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MICROPARTICLES FROM STREPTOCOCCUS PNEUMONIAE AS VACCINE ANTIGENS

Abstract

An isolated *Streptococcus pneumoniae* membrane vesicle microparticle (MP), wherein said MP comprises: the protein Ply at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; and/or the protein LytA at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; and/or the protein PspC at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP; and/or the protein RrgB at the level of ≥ 0.020 $\mu\text{g}/\text{mg}$ total protein in the MP. Compositions comprising such MPs. Uses thereof in particular in immunization, as well as methods of manufacture thereof.

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS [0001] This application is a continuation of U.S. application Ser. No. 18/212,857, filed on Jun. 22, 2023, which is a continuation of U.S. application Ser. No. 16/474,940, filed on Jun. 28, 2019, which is a National Stage application under 35 U.S.C. § 371 of International Application No. PCT/SE2017/051323, having an International Filing Date of Dec. 21, 2017, which claims the benefit of SE Application No. 1651746-8 filed Dec. 28, 2016. The contents of the aforementioned applications are hereby incorporated by reference in their entirety.

TECHNICAL FIELD

[0002] The present invention relates to the field of vaccines against *Streptococcus pneumoniae*.

BACKGROUND TO THE INVENTION

[0003] *Streptococcus pneumoniae* is a Gram-positive bacterium that is a major contributor to morbidity and mortality worldwide causing about 2 million deaths annually, i.e. the same range as for tuberculosis. Pneumococci are the major cause of common infections such as sinusitis, otitis, and community-acquired pneumonia (CAP), but also a common cause of severe invasive diseases (IPD) such as sepsis and meningitis. Young children and the elderly are the most susceptible age groups to acquire pneumococcal infections, but other factors such as underlying diseases, splenectomy, immunosuppression (including HIV), diabetes and a prior influenza-virus infection also sensitize for a pneumococcal infection. Pneumococcal infections such as CAP also predispose for cardiovascular diseases such as cardiac infarction, and meningitis patients may get neurological sequelae such as hearing loss and cognitive impairments.

[0004] Pneumococci have the human upper airways as their normal ecological niche, and up to 60% of preschool children may be colonized at any given time without symptoms. It is believed that most transmission events to the elderly come from young colonized children. *S. pneumoniae* is a highly genetically diverse species due to an efficient DNA exchange system resulting in a multitude of clonal lineages that may differ from one another in the presence and absence of genes and gene clusters, as well as in variations in individual genes.

[0005] Variations in the capsular locus, the major virulence factor and vaccine target of pneumococci, have resulted in at least 97 known capsular serotypes with different potential of causing disease.

[0006] Pneumococcal conjugate vaccines (PCVs), targeting a limited number (7, 10 or 13 in PCV7, PCV10 and PCV13 respectively) of the known 97 capsular serotypes, have been introduced in the childhood vaccination program in many countries. Vaccine introduction has led to a decrease in IPD caused by vaccine-types in vaccinated children, however, non-vaccine type pneumococci have rapidly expanded and replaced vaccine-types in childhood carriage and in IPD, but also in non-vaccinated population such as the elderly. Furthermore, protection against vaccine types differ, and for serotype 3, a serotype included in PCV13 and with a high mortality rate, there is more or less no evidence for protection against IPD so far. Data from Sweden, where both PCV10 and PCV 13 are used, show that serotype 3 was a dominating serotype among IPD cases during 2016, also in

countries using only PCV13. Hence, novel vaccine approaches are needed.

[0007] In Sweden the total number of patients with IPD has remained almost the same after vaccine introduction. Moreover, in a recent study we found that pneumococcal carriage rates remained around 30% in the youngest children 8 years after vaccine introduction in Stockholm, and non-vaccine types were found in over 90% of the isolates. Antibiotic resistance is emerging among clinical pneumococcal isolates and according to reports from the European Centre for Disease prevention and Control (ECDC), and the European Antimicrobial Resistance Surveillance Network (EARS-Net), reduced susceptibility to penicillin has increased during the last years in Sweden and now exceeds 5% among IPD cases. In addition, the need for multiple antigens covering various capsular serotypes lead to high production costs for the known vaccines.

[0008] Opsonophagocytosis is the primary mechanism for clearance of pneumococci from the host, and the measurement of opsonophagocytic antibodies appears to correlate with vaccine-induced protection. However, it has proven difficult to design vaccines that elicit an efficient response in form of opsonophagocytic antibodies.

[0009] Taken together, the above issues stress the need for novel vaccine approaches. Thus, an object of the present invention is the provision of improved pneumococcal antigens able to confer serotype-independent immunological responses and/or improved response in terms of opsonophagocytic antibodies and protection in experimental models.

Background for Pneumococcal Microparticles

[0010] Spherical membranous blebs, so called outer membrane vesicles (OMVs), are produced by Gram-negative bacteria. OMVs range in size from 10 to 300 nm and they have been shown to be formed by budding from the bacterial outer membrane, and to have many functions, such as influencing virulence by different mechanisms. In Gram-negative bacteria OMVs have been suggested to act as vehicle to deliver virulence factors to host cells. Only recently, membrane vesicles (MV.sub.L) were discovered also in Gram-positive bacteria and their formation and function is poorly understood compared to OMVs. Gram-positive bacteria do not contrain an outer membrane and the the cell wall differs from Gram-negative bacteria.

[0011] For *Streptococcus pneumoniae* MV.sub.L (membrane vesicle) were isolated from pneumococci belonging to different serotypes (2, 6B, 8 and 23F) grown in liquid culture medium (1). MV.sub.L from serotype 8 were shown to be protective against pneumococcal challenge with the same serotype in mice. Importantly, no cross protection was shown to pneumococci of other serotypes.

Definitions

[0012] The pneumococcal capsule. The pneumococcal capsule, consisting of polysaccharide, exists in at least 97 different variants, so called capsular serotypes. The pneumococcal capsule is a major virulence determinant of pneumococci, affecting phagocytosis of the bacteria by host cells, and influence bacterial interactions with the innate immune response.

[0013] Pneumolysin (termed Ply herein) is a 53 kDa cholesterol dependent cytolysin released by *Streptococcus pneumoniae* upon lysis. It is one of the major virulence factors of this bacterium. It forms pores in all eukaryotic cells that have cholesterol in their membranes. The formation of pores by Ply frequently results in host cell death as membrane integrity is destroyed. Ply plays a central role in protecting the pneumococcus from complement attack and aiding its spread to other tissues/organs. Ply is able to activate the classical complement pathway, even in the absence of Ply specific antibody (2). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 1.

[0014] LytA is the major autolysin of *Streptococcus pneumoniae*. Lysis is caused by cleaving the lactyl-amide bond between the stem peptides and the glycan strands of peptidoglycan, resulting in hydrolysis of the cell wall. The contribution of LytA to pneumococcal virulence is still unclear. It is possible that LytA-mediated lysis releases other virulence factors such as pneumolysin. LytA could also be released to lyse neighboring non-competent pneumococcal cells in a fratricidal manner. This would potentially facilitate genetic exchange between naturally competent pneumococcal

populations that easily take up and incorporate DNA by homologous recombination. A third possibility is that LytA mediates lysis to release proteins involved in immune evasion or cell wall components that may interfere with the host immune response (3). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 2.

[0015] PspC/CbpA (choline binding protein A) is a protein that binds the phosphocholine present in the teichoic acid and the lipoteichoic acid of the cell membrane and the cell wall. It is a major pneumococcal adhesin. It promotes pneumococcal adherence via a human-specific interaction with the ectodomain of the polymeric Ig receptor. It also prevents activation of C3b and complement-mediated opsonophagocytosis of pneumococci (4). The *pspC* locus is highly polymorphic and 11 major groups of this protein have been identified. Single PspC proteins are identified by sequential numbers separated from the group number by a dot (5) as follows: PspC1.1, PspC2.1, PspC2.2, PspC3.1, PspC3.4, PspC4.2/PspC10.1, PspC5.1, PspC6.1/PspC9.1, PspC7.1, PspC8.1, PspC11.1 and PspC11.4 (SEQ ID NOs: 3-14, respectively).

[0016] RrgB is the major subunit and stalk protein of the pneumococcal pilus. *S. pneumoniae* pilus 1 is encoded by a genetic islet (PI-1) present in 30 to 50% of the pneumococcal strains and is implicated in adhesion to epithelial cells, lung infection, and virulence. Pilus 1 is composed of the backbone subunit RrgB, the minor pilin subunits RrgA, and RrgC (6). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 15.

[0017] RrgA is a minor pilin subunit of the pneumococcal pilus. RrgA is the tip protein of the pilus and has been shown to mediate adhesion to epithelial cells (6, 7). Recently, RrgA was shown to promote meningitis development in mice (8). A reference sequence is presented in SEQ ID NO: 16.

[0018] PhtD is a polyhistidine triad protein of *S. pneumoniae*. Polyhistidine triad (Pht) proteins are streptococcal surface proteins that contain multiple copies of a characteristic HxxHxH sequence, designed as histidine triads, which were predicted to bind divalent metal cations. Despite the increasing amount of biochemical, structural and physiological data, the functional role of PhtD and the other Pht proteins remains unclear. PhtD could be involved in zinc uptake. Alternatively, Pht proteins could play a role in protecting the pneumococcus from toxic effects of high Zn.sup.2+ concentrations by scavenging, storing or trapping Zn.sup.2+ ions (9). A reference sequence from strain TIGR4 is presented in SEQ ID NO: 17.

[0019] IgA refers to Immunoglobulin A1 protease of *S. pneumoniae* (10). Said IgA1-protease is a highly specific enzyme that cleaves amino acid sequences of certain proteins. The natural substrate of the IgA protease is immunoglobulin A1, hence its name. The enzyme is in fact capable of cleavage of proteins with the amino acid sequence N—X-Z-Pro-Pro/-Y-Pro-C, where the X in the sequence is preferably a Proline or Serine; the Y=Threonine, Serine or Alanine; and Z preferably is Arginine or Threonine. Thus, the IgA1 protease acts by cleaving the proline-rich hinge region of the heavy chain of IgA1. Release of the IgA1 protease by the pathogen allows adherence to mucous membranes by degrading host immunoglobulin A. A reference sequence from strain TIGR4 is presented in SEQ ID NO: 18.

[0020] The term protective immunity in the present context refers to immunization measures resulting in any degree of reduction in the likelihood of developing the condition for which the protective immunity is relevant, including a minor, substantial or major reduction in likelihood of developing the condition as well as total prevention. Preferably, the degree of likelihood reduction is at least a minor reduction.

[0021] The term sequence identity expressed in percentage is defined as the value determined by comparing two optimally aligned sequences over a comparison window, wherein a portion of the sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of

comparison and multiplying the result by 100 to yield the percentage of sequence identity. Unless indicated otherwise, the comparison window is the entire length of the sequence being referred to. In this context, optimal alignment is the alignment produced by the BLASTP algorithm as implemented online by the US National Center for Biotechnology Information (see The NCBI Handbook [Internet], Chapter 16), with the following input parameters: Word length=3, Matrix=BLOSUM62, Gap cost=11, Gap extension cost=1.

Description

BRIEF DESCRIPTION OF THE FIGURES

[0022] FIGS. 1A through 1D: Membrane-derived vesicles and microparticles produced by *S. pneumoniae* TIGR4 (T4). (FIG. 1A) Electron micrographs and atomic force micrographs (insets) of membrane vesicles and microparticles after isolation and density gradient purification from liquid culture (MV.sub.L) and microparticles from plate-grown (MP) bacteria. (FIG. 1B) Size distribution of MV.sub.L MPs from TIGR4 and MPs from serotype 3. (FIG. 1C) Electron micrograph of a membrane budding from *S. pneumoniae* T4R and (FIG. 1D) the same electron micrograph in larger magnification. Released particle is indicated by arrow.

[0023] FIGS. 2A through 2D: Identification of proteins present in pneumococcal MV.sub.L and MP. (FIG. 1A) Preparations of MV.sub.L and MP were separated by SDS-PAGE and proteins visualized by coomassie staining. In comparison, proteins present in a whole cell lysate (WC) and supernatant (SN) of a liquid bacterial culture were visualized. (FIG. 1B) Venn diagram displaying the number of proteins identified by mass spectrometry (repeated twice) exclusively found in MV.sub.L and MP preparations and common to both. (FIG. 1C) Mass spectrometry identification of proteins from MV.sub.L and MP. Numbers indicate percentages of proteins based on their subcellular localization, defined as cytosolic proteins, membrane-associated proteins (lipoproteins), proteins with one transmembrane domain (N-terminally anchored), transmembrane proteins, cell wall associated proteins and secreted proteins. (FIG. 1D) Immunoblot detection of pneumococcal proteins and virulence factors present in MV.sub.L and MP isolated from *S. pneumoniae* T4 WT, pneumolysin-(T4 Δ ply) and LytA-deficient (T4 Δ lytA) strains.

[0024] FIG. 3: Pneumolysin associated with pneumococcal MV.sub.L and MP is biologically active. Quantification of in vitro hemolytic activity of purified MV.sub.L and MP isolated from *S. pneumoniae* T4 and T4 Δ ply. Erythrocytes from buffy coat blood were incubated for 60 minutes with different concentrations (0.1, 0.8, 2, 6 and 10 μ g protein) of MV.sub.L and MP. As control treatments, erythrocytes were incubated with PBS (-) or 1% Triton X-100 (+). Data are represented as means \pm SEM of three independent experiments. *=P<0.05; **=P<0.01; ***=P<0.001.

[0025] FIGS. 4A through 4E: Microparticles (MP) are internalized by A549 epithelial cells.

[0026] (FIGS. 4A, 4B and 4C) Immunofluorescence pictures of A549 cells treated with MP (10 μ g/ml) for 24 hours. Cells were stained for F-actin, MP are detected with anti-pneumolysin and LytA antibodies. (FIG. 4A) Z-stack image (nr. stacks=21) of MP-treated cells. Internalized MP are indicated by arrows. (FIGS. 4B and 4C) Orthogonal views of the same picture, taken at stack nr. 11, for the two indicated MP, further showing their intracellular localization.

[0027] (FIG. 4D) Immunoblot detection of anti-Ply, and anti- β -actin as loading control, in A549 cell lysates after incubation with increasing concentrations (1, 2, 3 μ g/ml) of MP. As control treatment, A549 cells were incubated with PBS (-). (FIG. 4E) Viability of A549 cells examined by flow cytometry of fixable viability dye (FVD) positive cells after 24 hours of incubation with MV.sub.L or MP. As control treatment, A549 cells were incubated with PBS (-) or 0.02% NP40 in PBS. Data are represented as means \pm SEM of three independent experiments.

[0028] FIGS. 5A through 5C: MV.sub.L and microparticles (MP) activate human monocyte-derived dendritic cells (DCs). (FIG. 5A) Percentages of FVD and Annexin V-positive cells analyzed by

flow cytometry. Cells were incubated for 24 hours with different concentrations (10, 25, 50 $\mu\text{g/ml}$) of MV.sub.L, MP, PBS (-) or the unencapsulated mutant of TIGR4 (T4R). Data are represented as means \pm SEM of three independent experiments. $\ast=P<0.05$. (FIG. 5B) Immunoblot detection of anti-Ply, and anti- β -actin as loading control, in cell lysates after incubation of DCs with MP (4 $\mu\text{g/ml}$) in presence or absence of the inhibitors Cytochalasin D and Wortmannin (C/W), to block active phagocytosis, or methyl- β -cyclodextrin (MBCD), to block lipid rafts and membrane fusion. Numbers represent the relative intensity of bands corresponding to Ply, adjusted to the loading control bands, in three independent experiments. (FIG. 5C) DC activation measured by flow cytometry of MHCII and CD86-positive cells after 24 hours incubation with different concentrations (2.5, 5 $\mu\text{g/ml}$) of MV.sub.L, MP, PBS (-) or LPS (1 $\mu\text{g/ml}$). Data are represented as means \pm SEM of three independent experiments. $\ast=P<0.05$; $\ast\ast\ast=P<0.001$.

[0029] FIGS. 6A through 6D: Cytokine release by human monocyte-derived dendritic cells upon incubation with MV.sub.L or MP. (FIG. 6A) IL-6, (FIG. 6B) IL-8, (FIG. 6C) IL-10 and (FIG. 6D) TNF released by dendritic cells after 24 hours of incubation with different concentrations (10, 25, 50 $\mu\text{g/ml}$) of MV.sub.L or MP. Control treatments include PBS (-), the unencapsulated mutant of TIGR4 (T4R) and LPS (1 $\mu\text{g/ml}$). Not detectable (n.d.) amounts of cytokines are indicated. Data are represented as means \pm SEM of three independent experiments. $\ast=P<0.05$; $\ast\ast=P<0.01$; $\ast\ast\ast=P<0.001$; $\ast\ast\ast\ast=P<0.0001$.

[0030] FIGS. 7A through 7E: Intranasal immunization of C57BL/6 mice with MV.sub.L or MP of *S. pneumoniae* TIGR4 increase survival against intranasal infection with serotype 1. [0031] (FIG. 7A) Percentage of mice survived after challenge. 20 mice per group. $\ast=P<0.05$; $\ast\ast=P<0.01$; $\ast\ast\ast\ast=P<0.0001$. (FIG. 7B) CFUs in lungs of mice after sacrifice. Each dot represents one mouse. $\ast\ast=P<0.01$; $\ast\ast\ast\ast=P<0.0001$. (C-E) CFUs in blood of mice. Each line represents one mouse.

[0032] FIGS. 8A through 8D: Intranasal immunization of mice with MV or MPs stimulate the production of pneumococcal-specific IgG. Detection of MV-reactive (FIG. 8A), MP-reactive (FIG. 8B), T4-reactive (FIG. 8C) and T4R-reactive (FIG. 8D) mouse IgG in immunized mice sera using ELISA assay. Each dot represents one mouse serum. $\ast=P<0.05$; $\ast\ast=P<0.01$; $\ast\ast\ast=P<0.001$; $\ast\ast\ast\ast=P<0.0001$.

[0033] FIGS. 9A and 9B: Staining of serotypes 1, 3 and 6B using antibodies raised against MP from a serotype 4 strain. (FIG. 9A) Immunofluorescence pictures of pneumococcal strains belonging to serotypes 1, 3 and 6B stained using sera, as primary antibodies, from mice immunized either with MV.sub.L+adjuvant or MP+adjuvant. The white spots on the bacterial surface indicates the presence of specific antibodies against pneumococci in the sera of the immunized mice. (FIG. 9B) Quantification of the signal (white spots) detected on the bacteria; the signal ratio was calculated by dividing the area of the signal detected on the bacteria (white spots) by the total area detected occupied by the bacteria. The area covered by the bacteria and the area covered by the signal detected on the bacteria after staining with sera were selected, defined and measured using the functions Image>Adjust>Threshold and Analyze>Measure of ImageJ. $\ast=P<0.05$; $\ast\ast=P<0.01$; $\ast\ast\ast=P<0.001$.

[0034] FIGS. 10A and 10B: Opsonophagocytic activity of antibodies in mice sera. (FIG. 10A) RAW mouse macrophages were incubated with *Streptococcus pneumoniae* type 1, pre-incubated with mouse sera from the adjuvant or MP+adjuvant groups. Indicated are the ratios of adherence of bacteria to cells, or uptake of bacteria inside cells. Data are represented as means \pm SEM of three independent experiments. $\ast=P<0.05$. (FIG. 10B) RAW mouse macrophages have been incubated with *Streptococcus pneumoniae* type 1, pre-incubated with mouse sera from the adjuvant or MP+adjuvant groups. Indicated is the percentage of taken up bacteria surviving inside the cells after 1 hour. Data are represented as means \pm SEM of three independent experiments. $\ast=P<0.05$.

[0035] FIGS. 11A through 11E: Intranasal immunization of C57BL/6 mice with MP of *S. pneumoniae* increases survival against intranasal infection with serotype 3. In particular,

immunization with MPs of serotype 3 confers 100% protection against serotype 3 infection in comparison to PCV13 immunization, which confers 75% protection. B-cell deficient mice immunized with MPs of serotype 3 are not protected against intranasal infection with serotype 3, strongly suggesting that protection provided by MPs is antibody-dependent.

[0036] (FIG. 11A) Percentage of mice that survived after pneumococcal challenge. 10 mice in the group of “B Cell-(deficient mice)”, 10 mice in the group “Adjuvant”, 20 mice per all other groups. $^{**}=P<0.05$; $^{****}=P<0.0001$. (FIG. 11B) CFUs in lungs of mice after sacrifice. Each dot represents one mouse. $^{***}=P<0.001$. (FIGS. 11C-11E) CFUs in blood of mice. Each line represents one mouse.

