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- (71) Applicant (for all designated States except US): PHARMACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): PEARSON, James, D. [US/US]; 3004 East Shore Drive, Portage, MI 49002 (US). SLIGHTOM, Jerry, L. [US/US]; 3305 Lorraine Avenue, Kalamazoo, MI 49008 (US). CHOSAY, John, G. [US/US]; 3580 Buckskin, Kalamazoo, MI 49004 (US). SHINABARGER, Dean, L. [US/US]; 10810 Cora Drive, Portage, MI 49002 (US).
- (74) Agent: REHBERG, Edward, F.; Intellectual Property Legal Services, Pharmacia & Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49001 (US).
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(54) Title: COMPLETE NUCLEOTIDE SEQUENCE OF *STAPHYLOCOCCUS AUREUS* RIBOSOMAL PROTEIN GENE, S16 AND METHODS FOR THE IDENTIFICATION OF ANITBACTERIAL SUBSTANCES

(57) Abstract: The invention provides an isolated *S. aureus* ribosomal polypeptide S16, and the isolated polynucleotide molecules that encode them, vectors and host cells comprising such polynucleotide molecules and also methods for the identification of agents that effect ribosomal assembly.

**Complete Nucleotide Sequence of *Staphylococcus aureus* Ribosomal Protein Gene, S16 and Methods for the Identification of Antibacterial Substances**

**CROSS REFERENCE TO RELATED APPLICATIONS**

5           The present application claims priority of Application Serial Number 60/219360 filed 19 July 2000 which is hereby incorporated by reference.

**FIELD OF THE INVENTION**

          The present invention provides an isolated *S. aureus* ribosomal polypeptide S16, and the isolated polynucleotide molecules that encode them, as well as vectors  
10   and host cells comprising such polynucleotide molecules. The invention also provides a method for the identification of agents that effect ribosomal assembly.

**BACKGROUND**

          The staphylococci, of which *Staphylococcus aureus* is the most important human pathogen, are hardy, gram-positive bacteria that colonize the skin of most  
15   humans. Staphylococcal strains that produce coagulase are designated *S. aureus* other clinically important coagulase-negative staphylococci are *S. epidermidis* and *S. saprophyticus*. When the skin or mucous membrane barriers are disrupted, staphylococci can cause localized and superficial infections that are commonly harmless and self-limiting. However, when staphylococci invade the lymphatics and  
20   the blood, potentially serious complications may result, such as bacteremia, septic shock, and serous metastatic infections, including endocarditis, arthritis, osteomyelitis, pneumonia and abscesses in virtually any organ. Certain strains of *S. aureus* produce toxins that cause skin rashes, food poisoning, or multisystem dysfunction (as in toxic shock syndrome). *S. aureus* and *S. epidermidis* together have  
25   become the most common cause of nosocomial non-urinary tract infection in U.S. hospitals. They are the most frequently isolated pathogens in both primary and secondary bacteremias and in cutaneous and surgical wound infections. See generally *Harrison's Principles of Internal Medicine*, 13<sup>th</sup> ed., Isselbacher et. al. eds. McGraw-Hill, New York (1994), particularly pages 611-617.

30           Transient colonization of the nose by *S. aureus* is seen in 70-90 percent of people, of which 20 to 30 percent carry the bacteria for relatively prolonged periods of time. Independent colonization of the perineal area occurs in 5-20 percent of people. Higher carriage rates of *S. aureus* have been documented in persons with atopic

dermatitis, hospital employees, hospitalized patients, patients whose care requires frequent puncture of the skin, and intravenous drug abusers.

Infection by staphylococci usually results from a combination of bacterial virulence factors and a diminution in host defenses. Important microbial factors include the ability of the staphylococcus to survive under harsh conditions, its cell wall constituents, the production of enzymes and toxins that promote tissue invasion, its capacity to persist intracellularly in certain phagocytes, and its potential to acquire resistance to antimicrobials. Important host factors include an intact mucocutaneous barrier, and adequate number of functional neutrophils, and removal of foreign bodies or dead tissue.

Once the skin or mucosa have been breached, local bacterial multiplication is accompanied by inflammation, neutrophil accumulation, tissue necrosis, thrombosis and fibrin deposition at the site of infection. Later, fibroblasts create a relatively avascular wall about the area. When host mechanisms fail to contain the cutaneous or submucosal infection, staphylococci may enter the lymphatics and the bloodstream. Common sites of metastatic spread include the lungs, kidneys, cardiac valves, myocardium, liver, spleen, bone and brain.

Antimicrobial resistance by staphylococci favors their persistence in the hospital environment. Over 90 percent of both hospital and community strains of *S. aureus* causing infection are resistant to penicillin. This resistance is due to the production of  $\beta$  lactamase enzymes. The genes for these enzymes are usually carried by plasmids. Infections due to organisms with such acquired resistance can sometimes be treated with  $\beta$  lactamase resistant penicillin derivatives. However the true penicillinase-resistant *S. aureus* organisms, called methicillin resistant *S. aureus* (MRSA), are resistant to all the  $\beta$  lactam antibiotics and the cephalosporins. MRSA resistance is chromosomally mediated and involves production of an altered penicillin-binding protein (PBP 2a or PBP 2') with a low binding for  $\beta$  lactams. MRSA frequently also have acquired plasmids mediating resistance to erythromycin, tetracycline, chloramphenicol, clindamycin, and aminoglycosides. MRSA have become increasingly common worldwide, particularly in tertiary-care referral hospitals. In the United States, approximately 32 percent of hospital isolates of *S. aureus* are methicillin resistant. Methicillin resistant staphylococci are a serious clinical and economic problem, since treatment of these infections often requires

vancomycin, an antibiotic that is more difficult to administer and more expensive than the penicillins. Quinolone antimicrobial agents have been used to treat methicillin-resistant staphylococcal infections. Unfortunately, resistance to these antibiotics has also developed rapidly. Sixty to 70% of methicillin resistant *S. aureus* isolates are  
5 also quinolone resistant.

A pressing need exists for new chemical entities that are effective in the treatment of staphylococcal infections. One fruitful area of research has been in the area of agents which inhibit protein synthesis. A large number of antibacterial agents, including many in current clinical use, inhibit protein synthesis in bacteria by  
10 interfering with essential functions of the ribosome. When ribosomal function is perturbed, protein synthesis may cease entirely or, alternatively, it may be sufficiently slowed so as to stop normal cell growth and metabolism. Differences between the prokaryotic 70S ribosomes (composed of 50S and 30S subunits) and the eukaryotic 80S ribosome (composed of 60S and 40S subunits) underlie the basis for the selective  
15 toxicity of many antimicrobial agents of this class. However, a limited subset of this class of antimicrobial agents exhibits some cross-reactivity with the 70S ribosomes of eukaryotic mitochondria. This cross-reactivity probably accounts for the host cells cytotoxicity effects observed with some agents and has limited their use as clinical antimicrobial agents. Other agents (e.g., tetracycline), which affect the function of  
20 eukaryotic 80S ribosomes in vitro, are still used clinically to treat bacterial infections as the concentrations employed during antimicrobial therapy are not sufficient to elicit host cell toxicity side-effects.

Moreover, protein biosynthesis inhibitors can be divided into a number of different classes based on differences in their mechanisms of action. The  
25 aminoglycoside agents (e.g., streptomycin) bind irreversibly to the 30S subunit of the ribosome, thereby slowing protein synthesis and causing mis-translation (i.e., mis-reading) of the mRNA. The resulting errors in the fidelity of protein synthesis are bacteriocidal, and the selective toxicity of this family of agents is increased by the fact that bacteria actively transport them into the cell.  
30 The tetracycline family of agents (e.g., doxycycline) also binds to the 30S ribosome subunit, but does so reversibly. Such agents are bacteriostatic and act by interfering with the elongation phase of protein synthesis by inhibiting the transfer of the amino acid moieties of the aminoacyl-tRNA substrates into the

growing polypeptide chain. However, inhibition mediated by the tetracyclines is readily reversible, with protein synthesis resuming once intracellular levels of the agent's decline. Chloramphenicol and the macrolide family of agents (e.g., erythromycin), in contrast, act on the function/activity of the 50S subunit of the ribosome. These agents are bacteriostatic in nature, and their effects are reversible. It has also been suggested that both chloramphenicol and the macrolides may have a second mode of action involved in ribosomal assembly. Champney and Burdine (1995). Finally, puromycin acts as a competitive inhibitor of the binding of aminoacyl-tRNA's to the so-called aminoacyl site (i.e., A-site) of the ribosome and acts as a chain-terminator of the elongation phase as a result of its incorporation into the growing peptide chain.

S16 is encoded by the *rpsP* gene in *E. coli*. Byström *et al.* 1983. It has been shown that S16 is required for efficient assembly of 30S ribosomal subunits but does not play a role in the functional activities of the assembled 30S subunit Held and Nomura (1975). Recently it has been shown that S16 is essential in *E. coli*. Persson *et al.* (1995). Essential genes in bacteria are attractive agents for antimicrobial agents.

This document discloses important new methods of identifying antibacterial substances related to the bacterial ribosomal assembly process, and to the *Staphylococcal* ribosomal protein S16 and it for the first time discloses the full nucleotide and amino acid sequence of *Staphylococcus aureus* S16 ribosomal polypeptide

#### Information Disclosure

U.S. Patent No. 3,940,475  
U.S. Patent No. 5,843,669  
U.S. Patent No. 6,083,924  
WO 97/09433, *Cell-Cycle Checkpoint Genes*

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#### Brief Description of the Sequence Listings

- 10 SEQ ID NO:1 Complete coding sequence of S16 ribosomal polypeptide  
 SEQ ID NO:2 Predicted polypeptide sequence of S16 ribosomal polypeptide  
 SEQ ID NO:3 Sequencing Primer  
 SEQ ID NO:4 Sequencing Primer  
 SEQ ID NO:5 Sequencing Primer
- 15 SEQ ID NO:6 Sequencing Primer  
 SEQ ID NO:7 Sequencing Primer  
 SEQ ID NO:8 PCR Primer  
 SEQ ID NO:9 PCR Primer  
 SEQ ID NO:10 DNA sequence for *Staphylococcus aureus* S4 ribosomal protein gene  
 20 (coding and flanking sequences)  
 SEQ ID NO:11 Polypeptide sequence for *Staphylococcus aureus* S4 ribosomal protein  
 SEQ ID NO:12 DNA sequence for *Staphylococcus aureus* S7 ribosomal protein gene  
 (coding and flanking sequences)  
 SEQ ID NO:13 Polypeptide sequence for *Staphylococcus aureus* S7 ribosomal protein
- 25 SEQ ID NO:14 DNA sequence for *Staphylococcus aureus* S8 ribosomal protein gene  
 (coding and flanking sequences)  
 SEQ ID NO:15 Polypeptide sequence for *Staphylococcus aureus* S8 ribosomal protein  
 SEQ ID NO:16 DNA sequence for *Staphylococcus aureus* S15 ribosomal protein gene  
 (coding and flanking sequences)
- 30 SEQ ID NO:17 Polypeptide sequence for *Staphylococcus aureus* S15 ribosomal  
 protein  
 SEQ ID NO:18 DNA sequence for *Staphylococcus aureus* S17 ribosomal protein gene  
 (coding and flanking sequences)  
 SEQ ID NO:19 Polypeptide sequence for *Staphylococcus aureus* S17 ribosomal  
 35 protein

- SEQ ID NO:20 DNA sequence for *Staphylococcus aureus* 16S ribosomal RNA gene  
(coding and flanking sequences)
- SEQ ID NO:21 DNA sequence for *Staphylococcus aureus* S1 ribosomal protein gene  
(coding and flanking sequences)
- 5 SEQ ID NO:22 Polypeptide sequence for *Staphylococcus aureus* S1 ribosomal protein  
gene
- SEQ ID NO:23 DNA sequence for *Staphylococcus aureus* S2 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:24 Polypeptide sequence for *Staphylococcus aureus* S2 ribosomal protein
- 10 SEQ ID NO:25 DNA sequence for *Staphylococcus aureus* S3 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:26 Polypeptide sequence for *Staphylococcus aureus* S3 ribosomal protein
- SEQ ID NO:27 DNA sequence for *Staphylococcus aureus* S5 ribosomal protein gene  
(coding and flanking sequences)
- 15 SEQ ID NO:28 Polypeptide sequence for *Staphylococcus aureus* S5 ribosomal protein
- SEQ ID NO:29 DNA sequence for *Staphylococcus aureus* S6 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:30 Polypeptide sequence for *Staphylococcus aureus* S6 ribosomal protein
- SEQ ID NO:31 DNA sequence for *Staphylococcus aureus* S9 ribosomal protein gene
- 20 (coding and flanking sequences)
- SEQ ID NO:32 Polypeptide sequence for *Staphylococcus aureus* S9 ribosomal protein
- SEQ ID NO:33 DNA sequence for *Staphylococcus aureus* S10 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:34 Polypeptide sequence for *Staphylococcus aureus* S10 ribosomal
- 25 protein
- SEQ ID NO:35 DNA sequence for *Staphylococcus aureus* S11 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:36 Polypeptide sequence for *Staphylococcus aureus* S11 ribosomal  
protein
- 30 SEQ ID NO:37 DNA sequence for *Staphylococcus aureus* S12 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:38 Polypeptide sequence for *Staphylococcus aureus* S12 ribosomal  
protein

