NO: 2 Polypeptide

Twenty BALB/c mice were immunized with three doses of a SEQ ID NO: 2 polypeptide (20 µg per dose) on aluminum hydroxyphosphate adjuvant (450 µg per dose). Aluminum
10 hydroxyphosphate adjuvant (AHP) is described by Klein *et al.*, *Journal of Pharmaceutical Sciences* 89, 311-321, 2000. 30 mice were vaccinated with three doses of AHP alone as an adjuvant control group. The doses were administered as two 50 µl intramuscular injections on days 0, 7 and 21. The mice were bled on day 28, and their sera were screened by ELSIA for reactivity to immunogen ORF2331.

On day 35 of the experiment the mice were challenged with *S. aureus* (7×10^8 CFU/ml)
15 and evaluated against the control set of 30 mice that had just been immunized with AHP. The mice were monitored over an 11 day period for survival. At the end of the experiment 8 of 20 (40%) mice survived the SEQ ID NO: 2 immunized group compared to 6 of 30 (20%) surviving in the AHP control group. The results are illustrated in Figure 4.

20 Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. A polypeptide immunogen comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1, wherein said polypeptide provides protective immunity against *S. aureus*,
5 provided that if one or more additional polypeptide regions are present said additional regions do not provide an amino terminus containing amino acids 1-20 of SEQ ID NO: 3.

2. The polypeptide of claim 1, wherein said amino acid sequence is at least 95% identical to SEQ ID NO: 1.
10

3. The polypeptide of claim 2, wherein said amino acid sequence consists essentially of SEQ ID NO: 1.

4. The polypeptide of claim 3, wherein said polypeptide consists of the amino acid
15 sequence of SEQ ID NO: 1, SEQ ID NO: 1 modified with an amino terminus methionine addition, or SEQ ID NO: 1 modified with an amino terminus methionine-glutamic acid addition.

5. An immunogen comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1, and one or more additional regions or moieties covalently joined to said amino acid
20 sequence at the carboxyl terminus or amino terminus, wherein each region or moiety is independently selected from a region or moiety having at least one of the following properties: enhances the immune response, facilitates purification, or facilitates polypeptide stability.

6. A composition able to induce a protective immune response in a patient
25 comprising an immunologically effective amount of an immunogen comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1 and a pharmaceutically acceptable carrier.

7. The composition of claim 6, wherein said composition further comprises an adjuvant.
30

8. A nucleic acid comprising a recombinant gene comprising a nucleotide sequence encoding the polypeptide of any one of claims 1-4.

9. The nucleic acid of claim 8, wherein said nucleic acid is an expression vector.
35

10. A recombinant cell comprising a recombinant gene comprising a nucleotide sequence encoding the polypeptide of any one of claims 1-4.

11. A method of making a *S. aureus* polypeptide that provides protective immunity comprising the steps of:

(a) growing the recombinant cell of claim 10 under conditions wherein said polypeptide is expressed; and

(b) purifying said polypeptide.

12. A method of inducing a protective immune response in a patient comprising the step of administering to said patient an immunologically effective amount of an immunogen comprising an amino acid sequence at least 85% identical to SEQ ID NO: 1.

13. The method of claim 12, wherein said immunogen is the immunogen of any one of claims 1-5.

14. The method of claim 12, wherein said patient is a human.

15. The method of claim 14, wherein said patient is treated prophylactically against *S. aureus* infection.

16. A method of inducing a protective immune response in a patient comprising the step of administering to said patient an immunologically effective amount of a polypeptide made by the method of claim 11.

1/2

NDTPKDETKSTESNTNQDTNTTKDVIALKDVKTSPE DAVKKAEEETYKGQKLKGISFENSNGEWAYKVTQQKSGEE
SEVLVADKNKKVINKKTEKEDTMNENDNFKYSDAIDYKKAKEGQKEFDGDIKEWSLEKDDGKLVYNIDLKKGK
KQEVTVDAKNGKVLKSEQDH

FIG. 1

SEQ ID NO: 5	MKLKSGGGGGLAVLSMSAVVLTACGNDTPKDETKSTESNTNQDTNTTKDVIALKDVKTSPE
SEQ ID NO: 4	MKLKSGGGGGLAVLSMSAVVLTACGNDTPKDETKSTESNTNQDTNTTKDVIALKDVKTSPE
SEQ ID NO: 3	MKLKSGGGGGLAVLSMSAVVLTACGNDTPKDETKSTESNTNQDTNTTKDVIALKDVKTSPE
SEQ ID NO: 2	MGLKSGGGGGLAVLSMSAVVLTACGNDTPKDETKSTESNTNQDTNTTKDVIALKDVKTSPE
SEQ ID NO: 5	DAVKKAEETYKGQKLKGISFENSNGEWAYKVTQQKSGEESVVLVADKNKKVINKKTEKED
SEQ ID NO: 4	DAVKKAEETYKGQKLKGISFENSNGEWAYKVTQQKSGEESVVLVADKNKKVINKKTEKED
SEQ ID NO: 3	DAVKKAEETYKGQKLKGISFENSNGEWAYKVTQQKSGEESVVLVADKNKKVINKKTEKED
SEQ ID NO: 2	DAVKKAEETYKGQKLKGISFENSNGEWAYKVTQQKSGEESVVLVADKNKKVINKKTEKED
SEQ ID NO: 5	TMNENDNFKYSDAIDYKKAKEGQKEFDGDIKEWSLEKDDGKLVYNIDLKKGKQEVTV
SEQ ID NO: 4	TMNENDNFKYSDAIDYKKAKEGQKEFDGDIKEWSLEKDDGKLVYNIDLKKGKQEVTV
SEQ ID NO: 3	TMNENDNFKYSDAIDYKKAKEGQKEFDGDIKEWSLEKDDGKLVYNIDLKKGKQEVTV
SEQ ID NO: 2	TMNENDNFKYSDAIDYKKAKEGQKEFDGDIKEWSLEKDDGKLVYNIDLKKGKQEVTV
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SEQ ID NO: 4	DAKNGKVLKSEQDH
SEQ ID NO: 3	DAKNGKVLKSEQDH
SEQ ID NO: 2	DAKNGKVLKSEQDH