SUMMARY OF THE INVENTION

[0037] The present invention relates to the following items. The subject matter disclosed in the items below should be regarded disclosed in the same manner as if the subject matter were disclosed in patent claims. [0038] 1. An isolated *Streptococcus pneumoniae* membrane vesicle microparticle (MP), wherein said MP comprises: [0039] i. the protein Ply at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; [0040] ii. the protein LytA at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; [0041] iii. the protein PspC at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP; or [0042] iv. the protein RrgB at the level of ≥ 0.020 $\mu\text{g}/\text{mg}$ total protein in the MP. [0043] 2. The microparticle according to item 1, further comprising a capsular polysaccharide of a capsular serotype of *Streptococcus pneumoniae*, preferably at a level of ≥ 0.001 , more preferably ≥ 0.01 , most preferably ≥ 0.1 $\mu\text{g}/\mu\text{g}$ total protein in the MP. [0044] 3. The microparticle according to any of the preceding items, comprising the protein Ply at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP. [0045] 4. The microparticle according to any of the preceding items, comprising the protein Ply at the level of ≥ 0.15 , preferably ≥ 0.2 , more preferably ≥ 0.3 , most preferably ≥ 0.35 $\mu\text{g}/\text{mg}$ total protein in the MP. [0046] 5. The microparticle according to any of the preceding items, comprising the protein LytA at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP. [0047] 6. The microparticle according to any of the preceding items, comprising the protein LytA at the level of ≥ 0.08 , preferably ≥ 0.09 , yet more preferably ≥ 0.10 , most preferably ≥ 0.20 $\mu\text{g}/\text{mg}$ total protein in the MP. [0048] 7. The microparticle according to any of the preceding items, comprising the protein PspC at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP. [0049] 8. The microparticle according to any of the preceding items, comprising the protein PspC at the level of ≥ 0.15 , preferably ≥ 0.18 , more preferably ≥ 0.20 , most preferably ≥ 0.3 $\mu\text{g}/\text{mg}$ total protein in the MP. [0050] 9. The microparticle according to any of the preceding items, comprising the protein RrgB at the level of ≥ 0.020 $\mu\text{g}/\text{mg}$ total protein in the MP. [0051] 10. The microparticle according to any of the preceding items, comprising the protein RrgB at the level of ≥ 0.022 , preferably ≥ 0.025 , most preferably ≥ 0.028 $\mu\text{g}/\text{mg}$ total protein in the MP. [0052] 11. The microparticle according to any of the preceding items, further comprising the protein PhtD. [0053] 12. The microparticle according to any of the preceding items, comprising the protein PhtD at a level being at least 2-fold, more preferably at least 2.5-fold compared to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of $\mu\text{g}/\text{mg}$ total protein in the particle or the vesicle, respectively. [0054] 13. The microparticle according to any of the preceding items, further comprising the protein RrgA. [0055] 14. The microparticle according to any of the preceding items, comprising the protein RrgA at the level of ≥ 0.02 , preferably ≥ 0.05 , more preferably ≥ 0.10 , most preferably ≥ 0.2 $\mu\text{g}/\text{mg}$ total protein in the MP. [0056] 15. The microparticle according to any of the preceding items, comprising the protein IgA. [0057] 16. The microparticle according to any of the preceding items, comprising the protein IgA at the level of >0.02 , preferably ≥ 0.05 , more preferably ≥ 0.10 , most preferably ≥ 0.2 $\mu\text{g}/\text{mg}$ total protein in the MP. [0058] 17. The microparticle according to any of the preceding items, wherein the protein Ply comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 1. [0059] 18. The microparticle according to any of the preceding items, wherein the protein LytA comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more

preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 2. [0060] 19. The microparticle according to any of the preceding items, wherein the protein PspC comprises a sequence having at least 40%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NOs: 3. [0061] 20. The microparticle according to any of the preceding items, wherein the protein PspC comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to any one of SEQ ID NOs: 3-14. [0062] 21. The microparticle according to any of the preceding items, wherein the protein RgrB comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 15. [0063] 22. The microparticle according to any of the preceding items, wherein the protein PhtD comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 16. [0064] 23. The microparticle according to any of the preceding items, comprising the protein RgrA, wherein the protein RgrA comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 17. [0065] 24. The microparticle according to any of the preceding items, comprising the protein IgA, wherein the protein IgA comprises a sequence having at least 80%, preferably 85%, more preferably 90%, yet more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 18. [0066] 25. The microparticle according to any of the preceding items, wherein the MP is 5-300 nm in diameter, preferably 15-175 nm. [0067] 26. The microparticle according to any of the preceding items, wherein the MP is 10-125 nm in diameter. [0068] 27. The microparticle according to any of the preceding items, wherein the MP is derived from a *Streptococcus pneumoniae* strain selected from any serotype 1 strain, any serotype 3 strain, TIGR4, P1031 and A66, preferably TIGR4. [0069] 28. The microparticle according to any of the preceding items, obtainable by: [0070] a. Culturing host cells of a *Streptococcus pneumoniae* strain on blood agar plates; [0071] b. harvesting the cultured host cells; [0072] c. centrifuging the harvested host cells at 17,000 g for 30 minutes at +4°C; [0073] d. subjecting the supernatant to filtration through a 0.22 µm filter; [0074] e. centrifuging the filtered supernatant at 120,000 g for 2 h at +4°C; [0075] f. washing the pellets from e) twice in phosphate-buffered saline with sedimentations at 120,000 g for 2 h at +4°C; [0076] g. resuspending the pellet in 1 ml phosphate-buffered saline; [0077] h. adjusting the resuspended pellet to 50% (w/v) Optiprep™ density gradient medium in a total volume of 2 ml and overlaying with one fraction of 30% (w/v) Optiprep™ (9 ml) followed by a fraction of 5% (w/v) Optiprep™ (3 ml); [0078] i. centrifuging the gradients at 250,000×g for 3 hours at 4° C.; [0079] j. collecting the 4 ml top fraction, containing the membrane microparticle; and [0080] k. washing the microparticle three times phosphate-buffered saline with sedimentations of 250,000×g for 2 hours at +4° C. [0081] 29. A composition comprising a microparticle according to any of items 1-28. [0082] 30. The composition according to item 29, wherein the composition is devoid of whole *Streptococcus pneumoniae* cells. [0083] 31. The composition according to any of items 29-30, further comprising capsular polysaccharides from *Streptococcus pneumoniae*. [0084] 32. The composition according to any of items 29-31, comprising MPs in an amount of 1 µg/ml, preferably 5 µg/ml, more preferably 10 µg/ml, most preferably 100 µg/ml. [0085] 33. The composition according to any of items 29-32, further comprising an adjuvant. [0086] 34. The composition according to any of items 29-33, further comprising an adjuvant comprising aluminium hydroxide. [0087] 35. The composition according to any of items 29-34, being an immunogenic composition. [0088] 36. The composition according to any of items 29-35, being an immunogenic composition capable of eliciting opsonophagocytic antibodies against *Streptococcus pneumoniae* when administered to a mammalian host. [0089] 37. The composition according to any of items 29-36, being an immunogenic composition is capable of eliciting serotype independent antibodies against *Streptococcus pneumoniae* when administered to a mammalian host. [0090] 38. The composition

according to any of items 29-37, being an immunogenic composition is capable of eliciting antibodies against *Streptococcus pneumoniae* serotype 3 when administered to a mammalian host. [0091] 39. The composition according to any of items 29-38, further comprising capsular polysaccharides of a capsular serotype of *Streptococcus pneumoniae*. [0092] 40. The composition according to any of items 29-39, being a vaccine. [0093] 41. The composition according to any of items 29-40, for use in a method for inducing protective immunity against *Streptococcus pneumoniae* in a subject. [0094] 42. The composition according to item 41, for use according to item 41, wherein the immunity is protective against a condition selected from pneumococcal sinusitis, pneumococcal otitis, pneumococcal pneumonia and invasive pneumococcal disease including but not limited to pneumococcal sepsis and pneumococcal meningitis, preferably invasive pneumococcal disease. [0095] 43. A use of a microparticle according to any of items 1-28 in an immunogenic composition. [0096] 44. The use according to item 43, wherein the immunogenic composition is a vaccine. [0097] 45. A use of a microparticle according to any of items 1-28 in the manufacture of a vaccine. [0098] 46. A method for manufacturing a vaccine, comprising: [0099] a. Providing a microparticle according to any of items 1-28; [0100] b. Providing an adjuvant; [0101] c. Mixing the microparticle and the adjuvant in a suitable vehicle in order to produce a vaccine. [0102] 47. A method for producing an isolated *Streptococcus pneumoniae* microparticle according to any of items 1-28, comprising: [0103] a. providing a *Streptococcus pneumoniae* host cell; [0104] b. culturing said host cell under conditions allowing the production of a microparticle according to any of items 1-28 by the host cell; and [0105] c. isolating the microparticle thus produced. [0106] 48. The method according to item 47, wherein the host cell is of a strain selected from any serotype 1 strain, any serotype 3 strain, TIGR4, P1031 and A66, preferably TIGR4. [0107] 49. The method according to any of items 47-48, wherein the culturing is carried out in solid phase. [0108] 50. The method according to any of items 47-49, wherein the culturing is carried out in solid phase on blood agar. [0109] 51. The method according to any of items 47-50, wherein isolating the microparticle comprises density gradient centrifugation.

DETAILED DESCRIPTION

[0110] The inventors isolated membrane vesicles (MV.sub.L) from *S. pneumoniae* of serotype 4 grown in liquid cultures as previously described in the literature (1). They also isolated a novel type of membrane particles termed microparticles (MP) using a method involving growth of the bacteria overnight on blood agar plates. FIG. 1 shows the appearance of the MV.sub.L and MP using electron microscopy and atomic force microscopy as well as their size distribution. Microparticles from *S. pneumoniae* TIGR4 grown on plates (MP) were smaller in size on average than MV.sub.L coming from liquid media (MV.sub.L). However, MP from *S. pneumoniae* serotype 3 display a different size distribution, containing less of the smallest particles and more of the largest ones.

[0111] Biochemical comparison of MV.sub.L and MP preparations from TIGR4 revealed different properties for the MP and MV.sub.L. MP isolated from plates carried certain pneumococcal proteins to a higher extent than liquid grown MV.sub.L. They were particularly enriched in pneumolysin, the major pore-forming toxin in *S. pneumoniae*, as shown in FIG. 2D.

[0112] The novel MP were characterized using in vitro cell assays, and it was shown that consistent with the quantitated levels, the MP contain more biological pneumolysin activity than the MV.sub.L (FIG. 3). It was further shown that the MP are taken up by epithelial cells (FIG. 4A-D) but are not cytotoxic (FIG. 4E). Using human monocyte-derived dendritic cells it was shown that both MP and MV.sub.L are internalized into DCs, induce maturation of DCs (FIG. 5), and elicit differential cytokine responses (FIG. 6).

[0113] Next, the inventors investigated the potential of MP to protect mice using experimental models of pneumonia and invasive disease (FIGS. 7-11). The results showed that 80% of mice (wild type C57BL/6 mice) immunized with MP from serotype 4 (combined with aluminium hydroxide as adjuvant) survived after an intranasal pneumococcal infection caused by serotype 1

(heterologous challenge, cross protection). ELISA analysis showed that mice immunized with MP had a significantly higher MP-specific IgG response in comparison to control mice (treated only with the adjuvant) (FIG. 8). Immunization with serotype 3 and then challenge with serotype 3 led to a 100% protection (FIG. 11). Immunization with serotype 4 MP and then challenge with serotype 3 (a serotype that has been problematic in current vaccines) gave a protection of ca 50% (FIG. 11). Furthermore, immunofluorescence stainings showed that sera from mice immunized with MP from serotype 4 contained antibodies specific against multiple other serotypes, such as serotypes 1, 3 and 6B (FIG. 9).

[0114] In conclusion, it was found that MP protect mice against pneumococcal infection, and, most importantly, such protection is serotype-independent to a significant degree, in contrast to the protection conferred by currently available vaccines (polysaccharide or conjugated vaccines) that are, as mentioned in the background section, strictly serotype-dependent. The MP immunisation furthermore elicited protection against serotype 3 which is a challenge for current vaccines. Indeed, immunization with the currently clinically used vaccine PCV13 provided significantly less protection to mice than MP isolated from serotype 3 (FIG. 11).

[0115] Moreover, for protein based vaccines that are under development today, there is a problem in obtaining opsonophagocytic capability. Importantly, the present data show that using MP as a vaccine affects opsonophagocytosis (Example 3, FIG. 10). Last but not least, available pneumococcal vaccines (PCVs) are extremely important to fight pneumococcal diseases in developing countries. However, the costs to produce current vaccines are very high. An important benefit with the present approach is a drop in production costs since isolation of microparticles does not require high-cost preparations with conjugations, instead the method of the present invention is more simple based on bacterial growth combined with high speed-centrifugation.

Isolated *Streptococcus pneumoniae* Membrane Vesicles and Microparticles

[0116] As discussed in the Background section, a few types of *Streptococcus pneumoniae* membrane vesicles have been obtained in liquid culture (1). The solid phase culture method used by the inventors resulted in a novel type of particles termed microparticles having different and advantageous properties as discussed above and shown in the Examples. As shown in Table 1, the microparticles differ from the known membrane vesicles, MV.sub.L in terms of relative protein expression.

TABLE-US-00001 TABLE 1 Quantification of proteins present in membrane vesicles, MV.sub.L, from serotype 4 strain TIGR4 and microparticles, MP, from serotype 4 and 3. Percentage sequence identities of protein homologues in reference strains for serotype 1 (P1031) and serotype 3 (A66) are shown. The amounts are indicated in µg/µg total protein or relative amount in percentage. MP Homology in Homology in Protein MV.sub.L (TIGR4) MP (Type 3) P1031* A66** Ply 0.055 µg 0.406 µg 0.314 µg 99% 99% LytA 0.050 µg 0.100 µg 0.266 µg 99% 100% PspC 0.118 µg 0.300 µg Not determined* 85% (PspC 45% (PspC (PspC3.4) 2.1) 11.4) RrgB 0.016 µg 0.028 µg Absent Absent PhtD 100% 266% 5793% 95% 87% *Because of the high genetic diversity of the pspC gene among pneumococcal serotypes it was not possible to use the anti TIGR4 PspC antibody (which was specific for the TIGR4 strain) to detect the serotype 3 PspC

[0117] Thus, in a first aspect of the present invention, there is provided isolated *Streptococcus pneumoniae* microparticle (MP) (a novel type of pneumococcal membrane vesicle particle), wherein said MP comprise either one, two, three or four of the following proteins at the designated levels: [0118] i. the protein Ply at the level of >0.070 µg/mg total protein in the MP; [0119] ii. the protein LytA at the level of ≥0.070 µg/mg total protein in the MP; [0120] iii. the protein PspC at the level of >0.130 µg/mg total protein in the MP; and/or [0121] iv. the protein RrgB at the level of >0.020 µg/mg total protein in the MP.

[0122] The microparticles may further comprise capsular polysaccharides of a capsular serotype of *Streptococcus pneumoniae*, preferably at a level of ≥0.001, more preferably ≥0.01, most preferably ≥0.1 µg/mg total protein in the MP.

- [0123] The microparticle may comprise the protein Ply at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP. The microparticle may comprise the protein Ply at the level of ≥ 0.15 , preferably ≥ 0.2 , more preferably ≥ 0.3 , most preferably ≥ 0.35 $\mu\text{g}/\text{mg}$ total protein in the MP.
- [0124] The microparticle may comprise the protein LytA at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP. The microparticle may comprise the protein LytA at the level of ≥ 0.08 , preferably ≥ 0.09 , yet more preferably ≥ 0.1 $\mu\text{g}/\text{mg}$, most preferably ≥ 0.2 $\mu\text{g}/\text{mg}$ total protein in the MP.
- [0125] The microparticle, MP, may comprise the protein PspC at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP. The microparticle may comprise comprising the protein PspC at the level of ≥ 0.15 , preferably ≥ 0.18 , more preferably ≥ 0.2 , most preferably ≥ 0.3 $\mu\text{g}/\text{mg}$ total protein in the MP.
- [0126] The microparticle may comprise the protein RrgB at the level of ≥ 0.02 $\mu\text{g}/\text{mg}$ total protein in the MP. The microparticle may comprise the protein RgrB at the level of ≥ 0.022 , preferably ≥ 0.025 , most preferably ≥ 0.028 $\mu\text{g}/\text{mg}$ total protein in the MP.
- [0127] The microparticle may further comprise the protein PhtD. The protein PhtD may be present at a level being at least 2-fold, more preferably at least 2.5-fold compared to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of $\mu\text{g}/\text{mg}$ total protein in the particle or the vesicle, respectively.
- [0128] The microparticle may further comprise the protein RrgA. The protein RrgA may be present at the level of ≥ 0.02 , preferably ≥ 0.05 , more preferably ≥ 0.10 , most preferably ≥ 0.2 $\mu\text{g}/\text{mg}$ total protein in the MP.
- [0129] The microparticle may further comprise the *Streptococcus pneumoniae* protein IgA. The protein IgA may be present at the level of ≥ 0.02 , preferably ≥ 0.05 , more preferably ≥ 0.10 , most preferably ≥ 0.2 $\mu\text{g}/\text{mg}$ total protein in the MP.
- [0130] The protein Ply may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 1.
- [0131] The protein LytA may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 2.
- [0132] The protein PspC may comprise a sequence having at least 40% or 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NOs: 3.
- [0133] The protein PspC may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to any one of SEQ ID NOs: 3-14.
- [0134] The protein RgrB may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 15.
- [0135] The protein PhtD may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 16.
- [0136] The protein RgrA may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 17.
- [0137] The protein IgA may comprise a sequence having at least 70%, preferably 80%, more preferably 85%, yet more preferably 90%, still more preferably 95%, most preferably 100% sequence identity to SEQ ID NO: 18.
- [0138] Thus, the first aspect encompasses (but is not limited to) embodiments disclosed in Table 2 below.

TABLE-US-00002 TABLE 2 Minimum levels of designated proteins in various embodiments of the first aspect. A blank cell indicates that the protein is optional i.e. may be absent or present at

any level. Units: $\mu\text{g}/\mu\text{g}$ total protein, except for PhtD, where the amount is in comparing to *Streptococcus pneumoniae* membrane vesicles obtained in liquid culture in terms of $\mu\text{g}/\mu\text{g}$ total protein in the particle or the vesicle, respectively. Embodiment# Ply LytA PspC PhtD RgrB 1 0.070 2 0.070 0.070 3 0.070 0.130 4 0.070 2-FOLD 5 0.070 0.070 0.130 6 0.070 0.070 2-FOLD 7 0.070 0.070 0.130 2-FOLD 8 0.070 0.130 2-FOLD 9 0.070 10 0.130 11 2-FOLD 12 0.070 0.130 13 0.070 2-FOLD 14 0.070 0.130 2-FOLD 15 0.130 2-FOLD 16 0.070 0.020 17 0.070 0.070 0.020 18 0.070 0.130 0.020 19 0.070 2-FOLD 0.020 20 0.070 0.070 0.130 0.020 21 0.070 0.070 2-FOLD 0.020 22 0.070 0.070 0.130 2-FOLD 0.020 23 0.070 0.130 2-FOLD 0.020 24 0.070 0.020 25 0.130 0.020 26 2-FOLD 0.020 27 0.070 0.130 0.020 28 0.070 2-FOLD 0.020 29 0.070 0.130 2-FOLD 0.020 30 0.130 2-FOLD 0.020 31 0.070 32 0.070 0.070 33 0.070 0.130 34 0.070 2-FOLD 35 0.070 0.070 0.130 36 0.070 0.070 2-FOLD 37 0.070 0.070 0.130 2-FOLD 38 0.070 0.130 2-FOLD 39 0.070 40 0.130 41 2-FOLD 42 0.070 0.130 43 0.070 2-FOLD 44 0.070 0.130 2-FOLD 45 0.130 2-FOLD 46 0.070 0.020 47 0.070 0.070 0.020 48 0.070 0.130 0.020 49 0.070 2-FOLD 0.020 50 0.070 0.070 0.130 0.020 51 0.070 0.070 2-FOLD 0.020 52 0.070 0.070 0.130 2-FOLD 0.020 53 0.070 0.130 2-FOLD 0.020 54 0.070 0.020 55 0.130 0.020 56 2-FOLD 0.020 57 0.070 0.130 0.020 58 0.070 2-FOLD 0.020 59 0.070 0.130 2-FOLD 0.020 60 0.130 2-FOLD 0.020

[0139] The microparticles may be 5-300 nm in diameter, preferably 10-125 nm in diameter.

[0140] The microparticles may be derived from any *Streptococcus pneumoniae* strain, but is preferably selected from a group consisting of all serotype 3 strains, all serotype 1 strains, TIGR4, P1031 and A66, most preferably TIGR4.

[0141] The microparticles according to the first aspect may be obtainable by: [0142] a. culturing host cells of a *Streptococcus pneumoniae* strain on blood agar plates or other plates; [0143] b. harvesting the cultured host cells; [0144] c. centrifuging the harvested host cells at 17,000 g for 30 minutes at +4° C.; [0145] d. subjecting the supernatant to filtration through a 0.22 μm filter; [0146] e. centrifuging the filtered supernatant at 120,000 g for 2 h at +4° C.; [0147] f. washing the pellets from e) twice in phosphate-buffered saline with sedimentations at 120,000 g for 2 h at +4° C.; [0148] g. resuspending the pellet in 1 ml phosphate-buffered saline; [0149] h. adjusting the resuspended pellet to 50% (w/v) Optiprep™ density gradient medium in a total volume of 2 ml and overlaying with one fraction of 30% (w/v) Optiprep™ (9 ml) followed by a fraction of 5% (w/v) Optiprep™ (3 ml); [0150] i. centrifuging the gradients at 250,000×g for 3 hours at 4° C.; [0151] j. collecting the 4 ml top fraction, containing the membrane microparticles; [0152] k. washing the microparticles three times phosphate-buffered saline with sedimentations of 250,000×g for 2 hours at +4° C.

Compositions and Uses

[0153] In a second aspect of the present invention, there is provided a composition comprising a microparticle according to the first aspect. The composition may be devoid of whole *Streptococcus pneumoniae* cells. The composition may optionally comprise capsular polysaccharides from *Streptococcus pneumoniae*.

[0154] The composition according to the second aspect may comprise MP in an amount of 1 $\mu\text{g}/\text{ml}$, preferably 5 $\mu\text{g}/\text{ml}$, more preferably 10 $\mu\text{g}/\text{ml}$, most preferably 100 $\mu\text{g}/\text{ml}$ (the concentrations refer to the total protein content of MP per ml).

[0155] The composition may further comprise an adjuvant. The adjuvant preferably comprises aluminium hydroxide.

[0156] The composition may be an immunogenic composition. Preferably, the immunogenic composition is capable of eliciting opsonophagocytic antibodies against *Streptococcus pneumoniae* when administered to a mammalian host. Preferably, the immunogenic composition is capable of eliciting serotype independent antibodies against *Streptococcus pneumoniae* when administered to a mammalian host. Preferably, the immunogenic composition is capable of eliciting antibodies against *Streptococcus pneumoniae* serotype 3 when administered to a mammalian host.

[0157] The composition may be formulated as a vaccine.

[0158] In a third aspect of the present invention, there is provided a composition according to the second aspect, for use in a method for inducing protective immunity against *Streptococcus pneumoniae* in a subject. The third aspect also encompasses a method for inducing protective immunity against *Streptococcus pneumoniae* in a subject in need thereof, comprising administering an effective amount of the composition according to the second aspect to the subject. The third aspect also encompasses the use of a composition according to the second aspect in the manufacture of a vaccine for immunization against *Streptococcus pneumoniae*.