- SEQ ID NO:39 DNA sequence for *Staphylococcus aureus* S13 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:40 Polypeptide sequence for *Staphylococcus aureus* S13 ribosomal  
protein
- 5 SEQ ID NO:41 DNA sequence for *Staphylococcus aureus* S14 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:42 Polypeptide sequence for *Staphylococcus aureus* S14 ribosomal  
protein
- SEQ ID NO:43 DNA sequence for *Staphylococcus aureus* S16 ribosomal protein gene  
10 (coding and flanking sequences)
- SEQ ID NO:44 Polypeptide sequence for *Staphylococcus aureus* S16 ribosomal  
protein
- SEQ ID NO:45 DNA sequence for *Staphylococcus aureus* S18 ribosomal protein gene  
(coding and flanking sequences)
- 15 SEQ ID NO:46 Polypeptide sequence for *Staphylococcus aureus* S18 ribosomal  
protein
- SEQ ID NO:47 DNA sequence for *Staphylococcus aureus* S19 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:48 Polypeptide sequence for *Staphylococcus aureus* S19 ribosomal  
20 protein
- SEQ ID NO:49 DNA sequence for *Staphylococcus aureus* S20 ribosomal polypeptide  
gene (coding and flanking sequences)
- SEQ ID NO:50 Polypeptide sequence for *Staphylococcus aureus* S20 ribosomal  
protein
- 25 SEQ ID NO:51 DNA sequence for *Staphylococcus aureus* S21 ribosomal protein gene  
(coding and flanking sequences)
- SEQ ID NO:52 Polypeptide sequence for *Staphylococcus aureus* S21 ribosomal  
protein
- SEQ ID NO:53 Exemplary S4 Forward PCR Primer
- 30 SEQ ID NO:54 Exemplary S4 Reverse PCR Primer
- SEQ ID NO:55 Exemplary S18 Forward PCR Primer
- SEQ ID NO:56 Exemplary S18 Reverse PCR Primer
- SEQ ID NO:57 Exemplary S6 Forward PCR Primer

SEQ ID NO:58 Exemplary S6 Reverse PCR Primer

### Brief Description of the Figures

Figure 1- DNA Coding Region and Amino Acid Sequence of the S16 ribosomal polypeptide

- 5 Figure 2 Graphic illustration of a simplified ribosomal assembly map incorporating direct binding S proteins (S4, S8, S7, S17, and S20) as well as S16. Arrows between proteins indicate the effect of a protein on another whose binding it enhances. Thick arrows indicate a principal contribution. Thin arrows indicate lesser contribution.

Based on Noller and Nomura (1987)

- 10 Figure 3 Graphic illustration of a ribosomal assembly map incorporating direct binding S proteins (S4, S8, S7, S17, and S20) as well as some proteins which integrate themselves into ribosomes by reliance on protein-protein interactions (non-direct binding proteins) (S3, S5, S9, S10, S12, S14, S16 and S19). Arrows between proteins indicate the effect of a protein on another whose binding it enhances. Thick arrows  
15 indicate a principal contribution. Thin arrows indicate lesser contribution. Noller and Nomura (1987)

- Figure 4 Graphical illustration of a ribosomal assembly assay incorporating direct binding S proteins (S4, S8, S7, S17, and S20) as well as proteins which integrate themselves into ribosomes by reliance on protein-protein interactions “non direct  
20 binding proteins”(S3, S5, S9, S10, S12, S14, S16 and S19).

### SUMMARY OF THE INVENTION

- The present invention provides an isolated *S aureus* S16 ribosomal polypeptide, and the isolated polynucleotide molecules that encode them, as well as vectors and host cells comprising such polynucleotide molecules. The DNA  
25 sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded polypeptide, upon expression, can be used as a target for the screening of antibacterial drugs. High-throughput assays for identifying inhibitors of ribosomal assembly  
are provided. Solid phase high throughput assays are provided, as are related assay  
30 compositions, integrated systems for assay screening and other features that will be evident upon review.

In one embodiment, the invention provides an isolated S16 ribosomal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

The DNA and predicted amino acid sequence of *Staphylococcus aureus* S16 ribosomal polypeptide is displayed below:

ATGGCAGTTAAAATTCGTTTAAACACGTTTAGGTTCAAAAAGAAATCCATTCTATCGTATC<sup>60</sup>  
 M A V K I R L T R L G S K R N P F Y R I  
 5 GTAGTAGCAGATGCTCGTTCTCCACGTGACGGACGTATCATCGAACAAATCGGTACTTAT<sup>120</sup>  
 V V A D A R S P R D G R I I E Q I G T Y  
 AACCCAACGAGCGCTAATGCTCCAGAAATTAAAGTTGACGAAGCGTTAGCTTTAAAATGG<sup>180</sup>  
 N P T S A N A P E I K V D E A L A L K W  
 10 TTAAATGATGGTGCGAAACCAACTGATACAGTTCACAATATCTTATCAAAAAGAAGGTATT<sup>240</sup>  
 L N D G A K P T D T V H N I L S K E G I  
 ATGAAAAATTTGACGAACAAAAGAAAAGCTAAGTAA<sup>276</sup>  
 M K K F D E Q K K A K \*

15 Although SEQ ID NOS:1 and 2 provide particular *S. aureus* sequences, the invention is intended to include within its scope other *S. aureus* allelic variants. Allelic variants are understood to mean naturally-occurring base changes in the species population which may or may not result in an amino acid change of the DNA sequences herein

20 The present invention also includes include variants of the aforementioned polypeptide, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics.

The nucleic acids of the invention include those nucleic acids coding for the  
 25 same amino acids in the S16 ribosomal polypeptide due to the degeneracy of the genetic code

In another embodiment, the invention provides isolated polynucleotides (e.g. RNA and DNA, both naturally occurring and synthetically derived, both single and double stranded) that comprise a nucleotide sequence encoding the amino acid  
 30 sequence of the polypeptides of the invention. Such polynucleotides are useful for recombinantly expressing the enzyme and also for detecting expression of the polypeptides in cells (e.g. using Northern hybridization and in situ hybridization assays). Specifically excluded from the definition of polynucleotides of the invention is the entire isolated chromosome of the native host cells. A preferred polynucleotide  
 35 of the invention set forth in SEQ ID NO:1 corresponds to the naturally occurring S16 ribosomal polypeptide encoding nucleic acid sequence. It will be appreciated that numerous other sequences exist that also encode S16 ribosomal polypeptide of SEQ ID NO:2 due to the well known degeneracy of the universal genetic code. In another

preferred embodiment the invention is directed to all isolated degenerate polynucleotides encoding the S16 ribosomal polypeptide.

In another embodiment the invention provides an isolated nucleic acid comprising the nucleotide sequence having least 70%, 80, 90%, 95% identity with SEQ ID NO:1. In one embodiment, the invention provides an isolated S16 ribosomal polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2.

In a related embodiment the invention provides vectors comprising a polynucleotide of the invention. Such vectors are useful, e.g. for amplifying the polynucleotides in host cells to create useful quantities thereof. In preferred 10 embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Such vectors are useful for recombinant production of polypeptides of the invention.

In another related embodiment, the invention provides host cells that are 15 transformed with polynucleotides or vectors of the invention. As stated above, such host cells are useful for amplifying the polynucleotides and also for expressing the S16 ribosomal polypeptide or a fragment thereof encoded by the polynucleotide.

In still another related embodiment, the invention provides a method for producing the S16 ribosomal polypeptide (or a fragment thereof) comprising the steps 20 of growing a host cell of the invention in a nutrient medium and isolating the S16 ribosomal polypeptide from the cells.

In still another related embodiment the invention provides a method for testing for inhibitors of ribosomal assembly comprising the steps of contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, 25 S8, S15, S17 and S20 with 16S ribosomal RNA to form a polyribonucleotide protein complex and; contacting said polyribonucleotide protein complex with at least one non- direct binding ribosomal polypeptide selected from the group consisting of S1, S2, S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S18, S19, and S21. in the presence and absence of a test agent; and then determining the amount of at 30 least one non- direct binding ribosomal polypeptide bound to the RNA in the presence and the absence of a test agent and then comparing the amount of least one non direct binding ribosomal polypeptide bound under both conditions.

A decrease in the amount of protein determined in the presence of test agent compared to that determined in the absence of the test agent indicates that said agent is an inhibitor of ribosomal assembly

In still another related embodiment the invention provides an isolated S16  
5 ribosomal polypeptide comprising an amino acid sequence at least 70%, 80, 90%,  
95% identical to the sequence of SEQ ID NO:2.

In addition to the foregoing, the invention includes as an additional aspect, all  
embodiments of the invention narrower in scope in any way than the variations  
specifically mentioned above. Although the applicant(s) invented the full scope of the  
10 claims appended hereto, the claims appended are not intended to encompass within  
their scope the prior art work of others. Therefore, in the event that statutory prior art  
within the scope of a claim is brought to the attention of the applicants by a Patent  
Office or other entity or individual, the applicant(s) reserve the right to exercise  
amendment rights under applicable patent laws to redefine the subject matter of such a  
15 claim to specifically exclude such statutory prior art or obvious variations of statutory  
prior art from the scope of such a claim. Variations of the invention defined by such  
amended claims also are intended as aspects of the invention.

#### **DETAILED DESCRIPTION OF THE INVENTION**

The foregoing is provided to further facilitate understanding of the applicant's  
20 invention but is not intended to limit the scope of applicant's invention.

#### **Definitions**

As used hereinafter "Isolated" means altered by the hand of man from the  
natural state. If an "isolated" composition or substance occurs in nature, it has been  
changed or removed from its original environment, or both. For example, a  
25 polynucleotide or a polypeptide naturally present in a living animal is not "isolated,"  
but the same polynucleotide or polypeptide separated from the coexisting materials of  
its natural state is "isolated", as the term is employed herein.

As used hereinafter "Polynucleotide" generally refers to any  
polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or  
30 DNA or modified RNA or DNA. "Polynucleotides" include, without limitation,  
single- and double-stranded DNA, DNA that is a mixture of single- and double-  
stranded regions, single- and double-stranded RNA, and RNA that is mixture of  
single- and double-stranded regions, hybrid molecules comprising DNA and RNA that

may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

As used hereinafter "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation,

gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, *Proteins-Structure and Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., *Post-translational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *Postranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan et al., "Protein Synthesis: Post-translational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:4842).

As used hereinafter "Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties.

A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

As used hereinafter "Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques (see, e.g.: *Computational Molecular Biology*,

Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A. M., and Griffin, H. G., eds., Humana Press, N.J., 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J Applied Math (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, and FASTA (Atschul, S. F. et al., *J Molec Biol* (1990) 215:403). The well known Smith Waterman algorithm may be used to determine identity. The Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison, Wisconsin) is one such program which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.* 2:482-489 (1981)).

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity (divided by

100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y)$$

5

wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1, and  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y)$$

30

wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in SEQ ID NO:2, and  $y$  is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the

nearest integer prior to subtracting it from  $x_a$ . Identity has been similarly defined in US Patent No. 6,083,924, which is hereby incorporated by reference.

The present invention provides isolated polynucleotides (e.g., DNA sequences and RNA transcripts, both sense and complementary antisense strands, both single and  
5 double stranded) encoding a *Staphylococcus aureus* ribosomal protein S16. The nucleic acids of the invention include those nucleic acids coding for the same amino acids in the S16 ribosomal polypeptide due to the degeneracy of the genetic code. DNA polynucleotides of the invention include genomic DNA and DNA that has been synthesized in whole or in part. "Synthesized" as used herein and understood in the  
10 art, refers to polynucleotides produced by purely chemical as opposed to enzymatic methods. "Wholly" synthesized DNA sequences are therefore produced entirely by chemical means, and "partially" synthesized DNAs embrace those wherein only portions of the resulting DNA were produced by chemical means.

Genomic DNA of the invention comprises the protein-coding region for a  
15 polypeptide of the invention and is also intended to include allelic variants. Allelic variants are understood to mean naturally-occurring base changes in the species population which may or may not result in an amino acid change of the DNA sequences herein.

"16S ribosomal RNA" is understood to mean an isolated small subunit RNA  
20 of any prokaryote whether isolated from ribosomes, made synthetically or prepared by transcription, "16S ribosomal RNA" can mean either the full length sequence or a fragment thereof.

As used herein, the term "contacting" means bringing together, either directly or indirectly, a compound into physical proximity to a polypeptide or polynucleotide  
25 of the invention. Additionally "contacting" may mean bringing a polypeptide of the invention into physical proximity with another polypeptide or polynucleotide (either another polypeptide or polynucleotide of the invention or a polypeptide or polynucleotide not so claimed) or bringing a polynucleotide of the invention into physical proximity with a polypeptide or polynucleotide (either a polypeptide or  
30 polynucleotide of the invention or a polypeptide or polynucleotide not so claimed).

As used herein, the term "polyribonucleotide protein complex" refers to a covalent or non-covalently associated molecular entity containing 16S ribosomal RNA and at least one small subunit ribosomal protein

“Small subunit ribosomal protein” as used herein refers to ribosomal proteins present in the small (30S) ribosomal subunit of the ribosome of derived from any prokaryotic species. Small subunit ribosomal proteins include: S1, S2 S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, and S21.

5 “Direct binding ribosomal polypeptide” or “direct binding S-protein” or “direct binding ribosomal protein” as used herein refers to a polypeptide derived from any prokaryotic species selected from the group consisting of S4, S7, S8, S17, S15 and S20.

10 “Non- Direct binding ribosomal polypeptide” or “non-direct binding S-protein” or “non-direct binding ribosomal protein” as used herein refers to a polypeptide derived from any prokaryotic species selected from the group consisting of S1, S2 S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S18, S19, and S21. These proteins are also referred to as “secondary binding proteins”.