FIG. 2

ATGGGCCATCATCATCATCATCATCATCACAGCAGCGGCCATATCGAAGGTCGTCATATGCTCGAGAAT
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FIG. 3

2/2

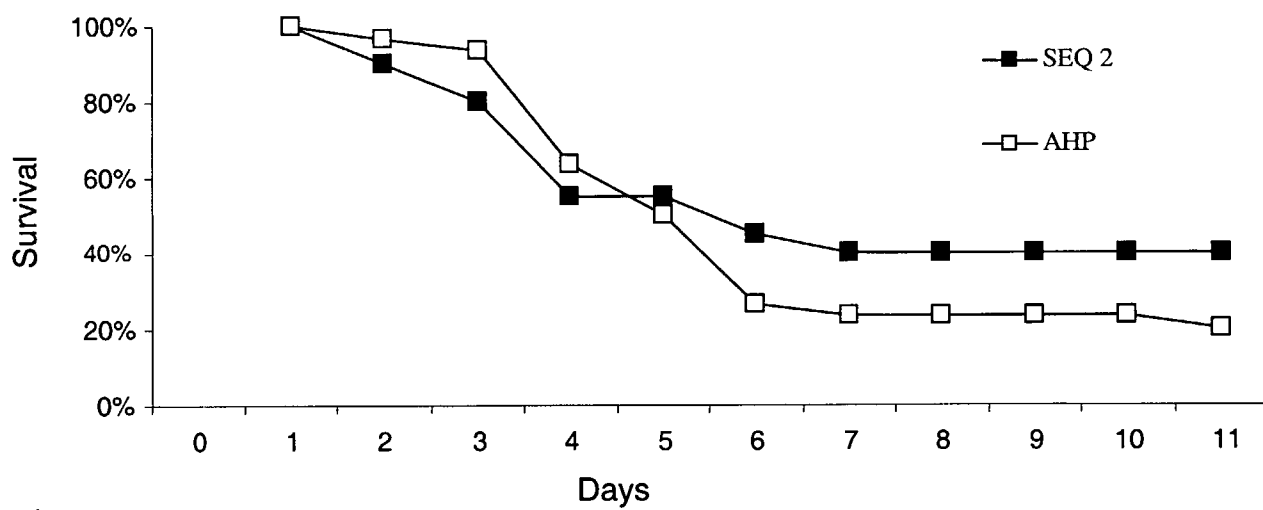


FIG. 4

SEQUENCE LISTING

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IMMUNE RESPONSE AGAINST STAPHYLOCOCCUS AUREUS

<130> 21667 PCT

<150> 60/585,698

<151> 2004-07-06

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			20					25					30		
Thr	Ser	Pro	Glu	Asp	Ala	Val	Lys	Lys	Ala	Glu	Glu	Thr	Tyr	Lys	Gly
		35					40					45			
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			85						90					95	
Thr	Met	Asn	Glu	Asn	Asp	Asn	Phe	Lys	Tyr	Ser	Asp	Ala	Ile	Asp	Tyr
		100						105					110		
Lys	Lys	Ala	Ile	Lys	Glu	Gly	Gln	Lys	Glu	Phe	Asp	Gly	Asp	Ile	Lys
		115					120					125			
Glu	Trp	Ser	Leu	Glu	Lys	Asp	Asp	Gly	Lys	Leu	Val	Tyr	Asn	Ile	Asp
	130					135					140				
Leu	Lys	Lys	Gly	Asn	Lys	Lys	Gln	Glu	Val	Thr	Val	Asp	Ala	Lys	Asn
145					150					155					160
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      35           40           45
Val Ile Ala Leu Lys Asp Val Lys Thr Ser Pro Glu Asp Ala Val Lys
      50           55           60
Lys Ala Glu Glu Thr Tyr Lys Gly Gln Lys Leu Lys Gly Ile Ser Phe
65           70           75           80
Glu Asn Ser Asn Gly Glu Trp Ala Tyr Lys Val Thr Gln Gln Lys Ser
      85           90           95
Gly Glu Glu Ser Glu Val Leu Val Ala Asp Lys Asn Lys Lys Val Ile
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Asn Lys Lys Thr Glu Lys Glu Asp Thr Met Asn Glu Asn Asp Asn Phe
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Gly Lys Leu Val Tyr Asn Ile Asp Leu Lys Lys Gly Asn Lys Lys Gln
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      35           40           45
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65           70           75           80
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      85           90           95
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Glu Lys Glu Asp Thr Met Asn Glu Asn Asp Asn Phe Lys Tyr Ser Asp
      115          120          125
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 Glu Lys Glu Asp Thr Val Asn Glu Asn Asp Asn Phe Lys Tyr Ser Asp
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 Gly Asp Ile Lys Glu Trp Ser Leu Glu Lys Asp Asp Gly Lys Leu Val
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 50 55 60
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 85 90 95

Glu Val Leu Val Ala Asp Lys Asn Lys Lys Val Ile Asn Lys Lys Thr
 100 105 110
 Glu Lys Glu Asp Thr Val Asn Glu Asn Asp Asn Phe Lys Tyr Ser Asp
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 130 135 140
 Gly Asp Ile Lys Glu Trp Ser Leu Glu Lys Asp Asp Gly Lys Leu Val
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