[0159] The protective immunity may be an immunity reducing the likelihood of a condition selected from pneumococcal sinusitis, pneumococcal otitis, pneumococcal pneumonia and invasive pneumococcal disease including but not limited to pneumococcal sepsis and pneumococcal meningitis, preferably invasive pneumococcal disease. Preferably, the subject to be immunized is a young child (e.g. less than 7 years of age) or an elderly person (e.g. over 65 years of age), but also other age groups could be targeted.

[0160] For immunization, the composition may be administered to the subject in various manners known in the art, including but not limited to by way of injection (e.g. intramuscular, intracutaneous, subcutaneous, intravenous), buccal, oral and intranasal administration as well as inhalation.

[0161] In a fourth aspect there is provided a use of a microparticle according to the first aspect in an immunogenic composition. The immunogenic composition may be a vaccine.

[0162] In a fifth aspect, there is provided a use of a microparticle according to the first aspect in the manufacture of a vaccine.

[0163] In a sixth aspect, there is provided a method for manufacturing a vaccine, comprising:

[0164] a. Providing a microparticle according to the first aspect [0165] b. Providing an adjuvant, such as aluminium hydroxide; [0166] c. Mixing the microparticle and the adjuvant in a suitable vehicle in order to produce a vaccine.

Methods for Producing *Streptococcus pneumoniae* Microparticles

[0167] In a sixth aspect of the present invention there is provided a method for producing an isolated *Streptococcus pneumoniae* microparticle according to the first aspect, comprising: [0168] a. providing a *Streptococcus pneumoniae* bacterial cell; [0169] b. culturing said bacterial cell under conditions allowing the production of a microparticle according to the first aspect by the bacterial cell; and [0170] c. isolating the microparticle thus produced.

[0171] The bacterial cell may be from any pneumococcal strain, but is preferably TIGR4, a serotype 1 and/or a serotype 3 strain.

[0172] The culturing may be carried out using plates or liquid culture. Preferably, the culturing is carried out in solid phase, most preferably on blood agar or other plates. Isolating the microparticles may comprise a density gradient centrifugation step.

General Aspects Relating to the Present Disclosure

[0173] The term “comprising” is to be interpreted as including, but not being limited to. All references are hereby incorporated by reference. The arrangement of the present disclosure into sections with headings and subheadings is merely to improve legibility and is not to be interpreted limiting in any way, in particular, the division does not in any way preclude or limit combining features under different headings and subheadings with each other.

EXAMPLES

[0174] The following examples are not to be regarded as limiting. For further information on the experimental details, the skilled reader is directed to a separate section titled Materials and Methods.

Example 1: Isolation and In Vitro Characterization of Novel Pneumococcal Microparticles
S. pneumoniae TIGR4 Produces Microparticles that Differ Depending on Bacterial Growth Conditions.

[0175] Membrane vesicles were isolated and purified from *Streptococcus pneumoniae* serotype 4

strain TIGR4 grown in liquid medium (MV.sub.L). Microparticels (MP) were isolated from pneumococci grown on blood agar plates. Preparations were analyzed by transmission electron microscopy (TEM) and atomic force microscopy, and revealed spherical bodies surrounded by membranous structures (FIG. 1A). The distribution of the size varied between MV.sub.L, MP from TIGR4 and MP from serotype 3 (FIG. 1B). In total 503 MV.sub.L, 378 MP from TIGR4 and 523 MP from serotype 3 were analyzed. As a control we also studied an isogenic pneumolysin mutant strain (TIGR4 Δ ply) and found a similar size distribution. To reveal shedding of MV.sub.L or MP from the surface of bacteria by TEM we then used an isogenic unencapsulated mutant in TIGR4 (T4R) grown in liquid culture. In the absence of the thick bacterial capsule, MV.sub.L were clearly visible, emanating from the plasma membrane of *S. pneumoniae* into the extracellular milieu (FIG. 1C-D).

Proteomic Analysis Revealed Differences in Protein Content Between MV.sub.L from Liquid Culture or MP from Plates

[0176] To analyze the protein content of both pneumococcal preparations, purified MV.sub.L and MP were subjected to SDS-PAGE analysis. Distinct protein patterns in MV.sub.L and MPs as compared with proteins present in whole cell lysates or bacterial supernatants (FIG. 2A), were identified. Interestingly, the proteomic content in MV.sub.L and MP fractions differed, suggesting a differential enrichment of proteins in the different types of particles. We further conducted proteomic analysis using tandem mass spectrometry. In total, in TIGR4, we identified 317 proteins in MV.sub.L (Table 3), and 216 in MP (Table 4), of which 184 proteins were found in both preparations. 133 proteins were unique to MV.sub.L and 32 to MP (FIG. 2B). In serotype 3 we identified 462 proteins in MV.sub.L (Table 5) and 344 in MP (Table 6). All detected proteins were further classified according to their subcellular localization based on the combination of LocateP and GO annotation (FIG. 2C) (11). A majority of the proteins identified in MV.sub.L and MP were cytosolic proteins, followed by multi-transmembrane proteins, lipoproteins, N-terminally anchored proteins, secretory proteins and cell wall-associated proteins. While MV.sub.L appeared to be more enriched in cytosolic protein and cell wall-associated proteins than MP from TIGR4, the latter harbored a higher percentage of multi-transmembrane proteins, lipoproteins, N-terminally anchored proteins and secretory proteins.

[0177] We further analyzed the mass spectrometry data in order to determine the presence of 30 known pneumococcal virulence factors in the particles. Both types of particles contained approximately half of these virulence factors present in TIGR4. Just two were unique to MV.sub.L CbpD (12) and BgaA (13), and one to MP, IgA (10).

[0178] Based on the proteomic results, we next performed immunoblot analysis on specific pneumococcal proteins and virulence factors (FIG. 2D). Among cytosolic proteins we investigated the presence of the cytotoxin pneumolysin (Ply), which is a pore-forming toxin and one of the most important virulence factors in pneumococcal pathogenesis (2), GAPDH as a cytosolic marker (14), and LytA, the major autolysin of *Streptococcus pneumoniae* (3). The most striking observation was the high enrichment of Ply especially in MP, which also contained more LytA than MV.sub.L. Smaller differences were also observed in the enrichment of virulence factors belonging to other subcellular localizations. While MV.sub.L displayed a higher amount of Pneumococcal surface antigen A (PsaA) (15) and Sortase A (SrtA) (16) than MP, the latter contained more Pneumococcal surface protein C (PspC) (17) and Polyhistidine triad protein D (PhtD) (9) (FIG. 2D). The major component of the pneumococcal pilus, RrgB (6), was equally found in both particle preparations. [0179] These findings suggest the presence of a still uncharacterized mechanism for the selective secretion and enrichment of specific proteins and virulence factors of *Streptococcus pneumoniae* in these particle preparations.

A Biologically Active Pneumolysin was Found in Membrane Microparticles

[0180] The enrichment of Ply in both MV.sub.L and MP prompted us to determine whether particle-associated Ply was functional and able to lyse erythrocytes in a hemolytic assay. For both

types of particles we observed a dose dependent hemolysis (FIG. 3), however, Ply from MP showed higher activity than from MV.sub.L, which is consistent with the observation that MP contain more Ply. Notably, hemolysis seemed to be entirely caused by Ply, and particles prepared from a TIGR4 mutant lacking Ply (TIGR4 Δ ply) exhibited no hemolytic activity.

MP are Taken Up by A549 Epithelial Cells, but are not Cytotoxic.

[0181] MV in other Gram-positive bacteria have been described to function as vehicle to deliver vesicle-associated proteins to human cells (18) (19). To test whether pneumococcal MP can deliver pneumococcal proteins, we incubated MP from TIGR4 with A549 lung epithelial cells for 24 hours and visualized Ply and LytA by immunofluorescence staining (FIG. 4A-C). By analyzing orthogonal views of A549 cells treated with MP, we showed by immunofluorescence that MP can be taken up, with Ply and LytA detectable inside the cells (FIG. 4B-C).

[0182] To confirm our observation we further analyzed lysates of A549 cells treated with MP by immunoblotting with anti-Ply antibody (FIG. 4D). In order to minimize extracellular associated particles, treated cells were excessively washed before lysates were made. Consistent with our immunofluorescence results, western blotting revealed a dose dependent increase in Ply further suggesting that MP can be internalized.

[0183] Next we asked whether proteins associated with MV.sub.L or MP, and in particular Ply, may contribute to cytotoxic effects on cultured human cells. To assess toxicity, A549 cells were treated with different concentrations of MV.sub.L and MP for 24 hours, stained with fixable viability dye and analyzed by flow cytometry (FIG. 4E). The difference in cell death between samples incubated with MV.sub.L or MPs and the negative control sample was not statistically significant, suggesting that Ply is not cytotoxic to the cells at concentrations present in MV.sub.L or MP.

MV.sub.L and MP are Internalized by Human Monocyte-Derived Dendritic Cells (DCs) and Induce their Maturation.

[0184] Since we found that MP can be internalized by epithelial cells, we then studied potential interactions of MV.sub.L or MP with innate immune cells, focusing on DCs. First we assessed cytotoxicity by incubating DCs with MV.sub.L or MP, stained with fixable viability dye and Annexin V, and then analyzed using flow cytometry (FIG. 5A). No significant increase in cytotoxicity was detected in samples incubated with MP, while addition of MV.sub.L displayed cytotoxic effects at the two highest concentrations used. While both preparations were considerably less cytotoxic than live bacteria, with 70-80% cytotoxic cells after 24 hours of incubation, more studies are needed to assess the mechanism behind the increased cytotoxicity of MV.sub.L from liquid cultures. Moreover, the percentage of apoptotic cells did not change after incubation with either of the particle preparations.

[0185] Then we investigated uptake of MP by DCs. First we incubated DCs with MP for 1 hour, lysed the cells and performed SDS-PAGE and immunoblotting on cell lysates to check for presence of Ply. Indeed, Ply was found in the cell lysates (FIG. 5B), suggesting that DCs internalize MP. Next, DCs were challenged for 30 minutes with inhibitors of actin polymerization and phosphatidylinositol 3-kinase (PI3K), cytochalasin and wortmannin, to block active phagocytosis, or with an inhibitor of lipid rafts, methyl- β -cyclodextrin, to block fusion of membranes. Subsequently, cells were incubated with MP for 1 hour, lysed, and the same protocol for immunoblotting was followed. Interestingly, blocking active phagocytosis or membrane fusion resulted in less Ply. However, when all inhibitors were used together, Ply still remained present in the lysates, suggesting either that the particles adhered to the cells or were internalized by another mechanism, not investigated in this study.

[0186] Taken together these data suggest that MP can function as a vehicle to deliver bacterial components to host cells. Since DCs are professional antigen presenting cells, we then assessed whether MV.sub.L or MP could influence DC maturation. Cells were incubated for 24 hours with MV.sub.L or MP, and then stained for MHCII and the co-stimulatory molecule CD86, and analyzed by flow cytometry (FIG. 5C). A dose-dependent increase in the presence of both markers on the

surface of DCs was detected when cells were incubated with the particles. MV.sub.L appeared to induce more maturation than MP, and similar levels of maturation were obtained with LPS.

MV.SUB.L .and MP Induce Pro-Inflammatory Cytokine Responses in DCs

[0187] Since OMVs from Gram-negative have been shown to trigger a potent innate immune response (20) (21), we studied whether also pneumococcal MV.sub.L or MP could affect pro-inflammatory cytokine responses. DCs we incubated with MV.sub.L or MP for 24 hours, and ELISA assays were used on supernatants to determine concentrations of IL-6 (FIG. 6A), IL-8 (FIG. 6B), IL-10 (FIG. 6C) and TNF (FIG. 6D). MP induced release of IL-8 and low levels of TNF, while no induction was seen for IL-6 and IL-10. In contrast, MV.sub.L induced all four cytokines tested to levels higher than live bacteria. None of the particles induced IL-1 β or IL-12 (data not shown).

TABLE-US-00003 TABLE 3 Proteins identified in MV.sub.L from TIGR4. The accession numbers refer to SwissProt on the date of filing. Accession Coverage # Peptides #AAs Score Description

Localization	Q97RH0	76.86	47	350	2615.53	Lipoprotein OS = <i>Streptococcus</i>	Lipid	<i>pneumoniae</i>	serotype 4 (strain ATCC anchored BAA-334/TIGR4)	GN = SP_0845	PE = 4	SV = 1	–									
	[Q97RH0_STRPN]	Q2MGF6	82.45	59	490	1620.74	Lysozyme OS = <i>Streptococcus</i>	Secretory	<i>pneumoniae</i> serotype 4 (strain ATCC (released) BAA-334/TIGR4)	GN = lytC	PE = 4 (with CS)	SV = 1	–									
	[Q2MGF6_STRPN]	Q97NK0	73.39	64	883	1364.43	Aldehyde-alcohol dehydrogenase	Intracellular OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = SP_2026	PE = 3	SV = 1	–	[Q97NK0_STRPN]									
	P18791	72.99	48	659	1307.17	Oligopeptide-binding protein AmiA Lipid OS = <i>Streptococcus pneumoniae</i> anchored serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = amiA	PE = 1	SV = 3	–	[AMIA_STRPN]	Q9L7Q2	55.82	91	1906	1166.89	Zinc metalloprotease ZmpB Multi- OS = <i>Streptococcus pneumoniae</i> trans- serotype 4 (strain ATCC BAA-334/ membrane TIGR4)	GN = zmpB	PE = 3	SV = 2	–	[ZMPB_STRPN]
	Q97T80	58.3	87	1856	1012.57	Zinc metalloprotease ZmpC LP \times TG OS = <i>Streptococcus pneumoniae</i> Cell-wall serotype 4 (strain ATCC BAA-334/ anchored TIGR4)	GN = zmpC	PE = 3	SV = 1	–	[ZMPC_STRPN]	Q97NQ8	59.59	75	1225	936.07	DNA-directed RNA polymerase subunit Intracellular beta' OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = rpoC	PE = 3	SV = 1	–	[RPOC_STRPN]
	P59213	74.47	48	423	838.2	Maltose/maltodextrin-binding protein Lipid OS = <i>Streptococcus pneumoniae</i> anchored serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = malX	PE = 1	SV = 1	–	[MALX_STRPN]	P64022	75.61	46	693	803.77	Elongation factor G OS = <i>Streptococcus</i> Intracellular <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	GN = fusA	PE = 3	SV = 1	–	[EFG_STRPN]
	P0C2J9	92.99	46	471	753.38	Pneumolysin OS = <i>Streptococcus</i> Intracellular <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	GN = ply	PE = 3	SV = 1	–	[TACY_STRPN]	Q97N55	75	27	392	729.12	Secreted 45 kd protein Secretory OS = <i>Streptococcus pneumoniae</i> (released) serotype 4 (strain ATCC BAA-334/ (with CS) TIGR4)	GN = usp45	PE = 4	SV = 1	–	[Q97N55_STRPN]
	Q97RY6	42.1	73	2140	715.85	Serine protease, subtilase family LP \times TG OS = <i>Streptococcus pneumoniae</i> Cell-wall serotype 4 (strain ATCC BAA-334/ anchored TIGR4)	GN = SP_0641	PE = 4	SV = 1	–	[Q97RY6_STRPN]	P64030	68.84	32	398	684.61	Elongation factor Tu OS = <i>Streptococcus</i> Intracellular <i>pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	GN = tuf	PE = 3	SV = 1	–	[EFTU_STRPN]
	Q97NQ7	66.42	61	1203	683.01	DNA-directed RNA polymerase subunit Intracellular beta OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = rpoB	PE = 3	SV = 1	–	[RPOB_STRPN]	Q97PE6	77.38	35	442	645.41	Sugar ABC transporter, sugar-binding Lipid protein OS = <i>Streptococcus pneumoniae</i> anchored serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = SP_1683	PE = 4	SV = 1	–	[Q97PE6_STRPN]
	Q97PT1	74.59	45	551	641.52	Uncharacterized protein Intracellular OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = SP_1518	PE = 4	SV = 1	–	[Q97PT1_STRPN]	Q97RQ0	81.35	26	386	599.79	Branched-chain amino acid ABC Lipid transporter, amino acid-binding anchored protein OS = <i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/ TIGR4)	GN = livJ	PE = 4	SV = 1	–	[Q97RQ0_STRPN]
	Q97SV2	62.45	19	277	575.15	50 S ribosomal protein L2 OS =																

Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplB PE = 3 SV = 1 – [RL2_STRPN] Q97R51 74.12 32 313 574.61 Foldase protein PrsA OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = prsA PE = 3 SV = 1 – [PRSA_STRPN] Q97SV5 59.62 17 208 571.92 50 S ribosomal protein L3 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIC PE = 3 SV = 1 – [RL3_STRPN] Q04707 64.12 38 719 564.47 Penicillin-binding protein 1 A OS = N-*Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = ponA PE = 1 SV = 2 – [PBPA_STRPN] (No CS) Q97NL1 88.36 26 335 546.25 Glyceraldehyde-3-phosphate Intracellular dehydrogenase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gap PE = 3 SV = 1 – [Q97NL1_STRPN] Q97PD6 64.87 46 837 501.57 Protein translocase subunit SecA 1 Intracellular OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = secA1 PE = 3 SV = 1 – [SECA1_STRPN] P35592 75.61 44 660 500.22 Oligopeptide-binding protein AliA OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = aliA PE = 3 SV = 4 – [ALIA_STRPN] P0A4G2 72.49 21 309 482.72 Manganese ABC transporter Lipid substrate-binding lipoprotein OS = anchored *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = psaA PE = 1 SV = 1 – [MITSA_STRPN] Q54970 61.59 38 591 482.11 Pyruvate oxidase OS = *Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = spxB PE = 3 SV = 2 – [POXB_STRPN] Q97R09 68.62 21 341 442.02 Iron-compound ABC transporter, iron Lipid compound-binding protein OS = anchored *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1032 PE = 1 SV = 1 – [Q97R09_STRPN] Q97PU3 67.63 31 278 434.28 Amino acid ABC transporter, amino Lipid acid-binding protein OS = *Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = aatB PE = 4 SV = 1 – [Q97PU3_STRPN] P0A4C3 72.35 20 217 427.97 30 S ribosomal protein S3 Intracellular OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = rpsC PE = 3 SV = 1 – [RS3_STRPN] Q97T39 36.42 28 744 407.46 Pneumococcal surface protein A N- OS = *Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = pspA PE = 4 SV = 1 – (with CS) [Q97T39_STRPN] I6L8V7 41.79 28 627 381.01 Choline binding protein E OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = cbpE PE = 4 SV = 1 – [I6L8V7_STRPN] P0A475 54.74 11 137 354.71 50 S ribosomal protein L16 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIP PE = 1 SV = 1 – [RL16_STRPN] P66112 51.26 9 119 346.49 50 S ribosomal protein L20 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpIT PE = 3 SV = 1 – [RL20_STRPN] Q97SV4 56.04 14 207 345.01 50 S ribosomal protein L4 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpID PE = 3 SV = 1 – [RL4_STRPN] Q97T12 78.99 26 276 332.96 ABC transporter, substrate-binding Lipid protein OS = *Streptococcus pneumoniae* anchored serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0148 PE = 4 SV = 1 – [Q97T12_STRPN] Q97N74 47.47 31 693 320.12 Choline binding protein A OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpA PE = 1 SV = 1 – (with CS) [Q97N74_STRPN] Q97QS2 58.06 22 434 315.73 Enolase OS = *Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = eno PE = 1 SV = 1 – [ENO_STRPN] Q97S57 49.58 34 958 315.37 Translation initiation factor IF-2 Intracellular OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = infB PE = 3 SV = 1 – [IF2_STRPN] Q97T11 75 27 284 313.6 Lipoprotein OS = *Streptococcus* Lipid *pneumoniae* serotype 4 (strain ATCC anchored BAA-334/TIGR4) GN = SP_0149 PE = 3 SV = 1 – [Q97T11_STRPN] P66359 55.12 10 127 299.97 30 S ribosomal protein S11 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsK PE = 3 SV = 1 – [RS11_STRPN] Q97PT6 68.8 24 468 285.18 ATP synthase subunit beta OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpD PE = 3 SV = 1 –