15 “Antibodies” as used herein includes monoclonal and polyclonal antibodies, chimeric, single chain, simianized antibodies and humanized antibodies, as well as Fab fragments, including the products of an Fab immunoglobulin expression library. The S16 ribosomal polypeptides of the invention or variants thereof, or cell expressing them can be used as an immunogen to produce antibodies immunospecific for such polypeptides.

#### 20 **Nucleic Acids of the Invention**

A preferred DNA sequence of the invention encoding the *Staphylococcus aureus* S16 ribosomal polypeptide is set out in SEQ ID NO:1. The worker of skill in the art will readily appreciate that the preferred DNA of the invention comprises a double stranded molecule, for example the molecule having the sequence set forth in SEQ ID  
25 NO:1 along with the complementary molecule (the “non-coding strand” or “complement”) having a sequence deducible from the sequence of SEQ ID NO:1 according to Watson-Crick base pairing rules for DNA. Also preferred are other polynucleotides encoding the S16 ribosomal polypeptide of SEQ ID NO:2, which differ in sequence from the polynucleotide of SEQ ID NO:1 by virtue of the well-  
30 known degeneracy of the universal genetic code. The determination of the nucleotide sequence is described in the following example.

### Example 1

#### Procedure for obtaining sequence information of the S16 gene directly from the 2.8 Mb *S. aureus* genome.

The *S. aureus* S16 gene was sequenced using an ABI377 fluorescence  
5 sequencer (Perkin Elmer Applied Biosystems, Foster City, CA) and the ABI  
BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with *AmpliTaq* FS  
DNA polymerase (PE Applied Biosystems, Foster City, CA). Each cycle sequencing  
reaction contained about 4 g of purified *S. aureus* DNA. Cycle-sequencing was  
performed using an initial denaturation at 98°C for 1 min, followed by 100 cycles:  
10 98°C for 30 sec, annealing at 50°C for 30 sec, and extension at 60°C for 4 min.  
Temperature cycles and times were controlled by a Perkin-Elmer 9700 thermocycler.  
Extension products were purified using Centriflex™ gel filtration cartridges (Edge  
BioSystems, Gaithersburg, MD). Each reaction product was loaded by pipette onto  
the column, which was then centrifuged in a swinging bucket centrifuge (Sorvall  
15 model RT6000B table top centrifuge) at 750 x g for 1.5 min at room temperature.  
Column-purified samples were dried under vacuum for about 40 min and then  
dissolved in 1.5 µl of a DNA loading solution (83% deionized formamide, 8.3 mM  
EDTA, and 1.6 mg/ml Blue Dextran). The samples were then heated to 90°C for  
three min and the complete sample was loaded into the gel sample well of the ABI377  
20 sequencer. Sequence chromatogram data files from the ABI377 were analyzed with  
the computer program Sequencher (Gene Codes, Ann Arbor, MI), for assembly of  
sequence fragments and correction of ambiguous base calls. Generally sequence reads  
of 600 bp were obtained. Sequence base call ambiguities were removed by obtaining  
the complete sequence of the S16 gene on both DNA strands.

25 **Sequencing of *S. aureus* S16 gene.** We located in the HGS *S. aureus* database a 175  
bp GST (Human Genome Sciences ID #btecc45r) which encodes about 16 amino  
acids of the S16 polypeptide. The DNA sequence corresponding to this coding region  
was used to design forward (SEQ ID NO:3, 5'- AACTGCCATTTATAAAAATCTCC)  
and reverse (SEQ ID NO:4, 5'- TAAAGGAGATTTTATAAATGGCAG) primers.

30 The primers were designed without the aid of the ABI profile, thus the quality of the  
HGS sequence data could not be assessed. New *S.aureus* sequence data was obtained  
only from the reverse primer (SEQ ID NO:4) which extended the sequence data  
upstream of the S16 gene. Using the sequence generated by primer SEQ ID NO:4 two

reverse primers were designed, SEQ ID NO:5 (5'-  
TTATATTGGGGGAACGTGTGCGG) and SEQ ID NO:6  
(5'AGTCTAATTTAGTAATCACATAG) to prime sequences starting about 150 and  
100 bp 5' of the start of the S16 gene, respectively). Both of these primers generated  
5 excellent ABI sequence profiles. The complete double-stranded sequence of this gene  
was obtained by using primer SEQ ID NO:7 (5'-  
TATTACTAACATGTGATATCCC) which was designed from sequence data  
located about 50 bp downstream from the 3'-end of the S16 gene. A total of 1.3 kb of  
sequence data was obtained within and around the S16 gene and analysis of this  
10 sequence revealed the complete S16 gene that encodes the complete S16 polypeptide.  
The *S.aureus* S16 polypeptide contains 91 amino acid residues, and this sequence  
shares about 66% identity with the S16 polypeptide from *B.subtilis*:

The invention further embraces species, which are homologs of the  
*Staphylococcus aureus* S16 ribosomal polypeptide encoding DNA. Species  
15 homologs, would encompass nucleotide sequences which share at least at least 70%,  
at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at  
least 99% identity with *Staphylococcus aureus* polynucleotide of the invention

The polynucleotide sequence information provided by the invention makes  
possible large scale expression of the encoded polypeptide by techniques well known  
20 and routinely practiced in the art. Polynucleotides of the invention also permit  
identification and isolation of polynucleotides encoding related ribosomal proteins,  
such as allelic variants and species homologs, by well known techniques including  
Southern and/or Northern hybridization, and polymerase chain reaction (PCR).

The disclosure herein of a full length polynucleotide encoding an S16  
25 ribosomal polypeptide makes readily available to the worker of ordinary skill in the art  
every possible fragment of the full length polynucleotide. The invention therefore  
provides fragments of the S16 ribosomal polypeptide encoding polynucleotides  
comprising at least 14-15, and preferably at least 18, 20, 25, 50, or 75 consecutive  
nucleotides of a polynucleotide encoding S16 ribosomal polypeptide. Preferably,  
30 fragment polynucleotides of the invention comprise sequences unique to the S16  
ribosomal polypeptide encoding polynucleotide sequence and therefore hybridize  
under highly stringent or moderately stringent conditions only (i.e. "specifically") to  
polynucleotides encoding S16 ribosomal polypeptide. Sequences unique to

polynucleotides of the invention are recognizable through sequence comparison to other known polynucleotides, and can be identified through use of alignment programs routinely utilized in the art, e.g. those made available in public sequence databases. Such sequences are also recognizable from Southern hybridization  
5 analyses to determine the number of fragments of genomic DNA to which a polynucleotide will hybridize. Polynucleotides of the invention can be labelled in a manner that permits their detection, including radioactive, fluorescent, and enzymatic labelling.

Fragment polynucleotides are particularly useful as probes for detection of full  
10 length or other fragment S16 ribosomal polypeptide polynucleotides or for the expression of fragments of S16 ribosomal polypeptide. One or more fragment polynucleotides can be included in kits that are used to detect variations in a polynucleotide sequence encoding S16 ribosomal polypeptide.

The invention also embraces DNAs encoding S16 ribosomal polypeptide  
15 polypeptides which DNAs hybridize under moderately stringent or high stringency conditions to the non-coding strand, or complement, of the polynucleotide in SEQ ID NO:1

Exemplary highly stringent hybridization conditions are as follows:  
hybridization at 42°C in a hybridization solution comprising 50% formamide, 1%  
20 SDS, 1M NaCl, 10% Dextran sulfate, and washing twice for 30 minutes at 60°C in a wash solution comprising 0.1 X SSC and 1% SDS. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), *Protocols in Molecular Biology*, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in  
25 hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, et al., (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

### 30 **Host Cells and Vectors of the Invention**

According to another aspect of the invention, host cells are provided, including prokaryotic and eukaryotic cells, comprising a polynucleotide of the invention (or vector of the invention) in a manner which permits expression of the encoded S16

ribosomal polypeptide. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell well known and routinely practiced in the art include transformation, transfection, 5 electroporation, nuclear injection, or fusion with carriers such as liposomes, micelles, ghost cells, and protoplasts. Expression systems of the invention include bacterial, yeast, fungal, plant, insect, invertebrate, and mammalian cells systems. Suitable host cells for expression of S16 ribosomal polypeptides include prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the 10 expression of human *Staphylococcus aureus* Ribosomal Protein Gene, S16 include bacteria of the genera *Escherichia*, *Bacillus*, and *Salmonella*, as well as members of the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*.

The isolated nucleic acid molecules of the invention are preferably cloned into a vector designed for expression in prokaryotic cells, rather than into a vector 15 designed for expression in eukaryotic cells. Prokaryotic cells are preferred for expression of genes obtained from prokaryotes because prokaryotic cells are more economical sources of protein production and because prokaryotic hosts grow to higher density and are typically grown in media which is less expensive than that used for the growth of eukaryotic hosts.

20 In the event a eukaryotic host were used the possibilities may include, but are not limited to, the following: insect cells, African green monkey kidney cells (COS cells), Chinese hamster ovary cells (CHO cells), human 293 cells, and murine 3T3 fibroblasts.

Expression vectors for use in prokaryotic hosts generally comprise one or 25 more phenotypic selectable marker genes. Such genes generally encode, *e.g.*, a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), and pQE vectors (Qiagen). A 30 representative cloning and expression scheme is provided by the following example.

### **Example 2**

#### **Isolation and Cloning of the S16 Coding Region**

Two primers were designed for PCR. One contains the ATG of S16 ribosomal protein with a Cla site on the end. This forward primer is a 37mer and is designated SEQ ID NO:8 and has the sequence 5' GTG TTA TCG ATA ATG CAG TTA AAA TTC GTT TAA CAC G. The downstream primer designated SEQ ID  
5 NO:9 is a 35mer and has the sequence 5' GTG TTG GAT CCT TAC TTA GCT TTC TTT TGT TCG TC

This sequence includes the stop codon of S16 ribosomal protein with a BamH1 site on the end. *Staphylococcus aureus* genomic DNA was used as a template. The buffer (N808-0006) and Amplitaq® (N8080-0101) were purchased from Perkin Elmer Cetus  
10 . The 10 mM dNTP mix was obtained from Gibco BRL (Gaithersburg, MD). The reaction mix was 5 µl of buffer, 1 µl of dNTP mix, 1 ng of each primer, 1 ng of genomic DNA and 0.5 µl (2.5 units) of amplitaq in a final volume of 50 µl. The program for PCR was 94°C for 10 minutes and then 40 cycles of 94°C for 1 minute, 57°C for 30 seconds, and 72°C for one minute. The final extension phase was  
15 at 72°C for 3 minutes and the reactions were allowed to stay at 4°C until they were removed from the thermocycler.

**Vector Construction and Expression** The PCR products were purified, digested with ClaI and BamHI and ligated to the expression vector pSR-Tac which contains Cla I and BamHI cloning sites. This vector contains a tac promoter, an AT rich  
20 synthetic ribosome binding site, two transcription terminators designated T1 and sib3 upstream of the tac promoter and downstream of the cloned gene, respectively, an ampicillin resistance gene derived from pBR322, and a ColE1 origin of replication. The Cla I restriction site is located immediately downstream of the ribosome binding site and the BamHI site is immediately upstream of the sib3 terminator. While this  
25 particular vector worked quite well it is expected that other vectors used in *E.coli* heterologous protein expression would be equally suitable.

After transformation into *E. coli* strain Top10 F' *lacI*<sup>q</sup>, the colonies were screened by DNA mini prep and restriction digestion to find the desired constructs. The constructs were sequenced and transformed into *E. coli* strain K12s F' *lacI*<sup>q</sup> for  
30 expression studies.

Cells harboring the construct pSRTac-S16 were grown in 50 ml LB with ampicillin at 37°C. The cultures were induced with 10<sup>-3</sup> M IPTG during the midlog phase of

growth and allowed to express for 3 hours. Then the cells were collected, sonicated and examined using gel electrophoresis.

Half a milliliter of the sonicated expression cultures were centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected as the soluble fraction and the pellet  
5 (insoluble fraction) was suspended in 10 mM Tris-HCl pH 8.0. These samples were electrophoresed on 20% acrylamide with DATD crosslinker. The S16 protein was expressed at moderate levels and observed to be in the soluble fraction.

### **Polypeptides of the Invention**

10 Overexpression in eukaryotic and prokaryotic hosts as described above facilitates the isolation of S16 polypeptides. The invention therefore includes isolated S16 polypeptides as set out in SEQ ID NO:2 and variants and conservative amino acid substitutions therein including labeled and tagged polypeptides.

The invention includes S16 polypeptides which are "labeled". The term  
15 "labeled" is used herein to refer to the conjugating or covalent bonding of any suitable detectable group, including enzymes (e.g., horseradish peroxidase, beta-glucuronidase, alkaline phosphatase, and beta-D-galactosidase), fluorescent labels (e.g., fluorescein, luciferase), and radiolabels (e.g.,  $^{14}\text{C}$ ,  $^{125}\text{I}$ ,  $^3\text{H}$ ,  $^{32}\text{P}$ , and  $^{35}\text{S}$ ) to the compound being labeled. Techniques for labeling various compounds, including  
20 proteins, peptides, and antibodies, are well known. See, e.g., Morrison, *Methods in Enzymology* 32b, 103 (1974); Syvanen et al., *J. Biol. Chem.* 284, 3762 (1973); Bolton and Hunter, *Biochem. J.* 133, 529 (1973). The termed labelled may also encompass a polypeptide which has covalently attached an amino acid tag as discussed below.