[ATPB_STRPN] Q97SP2 60.24 18 332 279.63 PTS system, mannose-specific IIB Intracellular components OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = manL PE = 4 SV = 1 – [Q97SP2_STRPN] Q97RZ7 60.08 15 238 278.06 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_0629 PE = 1 SV = 1 – [Q97RZ7_STRPN] Q97NL3 55.13 34 731 275.05 Penicillin-binding protein 2A OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = pbp2A PE = 4 SV = 1 – (No CS) [Q97NL3_STRPN] Q97PE1 62.92 29 445 269.34 ABC transporter, substrate-binding Lipid protein OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1690 PE = 4 SV = 1 – [Q97PE1_STRPN] Q97SX2 81.48 19 189 268.65 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_0191 PE = 1 SV = 1 – [Q97SX2_STRPN] Q97RN2 67.79 15 267 248.33 Peptidyl-prolyl cis-trans isomerase, Lipid cyclophilin-type OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0771 PE = 3 SV = 1 – [Q97RN2_STRPN] P65887 62.62 25 428 243.93 Adenylosuccinate synthetase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = purA PE = 1 SV = 1 – [PURA_STRPN] Q97QH2 33.7 23 721 242.51 Amino acid ABC transporter, amino Multi- acid-binding protein/permease trans-protein OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1241 PE = 1 SV = 1 – [Q97QH2_STRPN] O69076 50.46 25 652 240.51 ATP-dependent zinc metalloprotease Multi- FtsH OS = *Streptococcus pneumoniae* trans- serotype 4 (strain ATCC BAA-334/ membrane TIGR4) GN = ftsH PE = 3 SV = 3 – [FTSH_STRPN] Q97N37 62.6 22 393 236.55 Serine protease OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = SP_2239 PE = 1 anchored SV = 1 – [Q97N37_STRPN] (No CS) Q97T63 60.29 26 491 232.33 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0092 PE = 4 SV = 1 – [Q97T63_STRPN] Q97NQ0 68.79 19 330 230.07 Aspartate--ammonia ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = asnA PE = 3 SV = 1 – [ASNA_STRPN] Q97SV1 86.67 12 180 229.35 50 S ribosomal protein L5 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplE PE = 3 SV = 1 – [RL5_STRPN] Q97Q37 69.37 18 271 227.84 Amino acid ABC transporter, amino Lipid acid-binding protein OS = anchored *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1394 PE = 4 SV = 1 – [Q97Q37_STRPN] P66907 42.63 9 380 223.83 Queuine tRNA-ribosyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = tgt PE = 3 SV = 1 – [TGT_STRPN] P59205 43.77 20 658 217.64 Putative endo-beta-N- Secretory acetylglucosaminidase OS = (released) *Streptococcus pneumoniae* (strain (with CS) ATCC BAA-334/TIGR4) GN = lytB PE = 1 SV = 1 – [LYTB_STRPN] Q97SC6 49.35 27 774 216.35 Formate acetyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfl PE = 4 SV = 1 – [Q97SC6_STRPN] Q97NB5 42.19 22 621 215.44 Choline binding protein PcpA OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = pcpA PE = 1 SV = 1 – [Q97NB5_STRPN] P0A2U8 49.58 19 355 215.01 Oligopeptide transport ATP-binding Intracellular protein AmiE OS = -- *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiE PE = 3 SV = 1 – [AMIE_STRPN] Q97PA9 39 21 659 212.01 Serine/threonine-protein kinase StkP Intracellular/ OS = -*Streptococcus pneumoniae* TMH serotype 4 (strain ATCC BAA-334 start TIGR4) GN = stkP PE = 1 SV = 1 – AFTER 60 [STKP2_STRPN] Q97RF9 56.25 22 400 204.01 Ribosomal protein S1 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsA PE = 4 SV = 1 – [Q97RF9_STRPN] Q97SP4 43.89 12 303 202.88 PTS system, mannose-specific IID Multi- component OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC membrane BAA-33/TIGR4) GN = SP_0282 PE = 4 SV = 1 – [Q97SP4_STRPN] P0A4A7 58.39 11 137 202.86 30 S ribosomal protein S12 OS = Intracellular *Streptococcus*

pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsL PE = 3 SV = 1 – [RS12_STRPN] Q97Q31 55.82 13 292 202.43 Phosphate-binding protein PstS 1 OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = pstS1 PE = 1 SV = 1 – [PSTS1_STRPN] Q97N56 69.11 20 259 201.44 30 S ribosomal protein S2 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsB PE = 3 SV = 1 – [RS2_STRPN] Q97RC6 48.66 14 335 201.21 ATP-dependent 6- Intracellular phosphofructokinase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfkA PE = 3 SV = 1 – [PFKA_STRPN] Q97QX5 68.02 15 344 200.45 Uncharacterized protein OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1069 PE = 1 SV = 1 – [Q97QX5_STRPN] Q97NM6 60.64 17 404 197.5 Aminotransferase, class I OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1994 PE = 4 SV = 1 – [Q97NM6_STRPN] Q97N69 65.37 19 335 194.36 Putative ABC transporter, substrate- Lipid binding protein OS = - *Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2197 PE = 4 SV = 1 – [Q97N69_STRPN] Q97NS5 60.95 16 338 193.02 Putative transcriptional regulator OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1942 PE = 4 SV = 1 – [Q97NS5_STRPN] P0A4M7 38.35 20 498 189.98 Oligopeptide transport system Multi- permease protein AmiC OS = = trans- *Streptococcus pneumoniae* serotype 4 membrane (strain ATCC BAA-334/TIGR4) GN = amiC PE = 3 SV = 1 – [AMIC_STRPN] Q97QD2 56.41 20 523 188.15 Signal recognition particle protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ffh PE = 1 SV = 1 – [Q97QD2_STRPN] P14677 49.87 26 750 185.33 Penicillin-binding protein 2 × OS = = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = pbpX PE = 1 SV = 2 – [PBPX_STRPN] (No CS) Q97NQ3 51.88 14 345 183.26 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_1967 PE = 4 SV = 1 – (No CS) [Q97NQ3_STRPN] Q97RW9 59.34 16 332 182.68 Putative pneumococcal surface Lipid protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0667 PE = 1 SV = 1 – [Q97RW9_STRPN] Q97RL9 66.17 16 399 180.86 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_0785 PE = 4 SV = 1 – (No CS) [Q97RL9_STRPN] P0A3M9 47.26 14 328 180.34 L-lactate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = Idh PE = 3 SV = 2 – [LDH_STRPN] Q97PT4 39.72 17 501 180.17 ATP synthase subunit alpha OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = atpA PE = 3 SV = 1 – [ATPA_STRPN] Q97QH1 67.48 9 246 175.25 Amino acid ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1242 PE = 3 SV = 1 – [Q97QH1_STRPN] Q97NX9 72.9 19 321 174.95 Iron-compound ABC transporter, iron- Lipid compound-binding protein OS = anchored *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1872 PE = 4 SV = 1 – [Q97NX9_STRPN] Q97QC6 66.09 14 115 173.94 50 S ribosomal protein L19 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplS PE = 3 SV = 1 – [RL19_STRPN] Q97N99 63.14 18 274 172.57 SPFH domain/Band 7 family OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2156 PE = 4 SV = 1 – [Q97N99_STRPN] Q97PW1 50.97 17 463 169.74 Peptidoglycan N-acetylglucosamine N- deacetylase A OS = -*Streptococcus* terminally *pneumoniae* serotype 4 (strain ATCC anchored BAA-334/TIGR4) GN = pgdA PE = 4 (No CS) SV = 1 – [Q97PW1_STRPN] Q97SE1 36.4 14 555 169.45 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0443 PE = 1 SV = 1 – [Q97SE1_STRPN] Q97PM5 59.04 16 376 168.92 Sugar ABC transporter, ATP-binding Intracellular protein OS = - *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = msmK PE = 4 SV = 1

- [Q97PM5_STRPN] Q97SN5 85.81 15 148 165.86 50 S ribosomal protein L13 OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplM PE = 3 SV = 1 -
 [Q97SN5_STRPN] Q97PQ2 28.88 18 914 162.85 Cation-transporting ATPase, E1-E2 Multi-
 family OS = -*Streptococcus pneumoniae* trans- serotype 4 (strain ATCC BAA-334/ membrane
 TIGR4) GN = SP_1551 PE = 3 SV = 1 - [Q97PQ2_STRPN] Q97NE4 32.16 21 821 161.61
 Penicillin-binding protein 1 B OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain
 ATCC BAA-334/TIGR4) anchored GN = pbp1B PE = 4 SV = 1 - (No CS) [Q97NE4_STRPN]
 Q97Q62 54.48 20 424 161.35 Psr protein OS = -*Streptococcus* Intracellular/ *pneumoniae* serotype
 4 (strain ATCC TMH BAA-334/TIGR4) GN = SP_1368 PE = 4 start SV = 1 - [Q97Q62_STRPN]
 AFTER 60 P18766 50 13 308 160.11 Oligopeptide transport ATP-binding Intracellular protein
 AmiF OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiF PE
 = 3 SV = 2 - [AMIF_STRPN] I6L8U0 42.39 16 394 155.34 UDP-N-acetylglucosamine 2-
 Intracellular epimerase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = cps4L PE = 3 SV = 1 - [I6L8U0_STRPN] P0CC08 55.21 15 288 154.74
 Acetyl-coenzyme A carboxylase Intracellular carboxyl transferase subunit beta OS = *Streptococcus*
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = accD PE = 1 SV = 1 -
 [ACCD_STRPN] Q97RG9 51.27 21 511 154.04 Sugar ABC transporter, ATP-binding Intracellular
 protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =
 SP_0846 PE = 3 SV = 1 - [Q97RG9_STRPN] Q97SJ4 50.28 17 360 153.5 Uncharacterized protein
 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =
 SP_0355 PE = 4 SV = 1 - [Q97SJ4_STRPN] P72524 33.82 22 822 148.92 DNA gyrase subunit A
 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =
 gyrA PE = 3 SV = 3 - [GYRA_STRPN] P66565 56.16 17 203 148.91 30 S ribosomal protein S4
 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =
 rpsD PE = 3 SV = 1 - [RS4_STRPN] P0A4G0 41.1 20 652 146.87 Oligopeptide-binding protein
 AliB Lipid OS = = -*Streptococcus pneumoniae* anchored serotype 4 (strain ATCC BAA-334/
 TIGR4) GN = aliB PE = 3 SV = 1 - [ALIB_STRPN] Q97SG0 57.78 18 424 144.79 Serine--tRNA
 ligase OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4)
 GN = serS PE = 3 SV = 1 - [SYS_STRPN] Q97PF9 51.79 20 419 144.46 Cell division protein
 FtsZ OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN
 = ftsZ PE = 3 SV = 1 - [Q97PF9_STRPN] Q97Q34 64.04 17 267 144.16 Phosphate import ATP-
 binding protein Intracellular PstB 2 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC
 BAA-334/ TIGR4) GN = pstB2 PE = 3 SV = 1 - [PSTB2_STRPN] I6L8N1 47.25 14 455 141.81
 Acetyl-CoA carboxylase, biotin Intracellular carboxylase OS = -*Streptococcus pneumoniae*
 serotype 4 (strain ATCC BAA-334/TIGR4) GN = accC PE = 4 SV = 1 - [I6L8N1_STRPN]
 Q97SR1 45.54 19 617 140.84 Proline--tRNA ligase OS = = Intracellular *Streptococcus pneumoniae*
 serotype 4 (strain ATCC BAA-334/TIGR4) GN = proS PE = 3 SV = 1 - [SYP_STRPN] Q97SI4
 40.88 11 340 140.1 Choline binding protein C OS = N- *Streptococcus pneumoniae* serotype 4
 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpC PE = 4 SV = 1 -
 [Q97SI4_STRPN] (with CS) P0A4D7 40.84 17 524 138.63 DEAD-box ATP-dependent RNA
 Intracellular helicase CshA OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = cshA PE = 3 SV = 1 - [CSHA_STRPN] Q97RN3 44.44 17 513 136.66 ABC
 transporter, ATP-binding protein Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain
 ATCC BAA-334/ TIGR4) GN = SP_0770 PE = 1 SV = 1 - [Q97RN3_STRPN] Q97PR0 43.62 17
 447 136.28 Asparagine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4
 (strain ATCC BAA-334/TIGR4) GN = asnS PE = 1 SV = 1 - [SYN_STRPN] P22976 40.73 18 658
 132.04 Probable transketolase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain
 ATCC BAA-334/TIGR4) GN = tkt PE = 3 SV = 2 - [TKT_STRPN] Q97PX1 34.2 16 459 132
 NADH oxidase OS = = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCO BAA-
 334/TIGR4) GN = nox PE = 4 SV = 1 - [Q97PX1_STRPN] O05703 50.5 17 501 129.61 Zinc-

binding lipoprotein AdcA OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = adcA PE = 3 SV = 4 – [ADCA_STRPN] P66095 52.4 12 229 128.44 50 S ribosomal protein L1 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplA PE = 3 SV = 1 – [RL1_STRPN] Q97RP4 55.65 12 230 127.75 Cell division ABC transporter, ATP- Intracellular binding protein FtsE OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsE PE = 3 SV = 1 – [Q97RP4_STRPN] Q97RP3 37.66 12 308 126.99 Cell division protein FtsX OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = ftsX PE = 3 SV = 1 – [Q97RP3_STRPN] Q97N72 38.4 23 810 122.4 ATP-dependent Clp protease, ATP- Intracellular binding subunit OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2194 PE = 3 SV = 1 – [Q97N72_STRPN] Q97Q67 43.9 14 344 121.11 Conserved domain protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_1363 PE = 4 SV = 1 – [Q97Q67_STRPN] P95830 50.79 15 378 120.38 Chaperone protein DnaJ OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaJ PE = 1 SV = 2 – [DNAJ_STRPN] Q97SJ6 53.04 8 230 119.51 Capsular polysaccharide biosynthesis Multi- protein CpsC OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC membrane BAA-334/TIGR4) GN = cpsC PE = 3 SV = 1 – [CPSC_STRPN] P63373 71.03 13 252 116.79 Phosphate import ATP-binding protein Intracellular PstB 1 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = pstB1 PE = 3 SV = 1 – [PSTB1_STRPN] I6L8Q3 32.35 12 340 114.44 Choline binding protein F OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpF PE = 4 SV = 1 – [I6L8Q3_STRPN] (with CS) Q97RS8 62.96 16 378 110.09 Lactate oxidase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = IctO-2 PE = 4 SV = 1 – [Q97RS8_STRPN] Q97N53 70.96 11 272 109.3 Cell shape-determining protein MreC N- OS = -*Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = mreC PE = 3 SV = 1 – (No CS) [Q97N53_STRPN] P0A3M5 41.32 18 680 109.25 Penicillin-binding protein 2 B OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = penA PE = 3 SV = 1 – [PBP2_STRPN] (No CS) P0A4S1 49.15 9 293 109 Fructose-bisphosphate aldolase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = fba PE = 3 SV = 1 – [ALF_STRPN] Q97RK0 29.57 13 575 107.28 Septation ring formation regulator N- EzrA OS = -*Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = ezrA PE = 3 SV = 1 – (No CS) [EZRA_STRPN] Q97SI9 60.55 7 109 107.21 Cell cycle protein GpsB OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpsB PE = 3 SV = 1 – [GPSB_STRPN] P63413 36.36 12 396 106.75 Acetate kinase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ackA PE = 3 SV = 1 – [ACKA_STRPN] P0A2Z2 59.76 10 164 105.18 ATP synthase subunit b OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpF PE = 3 SV = 1 – [ATPF_STRPN] Q97SQ4 39.74 8 156 103.23 30 S ribosomal protein S7 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsG PE = 3 SV = 1 – [RS7_STRPN] Q97QK5 48.6 19 607 101.3 Elongation factor 4 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = lepA PE = 3 SV = 1 – [LEPA_STRPN] P65239 39.75 11 322 100.54 Ribose-phosphate pyrophosphokinase Intracellular 1 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = prs1 PE = 3 SV = 1 – [KPRS1_STRPN] P95829 36.41 14 607 100.51 Chaperone protein DnaK OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaK PE = 3 SV = 2 – [DNAK_STRPN] I6L8U5 55.47 11 411 100.01 3-oxoacyl-[acyl-carrier-protein] Intracellular synthase 2 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = fabF PE = 3 SV = 1 – [16L8U5_STRPN] Q97PV1 21.8 6 289 93.98 Putative glycerol uptake facilitator Multi- protein OS = -*Streptococcus pneumoniae* serotype 4 (strain

ATCC membrane BAA-334/TIGR4) GN = SP_1491 PE = 3 SV = 1 - [Q97PV1_STRPN]

Q2MGH6 15.73 18 1767 93.68 Endo-alpha-N-acetylgalactosaminidase LP × TG OS = -

Streptococcus pneumoniae Cell-wall anchored serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0368 PE = 1 SV = 1 - [GH101_STRPN] Q97SQ9 36.05 13 602 92.56 Glutamine--fructose-6-phosphate Intracellular aminotransferase [isomerizing] OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glmS PE = 3 SV = 3 - [GLMS_STRPN] Q97P40 53.96 9 202 92.31 Putative general stress protein 24 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1804 PE = 4 SV = 1 - [Q97P40_STRPN] Q97QE4 25.33 18 1058 90.95 Carbamoyl-phosphate synthase large Intracellular chain OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = carB PE = 3 SV = 1 - [CARB_STRPN] I6L8Q9 40.04 11 457 90.76 Cell division protein ftsA OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsA PE = 3 SV = 1 - [I6L8Q9_STRPN] I6L8S9 45.52 10 413 90.26 D-alanyl-D-alanine carboxypeptidase N- OS = -*Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = dacA PE = 3 SV = 1 - (with CS) [I6L859_STRPN] Q97Q48 27.52 18 872 89.81 Alanine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = alaS PE = 3 SV = 1 - [SYA_STRPN] Q97QD5 64.52 12 186 89.81 LemA protein OS = -*Streptococcus N- pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = lemA PE = 1 anchored SV = 1 - [Q97QD5_STRPN] (No CS) Q97NW2 48.69 13 419 88.69 Sugar ABC transporter, sugar-binding Lipid protein OS = -*Streptococcus anchored pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rafE PE = 1 SV = 1 - [Q97NW2_STRPN] I6L8N0 43.01 14 365 88.6 UDP-N-acetylglucosamine-2- Intracellular epimerase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4I PE = 3 SV = 1 - [I6L8N0_STRPN] Q97QW4 36.47 13 425 87.93 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1083 PE = 4 SV = 1 - [Q97QW4_STRPN] Q9FBB7 58.43 12 255 86.89 Acetyl-coenzyme A carboxylase Intracellular carboxyl transferase subunit alpha OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = accA PE = 1 SV = 1 - [ACCA_STRPN] P67266 42.42 4 99 86.55 Nucleoid-associated protein SP_1102 Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_1102 PE = 1 SV = 1 - [Y1102_STRPN] Q97QM4 24.06 13 719 86.48 Ribonucleoside-diphosphate Intracellular reductase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = nrde PE = 3 SV = 1 - [Q97QM4_STRPN] Q97PF5 44.17 8 283 85.34 Putative phosphosugar-binding Intracellular transcriptional regulator OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1674 PE = 4 SV = 1 - [Q97PF5_STRPN] P0A3Y3 50.87 10 230 84.95 2,3-bisphosphoglycerate-dependent Intracellular phosphoglycerate mutase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPN] Q97NE1 40.78 4 103 84.35 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2102 PE = 4 SV = 1 - [Q97NE1_STRPN] P67282 35.58 16 534 83.04 Ribonuclease Y OS = -*Streptococcus N- pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = rny PE = 3 SV = 1 - anchored [RNY_STRPN] (No CS) Q97QX6 22.61 14 898 82.38 Phosphoenolpyruvate carboxylase Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = ppc PE = 3 SV = 1 - [CAPP_STRPN] Q97SD1 32.63 12 521 81.72 Amino acid ABC transporter, amino Multi- acid-binding protein/permease trans- protein OS = -*Streptococcus membrane pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0453 PE = 4 SV = 1 - [Q97SD1_STRPN] P66419 50.56 8 89 78.12 30 S ribosomal protein S14 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsN PE = 3 SV = 1 - [RS14_STRPN] P61182 61.4 8 114 77.58 50 S ribosomal protein L22 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplV PE = 1 SV = 1 -

[RL22_STRPN] P66278 33.33 4 66 77.34 50 S ribosomal protein L35 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpml PE = 3 SV = 1 – [RL35_STRPN] Q97R14 34.57 10 324 76.27 Uncharacterized protein OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1027 PE = 4 SV = 1 – [Q97R14_STRPN] Q97T52 22.4 12 616 70.86 Putative capsular polysaccharide Multi- biosynthesis protein OS = trans- *Streptococcus pneumoniae* serotype 4 membrane (strain ATCC BAA-334/TIGR4) GN = SP_0103 PE = 4 SV = 1 – [Q97T52_STRPN] Q97SW2 26.53 13 735 70.68 Anaerobic ribonucleoside- Intracellular triphosphate reductase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = nrdD PE = 4 SV = 1 – [Q97SW2_STRPN] Q97SE4 32.3 12 514 70.62 Peptide chain release factor 3 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = prfC PE = 3 SV = 1 – [RF3_STRPN] Q97PC4 27.24 9 492 68.5 ABC transporter, ATP-binding protein Multi- OS = - *Streptococcus pneumoniae* trans- serotype 4 (strain ATCC BAA-334/ membrane TIGR4) GN = SP_1715 PE = 4 SV = 1 – [Q97PC4_STRPN] I6L8V8 47.33 7 243 68.48 3-oxoacyl-[acyl-carrier protein] Intracellular reductase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = fabG PE = 3 SV = 1 – [I6L8V8_STRPN] Q97S28 21.17 11 737 68.26 Polyribonucleotide Intracellular nucleotidyltransferase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pnp PE = 3 SV = 1 – [PNP_STRPN] Q97RC4 41.38 9 290 67.69 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_0899 PE = 4 SV = 1 – [Q97RC4_STRPN] Q97S86 21.65 7 448 65.38 Glutamine synthetase, type I OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glnA PE = 3 SV = 1 – [Q97S86_STRPN] Q97NH4 41.48 9 352 65.13 Alcohol dehydrogenase, zinc- Intracellular containing OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2055 PE = 3 SV = 1 – [Q97NH4_STRPN] P0A495 65.79 3 38 64.65 50 S ribosomal protein L36 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmJ PE = 3 SV = 1 – [RL36_STRPN] Q97ND6 25.72 13 587 64.48 Aspartate--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = aspS PE = 3 SV = 1 – [SYD_STRPN] P72525 18.1 10 823 63.42 DNA topoisomerase 4 subunit A OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = parC PE = 1 SV = 3 – [PARC_STRPN] Q97PH2 23.11 12 740 63.08 GTP pyrophosphokinase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = relA PE = 3 SV = 1 – [Q97PH2_STRPN] Q97T46 32.63 4 95 63.06 Putative bacteriocin OS = Secretory *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0109 PE = 4 SV = 1 – [Q97T46_STRPN] I6L8V0 42.17 11 351 60.21 Capsular polysaccharide biosynthesis Intracellular protein Cps4J OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cap4J PE = 4 SV = 1 – [I6L8V0_STRPN] Q97RQ6 23.84 6 281 60.06 DegV domain-containing protein Intracellular SP_0742 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0742 PE = 3 SV = 1 – [Y742_STRPN] Q97SR2 35.08 9 419 60.03 Putative zinc metalloprotease Multi- SP_0263 OS = -*Streptococcus* trans- *pneumoniae* serotype 4 (strain ATCC membrane BAA-334/TIGR4) GN = SP_0263 PE = 3 SV = 1 – [Y263_STRPN] Q97PB8 15.63 8 627 59.73 PTS system IIABC components OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_1722 PE = 4 SV = 1 – [Q97PB8_STRPN] Q97QE6 41.47 9 340 58.95 Alcohol dehydrogenase, zinc- Intracellular containing OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1270 PE = 4 SV = 1 – [Q97QE6_STRPN] Q97P07 34.56 10 408 58.93 Putative capsular polysaccharide Intracellular biosynthesis protein OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1837 PE = 3 SV = 1 – [Q97P07_STRPN] Q97T09 40.23 9 353 57.99 Methionine import ATP-binding Intracellular protein MetN OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = metN PE = 3 SV = 1 – [METN_STRPN]

Q97NP5 18.83 7 308 57.43 Membrane protein insertase YidC 1 Multi- OS = -*Streptococcus pneumoniae* trans- serotype 4 (strain ATCC BAA-334/ membrane TIGR4) GN = yidC1 PE = 3 SV = 1 – [YIDC1_STRPN] P0A451 41.49 12 388 57.14 Protein RecA OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = recA PE = 2 SV = 1 – [RECA_STRPN] Q97SU7 51.12 5 178 56.93 50 S ribosomal protein L6 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplF PE = 3 SV = 1 – [RL6_STRPN] Q97NE2 18.49 9 687 56.54 Cation-transporting ATPase, E1-E2 Intracellular family OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_2101 PE = 3 SV = 1 – [Q97NE2_STRPN] Q97SN4 32.31 6 130 56.22 30 S ribosomal protein S9 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsI PE = 3 SV = 1 – [RS9_STRPN] P66392 51.24 7 121 56.15 30 S ribosomal protein S13 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsM PE = 3 SV = 1 – [RS13_STRPN] Q97S10 23.51 11 553 55.98 Ribonuclease J OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rnj PE = 3 SV = 1 – [Q97S10_STRPN] Q97PG0 20.99 5 262 55.94 Cell division protein DivIVA OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = divIVA PE = 4 SV = 1 – [Q97PG0_STRPN] P35597 17.61 9 778 55.84 Probable cation-transporting ATPase Multi- exp7 OS = -*Streptococcus pneumoniae* trans- serotype 4 (strain ATCC BAA-334/ membrane TIGR4) GN = exp7 PE = 3 SV = 2 – [EXP7_STRPN] Q97SJ2 43.52 12 409 55.75 Capsular polysaccharide biosynthesis Intracellular protein Cps4K OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4K PE = 4 SV = 1 – [Q97SJ2_STRPN] Q97PI4 29.98 12 647 55.52 Threonine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = thrS PE = 1 SV = 1 – [SYT_STRPN] Q97QW8 25.81 8 434 55.41 GTPase Obg OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = obg PE = 3 SV = 1 – [OBG_STRPN] P67293 43.9 3 82 55.21 UPF0154 protein SP_1882 OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_1882 PE = 3 SV = 1 – (No CS) [Y1882_STRPN] P0A4L9 20.52 10 648 54.97 DNA gyrase subunit B OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gyrB PE = 3 SV = 1 – [GYRB_STRPN] Q97SJ8 33.2 11 494 54.78 UPF0371 protein SP_0341 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0341 PE = 3 SV = 1 – [Y341_STRPN] Q54869 24.16 10 563 54.43 Arginine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = argS PE = 1 SV = 2 – [SYR_STRPN] P35595 14.88 7 726 53.64 PTS system glucose-specific EIICBA Multi- component OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC membrane BAA-334/TIGR4) GN = exp5 PE = 3 SV = 2 – [PTG3C_STRPN] Q97SI7 22.41 7 464 53.36 Mid-cell-anchored protein Z OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = mapZ PE = 1 SV = 1 – [Q97SI7_STRPN] P0A4B5 55.91 7 93 52.72 30 S ribosomal protein S19 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsS PE = 3 SV = 1 – [RS19_STRPN] Q97SU3 23.29 4 146 52.09 50 S ribosomal protein L15 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplO PE = 3 SV = 1 – [RL15_STRPN] Q97RV5 27.57 11 613 51.74 Elongation factor Tu family protein Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0681 PE = 4 SV = 1 – [Q97RV5_STRPN] Q97NW9 8.4 5 655 50.97 Trehalose PTS system, IIABC Multi-components OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC membrane BAA-334/TIGR4) GN = SP_1884 PE = 4 SV = 1 – [Q97NW9_STRPN] Q97QE7 41.6 9 262 49.97 Choline kinase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pck PE = 4 SV = 1 – [Q97QE7_STRPN] P67595 31.96 8 341 48.09 Tryptophan--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = trpS PE = 3 SV = 1 – [SYW_STRPN] P66581 55.49 6 164 47.77 30 S

ribosomal protein S5 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsE PE = 3 SV = 1 - [RS5_STRPN] Q97R57 16.33 10 784 47.19 Ribonuclease R OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rnr PE = 3 SV = 1 - [Q97R57_STRPN] Q97RP0 21.03 7 447 46.86 DEAD-box ATP-dependent RNA Intracellular helicase CshB OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cshB PE = 3 SV = 1 - [Q97RP0_STRPN] P0A3R1 22.65 9 649 46.48 DNA mismatch repair protein HexB Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = hexB PE = 3 SV = 1 - [HEXB_STRPN] P0A3R3 18.48 11 844 45.87 DNA mismatch repair protein HexA Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = hexA PE = 3 SV = 1 - [HEXA_STRPN] Q97SF6 27.97 7 261 45.73 Enoyl-CoA hydratase/isomerase family Intracellular protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0415 PE = 4 SV = 1 - [Q97SF6_STRPN] P66708 45.98 9 311 45.6 DNA-directed RNA polymerase subunit alpha Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = rpoA PE = 3 SV = 1 - [RPOA_STRPN] I6L858 28.69 9 481 45.33 Capsular polysaccharide biosynthesis Multi- protein Cps4A OS = -*Streptococcus trans- pneumoniae* serotype 4 (strain ATCC membrane BAA-334/TIGR4) GN = cps4A PE = 4 SV = 1 - [I6L8S8_STRPN] Q97N43 19.51 7 492 45.08 Inosine-5'-monophosphate Intracellular dehydrogenase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = guaB PE = 3 SV = 1 - [Q97N43_STRPN] I6L8W8 16.87 6 332 44.57 Choline binding protein J OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpJ PE = 4 SV = 1 - [I6L8W8_STRPN] (with CS) Q2MGG2 27.62 7 467 44.4 Serine protease, subtilase family OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1954 PE = 4 SV = 1 - [Q2MGG2_STRPN] Q97SP0 42.22 7 270 44.36 Cof family protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0286 PE = 4 SV = 1 - [Q97SP0_STRPN] Q97PG9 38.75 7 240 44.16 Manganese ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = psaB PE = 3 SV = 1 - [Q97PG9_STRPN] Q97T72 31.67 6 221 44.14 Potassium uptake protein, Trk family N- OS = -*Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = SP_0079 PE = 4 SV = 1 - (No CS) [Q97T72_STRPN] Q97RS9 21.98 7 496 44.07 Lysine--tRNA ligase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = lysS PE = 1 SV = 2 - [SYK_STRPN] Q97PK5 27.22 2 158 43.99 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_1604 PE = 4 SV = 1 - (No CS) [Q97PK5_STRPN] Q97RV8 38.89 6 126 43.74 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_0678 PE = 4 SV = 1 - (No CS) [Q97RV8_STRPN] P06653 26.1 6 318 43.37 Autolysin OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytA PE = 1 SV = 2 - [ALYS_STRPN] P63791 27.8 8 410 42.54 ATP-dependent Clp protease ATP- Intracellular binding subunit ClpX OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = clpX PE = 3 SV = 1 - [CLPX_STRPN] Q97Q36 37.33 7 217 42.45 Phosphate-specific transport system Intracellular accessory protein PhoU OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1395 PE = 3 SV = 1 - [Q97Q36_STRPN] Q97R16 32.06 10 418 42.38 Serine hydroxymethyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glyA PE = 3 SV = 1 - [GLYA_STRPN] Q97NM1 38.99 9 336 42.33 Catabolite control protein A OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ccpA PE = 4 SV = 1 - [Q97NM1_STRPN] Q97PQ8 42.66 6 143 40.36 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1545 PE = 4 SV = 1 - [Q97PQ8_STRPN] Q97SU6 59.32 7 118 40.13 50 S ribosomal protein L18 OS =

Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplR PE = 3 SV = 1 – [RL18_STRPN] Q97RC9 30.77 3 156 40.01 Arginine repressor OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = argR PE = 3 SV = 1 – [Q97RC9_STRPN] Q97RC5 25.15 8 501 39.74 Pyruvate kinase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyk PE = 3 SV = 1 – [Q97RC5_STRPN] Q97PM1 45.04 7 262 38.81 GTP-sensing transcriptional pleiotropic Intracellular repressor CodY OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = codY PE = 1 SV = 1 – [CODY_STRPN] I6L8N6 28.91 5 211 38.64 Capsular polysaccharide biosynthesis N- protein Cps4E OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC anchored BAA-334/TIGR4) GN = cps4E PE = 4 (No CS) SV = 1 – [I6L8N6_STRPN] Q97R36 38.38 7 185 38.61 Thioredoxin family protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_1000 PE = 1 SV = 1 – [Q97R36_STRPN] Q97S93 17.76 7 535 37.74 CTP synthase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyrG PE = 3 SV = 1 – [PYRG_STRPN] Q97QW1 20.71 9 763 37.7 DNA helicase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pcrA PE = 4 SV = 1 – [Q97QW1_STRPN] P63544 51.76 6 170 37.59 Adenine phosphoribosyltransferase Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = apt PE = 1 SV = 1 – [APT_STRPN] P65241 18.18 5 319 37.57 Ribose-phosphate pyrophosphokinase Intracellular 2 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = prs2 PE = 3 SV = 1 – [KPRS2_STRPN] Q97RE5 17.08 6 650 37.17 PTS system, fructose specific IIABC Multi- components OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC membrane BAA-334/TIGR4) GN = SP_0877 PE = 4 SV = 1 – [Q97RE5_STRPN] P35596 21.38 9 608 37.12 Alpha-glycerophosphate oxidase OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = glpO PE = 3 SV = 2 – [GLPO_STRPN] (No CS) Q97R12 19.52 7 543 37.11 Uncharacterized RNA Intracellular methyltransferase SP_1029 OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1029 PE = 3 SV = 1 – [Y1029_STRPN] P64166 12.91 7 767 36.81 DNA translocase FtsK OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = ftsK PE = 3 SV = 1 – [FTSK_STRPN] P35594 17.15 10 752 36.7 ATP-dependent Clp protease ATP- Intracellular binding subunit ClpE OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = clpE PE = 3 SV = 2 – [CLPE_STRPN] P64072 53.33 8 195 36.63 Probable GTP-binding protein EngB Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = engB PE = 3 SV = 1 – [ENGB_STRPN] Q97TC4 31.11 5 180 36.56 Hypoxanthine-guanine Intracellular phosphoribosyltransferase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = hpt PE = 3 SV = 1 – [HPRT_STRPN] Q97PU2 37.8 4 209 36.45 Amino acid ABC transporter, ATP- Intracellular binding protein OS = - *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1501 PE = 3 SV = 1 – [Q97PU2_STRPN] P0A3S3 38.69 6 274 36.43 DNA-entry nuclease OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = endA PE = 1 SV = 1 – [NUCE_STRPN] (No CS) I6L8N9 25.49 8 506 36.2 Choline transporter OS = - *Streptococcus pneumoniae* serotype 4 (strain ATCC trans- BAA-334/TIGR4) GN = proWX PE = 3 membrane SV = 1 – [I6L8N9_STRPN] Q97PQ5 25.99 8 531 36.19 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_1548 PE = 4 SV = 1 – [Q97PQ5_STRPN] Q97NI7 28.66 6 328 36.13 Putative jag protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2040 PE = 4 SV = 1 – [Q97NI7_STRPN] P63742 19.32 7 502 36.12 Glycerol kinase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glpK PE = 3 SV = 1 – [GLPK_STRPN] Q97NT7 32.91 4 158 36 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC

BAA-334/TIGR4) membrane GN = SP_1926 PE = 4 SV = 1 - [Q97NT7_STRPN] P64297 23.85 7
 520 35.92 GMP synthase [glutamine-hydrolyzing] Intracellular OS = *-Streptococcus pneumoniae*
 serotype 4 (strain ATCC BAA-334/TIGR4) GN = guaA PE = 3 SV = 1 - [GUAA_STRPN]
 Q97R84 28.38 7 444 35.81 Methylenetetrahydrofolate--tRNA- Intracellular (uracil-5-)-
 methyltransferase TrmFO OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/
 TIGR4) GN = trmFO PE = 3 SV = 2 - [TRMFO_STRPN] Q97PK2 19.17 5 339 35.39 UDP-
 glucose 4-epimerase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = galE-1 PE = 3 SV = 1 - [Q97PK2_STRPN] Q97RP6 41.53 6 236 35.16
 Branched-chain amino acid ABC Intracellular transporter, ATP-binding protein OS = *Streptococcus*
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = livF PE = 3 SV = 1 -
 [Q97RP6_STRPN] Q97NM3 30.74 10 462 35.12 Cof family protein OS = *-Streptococcus*
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1997 PE = 4 SV =
 1 - [Q97NM3_STRPN] Q97S34 10.86 7 801 34.77 Phenylalanine--tRNA ligase beta Intracellular
 subunit OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pheT
 PE = 3 SV = 1 - [SYFB_STRPN] Q97NQ4 31.38 9 427 34.05 UDP-N-acetylglucosamine 1-
 Intracellular carboxyvinyltransferase 1 OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC
 BAA-334/TIGR4) GN = murA1 PE = 3 SV = 1 - [MURA1_STRPN] Q97PY0 33.2 6 247 34.04
 Amino acid ABC transporter, ATP- Intracellular binding protein OS = *-Streptococcus pneumoniae*
 serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1460 PE = 3 SV = 1 - [Q97PY0_STRPN]
 Q97RQ3 44.5 6 209 33.91 Uracil phosphoribosyltransferase OS = Intracellular *Streptococcus*
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = upp PE = 3 SV = 1 -
 [UPP_STRPN] Q97QP0 15.87 7 567 33.86 Dihydrolipoyl dehydrogenase OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1161 PE = 4 SV =
 1 - [Q97QP0_STRPN] P64062 24.77 8 436 33.49 GTPase Der OS = *-Streptococcus* Intracellular
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = der PE = 3 SV = 1 - [DER_STRPN]
 Q97N48 22.83 6 276 33.26 Uncharacterized protein OS = Intracellular/ *Streptococcus pneumoniae*
 serotype 4 TMH (strain ATCC BAA-334/TIGR4) start GN = SP_2223 PE = 4 SV = 1 - AFTER 60
 [Q97N48_STRPN] Q97SJ5 22.98 7 409 32.72 Capsular polysaccharide biosynthesis Multi- protein
 Cps4F OS = *-Streptococcus trans- pneumoniae* serotype 4 (strain ATCC membrane BAA-
 334/TIGR4) GN = cps4F PE = 4 SV = 1 - [Q97SJ5_STRPN] P65832 36.73 6 275 32.69 Pur
 operon repressor OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = purR PE = 3 SV = 1 - [PURR_STRPN] P63384 12.09 9 943 31.63 UvrABC
 system protein A OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = uvrA PE = 3 SV = 1 - [UVRA_STRPN] I6L8W3 33.88 8 242 31.53 Choline
 transporter OS = *-Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = proV PE = 3 SV = 1 - [I6L8W3_STRPN] Q9AHD2 40.97 8 227 31.44
 Tyrosine-protein kinase CpsD OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain
 ATCC BAA-334/TIGR4) GN = cpsD PE = 3 SV = 1 - [CPSD_STRPN] O08397 23.84 8 453 31.17
 Chromosomal replication initiator Intracellular protein DnaA OS = *-Streptococcus pneumoniae*
 serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaA PE = 3 SV = 2 - [DNAA_STRPN]
 Q97PT5 23.63 7 292 30.59 ATP synthase gamma chain OS = Intracellular *Streptococcus*
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpG PE = 3 SV = 1 -
 [ATPG_STRPN] Q97PJ1 22.09 5 249 30.29 Acyltransferase family protein OS = Multi-
Streptococcus pneumoniae serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN =
 SP_1624 PE = 4 SV = 1 - [Q97PJ1_STRPN] Q97NF6 14.36 6 564 30.07 ABC transporter, ATP-
 Multi- binding/permease protein OS = trans- *Streptococcus pneumoniae* serotype 4 membrane
 (strain ATCC BAA-334/TIGR4) GN = SP_2075 PE = 3 SV = 1 - [Q97NF6_STRPN] Q97PW8
 18.36 6 414 30.06 Putative oxidoreductase OS = Intracellular *Streptococcus pneumoniae* serotype 4
 (strain ATCC BAA-334/TIGR4) GN = SP_1472 PE = 4 SV = 1 - [Q97PW8_STRPN] Q97QT0
 15.61 5 474 29.86 Glyceraldehyde-3-phosphate Intracellular dehydrogenase, NADP-dependent OS

= *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gapN PE = 3 SV = 1 - [Q97QT0_STRPN] Q97PW6 12.24 6 678 29.5 Glycine--tRNA ligase beta subunit Intracellular OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glyS PE = 3 SV = 1 - [SYGB_STRPN] Q97T93 25.93 8 432 29.32 Adenylosuccinate lyase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = purB PE = 3 SV = 1 - [Q97T93_STRPN] Q97RG3 5.68 2 229 29.15 Putative membrane protein OS = Multi-*Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_0858 PE = 4 SV = 1 - [Q97RG3_STRPN] Q97TA6 12.42 3 330 28.21 Phosphate acyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = plsX PE = 3 SV = 1 - [PLSX_STRPN] Q97TA8 19.02 6 389 27.91 Aromatic amino acid aminotransferase Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = araT PE = 3 SV = 1 - [Q97TA8_STRPN] P0A3B7 23.99 5 346 27.48 Elongation factor Ts OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = tsf PE = 1 SV = 2 - [EFTS_STRPN] I6L8Q7 20.99 4 324 27.27 Enoyl- (Acyl-carrier-protein) reductase Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = fabK PE = 4 SV = 1 - [I6L8Q7_STRPN] Q97SG5 9.38 5 778 26.77 Endonuclease MutS2 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = mutS2 PE = 3 SV = 1 - [MUTS2_STRPN] Q97S79 15.4 5 487 26.69 Type I restriction-modification system, Intracellular M subunit OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = hsdM PE = 4 SV = 1 - [Q97S79_STRPN] Q97SF8 12.78 4 454 26.54 Aspartokinase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0413 PE = 3 SV = 1 - [Q97SF8_STRPN] Q97S89 19.1 5 398 26.46 Phosphoglycerate kinase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgk PE = 3 SV = 1 - [PGK_STRPN] Q97QH0 22.14 6 429 26.44 Signal recognition particle receptor Intracellular FtsY OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = ftsY PE = 1 SV = 1 - [Q97QH0_STRPN] P0CB59 5.83 3 326 26.31 Phospho-N-acetylmuramoyl- Multi- pentapeptide-transferase OS = trans- *Streptococcus pneumoniae* serotype 4 membrane (strain ATCC BAA-334/TIGR4) GN = mraY PE = 3 SV = 1 - [MRAY_STRPN] Q97P92 23.64 5 368 26.11 GTP-binding protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1749 PE = 4 SV = 1 - [Q97P92_STRPN] P63733 20.06 6 359 25.98 Carbamoyl-phosphate synthase small Intracellular chain OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = carA PE = 3 SV = 1 - [CARA_STRPN] Q97SP1 28.61 6 339 25.89 Alcohol dehydrogenase, zinc- Intracellular containing OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0285 PE = 3 SV = 1 - [Q97SP1_STRPN] Q97PP2 19.92 4 256 25.75 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_1561 PE = 1 SV = 1 - [Q97PP2_STRPN] P66339 48.04 4 102 25.74 30 S ribosomal protein S10 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsJ PE = 3 SV = 1 - [RS10_STRPN] P0A335 13.52 6 540 25.62 60 kDa chaperonin OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = groL PE = 1 SV = 1 - [CH60_STRPN] O07344 15.69 3 204 25.56 Signal peptidase I OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = lepB PE = 3 anchored SV = 2 - [LEP_STRPN] (No CS) Q97PP5 41.41 3 128 25.55 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1558 PE = 1 SV = 1 - [Q97PP5_STRPN] Q97RP7 15.75 2 254 25.33 Branched-chain amino acid ABC Intracellular transporter, ATP-binding protein OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = livG PE = 3 SV = 1 - [Q97RP7_STRPN] Q97Q12 13.37 5 486 24.99 Nicotinate phosphoribosyltransferase Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_1421 PE = 3 SV = 1 - [Q97Q12_STRPN] P67506 26.07 5 211 24.79 tRNA (guanine-N(7))- Intracellular

methyltransferase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = trmB PE = 1 SV = 1 - [TRMB_STRPN] P66155 17.74 2 62 24.21 50 S ribosomal protein L28 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmB PE = 3 SV = 1 - [RL28_STRPN] P66200 71.25 3 80 23.95 50 S ribosomal protein L31 type B OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmE2 PE = 3 SV = 1 - [RL31B_STRPN] Q97NH2 17.69 5 605 23.83 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_2057 PE = 4 SV = 1 - [Q97NH2_STRPN] Q97PP4 12.89 4 450 23.67 Phosphoglucosamine mutase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glmM PE = 3 SV = 1 - [GLMM_STRPN] Q97R44 50.43 4 115 23.65 Uncharacterized protein OS = Intracellular/ *Streptococcus pneumoniae* serotype 4 TMH (strain ATCC BAA-334/TIGR4) start GN = SP_0990 PE = 4 SV = 1 - AFTER 60 [Q97R44_STRPN] Q97QB4 15.18 4 448 23.57 Glutamate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gdhA PE = 3 SV = 1 - [Q97QB4_STRPN] Q97SW6 23.68 2 152 23.55 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_0198 PE = 1 SV = 1 - [Q97SW6_STRPN] Q97PW5 16.07 4 305 23.51 Glycine--tRNA ligase alpha subunit Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = glyQ PE = 3 SV = 1 - [SYGA_STRPN] Q97Q69 19.63 5 428 23.47 Homoserine dehydrogenase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = hom PE = 3 SV = 1 - [Q97Q69_STRPN] Q97QT2 12.27 6 652 23.37 DNA ligase OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ligA PE = 3 SV = 1 - [DNLJ_STRPN] P0A4B3 33.72 3 86 23.26 30 S ribosomal protein S17 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsQ PE = 3 SV = 1 - [RS17_STRPN] Q97NG0 14.92 4 449 23.07 Glucose-6-phosphate isomerase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgi PE = 3 SV = 1 - [G6PI_STRPN] Q97T58 14.41 3 354 22.73 Conserved domain protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_0097 PE = 4 SV = 1 - [Q97T58_STRPN] Q97P32 16.46 4 407 22.04 Tryptophan synthase beta chain OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = trpB PE = 3 SV = 1 - [TRPB_STRPN] Q97QX3 28.57 5 252 21.94 ABC transporter, ATP-binding protein Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_1071 PE = 3 SV = 1 - [Q97QX3_STRPN] Q97ST6 32.03 2 128 21.84 50 S ribosomal protein L17 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplQ PE = 3 SV = 1 - [RL17_STRPN] Q97RB6 8.74 5 709 21.38 Putative transcriptional regulator OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0908 PE = 4 SV = 1 - [Q97RB6_STRPN] Q97NB9 17.91 4 335 21.02 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 trans- (strain ATCC BAA-334/TIGR4) membrane GN = SP_2132 PE = 4 SV = 1 - [Q97NB9_STRPN] P66524 37.93 3 58 20.52 30 S ribosomal protein S21 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsU PE = 3 SV = 1 - [RS21_STRPN] Q97SI6 14.98 5 474 20.22 6-phosphogluconate dehydrogenase, Intracellular decarboxylating OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gnd PE = 3 SV = 1 - [Q97SI6_STRPN] Q97R47 30.45 4 266 20.09 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_0987 PE = 1 SV = 1 - (No CS) [Q97R47_STRPN]