25 In addition, the S16 polypeptides of the invention may be indirectly labeled. This involves the covalent addition of a moiety to the polypeptide and subsequent coupling of the added moiety to a label or labeled compound which exhibits specific binding to the added moiety. Possibilities for indirect labeling include biotinylation of the peptide followed by binding to avidin coupled to one of the above label  
30 groups. Another example would be incubating a radiolabeled antibody specific for a histidine tag with a S16 polypeptide comprising a polyhistidine tag. The net effect is to bind the radioactive antibody to the polypeptide because of the considerable affinity of the antibody for the tag.

The invention also embraces variants (or analogs) of the S16 protein. In one example, insertion variants are provided wherein one or more amino acid residues supplement a S16 amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the S16 protein amino acid sequence. Insertional variants with additional residues at either or both  
5 termini can include for example, fusion proteins and proteins including amino acid tags or labels. Insertion variants include S16 polypeptides wherein one or more amino acid residues are added to a S16 acid sequence, or to a biologically active fragment thereof.

10 Insertional variants therefore can also include fusion proteins wherein the amino and/or carboxy termini of S16 is fused to another polypeptide. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the influenza HA tag polypeptide and its antibody 12CA5 [Field  
15 et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag -peptide [Hopp et al.,  
20 BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an alpha -tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397(1990)]. In addition, the S16 polypeptide can be tagged with enzymatic proteins such as  
25 peroxidase and alkaline phosphatase.

In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a S16 polypeptide are removed. Deletions can be effected at one or both termini of the S16 polypeptide, or with removal of one or more residues within the S16 amino acid sequence. Deletion variants, therefore, include all  
30 fragments of the S16 polypeptide.

The invention also embraces polypeptide fragments of the sequence set out in SEQ ID NO: 2 wherein the fragments maintain biological (e.g., ligand binding or RNA binding and/or other biological activity) Fragments comprising at least 5, 10,

15, 20, 25, 30, 35, or 40 consecutive amino acids of SEQ ID NO: 2 are comprehended by the invention. Fragments of the invention having the desired biological properties can be prepared by any of the methods well known and routinely practiced in the art.

5           The present invention also includes include variants of the aforementioned polypeptide, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is substituted by another with like characteristics. Variant polypeptides include those wherein conservative substitutions have been introduced by modification of polynucleotides encoding polypeptides of the invention. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table A (from WO 97/09433, page 10, published March 13, 1997 (PCT/GB96/02197, filed 10  
15 9/6/96), immediately below.

**Table A**  
**Conservative Substitutions I**

20	<b>SIDE CHAIN CHARACTERISTIC</b>	<b>AMINO ACID</b>
	<b>Aliphatic</b>	
	Non-polar	G A P I L V
	Polar - uncharged	C S T M N Q
25	Polar - charged	D E K R
	Aromatic	H F W Y
	Other	N Q D E

30  
Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table B, immediately below

**Table B**  
**Conservative Substitutions II**

	<b>SIDE CHAIN</b>	
5	<b>CHARACTERISTIC</b>	<b>AMINO ACID</b>
	Non-polar (hydrophobic)	
	A. Aliphatic:	A L I V P
10	B. Aromatic:	F W
	C. Sulfur-containing:	M
	D. Borderline:	G
	Uncharged-polar	
	A. Hydroxyl:	S T Y
15	B. Amides:	N Q
	C. Sulfhydryl:	C
	D. Borderline:	G
	Positively Charged (Basic):	K R H
	Negatively Charged (Acidic):	D E
20		

As still an another alternative, exemplary conservative substitutions are set out in Table C, immediately below.

**Table C**  
**Conservative Substitutions III**

<u>Original Residue</u>	<u>Exemplary Substitution</u>
5	
Ala (A)	Val, Leu, Ile
Arg (R)	Lys, Gln, Asn
Asn (N)	Gln, His, Lys, Arg
Asp (D)	Glu
10 Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
His (H)	Asn, Gln, Lys, Arg
15 Ile (I)	Leu, Val, Met, Ala, Phe,
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, Gln, Asn
Met (M)	Leu, Phe, Ile
Phe (F)	Leu, Val, Ile, Ala
20 Pro (P)	Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser
25 Val (V)	Ile, Leu, Met, Phe, Ala

Generally it is anticipated that the S16 polypeptide will be found primarily intracellularly, the intracellular material can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation. The S16 polypeptide is found primarily in the supernatant after centrifugation of the cell

homogenate, and the S16 polypeptide can be isolated by way of non-limiting example by any of the methods below.

In those situations where it is preferable to partially or completely isolate the S16 polypeptide, purification can be accomplished using standard  
5 methods well known to the skilled artisan. Such methods include, without limitation, separation by electrophoresis followed by electroelution, various types of chromatography (immunoaffinity, molecular sieve, and/or ion exchange), and/or high pressure liquid chromatography. In some cases, it may be preferable to use more than one of these methods for complete purification.

10 Purification of S16 polypeptide can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (S16/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, Conn.) or myc (Invitrogen, Carlsbad, Calif.) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by  
15 passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the polypeptide directly (i.e., a monoclonal antibody specifically recognizing S16). For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen Registered  
TM nickel columns) can be used for purification of S16/polyHis. (See for example,  
20 Ausubel et al., eds., Current Protocols in Molecular Biology, Section 10.11.8, John Wiley & Sons, New York [1993]).

Even if the S16 polypeptide is prepared without a label or tag to facilitate purification. The S16 of the invention may be purified by immunoaffinity  
chromatography. To accomplish this, antibodies specific for the S16 polypeptide  
25 must be prepared by means well known in the art. Antibodies generated against the S16 polypeptides of the invention can be obtained by administering the polypeptides or epitope-bearing fragments, analogues or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any  
technique known in the art that provides antibodies produced by continuous cell line  
30 cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., Nature 256: 495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pg. 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985).

Where the S16 polypeptide is prepared without a tag attached, and no antibodies are available, other well known procedures for purification can be used. Such procedures include, without limitation, ion exchange chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined to achieve increased purity. A representative purification scheme is detailed below.

10

### Example 3

#### Large Scale Purification of S16 Protein

S16-expressing *E. coli* cell paste from about a 6 liters of fermentation is resuspended in ~ 70 mL Tris buffer pH 7.4 containing 1 mM MgCl<sub>2</sub> and 1 mM DTT. One Complete<sup>®</sup> EDTA-free protease inhibitor pellet (Boehringer Mannheim, Indianapolis, IN) is added to the suspended cells. The cells are lysed by passage three times through a French Press @ 10,000 PSI. A soluble fraction is prepared from the cellular lysate by ultracentrifugation @ 100,000 x g for 60 minutes @ 4<sup>0</sup> C. The soluble fraction is injected onto a HiPrep SP<sub>XL</sub> 16/10 cation exchange column which had been equilibrated in 50 mM Tris buffer pH 7.4, 1 mM MgCl<sub>2</sub>, and 1 mM DTT. The column flow rate is 4 mL/min. The column is washed with buffer until the Abs<sub>280</sub> of the column eluate is less then 0.01. Material is eluted off of the HiPrep SP<sub>XL</sub> column with a linear gradient of 0-700 mM NaCl in column buffer over 20 column volumes.

Fractions are collected and analyzed by SDS-PAGE using 4-12% Bis-Tris NuPage<sup>®</sup> gels (Novex, San Deigo, CA) employing a MES buffer system. S16-containing fractions are further analyzed by liquid chromatography electrospray mass spectrometry (LC/MS-ESI) performed on a Finnigan LC/Q instrument. The results of the LC/MS-ESI analysis are used to calculate an average mass of the isolated S16. The predicted average mass of the intact S16 is calculated to be around 10,000

In addition to preparing and purifying S16 polypeptide using recombinant DNA techniques, the S16 polypeptides, fragments, and/or derivatives thereof may be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield et al., (J. Am. Chem. Soc., 85:2149 [1963]), Houghten et al.

(Proc Natl Acad. Sci. USA, 82:5132 [1985]), and Stewart and Young (Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, Ill. [1984]). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized S16 polypeptides or fragments may be oxidized using methods set forth in these references to form disulfide bridges. The S16 polypeptides or fragments are expected to have biological activity comparable to S16 polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with recombinant or natural S16 polypeptide.

#### 10 **Ribosomal Assembly Assays**

70S ribosome particles in *E.coli* consist of 31 core ribosomal "L" proteins and two rRNAs (5S and 23S) in the 50S subunit and 21 "S" proteins and a single 16S rRNA in the 30S subunit. These particles constitute the basic machinery for bacterial protein translation. It is postulated that the *Staphylococcus aureus* ribosome is assembled in fashion to ribosomes in *E.coli*. The present invention provides several methods to study the *S.aureus* 30S subunit assembly and methods to screen for inhibitors of the assembly process.

Assembly of the 30S ribosomal subunit is an ordered process both *in vivo* and *in vitro*. Nomura, M. and Held, W.A. (1974), Noller and Nomura (1987). It is now well known that the 21 proteins which comprise the the *E. coli* 30S subunit assemble onto the the 16S rRNA in an ordered fashion *in vitro*. *Id.* These proteins have been defined as primary or secondary binders, according to whether they bind to the 16S RNA independently of other proteins or not. Proteins that bind directly to 16S rRNA include S4, S7, S8, S15, S17 and S20. Secondary binding proteins include S3, S5, S9, S10, S12, S14, S16 and S19.

Producing and purifying the *S.aureus* ribosomal "S" proteins which are most critical for the formation of functional 30S subunits including those that bind directly to 16S rRNA (i.e., S4, S7, S8, S15, S17 and S20) "direct binding S-proteins" and critical proteins that integrate themselves into the ribosome by reliance on protein-protein and/or protein-RNA interactions (S3, S5, S9, S10, S12, S14, S16 and S19) provides myriad choices in designing methods for testing inhibitors of ribosomal assembly.

### Simplified 30S Ribosomal Subunit Assembly Assay

It is recognized that the role of the S16 polypeptide in the assembly of complete ribosomal small subunits may, in part, be dependent on the interactions with the direct binding S-proteins which directly interact with the 16S ribosomal RNA.

It is likely that the incorporation of S16 into a complete 30S subunit is at least partially dependent on other secondary binding proteins include S3, S5, S9, S10, S12, S14, or S19. However, examination of published binding maps show that a incubation of the direct binding S proteins with S16 in the presence and absence of test compounds and subsequent measurement of relative incorporation of S16 into the polyribonucleotide protein complex provides a fruitful avenue for identification of small subunit ribosomal assembly inhibitors.

By way of non-limiting example one can envision numerous ways in which the presence of unbound or bound S16 could be detected. The S16 might be radiolabeled in any of a number of means including but not limited to, labeling in vitro by chemical or enzymatic means or vivo by metabolically labeling cells expressing S16.

As discussed above commonly used radioactive isotopes used for the radiolabeling of peptides and proteins and nucleic acids include but are not limited to  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ ,  $^{125}\text{I}$  and  $^{32}\text{P}$ . In addition, of course, if the S16 polypeptide or is tagged with an amino acid tag, as described above, the tag and the covalently attached S16 protein can be detected by means well known in the art. In addition, the S16 polypeptide or a polynucleotide can be tagged with enzymatic proteins such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) which are capable of being monitored for change in fluorescence intensity, wavelength shift, or fluorescence polarization (FP) or fluorescent resonance energy transfer (FRET). Another method of labeling polypeptides and nucleic acids includes biotinylation of the peptide of the peptide or nucleic acid followed by binding to avidin coupled to one of the above label groups or a solid support.

In another embodiment, all the direct binding S-proteins can be incubated with 16S RNA and the presence of bound or unbound S16 polypeptide determined. Indeed, the identity of all of the bound or unbound proteins can be determined. The identity of a bound or unbound S protein can be determined, for instance by a suitable

mass spectrometry technique, such as matrix-assisted laser desorption/ionization combined with time-of-flight mass analysis (MALDI-TOF MS) or electrospray ionization mass spectrometry (ESI MS). See Jensen et al., 1977, Protein Analysis By Mass Spectrometry, In Creighton (ed.), Protein Structure, A Practical Approach (Oxford University Press), Oxford, pp. 29-57; Patterson & Aebersold, 1995, Electrophoresis 16: 1791-1814; Figeys et al., 1996, Analyt. Chem. 68: 1822-1828 (each of which is incorporated herein by reference in its entirety). Preferably, a separation technique such as HPLC or capillary electrophoresis is directly or indirectly coupled to the mass spectrometer. See Ducret et al., 1996, Electrophoresis 17: 866-876; Gevaert et al., 1996, Electrophoresis 17: 918-924; Clauser et al., 1995, Proc. Natl. Acad. Sci. USA 92: 5072-5076 (each of which is incorporated herein by reference in its entirety).

#### Example 4

This assay is used to test for disruptions in interactions between the S16 polypeptide, the direct binding S proteins, and the 16S RNA.

#### Preparation of Starting Materials

##### *Preparation of Direct Binding Ribosomal Proteins*

The starting material proteins are preferably prepared by recombinant means and over-expression in a suitable host essentially as described in Examples 1, 2 and 3 for S16 with obvious modifications to reflect the differing sequences of the proteins involved. The nucleotide sequences of cDNA's encoding *S. aureus* direct binding ribosomal proteins S4, S7, S8, S15, S17 and S20 are presented in SEQ ID NOS:10, 12, 14, 16, 18 and 49 respectively. Sequences encoding S4, S7, S8, S15, and S17 can be isolated by means of the polymerase chain reaction. Primers are selected such that entire coding region is isolated. The complete amino acid sequences of S4, S7, S8, S15, S17 and S20 polypeptides are presented in SEQ ID NOS:11, 13, 15, 17, 19 and 50. Sequences encoding S4, S7, S8, S15, S17 and S20 can be isolated by means of probing a genomic *Staphylococcus aureus* library with probes designed from SEQ ID NOS: 10, 12, 14, 16, 18 and 49 as well. The polymerase chain reaction would be a preferred method because it generally allows the isolation of a complete coding sequence in one experiment.