TABLE-US-00004 TABLE 4 Proteins identified in MP from TIGR4. The accession numbers refer to SwissProt on the date of filing. Accession # Coverage # Peptides #AAs Score Description Localization

Q97RH0 76.86 46 350 2085.84 Lipoprotein OS = -*Streptococcus* Lipid *pneumoniae* serotype 4 (strain ATCC anchored BAA-334/TIGR4) GN = SP_0845 PE = 4 SV = 1 -

[Q97RH0_STRPN] Q97PE6 90.27 56 442 1745.9 Sugar ABC transporter, sugar-binding Lipid protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1683 PE = 4 SV = 1 - [Q97PE6_STRPN] Q97T39 61.29 50 744 1569.47 Pneumococcal surface protein A OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = pspA PE = 4 SV = 1 - (with CS) [Q97T39_STRPN] P59213 82.74 62 423 1500.08 Maltose/maltodextrin-binding protein Lipid OS = -*Streptococcus pneumoniae* anchored serotype 4 (strain ATCC BAA-334/ TIGR4) GN = malX PE = 1 SV = 1 - [MALX_STRPN] Q97N74 53.25 47 693 1294.91 Choline binding protein A OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpA PE = 1 SV = 1 - (with CS) [Q97N74_STRPN] Q97R51 87.86 45 313 1201.93 Foldase protein PrsA OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = prsA PE = 3 SV = 1 - [PRSA_STRPN] Q54970 70.05 54 591 1000.77 Pyruvate oxidase OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = spxB PE = 3 SV = 2 - [POXB_STRPN] Q2MGF6 75.51 50 490 957.09 Lysozyme OS = - *Streptococcus* Secretory *pneumoniae* serotype 4 (strain ATCC (released) BAA-334/TIGR4) GN = lytC PE = 4 SV = 1 - (with CS) [Q2MGF6_STRPN] Q97PT1 77.13 52 551 947.11 Uncharacterized protein OS = Intracellular/ *Streptococcus pneumoniae* serotype 4 TMH start (strain ATCC BAA-334/TIGR4) AFTER 60 GN = SP_1518 PE = 4 SV = 1 - [Q97PT1_STRPN] Q97QS2 75.35 31 434 783.92 Enolase OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = eno PE = 1 SV = 1 - [ENO_STRPN] P64022 76.77 48 693 749.91 Elongation factor G OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = fusA PE = 3 SV = 1 - [EFG_STRPN] Q04707 72.6 47 719 715.79 Penicillin-binding protein 1A OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = ponA PE = 1 SV = 2 - [PBPA_STRPN] (No CS) P0C2J9 87.47 49 471 703.79 Pneumolysin OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ply PE = 3 SV = 1 - [TACY_STRPN] Q97PE1 73.93 35 445 655.97 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1690 PE = 4 SV = 1 - [Q97PE1_STRPN] Q97NQ8 61.8 68 1225 650.84 DNA-directed RNA polymerase subunit Intracellular beta' OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPN] Q97NL1 79.1 29 335 611.32 Glyceraldehyde-3-phosphate Intracellular dehydrogenase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gap PE = 3 SV = 1 - [Q97NL1_STRPN] Q97T12 82.97 33 276 589.52 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0148 PE = 4 SV = 1 - [Q97T12_STRPN] Q97T63 83.3 36 491 576.45 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0092 PE = 4 SV = 1 - [Q97T63_STRPN] I6L8V7 44.02 33 627 457.19 Choline binding protein E OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = cbpE PE = 4 SV = 1 - [I6L8V7_STRPN] Q97NQ7 55.61 50 1203 449.03 DNA-directed RNA polymerase subunit Intracellular beta OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = rpoB PE = 3 SV = 1 - [RPOB_STRPN] Q97SV2 64.62 18 277 395.51 50S ribosomal protein L2 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplB PE = 3 SV = 1 - [RL2_STRPN] P59205 60.33 29 658 388.39 Putative endo-beta-N- Secretory acetylglucosaminidase OS = (released) *Streptococcus pneumoniae* serotype 4 (with CS) (strain ATCC BAA-334/TIGR4) GN = lytB PE = 1 SV = 1 - [LYTB_STRPN] O69076 55.98 31 652 385.64 ATP-dependent zinc metalloprotease Multi- FtsH OS = -*Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/ TIGR4) GN = ftsH PE = 3 SV = 3 - [FTSH_STRPN] Q97SP2 67.47 25 332 369.57 PTS system, mannose-specific IIAB Intracellular components OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =

manL PE = 4 SV = 1 - [Q97SP2_STRPN] Q97T11 66.55 23 284 361.41 Lipoprotein OS = -
Streptococcus pneumoniae serotype 4 (strain ATCC anchored BAA-334/TIGR4) GN =
 SP_0149 PE = 3 SV = 1 - [Q97T11_STRPN] I6L8Q3 52.65 17 340 354.19 Choline binding protein
 F OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4)
 anchored GN = cbpF PE = 4 SV = 1 - [I6L8Q3_STRPN] (with CS) Q97NX9 80.69 25 321 336.18
 Iron-compound ABC transporter, iron- Lipid anchored compound-binding protein OS =
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1872 PE = 4 SV
 = 1 - [Q97NX9_STRPN] P64030 57.29 25 398 336.06 Elongation factor Tu OS = -*Streptococcus*
Intracellular pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = tuf PE = 3 SV = 1 -
 [EFTU_STRPN] Q97NW2 64.44 25 419 331.55 Sugar ABC transporter, sugar-binding Lipid
 protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN
 = rafE PE = 1 SV = 1 - [Q97NW2_STRPN] Q97QH2 42.86 29 721 330.61 Amino acid ABC
 transporter, amino Multi- acid-binding protein/permease protein transmembrane OS = -
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_1241 PE = 1 SV
 = 1 - [Q97QH2_STRPN] Q97RS8 78.31 29 378 328.02 Lactate oxidase OS = -*Streptococcus*
Intracellular pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = lctO-2 PE = 4 SV = 1 -
 [Q97RS8_STRPN] Q97PW1 63.71 25 463 316.66 Peptidoglycan N-acetylglucosamine N-
 deacetylase A OS = -*Streptococcus* terminally *pneumoniae* serotype 4 (strain ATCC anchored
 BAA-334/TIGR4) GN = pgdA PE = 4 SV = 1 - (No CS) [Q97PW1_STRPN] Q97PT6 75.85 26
 468 315.76 ATP synthase subunit beta OS = Intracellular *Streptococcus pneumoniae* serotype 4
 (strain ATCC BAA-334/TIGR4) GN = atpD PE = 3 SV = 1 - [ATPB_STRPN] Q97NQ3 58.55 22
 345 310 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain
 ATCC BAA-334/TIGR4) anchored GN = SP_1967 PE = 4 SV = 1 - (No CS) [Q97NQ3_STRPN]
 Q97R09 58.94 17 341 309.62 Iron-compound ABC transporter, iron Lipid compound-binding
 protein OS = anchored *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN
 = SP_1032 PE = 1 SV = 1 - [Q97R09_STRPN] P18791 69.8 28 659 298.67 Oligopeptide-binding
 protein AmiA Lipid OS = -*Streptococcus pneumoniae* anchored serotype 4 (strain ATCC BAA-334/
 TIGR4) GN = amiA PE = 1 SV = 3 - [AMIA_STRPN] Q97RZ7 63.03 15 238 283.45
 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC
 BAA-334/TIGR4) GN = SP_0629 PE = 1 SV = 1- [Q97RZ7_STRPN] P0A4G2 65.05 18 309
 281.58 Manganese ABC transporter substrate- Lipid binding lipoprotein OS = -*Streptococcus*
 anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = psaA PE = 1 SV = 1 -
 [MTSA_STRPN] P66112 60.5 10 119 280.56 50S ribosomal protein L20 OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplT PE = 3 SV = 1 -
 [RL20_STRPN] Q97PM5 63.3 23 376 278.8 Sugar ABC transporter, ATP-binding Intracellular
 protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = msmK
 PE = 4 SV = 1 - [Q97PM5_STRPN] P0A4M7 41.57 23 498 271.04 Oligopeptide transport system
 Multi- permease protein AmiC OS = transmembrane *Streptococcus pneumoniae* serotype 4 (strain
 ATCC BAA-334/TIGR4) GN = amiC PE = 3 SV = 1 - [AMIC_STRPN] Q97SP4 48.18 12 303
 262.56 PTS system, mannose-specific IID Multi- component OS = -*Streptococcus* transmembrane
pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0282 PE = 4 SV = 1 -
 [Q97SP4_STRPN] Q97NL3 60.74 34 731 258.29 Penicillin-binding protein 2A OS = N-
Streptococcus pneumoniae serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN =
 pbp2A PE = 4 SV = 1 - (No CS) [Q97NL3_STRPN] P14677 61.33 32 750 257.72 Penicillin-
 binding protein 2x OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-
 334/TIGR4) anchored GN = pbpX PE = 1 SV = 2 - [PBPX_STRPN] (No CS) Q97PT4 38.92 21
 501 249.37 ATP synthase subunit alpha OS = Multi- *Streptococcus pneumoniae* serotype 4
 transmembrane (strain ATCC BAA-334/TIGR4) GN = atpA PE = 3 SV = 1 - [ATPA_STRPN]
 Q97NB5 52.66 30 621 236.54 Choline binding protein PcpA OS = Secretory *Streptococcus*
pneumoniae serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = pcpA PE = 1

SV = 1 - [Q97NB5_STRPN] Q97PQ2 33.26 21 914 236.23 Cation-transporting ATPase, E1-E2 Multi-family OS = *-Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1551 PE = 3 SV = 1 - [Q97PQ2_STRPN] P0A475 50.36 8 137 229.6 50S ribosomal protein L16 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplP PE = 1 SV = 1 - [RL16_STRPN] Q97PU3 64.03 23 278 227.7 Amino acid ABC transporter, amino Lipid acid-binding protein OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = aatB PE = 4 SV = 1 - [Q97PU3_STRPN] Q97RC5 50.3 20 501 223.13 Pyruvate kinase OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyk PE = 3 SV = 1 - [Q97RC5_STRPN] Q97NS5 65.68 19 338 221.3 Putative transcriptional regulator OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1942 PE = 4 SV = 1 - [Q97NS5_STRPN] Q97RP4 75.22 14 230 213.93 Cell division ABC transporter, ATP- Intracellular binding protein FtsE OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsE PE = 3 SV = 1 - [Q97RP4_STRPN] Q97RN2 66.67 13 267 207.19 Peptidyl-prolyl cis-trans isomerase, Lipid cyclophilin-type OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0771 PE = 3 SV = 1 - [Q97RN2_STRPN] P66565 61.58 21 203 205.82 30S ribosomal protein S4 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsD PE = 3 SV = 1 - [RS4_STRPN] Q97QH1 61.79 10 246 202.96 Amino acid ABC transporter, ATP- Intracellular binding protein OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1242 PE = 3 SV = 1 - [Q97QH1_STRPN] I6L8N9 40.91 18 506 201.9 Choline transporter OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC transmembrane BAA-334/TIGR4) GN = proWX PE = 3 SV = 1 - [I6L8N9_STRPN] Q97N69 55.22 15 335 201.71 Putative ABC transporter, substrate- Lipid binding protein OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2197 PE = 4 SV = 1 - [Q97N69_STRPN] P65887 53.97 23 428 199.01 Adenylosuccinate synthetase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = purA PE = 1 SV = 1 - [PURA_STRPN] P18766 60.71 13 308 198.48 Oligopeptide transport ATP-binding Intracellular protein AmiF OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiF PE = 3 SV = 2 - [AMIF_STRPN] Q97QB4 61.83 23 448 197.42 Glutamate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gdhA PE = 3 SV = 1 - [Q97QB4_STRPN] Q97SP5 55.18 19 444 196.27 Aminopeptidase C OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pepC PE = 4 SV = 1 - [Q97SP5_STRPN] Q97RP3 40.58 17 308 193.01 Cell division protein FtsX OS = Multi-*Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = ftsX PE = 3 SV = 1 - [Q97RP3_STRPN] Q97T46 36.84 5 95 189.78 Putative bacteriocin OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC (released) BAA-334/TIGR4) GN = SP_0109 PE = 4 (no CS) SV = 1 - [Q97T46_STRPN] Q97PD6 51.85 31 837 188.69 Protein translocase subunit SecA 1 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = secA1 PE = 3 SV = 1 - [SECA1_STRPN] P0A4A7 60.58 13 137 187.87 30S ribosomal protein S12 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsL PE = 3 SV = 1 - [RS12_STRPN] P0A2U8 52.96 20 355 185.88 Oligopeptide transport ATP-binding Intracellular protein AmiE OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = amiE PE = 3 SV = 1 - [AMIE_STRPN] Q97RQ0 70.21 18 386 182.7 Branched-chain amino acid ABC Lipid transporter, amino acid-binding anchored protein OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = livJ PE = 4 SV = 1 - [Q97RQ0_STRPN] P0A3M9 67.68 16 328 176.61 L-lactate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ldh PE = 3 SV = 2 - [LDH_STRPN] Q97RG9 49.51 20 511 174.4 Sugar ABC transporter, ATP-binding Intracellular protein OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4)

GN = SP_0846 PE = 3 SV = 1 - [Q97RG9_STRPN] P0A3M5 43.38 22 680 166.84 Penicillin-binding protein 2B OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = penA PE = 3 SV = 1 - [PBP2_STRPN] (No CS) Q97N53 63.24 12 272 163.14 Cell shape-determining protein MreC N- OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = mreC PE = 3 SV = 1 - (No CS) [Q97N53_STRPN] P95829 49.26 20 607 161.66 Chaperone protein DnaK OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = dnaK PE = 3 SV = 2 - [DNAK_STRPN] Q97S19 67.89 7 109 156.35 Cell cycle protein GpsB OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpsB PE = 3 SV = 1 - [GPSB_STRPN] Q97NE4 36.3 26 821 153.09 Penicillin-binding protein 1B OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = pbp1B PE = 4 SV = 1 - (No CS) [Q97NE4_STRPN] I6L8W3 71.49 15 242 152.01 Choline transporter OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = proV PE = 3 SV = 1 - [I6L8W3_STRPN] P0A335 52.04 21 540 151.44 60 kDa chaperonin OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = groL PE = 1 SV = 1 - [CH60_STRPN] Q97NG0 44.77 17 449 145.04 Glucose-6-phosphate isomerase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgi PE = 3 SV = 1 - [G6PI_STRPN] Q97Q31 57.53 12 292 143.85 Phosphate-binding protein PstS 1 OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = pstS1 PE = 1 SV = 1 - [PSTS1_STRPN] Q97RW9 51.81 16 332 139.95 Putative pneumococcal surface protein Lipid OS = -*Streptococcus pneumoniae* anchored serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0667 PE = 1 SV = 1 - [Q97RW9_STRPN] P22976 44.98 18 658 139.63 Probable transketolase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = tkt PE = 3 SV = 2 - [TKT_STRPN] Q97SQ9 42.52 17 602 138.65 Glutamine--fructose-6-phosphate Intracellular aminotransferase [isomerizing] OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glmS PE = 3 SV = 3 - [GLMS_STRPN] Q97PA9 33.84 18 659 132.79 Serine/threonine-protein kinase StkP Intracellular/ OS = -*Streptococcus pneumoniae* TMH start serotype 4 (strain ATCC BAA-334/ AFTER 60 TIGR4) GN = stkP PE = 1 SV = 1 - [STKP2_STRPN] Q97Q34 70.41 18 267 131.91 Phosphate import ATP-binding protein Intracellular PstB 2 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = pstB2 PE = 3 SV = 1 - [PSTB2_STRPN] Q97QX5 55.81 14 344 130.66 Uncharacterized protein OS = Secretory(*Streptococcus pneumoniae* serotype 4 released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1069 PE = 1 SV = 1 - [Q97QX5_STRPN] I6L8V0 55.84 18 351 130.62 Capsular polysaccharide biosynthesis Intracellular protein Cps4J OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cap4J PE = 4 SV = 1 - [I6L8V0_STRPN] Q97SD1 43.38 17 521 119.96 Amino acid ABC transporter, amino Multi- acid-binding protein/permease protein transmembrane OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0453 PE = 4 SV = 1 - [Q97SD1_STRPN] Q97SR1 50.73 21 617 119.02 Proline--tRNA ligase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = proS PE = 3 SV = 1 - [SYP_STRPN] P0A4D7 36.07 14 524 117.93 DEAD-box ATP-dependent RNA Intracellular helicase CshA OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cshA PE = 3 SV = 1 - [CSHA_STRPN] Q97P40 68.81 12 202 117.92 Putative general stress protein 24 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1804 PE = 4 SV = 1 - [Q97P40_STRPN] Q97NK0 41 21 883 117.4 Aldehyde-alcohol dehydrogenase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2026 PE = 3 SV = 1 - [Q97NK0_STRPN] Q97N99 51.82 14 274 115.56 SPFH domain/Band 7 family OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_2156 PE = 4 SV = 1 - [Q97N99_STRPN] Q9L7Q2 17.63 23 190 6 114.8 Zinc

metalloprotease ZmpB OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = zmpB PE = 3 SV = 2 - [ZMPB_STRPN] P67293 43.9 4 82 114.56
 UPF0154 protein SP_1882 OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_1882 PE = 3 SV = 1 - (No CS) [Y1882_STRPN] Q97N37
 61.83 14 393 114.16 Serine protease OS = -*Streptococcus* N- *pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = SP_2239 PE = 1 anchored SV = 1 - [Q97N37_STRPN] (No CS) P66359 55.12 8 127 112.68 30S ribosomal protein S11 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsK PE = 3 SV = 1 - [RS11_STRPN] Q97PX1 30.72 12 459 110.91 NADH oxidase OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = nox PE = 4 SV = 1 - [Q97PX1_STRPN] P35592 43.64 21 660 109.44 Oligopeptide-binding protein AliA OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = aliA PE = 3 SV = 4 - [ALIA_STRPN] Q97PC4 28.66 11 492 108.41 ABC transporter, ATP-binding protein Multi- OS = -*Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1715 PE = 4 SV = 1 - [Q97PC4_STRPN] Q97P68 69.23 8 104 104.87 Thioredoxin OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = trx PE = 3 SV = 1 - [Q97P68_STRPN] Q97PL8 41.55 12 438 101 Oxidoreductase, pyridine nucleotide- Intracellular disulfide, class I OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1588 PE = 3 SV = 1 - [Q97PL8_STRPN] Q975X2 68.78 17 189 97.17 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_0191 PE = 1 SV = 1 - [Q97SX2_STRPN] P63413 43.94 14 396 96.43 Acetate kinase OS = -*Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ackA PE = 3 SV = 1 - [ACKA_STRPN] Q97SJ6 52.61 11 230 96.34 Capsular polysaccharide biosynthesis Multi- protein CpsC OS = -*Streptococcus* transmembrane *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cpsC PE = 3 SV = 1 - [CPSC_STRPN] Q97Q67 38.08 11 344 95.87 Conserved domain protein OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = SP_1363 PE = 4 SV = 1 - [Q97Q67_STRPN] P0A4S1 49.15 10 293 93.48 Fructose-bisphosphate aldolase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = fba PE = 3 SV = 1 - [ALF_STRPN] Q97SR2 37.71 11 419 93.31 Putative zinc metalloprotease SP_0263 Multi- OS = -*Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0263 PE = 3 SV = 1 - [Y263_STRPN] Q97NP5 25.65 9 308 92.53 Membrane protein insertase YidC 1 Multi- OS = -*Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/ TIGR4) GN = yidC1 PE = 3 SV = 1 - [YIDC1_STRPN] Q97SJ4 44.44 15 360 91.77 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0355 PE = 4 SV = 1 - [Q97SJ4_STRPN] Q97RK0 19.83 10 575 91.07 Septation ring formation regulator N- EzrA OS = -*Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = ezrA PE = 3 SV = 1 - (No CS) [EZRA_STRPN] P63373 71.43 11 252 91.01 Phosphate import ATP-binding protein Intracellular PstB 1 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = pstB1 PE = 3 SV = 1 - [PSTB1_STRPN] Q97S89 51.01 13 398 89.47 Phosphoglycerate kinase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgk PE = 3 SV = 1 - [PGK_STRPN] Q97PT5 47.26 12 292 85.91 ATP synthase gamma chain OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpG PE = 3 SV = 1 - [ATPG_STRPN] Q97514 32.65 9 340 85.66 Choline binding protein C OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpC PE = 4 SV = 1 - [Q97SI4_STRPN] (with CS) I6L858 39.71 14 481 85.62 Capsular polysaccharide biosynthesis Multi- protein Cps4A OS = -*Streptococcus* transmembrane *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4A PE = 4 SV = 1 - [I6L8S8_STRPN] Q97PY0 65.59 13 247 85.07

Amino acid ABC transporter, ATP-binding protein OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1460 PE = 3 SV = 1 - [Q97PYO_STRPN] Q97SQ4 46.79 9 156 83.39 30S ribosomal protein S7 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsG PE = 3 SV = 1 - [RS7_STRPN] P0A3Y3 60 10 230 82.54 2,3-bisphosphoglycerate-dependent Intracellular phosphoglycerate mutase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPN] P0A4C3 59.91 11 217 82.05 30S ribosomal protein S3 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsC PE = 3 SV = 1 - [RS3_STRPN] P65607 38.46 11 338 79.74 Ornithine carbamoyltransferase, Intracellular catabolic OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = arcB PE = 3 SV = 1 - [OTCC_STRPN] O05703 39.92 12 501 79.68 Zinc-binding lipoprotein AdcA OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = adcA PE = 3 SV = 4 - [ADCA_STRPN] I6L8U0 27.16 11 394 79.39 UDP-N-acetylglucosamine 2-epimerase Intracellular OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = cps4L PE = 3 SV = 1 - [I6L8U0_STRPN] Q97SI7 28.23 8 464 78.19 Mid-cell-anchored protein Z OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = mapZ PE = 1 SV = 1 - [Q97SI7_STRPN] Q97N56 58.3 15 259 78.0 30S ribosomal protein S2 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsB PE = 3 SV = 1 - [RS2_STRPN] Q97SI6 35.44 13 474 77.15 6-phosphogluconate dehydrogenase, Intracellular decarboxylating OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = gnd PE = 3 SV = 1 - [Q97SI6_STRPN] Q97PG0 27.1 7 262 77.07 Cell division protein DivIVA OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = divIVA PE = 4 SV = 1 - [Q97PG0_STRPN] Q97RF9 32 11 400 76.46 Ribosomal protein S1 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsA PE = 4 SV = 1 - [Q97RF9_STRPN] Q97T80 15.68 17 1856 76.28 Zinc metalloprotease ZmpC OS = LPXTG *Streptococcus pneumoniae* serotype 4 Cell-wall (strain ATCC BAA-334/TIGR4) anchored GN = zmpC PE = 3 SV = 1 - [ZMPC_STRPN] P0A2Z2 62.8 9 164 75.56 ATP synthase subunit b OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpF PE = 3 SV = 1 - [ATPF_STRPN] I6L859 47.7 10 413 73.22 D-alanyl-D-alanine carboxypeptidase N- OS = *-Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = dacA PE = 3 SV = 1 - (with CS) [I6L859_STRPN] Q97PU4 80 7 115 71.8 Bacterocin transport accessory protein Intracellular OS = *-Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = bta PE = 4 SV = 1 - [Q97PU4_STRPN] Q97SU6 70.34 7 118 71.64 50S ribosomal protein L18 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplR PE = 3 SV = 1 - [RL18_STRPN] P63742 35.46 11 502 71.21 Glycerol kinase OS = *-Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = glpK PE = 3 SV = 1 - [GLPK_STRPN] Q97SN4 32.31 6 130 68.89 30S ribosomal protein S9 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsI PE = 3 SV = 1 - [RS9_STRPN] P66907 31.84 6 380 68.13 Queuine tRNA-ribosyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = tgt PE = 3 SV = 1 - [TGT_STRPN] Q97SF8 32.16 12 454 65.06 Aspartokinase OS = *-Streptococcus* Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0413 PE = 3 SV = 1 - [Q97SF8_STRPN] P0A471 82.79 9 122 62.07 50S ribosomal protein L7/L12 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplL PE = 1 SV = 2 - [RL7_STRPN] I6L8W8 21.08 8 332 61.96 Choline binding protein J OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = cbpJ PE = 4 SV = 1 - [I6L8W8_STRPN] (with CS) P0A4J6 40.8 5 201 61.71 Superoxide dismutase [Mn] OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = soda PE = 1 SV = 2 - [SODM_STRPN] Q97T52 30.03 14 616 60.5 Putative capsular polysaccharide Multi-

biosynthesis protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0103 PE = 4 SV = 1 - [Q97T52_STRPN] Q97RE5 17.08 6 650 60.23 PTS system, fructose specific IIABC Multi- components OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0877 PE = 4 SV = 1 - [Q97RE5_STRPN] Q97SV4 54.11 9 207 60.13 50S ribosomal protein L4 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplD PE = 3 SV = 1 - [RL4_STRPN] Q97RK7 19.46 12 848 59.83 Aminopeptidase N OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pepN PE = 4 SV = 1 - [Q97RK7_STRPN] Q97Q62 34.67 11 424 59.21 Psr protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC TMH start BAA-334/TIGR4) GN = SP_1368 PE = 4 AFTER 60 SV = 1 - [Q97Q62_STRPN] O07344 26.96 5 204 58.64 Signal peptidase I OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = lepB PE = 3 SV = 2 - anchored [LEP_STRPN] (No CS) P0A3S3 34.67 6 274 57.63 DNA-entry nuclease OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = endA PE = 1 SV = 1 - anchored [NUCE_STRPN] (No CS) Q97PU5 29.55 10 572 57.38 Phosphoglucosyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pgm PE = 3 SV = 1 - [Q97PU5_STRPN] Q97RC6 31.34 8 335 57.3 ATP-dependent 6- Intracellular phosphofructokinase OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPN] Q97SV5 37.5 6 208 56.96 50S ribosomal protein L3 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplC PE = 3 SV = 1 - [RL3_STRPN] Q97RL9 25.56 6 399 56.21 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_0785 PE = 4 SV = 1 - (No CS) [Q97RL9_STRPN] P66278 18.18 2 66 55.84 50S ribosomal protein L35 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpmI PE = 3 SV = 1 - [RL35_STRPN] I6L8N0 40.82 12 365 55.32 UDP-N-acetylglucosamine-2-epimerase Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = cps4I PE = 3 SV = 1 - [I6L8N0_STRPN] Q97PQ8 57.34 6 143 54.74 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1545 PE = 4 SV = 1 - [Q97PQ8_STRPN] Q97TA6 33.64 10 330 52.71 Phosphate acyltransferase OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = plsX PE = 3 SV = 1 - [PLSX_STRPN] Q97PK5 27.22 2 158 52.32 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_1604 PE = 4 SV = 1 - (No CS) [Q97PK5_STRPN] Q97SN5 67.57 8 148 51.12 50S ribosomal protein L13 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplM PE = 3 SV = 1 - [Q97SN5_STRPN] Q97RV8 60.32 8 126 49.28 Uncharacterized protein OS = N- *Streptococcus pneumoniae* serotype 4 terminally (strain ATCC BAA-334/TIGR4) anchored GN = SP_0678 PE = 4 SV = 1 - (No CS) [Q97RV8_STRPN] Q97R14 30.25 7 324 48.6 Uncharacterized protein OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_1027 PE = 4 SV = 1 - [Q97R14_STRPN] P35597 15.04 7 778 48.34 Probable cation-transporting ATPase Multi- exp7 OS = -*Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/ TIGR4) GN = exp7 PE = 3 SV = 2 - [EXP7_STRPN] P65239 41.61 9 322 48.08 Ribose-phosphate pyrophosphokinase Intracellular 1 OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = prs1 PE = 3 SV = 1 - [KPRS1_STRPN] Q97Q37 46.13 7 271 47.67 Amino acid ABC transporter, amino Lipid acid-binding protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1394 PE = 4 SV = 1 - [Q97Q37_STRPN] Q97R36 52.43 8 185 47.65 Thioredoxin family protein OS = Lipid *Streptococcus pneumoniae* serotype 4 anchored (strain ATCC BAA-334/TIGR4) GN = SP_1000 PE = 1 SV = 1 - [Q97R36_STRPN] Q97N48 26.81 5 276 45.87 Uncharacterized protein OS = Intracellular/ *Streptococcus pneumoniae* serotype 4 TMH start

(strain ATCC BAA-334/TIGR4) AFTER 60 GN = SP_2223 PE = 4 SV = 1 - [Q97N48_STRPN]

I6L8R1 22.79 4 215 45.06 ABC transporter, ATP-binding protein Intracellular Vexp2 OS = -
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = vex2 PE = 3 SV = 1 -
 [I6L8R1_STRPN] Q97SC6 20.93 10 774 44.93 Formate acetyltransferase OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = pfl PE = 4 SV = 1 -
 [Q97SC6_STRPN] Q97QD5 51.08 8 186 44.75 LemA protein OS = -*Streptococcus N-pneumoniae*
 serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = lemA PE = 1 SV = 1 - anchored
 [Q97QD5_STRPN] (No CS) Q97PF9 37.71 9 419 44.71 Cell division protein FtsZ OS =
 Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = ftsZ PE =
 3 SV = 1 - [Q97PF9_STRPN] P0A3B7 36.71 7 346 44.56 Elongation factor Ts OS = -
Streptococcus Intracellular *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = tsf PE =
 1 SV = 2 - [EFTS_STRPN] P72524 20.56 10 822 44.46 DNA gyrase subunit A OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = gyrA PE = 3 SV = 3 -
 [GYRA_STRPN] P67282 26.22 11 534 43.28 Ribonuclease Y OS = -*Streptococcus N-pneumoniae*
 serotype 4 (strain ATCC terminally BAA-334/TIGR4) GN = rny PE = 3 SV = 1 - anchored
 [RNY_STRPN] (No CS) P66419 38.2 6 89 42.98 30S ribosomal protein S14 OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsN PE = 3 SV = 1 -
 [RS14_STRPN] Q97SV1 48.89 6 180 42.64 50S ribosomal protein L5 OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplE PE = 3 SV = 1 -
 [RL5_STRPN] Q97NW9 7.48 5 655 42.08 Trehalose PTS system, IIABC Multi- components OS =
 -*Streptococcus* transmembrane *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =
 SP_1884 PE = 4 SV = 1 - [Q97NW9_STRPN] Q97Q18 91.06 6 123 40.86 Uncharacterized protein
 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN =
 SP_1218 PE = 4 SV = 1 - [Q97Q18_STRPN] Q97T04 11.92 2 193 39.59 Uncharacterized protein
 OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4)
 GN = SP_0157 PE = 4 SV = 1 - [Q97T04_STRPN] Q97PB8 9.09 4 627 39.49 PTS system IIABC
 components OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC
 BAA-334/TIGR4) GN = SP_1722 PE = 4 SV = 1 - [Q97PB8_STRPN] Q97P19 57.3 5 89 39.48
 30S ribosomal protein S15 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC
 BAA-334/TIGR4) GN = rpsO PE = 3 SV = 1 - [RS15_STRPN] P61182 40.35 4 114 39.35 50S
 ribosomal protein L22 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC
 BAA-334/TIGR4) GN = rplV PE = 1 SV = 1 - [RL22_STRPN] Q97SU7 51.69 6 178 38.14 50S
 ribosomal protein L6 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-
 334/TIGR4) GN = rplF PE = 3 SV = 1 - [RL6_STRPN] Q97NB9 39.1 8 335 38.04
 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain
 ATCC BAA-334/TIGR4) GN = SP_2132 PE = 4 SV = 1 - [Q97NB9_STRPN] Q97QP7 5.34 8
 2004 37.84 Immunoglobulin A1 protease OS = Multi- *Streptococcus pneumoniae* serotype 4
 transmembrane (strain ATCC BAA-334/TIGR4) GN = iga PE = 3 SV = 1 - [IGA1A_STRPN]
 Q97N55 23.72 6 392 37.16 Secreted 45 kd protein OS = Secretory *Streptococcus pneumoniae*
 serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = usp45 PE = 4 SV = 1 -
 [Q97N55_STRPN] P66942 18.25 4 252 36.63 Triosephosphate isomerase OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = tpiA PE = 3 SV = 1 -
 [TPIS_STRPN] Q97QC6 66.09 7 115 35.81 50S ribosomal protein L19 OS = Intracellular
Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplS PE = 3 SV = 1 -
 [RL19_STRPN] I6L8S7 14.6 5 459 35.54 Transmembrane protein Vexp3 OS = Multi-
Streptococcus pneumoniae serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = vex3
 PE = 4 SV = 1 - [I6L8S7_STRPN] P35595 12.4 6 726 35.44 PTS system glucose-specific EIICBA
 Multi- component OS = -*Streptococcus* transmembrane *pneumoniae* serotype 4 (strain ATCC
 BAA-334/TIGR4) GN = exp5 PE = 3 SV = 2 - [PTG3C_STRPN] Q97PV1 14.88 3 289 34.89
 Putative glycerol uptake facilitator Multi- protein OS = -*Streptococcus* transmembrane *pneumoniae*

serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1491 PE = 3 SV = 1 - [Q97PV1_STRPN]

Q97NM6 21.04 6 404 33.79 Aminotransferase, class I OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1994 PE = 4 SV = 1 - [Q97NM6_STRPN]

Q97SD2 45.93 5 246 33.44 Amino acid ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0452 PE = 3 SV = 1 - [Q97SD2_STRPN]

Q97NN3 36.15 6 213 33.1 ABC transporter, ATP-binding protein Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1987 PE = 1 SV = 1 - [Q97NN3_STRPN]

Q97RJ0 23.77 5 244 33.09 Amino acid ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0824 PE = 3 SV = 1 - [Q97RJ0_STRPN]

Q97NJ7 27.27 3 99 32.76 Preprotein translocase, YajC subunit N- OS = -*Streptococcus pneumoniae* terminally serotype 4 (strain ATCC BAA-334/ anchored TIGR4) GN = yajC-2 PE = 4 SV = 1 - (No CS) [Q97NJ7_STRPN]

P66095 34.5 8 229 32.4 50S ribosomal protein L1 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplA PE = 3 SV = 1 - [RL1_STRPN]

Q97NT7 38.61 6 158 32.3 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = SP_1926 PE = 4 SV = 1 - [Q97NT7_STRPN]

P63667 29.5 4 139 31.7 ATP synthase epsilon chain OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = atpC PE = 3 SV = 1 - [ATPE_STRPN]

Q97T58 18.08 4 354 31.6 Conserved domain protein Multi- OS = *Streptococcus pneumoniae* transmembrane serotype 4 (strain ATCC BAA-334/ TIGR4) GN = SP_0097 PE = 4 SV = 1 - [Q97T58_STRPN]

Q97R06 36.74 6 264 31.17 Iron-compound ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1035 PE = 3 SV = 1 - [Q97R06_STRPN]

Q97PQ5 20.34 6 531 30.81 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = SP_1548 PE = 4 SV = 1 - [Q97PQ5_STRPN]

Q97R44 33.91 2 115 30.62 Uncharacterized protein OS = Intracellular/ *Streptococcus pneumoniae* serotype 4 TMH start (strain ATCC BAA-334/TIGR4) AFTER 60 GN = SP_0990 PE = 4 SV = 1 - [Q97R44_STRPN]

Q97SW3 47.73 3 88 29.98 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0201 PE = 4 SV = 1 - [Q97SW3_STRPN]

I6L8N6 27.01 4 211 29.5 Capsular polysaccharide biosynthesis N- protein Cps4E OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC anchored BAA-334/TIGR4) GN = cps4E PE = 4 SV = 1 - (No CS) [I6L8N6_STRPN]

Q97SU3 34.25 5 146 28.53 50S ribosomal protein L15 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplO PE = 3 SV = 1 - [RL15_STRPN]

Q97QS6 25.86 6 379 28.15 Glycogen biosynthesis protein GlgD Intracellular OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/ TIGR4) GN = glgD PE = 4 SV = 1 - [Q97QS6_STRPN]

Q97P39 52.24 2 67 27.23 UPF0337 protein SP_1805 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1805 PE = 3 SV = 1 - [Y1805_STRPN]

P06653 20.44 6 318 25.81 Autolysin OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = lytA PE = 1 SV = 2 - [ALYS_STRPN]

Q97PX9 12.78 5 266 25.41 Amino acid ABC transporter, permease Multi- protein OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1461 PE = 3 SV = 1 - [Q97PX9_STRPN]

Q97QW4 17.65 5 425 24.84 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1083 PE = 4 SV = 1 - [Q97QW4_STRPN]

Q97S93 13.27 7 535 23.79 CTP synthase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = pyrG PE = 3 SV = 1 - [PYRG_STRPN]

Q97SX1 26.14 2 88 22 UPF0297 protein SP_0192 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_0192 PE = 1 SV = 1 - [Y192_STRPN]

Q97RH7 50 4 78 21.34 30S ribosomal protein S20 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsT PE = 3 SV = 1 - [RS20_STRPN]

P66050 45.78 5 166 20.98 50S

ribosomal protein L10 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rplJ PE = 3 SV = 2 - [RL10_STRPN] Q97RW6 32.14 3 56 20.85
 Uncharacterized protein OS = Secretory *Streptococcus pneumoniae* serotype 4 (released) (strain ATCC BAA-334/TIGR4) (with CS) GN = SP_0670 PE = 4 SV = 1 - [Q97RW6_STRPN] Q9FBB7 29.41 4 255 20.65 Acetyl-coenzyme A carboxylase Intracellular carboxyl transferase subunit alpha OS = *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = accA PE = 1 SV = 1 - [ACCA_STRPN] I6L8V8 30.45 4 243 20.58 3-oxoacyl-[acyl-carrier protein] Intracellular reductase OS = -*Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = fabG PE = 3 SV = 1 - [I6L8V8_STRPN] P66581 41.46 4 164 20.46 30S ribosomal protein S5 OS = Intracellular *Streptococcus pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = rpsE PE = 3 SV = 1 - [RS5_STRPN] Q97T45 11.82 5 694 20.39 Uncharacterized protein OS = Multi-*Streptococcus pneumoniae* serotype 4 transmembrane (strain ATCC BAA-334/TIGR4) GN = SP_0110 PE = 4 SV = 1 - [Q97T45_STRPN] Q97P18 18.31 4 355 20.06 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus* anchored *pneumoniae* serotype 4 (strain ATCC BAA-334/TIGR4) GN = SP_1826 PE = 4 SV = 1 - [Q97P18_STRPN]
 TABLE-US-00005 TABLE 5 Proteins identified in MV.sub.L from serotype 3. The accession numbers refer to SwissProt on the date of filing. Accession # Coverage # Peptides #AAs Score
 Description Localization B2IQV2 91.09 50 404 5251.94 Elongation factor Tu OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tuf PE = 3 SV = 1 - [B2IQV2_STRPS] B2IPX8 95.62 40 434 2291.84 Enolase OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = eno PE = 3 SV = 1 - [ENO_STRPS] B2INW2 70.86 42 374 2221.7 Lipoprotein OS = -*Streptococcus* Lipid *pneumoniae* (strain CGSP14) anchored GN = SPCG_0787 PE = 4 SV = 1 - [B2INW2_STRPS] B2ISJ9 85.28 67 693 1851.66 Elongation factor G OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = fusA PE = 3 SV = 1 - [EFG_STRPS] B2IMI7 73.82 33 359 1675.28 Glyceraldehyde-3-phosphate Intracellular dehydrogenase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gapA PE = 3 SV = 1 - [B2IMI7_STRPS] B2IM39 82.94 101 1225 1348.46 DNA-directed RNA polymerase Intracellular subunit beta' OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPS] B2IMJ9 86.85 68 890 1321.16 Aldehyde-alcohol dehydrogenase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = adhE PE = 3 SV = 1 - [B2IMJ9_STRPS] B2IPD4 73.16 38 313 1171.98 Foldase protein PrsA OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = prsA PE = 3 SV = 1 - [PRSA_STRPS] B2INY1 91 53 400 1141.84 30S ribosomal protein S1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsA PE = 4 SV = 1 - [B2INY1_STRPS] B2IP53 87.46 27 335 830.14 ATP-dependent 6- Intracellular phosphofructokinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPS] B2IQX0 90.6 38 468 772.66 ATP synthase subunit beta OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = atpD PE = 3 SV = 1 - [ATPB_STRPS] B2IRV7 72.76 62 837 760.12 Protein translocase subunit SecA Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = secA PE = 3 SV = 1 - [B2IRV7_STRPS] B2IN25 78.22 51 450 751.01 Maltose/maltodextrin ABC Lipid transporter, maltose/maltodextrin- anchored binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2072 PE = 4 SV = 1 - [B2IN25_STRPS] B2INB2 72.76 41 591 749.62 Pyruvate oxidase OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = spxB PE = 3 SV = 1 - [B2INB2_STRPS] B2ISZ0 69.04 47 659 746.61 Oligopeptide ABC transporter, Lipid oligopeptide-binding protein AmiA anchored OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = amiA PE = 4 SV = 1 - [B2ISZ0_STRPS] B2ISK8 56.44 22 303 734.11 PTS system, mannose-specific IID Multi-component OS = -*Streptococcus* transmembrane *pneumoniae* (strain CGSP14) GN = manN PE = 4 SV = 1 - [B2ISK8_STRPS] B2INN2 85.33 30 259 682.23 30S ribosomal protein S2 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsB PE = 3 SV = 2 - [RS2_STRPS] B2IP54 76.05 39 501 676.55 Pyruvate kinase OS = -*Streptococcus* Intracellular