Methods for preparing and using probes and primers are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed.,

vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1987 (with periodic updates); and Innis et al., PCR Protocols: A Guide to Methods and Applications, 5 Academic Press: San Diego, 1990.

Primers are selected to have low self- or cross-complementarity, particularly at the 3' ends of the sequence. Long homopolymer tracts and high GC content are avoided to reduce spurious primer extension. Primers are typically about 20 to 30 residues in length, but this length can be modified as well known in the art, in view 10 of the particular sequence to be amplified. Computer programs are available to aid in these aspects of the design. One widely used computer program for designing PCR primers is (OLIGO 4.0 by National Biosciences, Inc., 3650 Annapolis Lane, Plymouth, Mich.). Another is Primer (Version 0.5,(c) 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.).

#### 15 *Cloning of 16S Ribosomal RNA*

The complete 16S-rRNA gene was identified in the HGS data base on contig 168268 by homology to the *B. subtilis* sequence. Five prime sequence of 5'-TTTATGGAGAGTTTGATCCTGGC-3' and the 3' sequence of 5'-GCGGCTGGATCACCTCCTTTCT-3' were used to amplify the entire 16S-rRNA 20 gene from *S. aureus* (Oligo Etc; Wilsonville, OR). The amplified gene was cloned into pT7Blue using Novagen's (Madison, WI) Perfectly Blunt Cloning Kit. DNA template was created by PCR using a primer that had the T7 promoter on the 5' end sequence of the 16S-rRNA gene (5'-TAATACGACTCACTATAGTTTTATGGA-GAGTTTGATCCTGGC-3'). The length of the amplified 16S-rRNA fragment can be 25 altered by the selection of the 3' primer. <sup>3</sup>H-UTP or <sup>35</sup>S-ATP are used to label the RNA if labeled RNA is desired. Resulting RNAs are characterized by electrophoresis on acrylamide-urea gels, and RNA concentrations are determined by UV spectroscopy using A<sub>260</sub> unit = 40 ug/ml. The entire S16 ribosomal RNA gene sequence has been reported (Genbank Accession # X68417 also US Patent No. 5,843,669 Sequence # 30 160). The sequence of the gene is included in this document as SEQ ID NO:21

In this assay all six of the S-proteins that bind directly to 16S RNA are added together followed by S16 in the presence and absence of a test compound. Unbound S-proteins are then removed by size-separation or filtration. Automated LC/ESI ion-trap or MALDI-tof-MS is then used to determine if a particular S-protein is inhibited

in its binding to 16S RNA. Mass spectrometry is an ideal detection tool since all of the S-protein average masses are known and unique. An example illustrates how specific inhibition of S16 protein binding to RNA is detected. The concept is illustrated in Figure 2.

5 RNA:protein assembly is assayed in 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 330 mM NaCl at 42 °C. The procedure is based on the conditions of Culver and Noller (RNA, 1999, 5: 832-843) except that 0.01% Nikkol detergent is removed because it significantly complicates the LC/MS analysis. Primary ribosomal binding proteins S4, S7, S8, S15, S17, and S20 are dialyzed overnight against 80 mM K<sup>+</sup>-  
10 HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 1 M NaCl. In the reconstitution, 200 pmol *in vitro* transcribed 16S RNA is incubated at 42 °C for 15 minutes. Then, 800 pmol S7, S8, S15, S17, S4 and S20 each are added to the RNA. 400 pmol S16 is then added to the mixture. The NaCl concentration is then adjusted to 330 mM by adding 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>. The mixture is then incubated at 42 °C for 20 more  
15 minutes. The protein:RNA complex is then separated from the free proteins by spinning in a YM-100 Microcon at 500 xg for 20 minutes. The RNA is precipitated from the retentate by adding 2 volumes of acetic acid and incubating on ice for 45 minutes. Proteins from both the flow-through and retentate are analyzed by LC/ESI ion trap mass spectrometry. The proteins are first separated on a C4 reversed phase  
20 column (Vydac) using a gradient from 98% of 0.1% TFA, 2% of 90% acetonitrile/0.1% TFA to 100% of 90% acetonitrile/0.1 % TFA. The intact mass of each protein is observed by electrospray mass spectrometry as it eluted from the column. Relative amounts of each protein are accessed in the presence and absence of test compounds.

25

### Example 5

#### Scintillation Proximity Assay (SPA)

As in the previous example all six of the S-proteins that bind directly to 16S RNA are added together followed by S16 ribosomal polypeptide in the presence and absence of a test compound. In this example the 16S ribosomal RNA is end labeled  
30 with biotin and the S16 ribosomal polypeptide is radioactively labeled.

Primary ribosomal binding proteins S4, S7, S8, S15, S17, and S20 are dialyzed overnight against 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 1 M NaCl. In the reconstitution, 200 pmol *in vitro* transcribed biotin end labeled 16S RNA is incubated

at 42 °C for 15 minutes. Then, 800 pmol S7, S8, S15, S17, S4 and S20 each are added to the RNA. 400 pmol radioactively labeled S16 is then added to the mixture. The NaCl concentration is then adjusted to 330 mM by adding 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>. Fifty µl streptavidin coated SPA beads (20 mg/ml) is added to the 50 µl of of the reaction mixture in a Dynatech Microlite plate and counted in a Topcount<sup>TM</sup> Microplate Scintillation Counter. To identify potential inhibitors of S16 incorporation into the polyribonucleotide protein complex, the assay is run in the presence and absence of potential inhibitors and the effect on binding is assessed.

### **Protein-protein Interaction Assembly Screen**

The isolated S16 polypeptide of the invention also makes possible an assay through which one may detect all possible protein-protein disruptions in the 30S assembly process. This is important since published assembly maps are not based on the myriad of possible protein-protein interactions that may occur. In practice these maps are based on limited S-protein combinations that were tested *in vitro*. This assay makes use of the fact that the assembly of ribosomes in general and the 30S subunit in particular, is an ordered process and makes use of all 21 small subunit ribosomal proteins or a limited subset of those proteins. The S3 ribosomal protein is known to integrate itself last or very late in the ribosomal assembly process. Its efficient integration is known to be dependent upon the proper integration of the direct binding ribosomal proteins as well non-direct binding proteins. Proper partial assembly is monitored by measuring the incorporation of S3 ribosomal polypeptide into the partially or fully assembled ribosome. In the alternative, improper or disrupted assembly can be assayed by exclusion of S3 ribosomal polypeptide from the ribosome

The S3 ribosomal protein may be labeled as discussed hereinbefore for ease of detection. The 16S ribosomal RNA or a direct binding ribosomal peptide may be immobilized or the entire assay may be performed with all components in solution phase. The starting materials for the assays are preferably prepared by recombinant means. The DNA sequences encoding all 21 30S subunit proteins are provided in the sequence listings as well as the amino acids sequences encoded by each. The invention provides ribosomal assembly assays utilizing all 21 small subunit ribosomal proteins as well as a select subset of proteins readily apparent to one skilled in the art. Sequences encoding each protein can be isolated by means of the polymerase chain reaction. Primers are selected as discussed previously. Primers

are selected such that entire coding region is isolated. Methods for preparing and using probes and primers are discussed above.

Exemplary forward and reverse primers suitable for amplification of S4, S6, and S18 are described listed here by way of example. One skilled in the art would  
5 recognize that other primers may be equally suitable.

S4 Forward 5'-TATATTATCGATAATGGCTCGATTCAGAGGT-3' (SEQ ID NO:53)

S4 Reverse 5'-TATAGGATCCTTAACGGATTAATTGTTCGTTAATTT-3' (SEQ ID NO:54)

10 S18 Forward 5'-TATATTATCGATAATGGCAGGTGGACCAAGAAG-3' (SEQ ID NO:55)

S18 Reverse 5'-TATAGGATCCTTATTGTTCTTCTTTAACAT-3' (SEQ ID NO:56)

S6 Forward 5'-TATATTATCGATAATGAAGAAACATATGAAGTTAT-3' (SEQ ID NO:57)

15 S6 Reverse 5'-TATAGGATCCTTACTTGTCTTCGTCTTCAC-3' (SEQ ID NO:58)

### Example 6

#### Partial Ribosomal Assembly Assay

In this assay format several S-proteins are allowed to interact with 16S RNA in the presence of a test compound (Fig.3). The assay makes use of all of the direct  
20 binding ribosomal proteins except S15 (S4, S7, S8, S17 and S20) and a select group of *S. aureus* ribosomal proteins which integrate themselves into the ribosome by reliance on protein-protein or protein-RNA interactions (non-direct binding ribosomal proteins) (S3, S5, S9, S10, S12, S14, S16 and S19)

The starting material proteins are prepared by recombinant means and over-  
25 expression in a suitable host essentially as described in Examples 1, 2 and 3 for the S16 polypeptide of the invention with obvious modifications to reflect the differing sequences of the proteins involved. The nucleotide sequences of cDNA's encoding *S. aureus* direct binding ribosomal proteins S4, S7, S8, S17 and S20 are presented in SEQ ID NOS:10, 12, 14, 19 and 49 respectively.

30 The nucleotide sequences of cDNA's encoding *S. aureus* ribosomal proteins which integrate themselves into the ribosome by reliance on protein-protein or protein-RNA interactions S3, S5, S9, S10, S12, S14, and S19 are presented in SEQ ID NOS: 25, 27, 31, 33, 37, 41, 43, and 48 respectively. Nucleotide sequences encoding

*S. aureus*. S3, S4, S5, S7, S8, S9, S10, S12, S14, S17 and S19 can be isolated by means of the polymerase chain reaction. Primers are selected as discussed previously, such that the entire amino acid coding region is isolated. The complete amino acid sequences of *S. aureus* S3, S4, S5, S7, S8, S9, S10, S12, S14, S17 and S19 polypeptides are presented in SEQ ID NOS:26, 11, 28, 13, 15, 31, 34, 38, 42, and 19 respectively. The production of the isolated S16 polypeptide of the invention is described hereinbefore. Sequences encoding S3, S4, S5, S7, S8, S9, S10, S12, S14, S17 and S19 can be isolated by means of probing a genomic *Staphylococcus aureus* library with probes designed from SEQ ID NOS: 25, 27, 31, 33, 37, 41, 43, and 48 as well. The polymerase chain reaction would be a preferred method because it generally allows the isolation of a complete coding sequence in one experiment. The S3 protein is labeled, preferably radiolabeled.

RNA:protein assembly is assayed in 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 330 mM NaCl at 42 °C. The procedure is based on the conditions of Culver and Noller (RNA, 1999, 5: 832-843) except that 0.01% Nikkol detergent is removed because it significantly complicates the LC/MS analysis. Ribosomal proteins S3, S4, S5, S7, S8, S9, S10, S12, S14, S16, S17, S19 and S20 are dialyzed overnight against 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>, 1 M NaCl. In the reconstitution, 200 pmol *in vitro* transcribed 16S RNA is incubated at 42 °C for 15 minutes. Then, 800 pmol ribosomal proteins S4, S7, S8, S17, and S20 added to the RNA, followed by ribosomal proteins, S5, S9, S10, S12, S14, S16 and S19. The NaCl concentration is then adjusted to 330 mM by adding 80 mM K<sup>+</sup>-HEPES, pH 7.6, 20 mM MgCl<sub>2</sub>. The mixture is then incubated at 42 °C for 20 more minutes. 800 pmol labeled ribosomal protein S3 is then added.

Unbound S-proteins are removed by size-separation or filtration. If the labelled S3 protein is present in the RNA:multiprotein complex then the compound does not inhibit any specific protein-protein interactions during the assembly process. If the compound prevents the incorporation of labelled S3 protein then the assay reveals that the test compound inhibits a protein-protein interaction.

The partially assembled RNA:multiprotein complex is then analyzed by LC/ion-trap electrospray analysis to determine the S-protein components in the partially assembled complex. Alternatively MALDI-tof-MS can be used. Knowing the identity of S-proteins in the partially assembled complex and published knowledge of

how the 30S subunit is assembled *in vitro* (Noller and Nomura (1987) the protein-protein interaction that is disrupted by the test compound may be determined. The exact protein-protein interaction that is disrupted can be determined using selective combinations of S-proteins added to 16S RNA and compound. As stated above, this is  
5 an important confirmation process since published *in vitro* assembly maps are based on a limited data set. Assembly disruption by the test compound can be independently verified by analytical ultracentrifugation analysis (Fig.4). In this process the partially assembled 30S complex is differentiated from intact complex by displaying a lower rate of sedimentation in a given centrifugal field (i.e., as measured by a lower  
10 sedimentation constant, expressed in Svedberg units or S). The contents of sedimentation clusters can be verified by mass spectrometry.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in  
15 light of the above teachings and, therefore, are within the scope of the invention.

The entire disclosure of all publications cited herein are hereby incorporated by reference.

## CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence that encodes an amino  
5 acid sequence having at least 85% identity with SEQ ID NO:2
2. An isolated nucleic acid comprising the nucleotide sequence having least 85%  
identity with SEQ ID NO:1
- 10 3. An isolated nucleic acid comprising a nucleotide sequence that encodes the amino  
acid sequence of SEQ ID NO:2
4. An isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO:1
- 15 5. An isolated S16 ribosomal polypeptide comprising an amino acid sequence having  
least 85% identity to the sequence of SEQ ID NO:2
6. An isolated S16 ribosomal polypeptide comprising the amino acid sequence of  
SEQ ID NO:2
- 20 7. The isolated S16 ribosomal polypeptide of claim 6 which is labeled
8. The isolated S16 ribosomal polypeptide of claim 7 wherein the label is selected  
from the group consisting of: radiolabels, fluorescent labels, amino acid tags and  
25 biotin
9. The isolated S16 ribosomal polypeptide of claim 8 wherein said S16 ribosomal  
polypeptide comprises a radiolabel
- 30 10. The isolated S16 ribosomal polypeptide of claim 8 wherein said S16 ribosomal  
polypeptide comprises a fluorescent label.
11. The isolated S16 ribosomal polypeptide of claim 8 wherein said S16 ribosomal  
polypeptide comprises an amino acid tag.

12. The isolated S16 ribosomal polypeptide of claim 8 wherein said S16 ribosomal polypeptide comprises a biotin molecule
- 5 13. A vector comprising the nucleic acid of claim 1
14. A host cell comprising the vector of claim 13
15. A method of making isolated an S16 ribosomal polypeptide comprising:
- 10 a) introducing the nucleic acid of claim 1 into a host cell  
b) maintaining said host cell under conditions whereby said nucleic acid is expressed to produce said S16 ribosomal polypeptide  
c) purifying said S16 ribosomal polypeptide
- 15 16. A method for testing for inhibitors of ribosomal assembly comprising the steps of:
- a.) contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15, S17 and S20 with 16S ribosomal RNA to form a polyribonucleotide protein complex and;
- b) contacting said polyribonucleotide protein complex with S16 ribosomal polypeptide
- 20 (i) in the presence of a test agent; and  
(ii) in the absence of said test agent; and
- c) determining the amount of S16 ribosomal polypeptide bound to the polyribonucleotide protein complex
- 25 (i) in the presence of a test agent; and  
(ii) in the absence of said test agent; and
- d) comparing the amount of S16 ribosomal polypeptide determined in step (c)(i) to the amount of S16 ribosomal polypeptide determined in step (c)(ii);
- 30 17. The method of claim 16 wherein the direct binding ribosomal proteins comprise S4, S7, S8.

18. The method of claim 16 wherein the direct binding ribosomal proteins comprise S4, S7, S8 and S17.
19. The method of claim 16 wherein the direct binding ribosomal proteins comprise  
5 S4, S7, S8, S17, S15.
20. The method of claim 16 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17, S15 and S20
- 10 21. The method of claim 16 wherein the S16 ribosomal polypeptide is labelled
22. The method of claim 16 wherein S16 ribosomal polypeptide comprises a radiolabel
- 15 23. The method of claim 16 wherein S16 ribosomal polypeptide comprises an amino acid tag.
24. The method of claim 16 wherein wherein S16 ribosomal polypeptide comprises  
comprises a biotin molecule  
20
25. The method of claim 16 wherein said 16S ribosomal RNA is labeled
26. The method of claim 16 wherein said 16S ribosomal RNA comprises a radiolabel
- 25 27. The method of claim 16 wherein said 16S ribosomal RNA comprises a biotin molecule
28. The method of claim 16 wherein wherein S16 ribosomal polypeptide is attached to  
a solid support.  
30
29. The method of claim 16 wherein said 16S ribosomal RNA is attached to a solid support

30. A method for testing for inhibitors of ribosomal assembly comprising the steps of:
- 5 a.) contacting at least one direct binding ribosomal polypeptide selected from the group consisting of S4, S7, S8, S15, S17 and S20 with 16S ribosomal RNA to form a polyribonucleotide protein complex and;
- b) contacting said polyribonucleotide protein complex with at least one non-direct binding ribosomal polypeptide selected from the group consisting of S1, S2, S3, S5, S6, S9, S10, S11, S12, S13, S14, S16, S18, S19, and S21.
- 10 (iii) in the presence of a test agent; and  
(iv) in the absence of said test agent; and
- c) determining the amount of at least one non- direct binding ribosomal polypeptide bound to the RNA
- (i) in the presence of a test agent; and  
15 (ii) in the absence of said test agent; and
- d) comparing the amount of least one non direct binding ribosomal polypeptide in step (c)(i) to the amount of non-direct binding ribosomal polypeptide protein determined in step (c)(ii);
- 20
31. The method of claim 30 wherein the direct binding ribosomal proteins comprise S4, S7, S8.
32. The method of claim 30 wherein the direct binding ribosomal proteins comprise  
25 S4, S7, S8 and S17.
33. The method of claim 30 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17, S15.
- 30 34. The method of claim 30 wherein the direct binding ribosomal proteins comprise S4, S7, S8, S17, S15 and S20

35. The method of claim 30 wherein the non-direct binding ribosomal proteins  
comprise S16
36. The method of claim 30 wherein the non-direct binding ribosomal proteins  
comprise S3, S5, S9, S10, S12, S14, S16 and S19
- 5
37. The method of claim 30 wherein said direct binding or non-direct binding  
ribosomal polypeptide is labeled
38. The method of claim 30 wherein said direct binding or non-direct binding  
10 ribosomal polypeptide comprises a radiolabel
39. The method of claim 30 wherein said direct binding or non-direct binding  
ribosomal polypeptide comprises an amino acid tag.
- 15 40. The method of claim 30 wherein said said direct binding or non-direct binding  
ribosomal polypeptide comprises a biotin molecule
41. The method of claim 30 wherein said 16S ribosomal RNA is labeled
- 20 42. The method of claim 30 wherein said 16S ribosomal RNA comprises a radiolabel
43. The method of claim 30 wherein said 16S ribosomal RNA comprises a biotin  
molecule
- 25 44. The method of claim 30 wherein said direct binding or non-direct binding  
ribosomal polypeptide is attached to a solid support.
45. The method of claim 30 wherein said 16S ribosomal RNA is attached to a solid  
support
- 30
46. A method for testing for inhibitors of ribosomal assembly comprising the steps of:  
a.) contacting S4, S7, S8, S17 and S20 ribosomal polypeptides with 16S  
ribosomal RNA to form a polyribonucleotide protein complex and;

b) contacting said polyribonucleotide protein complex with non- direct binding ribosomal polypeptides S3, S5, S9, S10, S12, S14, S16 and S19 to form a resultant polyribonucleotide protein complex

(v) in the presence of a test agent; and

5

(vi) in the absence of said test agent; and

b) contacting non-direct binding ribosomal polypeptide S3 with said resultant polyribonucleotide protein complex; and

c) determining the amount of said non-direct binding ribosomal polypeptide S3 bound to said resultant polyribonucleotide protein complex;

10

(i) formed in the presence of said test agent; and

(ii) formed in the absence of said test agent; and

e) comparing the amount of S3 determined in step (c)(i) to the amount of S3 determined in step (c)(ii)

47. The method of claim 46 wherein said non-direct binding ribosomal polypeptide S3 is labeled.

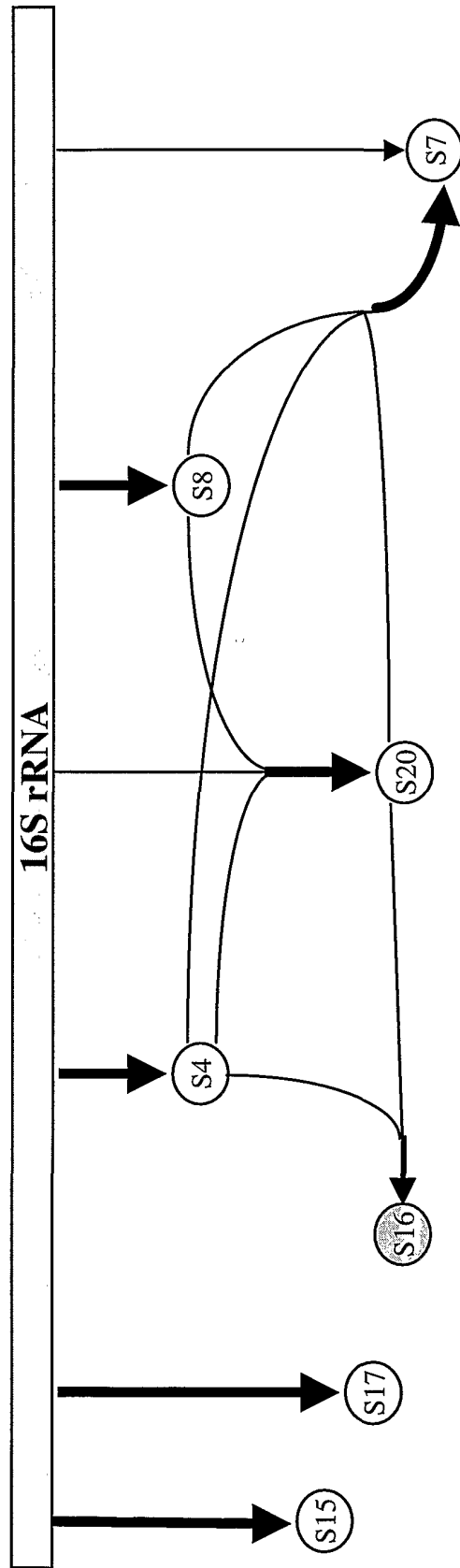
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48. The method of claim 47 wherein said non-direct binding ribosomal polypeptide S3 is radiolabeled

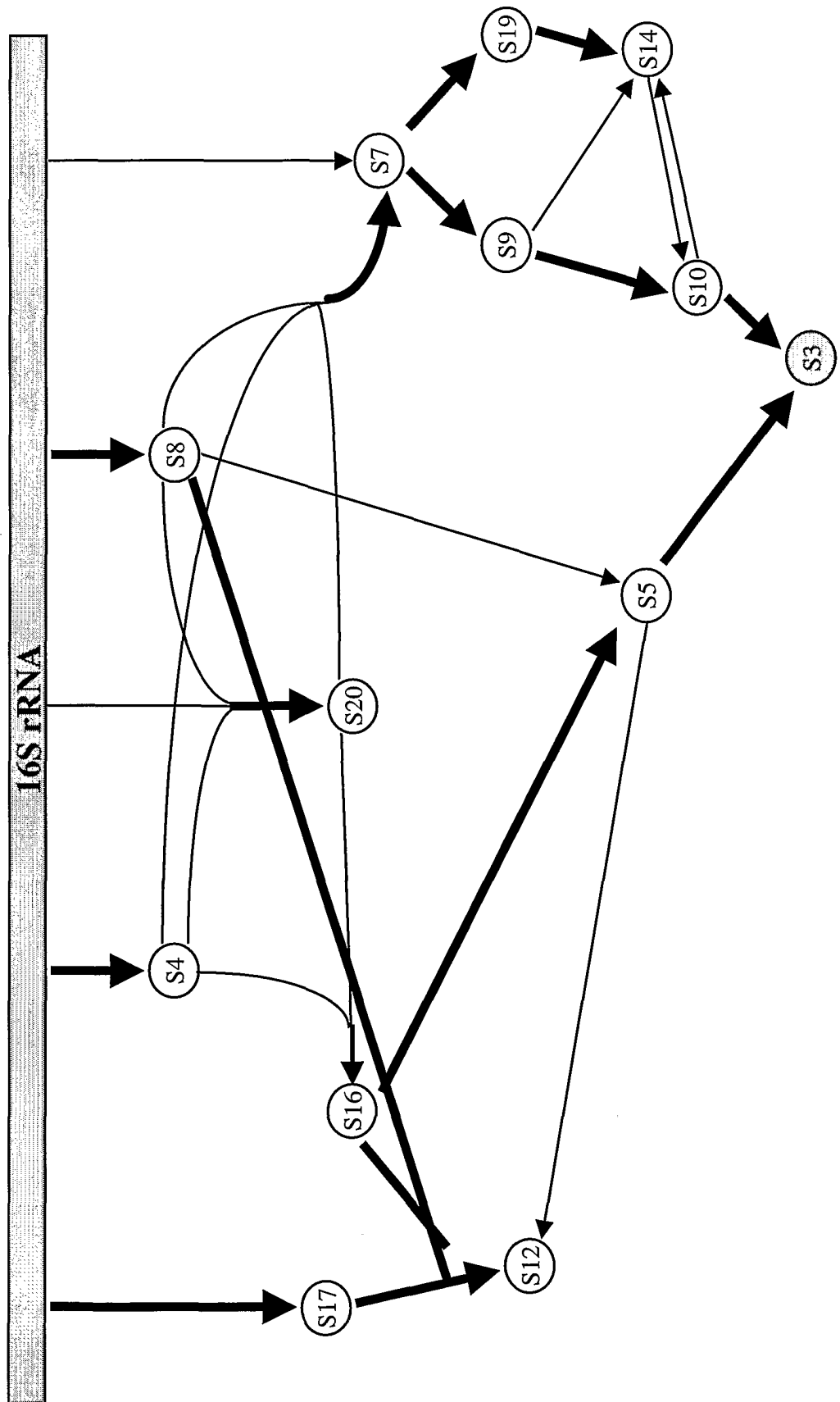
*Figure 1*

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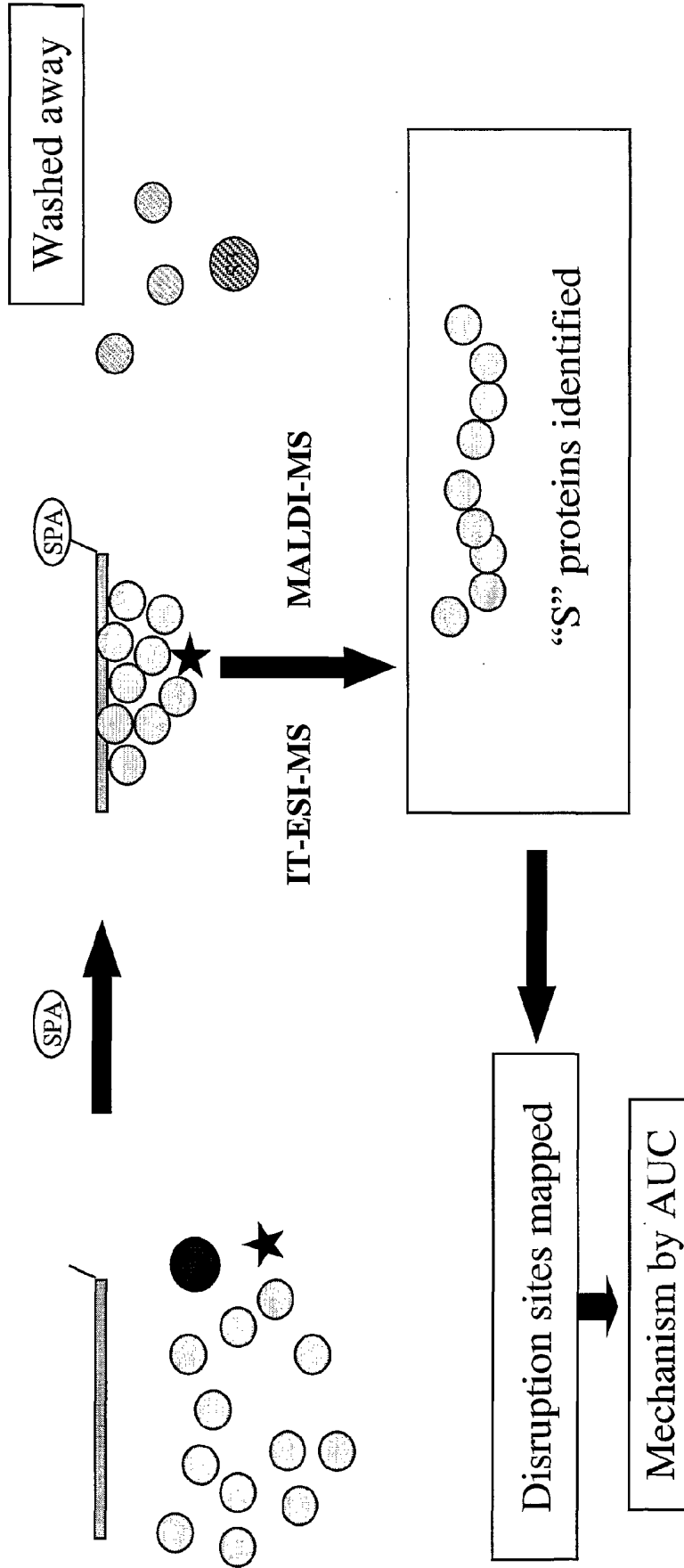
*Figure 2*



*Figure 3*



*Figure 4*



SEQUENCE LISTING

<110> Pearson, James D.  
 Slightom, Jerry  
 Chosay, John G.  
 Shinabarger, Dean L.

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 gtggttggtat tatccgtgat tgggaagcta aatggatgac tgaaaaagac ttcgcttcac 180  
 ttttacacga agatttaaaa atccgtaaat ttattgataa tgaattaaaa gaagcatcag 240  
 tttctcacgt agagattgaa cgtgctgcaa accgatatcaa cattgcaatt catactggta 300  
 aacctggtat ggtaattggg aaagcgggtt cagaaatcga aaaattacgc aacaaattaa 360  
 atgcggttaac tgataaaaaa gtacacatca acgtaattga aatcaaaaaa gttgatcttg 420  
 acgctcgttt agtagctgaa aacatcgcac gtcaattaga aaaccgtgct tcattccgctc 480  
 gtgtacaaaa acaagcaatc actagagcta tgaacttgg tgctaaaggt atcaaaaactc 540  
 aagtatctgg tcgttttaggc ggagctgaca tcgctcgtgc tgaacaatat tcagaaggaa 600  
 ctgttccact tcatacgtta cgtgctgaca tcgattatgc acacgctgaa gctgacacta 660  
 cttacggtaa attagcgtt aaagtatgga tttatcgtgg agaagttctt cctactaaga 720  
 aactagtgg aggaggaaaa taataatggt actacaaaa cgtgtaaaat atcgtcgtca 780  
 acatcgtcct aaaacaactg 800

<210> 26  
 <211> 221  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 26  
 Met Gly Asn Thr Val Gly Gln Lys Ile Asn Pro Ile Gly Leu Arg Val  
 1 5 10 15  
 Gly Ile Ile Arg Asp Trp Glu Ala Lys Trp Tyr Ala Glu Lys Asp Phe  
 20 25 30  
 Ala Ser Leu Leu His Glu Asp Leu Lys Ile Arg Lys Phe Ile Asp Asn  
 35 40 45  
 Glu Leu Lys Glu Ala Ser Val Ser His Val Glu Ile Glu Arg Ala Ala  
 50 55 60  
 Asn Arg Ile Asn Ile Ala Ile His Thr Gly Lys Pro Gly Met Val Ile  
 65 70 75 80  
 Gly Lys Gly Gly Ser Glu Ile Glu Lys Leu Arg Asn Lys Leu Asn Ala  
 85 90 95  
 Leu Thr Asp Lys Lys Val His Ile Asn Val Ile Glu Ile Lys Lys Val  
 100 105 110  
 Asp Leu Asp Ala Arg Leu Val Ala Glu Asn Ile Ala Arg Gln Leu Glu  
 115 120 125

Asn Arg Ala Ser Phe Arg Arg Val Gln Lys Gln Ala Ile Thr Arg Ala  
 130 135 140

Met Lys Leu Gly Ala Lys Gly Ile Lys Thr Gln Val Ser Gly Arg Leu  
 145 150 155 160

Gly Gly Ala Asp Ile Ala Arg Ala Glu Gln Tyr Ser Glu Gly Thr Val  
 165 170 175

Pro Leu His Thr Leu Arg Ala Asp Ile Asp Tyr Ala His Ala Glu Ala  
 180 185 190

Asp Thr Thr Tyr Gly Lys Leu Gly Val Lys Val Trp Ile Tyr Arg Gly  
 195 200 205

Glu Val Leu Pro Thr Lys Asn Thr Ser Gly Gly Gly Lys  
 210 215 220

<210> 27  
 <211> 639  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 27  
 tcacggacgt gttaaagcat tagctgaagc agcaagagaa agcggattag aattttaatt 60  
 taaaggaggg acaaatacat ggctcgtaga gaagaagaga cgaaagaatt tgaagaacgc 120  
 gttgttaciaa tcaaccgtgt agcaaaagtt gtaaaagggtg gtcgtcgttt ccgtttcact 180  
 gcattagttg tagttggaga caaaaatggt cgtgtaggtt tcgggtactgg taaagctcaa 240  
 gaggtaccag aagcaatcaa aaaagctggt gaagcagcta aaaaagattt agtagttggt 300  
 ccacgtggtg aaggtacaac tccacacaca attactggcc gttacggttc aggaagcgta 360  
 tttatgaaac cggctgcacc tggtagagga gttatcgtctg gtggtcctgt tcgtgccgta 420  
 cttgaattag caggtatcac tgatatctta agtaaatcat taggatcaaa cacaccaatc 480  
 aacatggttc gtgctacaat cgatggttta caaacctta aaaatgctga agatggtgcg 540  
 aaattacgtg gcaaaacagt agaagaatta tacaattaag gagggaaaac tagttatggc 600  
 taaattacaa attacctca ctcgtagtgt tattggtcg 639

<210> 28  
 <211> 166  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 28  
 Met Ala Arg Arg Glu Glu Glu Thr Lys Glu Phe Glu Glu Arg Val Val  
 1 5 10 15

Thr Ile Asn Arg Val Ala Lys Val Val Lys Gly Gly Arg Arg Phe Arg  
 20 25 30

Phe Thr Ala Leu Val Val Val Gly Asp Lys Asn Gly Arg Val Gly Phe  
 35 40 45

Gly Thr Gly Lys Ala Gln Glu Val Pro Glu Ala Ile Lys Lys Ala Val  
 50 55 60

Glu Ala Ala Lys Lys Asp Leu Val Val Val Pro Arg Val Glu Gly Thr  
 65 70 75 80

Thr Pro His Thr Ile Thr Gly Arg Tyr Gly Ser Gly Ser Val Phe Met  
 85 90 95

Lys Pro Ala Ala Pro Gly Thr Gly Val Ile Ala Gly Gly Pro Val Arg  
 100 105 110

Ala Val Leu Glu Leu Ala Gly Ile Thr Asp Ile Leu Ser Lys Ser Leu  
 115 120 125

Gly Ser Asn Thr Pro Ile Asn Met Val Arg Ala Thr Ile Asp Gly Leu  
 130 135 140

Gln Asn Leu Lys Asn Ala Glu Asp Val Ala Lys Leu Arg Gly Lys Thr  
 145 150 155 160

Val Glu Glu Leu Tyr Asn  
 165

<210> 29  
 <211> 499  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 29  
 ggcgatgata taattcttta ttgtgagtaa tgaaaattat tccttgctta tctgttttaa 60  
 gattgataag ccgtatagac cacaaggagg tgcaaataaa aaatgagaac atatgaagtt 120  
 atgtacatcg tacgcccata cattgaggaa gatgctaaaa aagcgtagt tgaacgtttc 180  
 aacggtatct tagctactga aggtgcagaa gttttagaag caaaagactg gggtaaactg 240  
 cgcttagctt atgaaatcaa tgatttcaaa gatggcttct acaacatcgt acgtgtttaa 300  
 tctgataaca acaaagctac tgacgaattc caacgtctag ctaaaatcag tgacgatatc 360  
 attcgttaca tggttattcg tgaagacgaa gacaagtaat aattagaggg ggcgttttaa 420  
 tgctaaatag agttgtatta gtaggtcgtt taacgaaaga tccggaatac agaaccactc 480  
 cctcaggtgt gtagttagc 499

<210> 30  
 <211> 98  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 30  
 Met Arg Thr Tyr Glu Val Met Tyr Ile Val Arg Pro Asn Ile Glu Glu  
 1 5 10 15

Asp Ala Lys Lys Ala Leu Val Glu Arg Phe Asn Gly Ile Leu Ala Thr  
 20 25 30

Glu Gly Ala Glu Val Leu Glu Ala Lys Asp Trp Gly Lys Arg Arg Leu  
 35 40 45

Ala Tyr Glu Ile Asn Asp Phe Lys Asp Gly Phe Tyr Asn Ile Val Arg  
 50 55 60

Val Lys Ser Asp Asn Asn Lys Ala Thr Asp Glu Phe Gln Arg Leu Ala  
 65 70 75 80

Lys Ile Ser Asp Asp Ile Ile Arg Tyr Met Val Ile Arg Glu Asp Glu  
 85 90 95

Asp Lys

<210> 31

<211> 462  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 31  
 gtgcacaaca accagaaaac tacgaattac gtgggtaatt agaaggagga aatgactttg 60  
 gcacaagttg aatatagagg cacaggccgt cgtaaaaact cagtagcacg tgtacgttta 120  
 gtaccagggtg aaggtaacat cacagttaat aaccgtgacg tacgcgaata ctaccattc 180  
 gaatcattaa ttttagactt aaaccaacca tttgatgtaa ctgaaactaa aggtaactat 240  
 gatgttttag ttaacgttca tgggtggtgt ttcactggac aagctcaagc tatccgtcac 300  
 ggaatcgctc gtgcattatt agaagcagat cctgaatata gaggttcttt aaaacgcgct 360  
 ggattactta ctcgtgaccc acgtatgaaa gaacgtaaaa aaccaggctc taaagcagct 420  
 cgtcgttcac ctcaattctc aaaacgttaa ttgtcggacg at 462

<210> 32  
 <211> 132  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 32  
 Met Thr Leu Ala Gln Val Glu Tyr Arg Gly Thr Gly Arg Arg Lys Asn  
 1 5 10 15  
 Ser Val Ala Arg Val Arg Leu Val Pro Gly Glu Gly Asn Ile Thr Val  
 20 25 30  
 Asn Asn Arg Asp Val Arg Glu Tyr Leu Pro Phe Glu Ser Leu Ile Leu  
 35 40 45  
 Asp Leu Asn Gln Pro Phe Asp Val Thr Glu Thr Lys Gly Asn Tyr Asp  
 50 55 60  
 Val Leu Val Asn Val His Gly Gly Gly Phe Thr Gly Gln Ala Gln Ala  
 65 70 75 80  
 Ile Arg His Gly Ile Ala Arg Ala Leu Leu Thr Arg Asp Pro Glu Tyr  
 85 90 95  
 Arg Gly Ser Leu Lys Arg Ala Gly Leu Leu Thr Arg Asp Pro Arg Met  
 100 105 110  
 Lys Glu Arg Lys Lys Pro Gly Leu Lys Ala Ala Arg Arg Ser Pro Gln  
 115 120 125  
 Phe Ser Lys Arg  
 130

<210> 33  
 <211> 441  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 33  
 aggttactga cacaccggc cgctttgcc tggcgctgtg taagatagtt ttcgtggaga 60  
 agtctatcac taaatgtaga cgaataagga gggaaaatta tggcaaaaca aaaaatcaga 120  
 atcagattaa aagcttatga tcaccgcgta attgatcaat cagcagagaa gattgtagaa 180  
 acagcgaaac gttctggtgc agatgtttct ggaccaattc cgttaccaac tgagaaatca 240  
 cgtacacaca aacgtttaat cgatattgta aaccaaacac caaaaacagt tgacgcttta 300  
 atgggcttaa acttaccatc tgggtgtagac atcgaaatca aattataata gacaatttta 360  
 ggaggtggac tttcgtatgac caaaggaatc ttaggaagaa aaattgggat gacacaagta 420

ttcggagaaa acggtgaatt a

441

<210> 34  
 <211> 102  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 34  
 Met Ala Lys Gln Lys Ile Arg Ile Arg Leu Lys Ala Tyr Asp His Arg  
 1 5 10 15  
 Val Ile Asp Gln Ser Ala Glu Lys Ile Val Glu Thr Ala Lys Arg Ser  
 20 25 30  
 Gly Ala Asp Val Ser Gly Pro Ile Pro Leu Pro Thr Glu Lys Ser Val  
 35 40 45  
 Tyr Thr Ile Ile Arg Ala Val His Lys Tyr Lys Asp Ser Arg Glu Gln  
 50 55 60  
 Phe Glu Gln Arg Thr His Lys Arg Leu Ile Asp Ile Val Asn Pro Thr  
 65 70 75 80  
 Pro Lys Thr Val Asp Ala Leu Met Gly Leu Asn Leu Pro Ser Gly Val  
 85 90 95  
 Asp Ile Glu Ile Lys Leu  
 100

<210> 35  
 <211> 594  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 35  
 agttcgtggt caaaaaacga aaaacmacgc gcgtactcgt aaaggaccag ttaaaaacggt 60  
 agctaacaag aaaaaatmat aggtaaagga ggcaaatttt aaatggcacg taaacaagta 120  
 tctcgtaaac gtagagtgaa aaagaatatt gaaaatgggtg tagcacacat ccgttcaaca 180  
 ttcaacaaca ctattgtaac taccactgat gagttcggta atgctttatc atgggcatca 240  
 gctgggtgcat taggattcaa aggatctaaa aaatcaaacac catttgacgc acaaatggct 300  
 tctgaaactg catctaaatc agctatggag catgggtttaa aaacagttga agtaacagtt 360  
 aaaggacctg gtccaggctc tgaatcagct attcgtgcat tacaatctgc aggttttagaa 420  
 gtaactgcga tcagagacgt tactccagta ctcataacg gttgtcgtcc accaaaacgt 480  
 cgtcgtgtat aatttatgat ggtattgtta caggtcactg agcaaacatt ttaaattaag 540  
 tcgacgtata taaggaggat atttaaataga tagaaatcga aaaacctaga attg 594

<210> 36  
 <211> 129  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 36  
 Met Ala Arg Lys Gln Val Ser Arg Lys Arg Arg Val Lys Lys Asn Ile  
 1 5 10 15  
 Glu Asn Gly Val Ala His Ile Arg Ser Thr Phe Asn Asn Thr Ile Val  
 20 25 30  
 Thr Ile Thr Asp Glu Phe Gly Asn Ala Leu Ser Trp Ser Ser Ala Gly

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

<210> 37  
 <211> 620  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 37  
 ttaaagtgaga attagtaagt gttttactta ctaaatttta tttaacctaa aatgaacca 60  
 cctggatgtg tgggattaaa aagtgaagag aggaggacat atcacatgcc aactattaac 120  
 caattagtac gtaaaccaag acaagcaaa atcaaaaaat cagattctcc agctttaaat 180  
 aaaggtttca acagtaaaaa gaaaaaattt actgacttaa actcaccaca aaaacgtggg 240  
 gtatgtactc gtgtaggtagc aatgacacct aaaaaacctt actcagcgtt acgtaaatat 300  
 gcacgtgtgc gtttatcaaa caacatcgaa attaacgcat acatccctgg tatoggacat 360  
 aacttacaag aacacagtggt tgtacttgta cgtgggtggac gtgtaaaaga cttaccaggt 420  
 gtgcgttacc atattgtacg tggagcactt gatacttcag gtgttgacgg acgtagacaa 480  
 ggtcgttcat tatacgggaac taagaaacct aaaaactaag aatttagttt ttaattaaat 540  
 cttaaactta aatatattta tataaggaag ggaggattta cattatgcct cgtaaaggat 600  
 cagtaccta aagagacgta 620

<210> 38  
 <211> 137  
 <212> PRT  
 <213> Staphylococcus aureus

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

85 90 95  
 Gly Gly Arg Val Lys Asp Leu Pro Gly Val Arg Tyr His Ile Val Arg  
 100 105 110  
 Gly Ala Leu Asp Thr Ser Gly Val Asp Gly Arg Arg Gln Gly Arg Ser  
 115 120 125  
 Leu Tyr Gly Thr Lys Lys Pro Lys Asn  
 130 135

<210> 39  
 <211> 633  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 39  
 gtataaaaat gaaagtaaga ccatcagtaa aacctatttg cgaaaaatgt aaagtcatta 60  
 aacgtaaagg taaagtaatg gtaatttgta aaaatccaaa acacaaacaa agacaagggtt 120  
 aataaaagag aggtgtaaata taatatggca cgtattgcag gagtagatat tccacgtgaa 180  
 aaacgcgtag ttatctcatt aacttatata tacggatcgc gtacgtcaac tgctcaaaaa 240  
 attcttgaag aagctaacgt atcagctgat actcgtgtga aagatttaac tgatgacgaa 300  
 ttaggtcgca tccgtgaagt tgtagacggt tataaagtcg aaggtgactt acgtcgtgaa 360  
 actaacttaa atatcaaacg tttaatggaa atttcatcat accgtggtat ccgtcaccgt 420  
 cgtggtttac cagttcgtgg tcaaaaaacg aaaaacaacg cgcgtactcg taaaggacca 480  
 gttaaaacgg tagctaacaa gaaaaaataa taggtaaagg aggcaaattt taaatggcac 540  
 gtaaacaagt atctcgtaaa cgtagagtga aaaagaatat tgaaaatggt gtagcacaca 600  
 tccgttcaac attcaacaac actattgtaa cta 633

<210> 40  
 <211> 121  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 41  
 Met Ala Arg Ile Ala Gly Val Asp Ile Pro Arg Glu Lys Arg Val Val  
 1 5 10 15  
 Ile Ser Leu Thr Tyr Ile Tyr Gly Ile Gly Thr Ser Thr Ala Gln Lys  
 20 25 30  
 Ile Leu Glu Glu Ala Asn Val Ser Ala Asp Thr Arg Val Lys Asp Leu  
 35 40 45  
 Thr Asp Asp Glu Leu Gly Arg Ile Arg Glu Val Val Asp Gly Tyr Lys  
 50 55 60  
 Val Glu Gly Asp Leu Arg Arg Glu Thr Asn Leu Asn Ile Lys Arg Leu  
 65 70 75 80  
 Met Glu Ile Ser Ser Tyr Arg Gly Ile Arg His Arg Arg Gly Leu Pro  
 85 90 95  
 Val Arg Gly Gln Lys Thr Lys Asn Asn Ala Arg Thr Arg Lys Gly Pro  
 100 105 110  
 Val Lys Thr Val Ala Asn Lys Lys Lys  
 115 120

<210> 41  
 <211> 311  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 41  
 ctcgtaatt gttagctaac ttcggtatgc cattccgtaa ataattattt aaaggaggct 60  
 aattaagtgg ctaaaacttc aatggttgct aagcaacaaa aaaaacaaaa atatgcagtt 120  
 cgtgaataca ctcgttgtga acgttgtggc cgtccacatt ctgtatatcg taaatttaaa 180  
 ttatgccgta tttgtttccg tgaattagct tacaaggcc aaatccctgg cgttcgtaaa 240  
 gctagctggg aataaaaaag agtctgaaag gaggcaacaa tcaatgacaa tgacagatcc 300  
 aatcgcatg a 311

<210> 42  
 <211> 61  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 42  
 Met Ala Lys Thr Ser Met Val Ala Lys Gln Gln Lys Lys Gln Lys Tyr  
 1 5 10 15  
 Ala Val Arg Glu Tyr Thr Arg Cys Glu Arg Cys Gly Arg Pro His Ser  
 20 25 30  
 Val Tyr Arg Lys Phe Lys Leu Cys Arg Ile Cys Phe Arg Glu Leu Ala  
 35 40 45  
 Tyr Lys Gly Gln Ile Pro Gly Val Arg Lys Ala Ser Trp  
 50 55 60

<210> 43  
 <211> 710  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 43  
 aacattcata cacctgttaa tattatttct tgtagaaaat aaaaatttaa acatgactta 60  
 aaggagattdt tataaatggc agttaaatt cgtttaacac gtttaggttc aaaaagaaat 120  
 ccattctatc gtatcgtagt agcagatgct cgttctccac gtgacggacg tatcatcgaa 180  
 caaatcggta cttataacc aacgagcgt aatgctccag aaattaaagt tgacgaagcg 240  
 ttagctttaa aatggttaaa tgatggtgcg aaaccaactg atacagttca caatatctta 300  
 tcaaaagaag gtattatgaa aaaatttgac gaacaaaaga aagctaagta atttagcgta 360  
 aaattgttct aacaataaga ataactcgtt tacactgaca gttattactc aatgatacgt 420  
 tgggaatata acatgtagt aatatagaac gtttgggtac cataatggtg ccctttttct 480  
 ttgaattatt ttcaattaaa atagaagtgg tcaaagcata gagttggagg taatagaatg 540  
 agagttgaag ttggtcaaaa ttgtttacac acacggggtt taaaaggtgg aaattaaag 600  
 taaatccatt tcagacctt tacagaccgg ttcggttttc aaccccggtc caaagatgcc 660  
 tgaccagttg ggccttaaac caaattaaac cgacccctt ggaaatatta 710

<210> 44  
 <211> 92  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 44  
 Met Ala Val Lys Ile Arg Leu Thr Arg Leu Gly Ser Lys Arg Asn Pro  
 1 5 10 15



<400> 47  
 aaacttatcg ttcgtggacg taagaaaaaa taatataatc aacttatttg ggtgtgcggc 60  
 ttaaagctgc acgcacataa taagaagga ggcgcccaaa tggctcgtag tattaataaa 120  
 ggacctttcg tcgatgagca tttaatgaaa aaagttgaag ctcaagaagg aagcgaaaag 180  
 aaacaagtaa tcaaaacatg gtcacgtcgt tctacaattt tccctaattt catcggacat 240  
 actttttgcag tatacgacgg acgtaaacac gtacctgtat atgtaactga agatatggta 300  
 ggtcataaat taggtgagtt tgctcctact cgtacattca aaggacacgt tgcagacgac 360  
 aagaaaacaa gaagataata tctattaagt agaggaggac atcctaattg aagcaaaagc 420  
 ggttgctaga acaataagaa tcgcacctcg taaagtaaga ctagttcttg acttaatc 478

<210> 48  
 <211> 92  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 48  
 Met Ala Arg Ser Ile Lys Lys Gly Pro Phe Val Asp Glu His Leu Met  
 1 5 10 15  
 Lys Lys Val Glu Ala Gln Glu Gly Ser Glu Lys Lys Gln Val Ile Lys  
 20 25 30  
 Thr Trp Ser Arg Arg Ser Thr Ile Phe Pro Asn Phe Ile Gly His Thr  
 35 40 45  
 Phe Ala Val Tyr Asp Gly Arg Lys His Val Pro Val Tyr Val Thr Glu  
 50 55 60  
 Asp Met Val Gly His Lys Leu Gly Glu Phe Ala Pro Thr Arg Thr Phe  
 65 70 75 80  
 Lys Gly His Val Ala Asp Asp Lys Lys Thr Arg Arg  
 85 90

<210> 49  
 <211> 520  
 <212> DNA  
 <213> Staphylococcus aureus

<400> 49  
 tgcaaaattt taagctaacc ccatcaaata aatgattgca caacggttag actttttgta 60  
 aaatatttct tgttgtaatc aaataaaatt ttgataagat gaactcactt ttagggaggtg 120  
 gcagaaatgg caaatatcaa atctgcaatt aaacgtgtaa aaacaactga aaaagctgaa 180  
 gcacgcaaca tttcacaaaa gagtgcaatg cgtacagcag ttaaaaacgc taaaacagct 240  
 gtttcaata acgctgataa taaaaatgaa ttagtaagct tagcagttaa gttagtagac 300  
 aaagctgctc aaagtaattt aatacattca acaaaagctg accgtattaa atcacaatta 360  
 atgactgcaa ataaataatc tttttaaata aaagttcaag cgcattgctt aacttttatt 420  
 ttttataaag atagaatgaa taattccagt attaactggt tatccatata tgatgattta 480  
 agtttataat cagttttcgc acaagcatct ataatttca 520

<210> 50  
 <211> 83  
 <212> PRT  
 <213> Staphylococcus aureus

<400> 50  
 Met Ala Asn Ile Lys Ser Ala Ile Lys Arg Val Lys Thr Thr Glu Lys  
 1 5 10 15



<210> 54  
 <211> 36  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:PCR Primer  
  
 <400> 54  
 tataggatcc ttaacggatt aattgttcgt taattt 36

<210> 55  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:PCR Primer  
  
 <400> 55  
 tatattatcg ataatggcag gtggaccaag aag 33

<210> 56  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:PCR Primer  
  
 <400> 56  
 tataggatcc ttattgttct tctttaacat 30

<210> 57  
 <211> 35  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:PCR Primer  
  
 <400> 57  
 tatattatcg ataatgaaga aacatatgaa gttat 35

<210> 58  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:PCR Primer  
  
 <400> 58  
 tataggatcc ttacttgtct tegtcttcac 30