pneumoniae (strain CGSP14) GN = SPCG_0873 PE = 3 SV = 1 - [B2IP54_STRPS] B2IR47 69.48
 50 652 673.91 Oligopeptide ABC transporter, Lipid oligopeptide-binding protein AliB anchored
 OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = aliB PE = 4 SV = 1 - [B2IR47_STRPS]
 B2IR00 66.26 44 652 636.95 ATP-dependent zinc Multi- metalloprotease FtsH OS =
 transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = ftsH PE = 3 SV = 1 -
 [B2IR00_STRPS] B2IM40 77.67 50 824 633.52 DNA-directed RNA polymerase Intracellular
 subunit beta OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rpoB PE = 3 SV = 1 -
 [B2IM40_STRPS] B2IS43 70.76 23 277 575.93 50S ribosomal protein L2 OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = rplB PE = 3 SV = 1 - [RL2_STRPS] B2IS46
 79.26 27 217 569.56 30S ribosomal protein S3 OS = Intracellular *Streptococcus pneumoniae*
 (strain CGSP14) GN = rpsC PE = 3 SV = 1 - [RS3_STRPS] B2IME4 64.95 51 930 553.42
 Translation initiation factor IF-2 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14)
 GN = infB PE = 3 SV = 1 - [IF2_STRPS] B2IQY1 73.14 43 551 550.86 Uncharacterized protein
 OS = Intracellular/ *Streptococcus pneumoniae* (strain TMH start CGSP14) GN = SPCG_1503 PE =
 4 SV = 1 - AFTER 60 [B2IQY1_STRPS] B2INT1 58.9 34 584 531.45 Septation ring formation
 regulator N- EzrA OS = -*Streptococcus pneumoniae* terminally (strain CGSP14) GN = ezrA PE = 3
 SV = 1 - anchored [B2INT1_STRPS] (No CS) B2IPY8 67.93 32 474 511.28 Glyceraldehyde-3-
 phosphate Intracellular dehydrogenase, NADP-dependent OS = -*Streptococcus pneumoniae* (strain
 CGSP14) GN = gapN PE = 3 SV = 1 - [B2IPY8_STRPS] B2IRQ6 79.23 20 313 461.56
 Manganese ABC transporter, Lipid manganese-binding adhesion anchored liprotein OS = -
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_1623 PE = 3 SV = 1 - [B2IRQ6_STRPS]
 B2IPU8 63.14 36 719 457.32 Ribonucleoside-diphosphate Intracellular reductase OS = -
Streptococcus pneumoniae (strain CGSP14) GN = nrdE PE = 3 SV = 1 - [B2IPU8_STRPS]
 B2IM49 72.42 27 330 449.87 Aspartate--ammonia ligase OS = Intracellular *Streptococcus*
pneumoniae (strain CGSP14) GN = asnA PE = 3 SV = 1 - [ASNA_STRPS] B2IQX2 68.86 31 501
 447.54 ATP synthase subunit alpha OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14)
 GN = atpA PE = 3 SV = 1 - [ATPA_STRPS] B2IMK3 70.36 32 658 435.4 Transketolase OS = -
Streptococcus Intracellular *pneumoniae* (strain CGSP14) GN = tktA PE = 3 SV = 1 -
 [B2IMK3_STRPS] B2IRH9 64.45 36 616 434.01 Capsular polysaccharide Multi- biosynthesis
 protein, putative OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN =
 SPCG_0098 PE = 4 SV = 1 - [B2IRH9_STRPS] B2IN95 82.06 37 496 432.16 Lysine--tRNA ligase
 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = lysS PE = 3 SV = 1 -
 [SYK_STRPS] B2IS90 71.32 40 537 413.57 Ribonuclease Y OS = -*Streptococcus* Intracellular
pneumoniae (strain CGSP14) GN = rny PE = 3 SV = 1 - [B2IS90_STRPS] B2IRT9 66.06 32 442
 412.96 Sugar ABC transporter, sugar- Lipid binding protein OS = -*Streptococcus* anchored
pneumoniae (strain CGSP14) GN = SPCG_1656 PE = 4 SV = 1 - [B2IRT9_STRPS] B2IN10 73.37
 15 338 401 Glycerol-3-phosphate Intracellular dehydrogenase [NAD(P)+] OS = *Streptococcus*
pneumoniae (strain CGSP14) GN = gpsA PE = 3 SV = 1 - [GPDA_STRPS] B2IP07 38.46 23 650
 398.03 PTS system, fructose specific IIABC Multi- components OS = -*Streptococcus*
 transmembrane *pneumoniae* (strain CGSP14) GN = fruA PE = 4 SV = 1 - [B2IP07_STRPS]
 B2IPB5 52.94 10 119 388.03 50S ribosomal protein L20 OS = Intracellular *Streptococcus*
pneumoniae (strain CGSP14) GN = rplT PE = 3 SV = 1 - [RL20_STRPS] B2IS19 66.29 35 617
 385.71 Proline--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN =
 proS PE = 3 SV = 1 - [SYP_STRPS] B2INH5 83.95 31 299 370.13 SPFH domain/Band 7 family
 OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_2124 PE = 4 SV = 1
 - anchored [B2INH5_STRPS] (No CS) B2IQ40 64.14 46 898 367.23 Phosphoenolpyruvate
 carboxylase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = ppc PE = 3 SV
 = 1 - [B2IQ40_STRPS] B2IML8 76.35 25 406 358.33 Acetate kinase OS = -*Streptococcus*
 Intracellular *pneumoniae* (strain CGSP14) GN = ackA PE = 3 SV = 1 - [B2IML8_STRPS] B2IRS1
 53.94 28 419 341.4 Cell division protein FtsZ OS = Intracellular *Streptococcus pneumoniae* (strain

CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS1_STRPS] B2IMN0 96.59 22 352 339.61 Alcohol dehydrogenase, zinc- Intracellular containing OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = adh PE = 3 SV = 1 - [B2IMNO_STRPS] B2ISJ7 51.09 13 137 337.74 30S ribosomal protein S12 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsL PE = 3 SV = 1 - [RS12_STRPS] B2IS41 63.29 16 207 337.71 50S ribosomal protein L4 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplD PE = 3 SV = 1 - [RL4_STRPS] B2ILW7 80.68 18 414 332.02 3-oxoacyl-[acyl-carrier-protein] Intracellular synthase 2 OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fabF PE = 3 SV = 1 - [B2ILW7_STRPS] B2IRM9 70.29 22 276 330.56 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0152 PE = 4 SV = 1 - [B2IRM9_STRPS] B2ISY9 47.99 21 498 319.21 Oligopeptide ABC transporter, Multi- permease protein Amic OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = amiC PE = 3 SV = 1 - [B2ISY9_STRPS] B2IQT6 62.75 22 459 313.52 NADH oxidase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = nox PE = 4 SV = 1 - [B2IQT6_STRPS] B21PP2 47.02 32 721 312 Amino acid ABC transporter, amino Multi- acid-binding protein/permease transmembrane protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1062 PE = 3 SV = 1 - [B2IPP2_STRPS] B2IS47 63.5 13 137 300.82 50S ribosomal protein L16 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplP PE = 3 SV = 1 - [RL16_STRPS] B2IRC1 66.67 19 339 299.62 UDP-glucose 4-epimerase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = galE PE = 4 SV = 1 - [B2IRC1_STRPS] B2IQW3 71.22 25 278 296.15 Amino acid ABC transporter, amino Lipid acid-binding protein OS = anchored *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1485 PE = 4 SV = 1 - [B2IQW3_STRPS] B2IRR1 67.83 19 230 295.06 2,3-bisphosphoglycerate- Intracellular dependent phosphoglycerate mutase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPS] B2IS34 61.87 39 737 290.83 Anaerobic ribonucleoside Intracellular triphosphate reductase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0212 PE = 4 SV = 1 - [B2IS34_STRPS] B2IS52 87.78 18 180 288.42 50S ribosomal protein L5 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplE PE = 3 SV = 1 - [RL5_STRPS] B2IQU1 65.63 33 678 288.36 Glycine--tRNA ligase beta subunit Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glyS PE = 3 SV = 1 - [SYGB_STRPS] B2ILY5 60.36 21 555 284.08 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0438 PE = 4 SV = 1 - [B2ILY5_STRPS] B2IS40 59.62 14 208 283.69 50S ribosomal protein L3 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplC PE = 3 SV = 1 - [RL3_STRPS] B21M73 32.44 18 521 281.46 Amino acid ABC transporter, amino Multi- acid-binding protein/permease transmembrane protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glnH PE = 3 SV = 1 - [B2IM73_STRPS] B2IR19 52.65 17 302 275.76 Amino acid ABC transporter, Lipid periplasmic amino acid-binding anchored protein, putative OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0108 PE = 4 SV = 1 - [B2IR19_STRPS] B2IRN0 57.75 22 284 275.2 Lipoprotein OS = -*Streptococcus pneumoniae* (strain CGSP14) anchored GN = SPCG_0153 PE = 3 SV = 1 - [B2IRNO_STRPS] B2IM81 70.93 36 774 270.81 Formate acetyltransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pfl PE = 4 SV = 1 - [B2IM81_STRPS] B2IRN7 65.61 30 660 269.02 Threonine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = thrS PE = 3 SV = 1 - [B2IRN7_STRPS] B2IMN6 63.36 22 494 268.42 Threonine synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = thrC PE = 4 SV = 1 - [B2IMN6_STRPS] B2IR98 69.68 20 376 261.66 Sugar ABC transporter, ATP-binding Intracellular protein OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1565 PE = 3 SV = 1 - [B2IR98_STRPS] B2IMG8 58.42 23 404 253.09 Aspartate aminotransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = aspC PE = 4 SV = 1 - [B2IMG8_STRPS] B2ILW6 65.43 13 243 252.82 3-ketoacyl-(Acyl-carrier-protein) Intracellular reductase OS = -*Streptococcus*

pneumoniae (strain CGSP14) GN = fabG PE = 4 SV = 1 - [B2ILW6_STRPS] B2INF2 70.18 25
 513 248.48 ABC transporter, ATP-binding Intracellular protein OS = *-Streptococcus pneumoniae*
 (strain CGSP14) GN = SPCG_0719 PE = 4 SV = 1 - [B2INF2_STRPS] B2IRS2 78.12 20 457
 247.37 Cell division protein FtsA OS = Multi- *Streptococcus pneumoniae* (strain transmembrane
 CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS2_STRPS] B2IM41 70.82 22 377 246.54
 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN =
 SPCG_1926 PE = 3 SV = 1 - [B2IM41_STRPS] B2IQ88 66.09 13 115 240.71 50S ribosomal
 protein L19 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplS PE = 3 SV =
 1 - [RL19_STRPS] B2IQ10 56.1 22 344 240.32 Uncharacterized protein OS = Multi-
Streptococcus pneumoniae (strain transmembrane CGSP14) GN = SPCG_1352 PE = 4 SV = 1 -
 [B2IQ10_STRPS] B2IND2 63.73 18 386 236.96 Branched-chain amino acid ABC Lipid
 transporter, amino acid-binding anchored protein OS = *-Streptococcus pneumoniae* (strain
 CGSP14) GN = SPCG_0699 PE = 4 SV = 1 - [B2IND2_STRPS] B2INP5 71.34 24 492 236.94
 Inosine-5'-monophosphate Intracellular dehydrogenase OS = *-Streptococcus pneumoniae* (strain
 CGSP14) GN = imdH PE = 3 SV = 1 - [B2INP5_STRPS] B2ISL2 67.26 18 339 235.53 Alcohol
 dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = adhP PE = 3
 SV = 1 - [B2ISL2_STRPS] B2ISM4 59.23 9 130 228.51 30S ribosomal protein S9 OS =
 Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsI PE = 3 SV = 1 - [RS9_STRPS]
 B2IR71 40.26 23 914 227.01 Cation-transporting ATPase, E1-E2 Multi- family OS = -
Streptococcus pneumoniae transmembrane (strain CGSP14) GN = pacL PE = 3 SV = 1 -
 [B2IR71_STRPS] B2IPP1 85.77 17 246 225.41 Amino acid ABC transporter, ATP- Intracellular
 binding protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1061 PE = 4 SV =
 1 - [B2IPP1_STRPS] B2ISY7 58.31 18 355 221.22 Oligopeptide ABC transporter, ATP-
 Intracellular binding protein AmiE OS = *Streptococcus pneumoniae* (strain CGSP14) GN = amiE
 PE = 3 SV = 1 - [B2ISY7_STRPS] B2IQL1 56.83 16 271 219.73 Amino acid ABC transporter,
 amino Lipid acid-binding protein OS = anchored *Streptococcus pneumoniae* (strain CGSP14) GN =
 SPCG_1383 PE = 4 SV = 1 - [B2IQL1_STRPS] B2ISG3 77.23 19 202 219.45 General stress
 protein 24, putative Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN =
 SPCG_1781 PE = 4 SV = 1 - [B2ISG3_STRPS] B2IMA2 70.1 20 398 216.35 Phosphoglycerate
 kinase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pgk PE = 3 SV = 1 -
 [PGK_STRPS] B2IN71 49.03 25 620 212.01 Elongation factor Tu family protein Intracellular OS
 = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0638 PE = 4 SV = 1 -
 [B2IN71_STRPS] B2IPR1 75.6 16 332 210.14 L-lactate dehydrogenase OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = Idh PE = 3 SV = 1 - [B2IPR1_STRPS] B2IQK0
 51.83 30 872 209.16 Alanine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain
 CGSP14) GN = alaS PE = 3 SV = 1 - [SYA_STRPS] B2IS44 61.29 12 93 208.67 30S ribosomal
 protein S19 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsS PE = 3 SV =
 1 - [RS19_STRPS] B2IMN9 48.47 17 425 208.24 Uncharacterized protein OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_2034 PE = 4 SV = 1 -
 [B2IMN9_STRPS] B2IR17 72.27 16 339 208.13 Ribose-phosphate Intracellular
 pyrophosphokinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = prsA PE = 3 SV = 1 -
 [B2IR17_STRPS] B2IQR1 62.69 25 520 208 GMP synthase [glutamine- Intracellular hydrolyzing]
 OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = guaA PE = 3 SV = 1 - [GUAA_STRPS]
 B2INL3 50.25 23 810 202.66 ATP-dependent Clp protease, ATP- Intracellular binding subunit OS
 = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2162 PE = 3 SV = 1 -
 [B2INL3_STRPS] B2IN11 58.53 17 299 197.31 UTP--glucose-1-phosphate Secretory
 uridylyltransferase OS = (released) *Streptococcus pneumoniae* (strain (with CS) CGSP14) GN =
 galU PE = 3 SV = 1 - [B2IN11_STRPS] B2IQU5 44.38 17 480 197.17 Peptidoglycan N-
 acetylglucosamine N- deacetylase A OS = *-Streptococcus terminally pneumoniae* (strain CGSP14)
 anchored GN = pgdA PE = 4 SV = 1 - (with CS) [B2IQU5_STRPS] B2INE4 57.49 19 447 196.45

DEAD-box ATP-dependent RNA Intracellular helicase CshB OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = rheB PE = 3 SV = 1 - [B2INE4_STRPS] B2IMU6 65.97 15 238 196.17 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_0590 PE = 4 SV = 1 - [B2IMU6_STRPS] B2INT7 54.92 27 752 195.47 ATP-dependent Clp protease, ATP- Intracellular binding subunit ClpE OS = *Streptococcus pneumoniae* (strain CGSP14) GN = clpE PE = 3 SV = 1 - [B2INT7_STRPS] B2IM28 44.59 17 388 194.98 Protein RecA OS = *-Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = recA PE = 3 SV = 1 - [RECA_STRPS] B2IQL4 79.78 18 267 192.86 Phosphate import ATP-binding Intracellular protein PstB OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL4_STRPS] B2ISL0 43.67 13 332 190.43 PTS system, mannose-specific IIAB Intracellular components OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = manL PE = 4 SV = 1 - [B2ISLO_STRPS] B2INW3 54.6 19 511 188.38 Sugar ABC transporter, ATP-binding Intracellular protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0788 PE = 4 SV = 1 - [B2INW3_STRPS] B2INT0 40.9 22 648 185.21 DNA gyrase subunit B OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gyrB PE = 3 SV = 1 - [B2INT0_STRPS] B2IP17 57.18 13 341 184.52 Iron-compound ABC transporter, Lipid iron compound-binding protein OS = anchored *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1009 PE = 4 SV = 1 - [B2IPI7_STRPS] B2IPT0 48.09 22 628 183.98 Elongation factor 4 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = lepA PE = 3 SV = 1 - [B2IPT0_STRPS] B2IN23 39.89 17 752 181.97 Alpha-1,4 glucan phosphorylase Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = maIP PE = 3 SV = 1 - [B2IN23_STRPS] B2IQZ2 64.71 20 374 180.03 Ribosome-binding ATPase YchF OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ychF PE = 3 SV = 1 - [B2IQZ2_STRPS] B2IMU8 66.81 17 229 179.85 50S ribosomal protein L1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplA PE = 3 SV = 1 - [RL1_STRPS] B2ISY6 51.06 14 331 178.98 Oligopeptide ABC transporter, ATP- Intracellular binding protein AmiF OS = *Streptococcus pneumoniae* (strain CGSP14) GN = amiF PE = 3 SV = 1 - [B2ISY6_STRPS] B2IR87 54.15 18 410 170.5 ATP-dependent Clp protease ATP- Intracellular binding subunit ClpX OS = *Streptococcus pneumoniae* (strain CGSP14) GN = clpX PE = 3 SV = 1 - [CLPX_STRPS] B2IR07 58.6 24 442 169.98 Adenylosuccinate synthetase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = purA PE = 3 SV = 1 - [B2IR07_STRPS] B2ILV9 61.23 20 454 169.93 Aspartokinase OS = *-Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = lysC PE = 3 SV = 1 - [B2ILV9_STRPS] B2IMZ6 42.12 25 857 169.84 DNA mismatch repair protein MutS Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = hexA PE = 3 SV = 1 - [B2IMZ6_STRPS] B2IS57 84.76 17 164 169.34 30S ribosomal protein S5 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsE PE = 3 SV = 1 - [B2IS57_STRPS] B2ILR1 29.62 20 719 166.63 Penicillin-binding protein 1A OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = pbp1A PE = 4 SV = 1 - anchored [B2ILR1_STRPS] (with CS) B2IQ78 53.73 22 523 165.23 Signal recognition particle protein Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = ffh PE = 3 SV = 1 - [B2IQ78_STRPS] B2ISM3 78.38 17 148 163.82 50S ribosomal protein L13 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplM PE = 3 SV = 1 - [B2ISM3_STRPS] B2INJ2 70.02 21 427 163.61 DltD protein OS = *-Streptococcus* N- *pneumoniae* (strain CGSP14) terminally GN = dltD PE = 4 SV = 1 - anchored [B2INJ2_STRPS] (with CS) B2ISJ1 48.84 19 602 159.21 Glutamine--fructose-6-phosphate Intracellular aminotransferase [isomerizing] OS = *Streptococcus pneumoniae* (strain CGSP14) GN = glmS PE = 3 SV = 1 - [B2ISJ1_STRPS] B2IPM7 52.69 18 577 159.04 Phosphoenolpyruvate-protein Intracellular phosphotransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1121 PE = 3 SV = 1 - [B2IPM7_STRPS] B2IQ29 61.01 17 436 157.98 GTPase Obg OS = *-Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = obg PE = 3 SV = 1 - [OBG_STRPS] B2IS66 74.8 9 127 157.55 30S ribosomal protein S11 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsK PE = 3 SV

= 1 - [RS11_STRPS] B2IQ67 33.36 24 1058 157.35 Carbamoyl-phosphate synthase Intracellular
 large chain OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = carB PE = 3 SV = 1 -
 [CARB_STRPS] B2IQ25 62.29 16 419 154.75 UDP-N-acetylglucosamine 1- Intracellular
 carboxyvinyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = murZ PE = 3 SV
 = 1 - [B2IQ25_STRPS] B2IQX4 82.93 14 164 154.18 ATP synthase subunit b OS = N-
Streptococcus pneumoniae (strain terminally CGSP14) GN = atpF PE = 3 SV = 1 - anchored
 [ATPF_STRPS] (with CS) B2IM72 69.51 10 246 154.11 Amino acid ABC transporter, ATP-
 Intracellular binding protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = glnQ PE = 4
 SV = 1 - [B2IM72_STRPS] B2INR2 35.79 19 679 153.3 Methionine--tRNA ligase OS =
 Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = metG PE = 3 SV = 1 -
 [B2INR2_STRPS] B2IS42 81.63 6 98 152.99 50S ribosomal protein L23 OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = rplW PE = 3 SV = 1 - [RL23_STRPS] B2IPI0
 44.02 18 418 152.53 Serine hydroxymethyltransferase Intracellular OS = *-Streptococcus*
pneumoniae (strain CGSP14) GN = glyA PE = 3 SV = 1 - [GLYA_STRPS] B2IRG4 73.89 21 203
 151.28 30S ribosomal protein S4 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14)
 GN = rpsD PE = 3 SV = 1 - [RS4_STRPS] B2ILV7 59.29 21 452 150.33 Serine--tRNA ligase OS =
 Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = serS PE = 3 SV = 1 -
 [B2ILV7_STRPS] B2INC8 60.65 13 216 146.88 Uracil phosphoribosyltransferase Intracellular OS
 = *-Streptococcus pneumoniae* (strain CGSP14) GN = upp PE = 3 SV = 1 - [B2INC8_STRPS]
 B2IQL3 70.63 16 252 145.61 Phosphate import ATP-binding Intracellular protein PstB OS = -
Streptococcus pneumoniae (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL3_STRPS]
 B2IMZ5 31.91 19 564 144.21 ABC transporter, ATP- Multi- binding/permease protein OS =
 transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2042 PE = 4 SV = 1 -
 [B2IMZ5_STRPS] B2IS39 59.8 8 102 143.18 30S ribosomal protein S10 Intracellular OS =
Streptococcus pneumoniae (strain CGSP14) GN = rpsJ PE = 3 SV = 1 - [RS10_STRPS] B2IP78
 53.87 13 375 141.59 Carboxynorspermidine Intracellular decarboxylase OS = *-Streptococcus*
pneumoniae (strain CGSP14) GN = nspC PE = 4 SV = 1 - [B2IP78_STRPS] B2IM60 54.81 13 416
 139.41 Diaminopimelate decarboxylase Intracellular OS = *-Streptococcus pneumoniae* (strain
 CGSP14) GN = lysA PE = 3 SV = 1 - [B2IM60_STRPS] B2IMH3 65.03 14 346 139.16 Catabolite
 control protein A OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ccpA PE = 4
 SV = 1 - [B2IMH3_STRPS] B2IS68 57.03 8 128 139.07 50S ribosomal protein L17 OS =
 Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplQ PE = 3 SV = 1 -
 [RL17_STRPS] B2INE0 34.95 13 329 136.77 Cell division protein FtsX OS = Multi-
Streptococcus pneumoniae (strain transmembrane CGSP14) GN = ftsX PE = 3 SV = 1 -
 [B2INE0_STRPS] B2ILV2 41.65 20 778 134.89 Endonuclease MutS2 OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = mutS2 PE = 3 SV = 1 - [MUTS2_STRPS]
 B2IQU4 63.93 15 280 133.96 Oxidoreductase, aldo/keto Intracellular reductase family OS = -
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_1466 PE = 4 SV = 1 - [B2IQU4_STRPS]
 B2IS55 71.35 14 178 129.78 50S ribosomal protein L6 OS = Intracellular *Streptococcus*
pneumoniae (strain CGSP14) GN = rplF PE = 3 SV = 1 - [RL6_STRPS] B2ISJ8 67.95 15 156
 129.16 30S ribosomal protein S7 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14)
 GN = rpsG PE = 3 SV = 1 - [RS7_STRPS] B2IRR6 24.81 8 266 128.96 Cell division protein
 DivIVA OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1633 PE = 4
 SV = 1 - [B2IRR6_STRPS] B2IS18 43.68 14 419 128.49 Zinc metalloprotease OS = Multi-
Streptococcus pneumoniae (strain transmembrane CGSP14) GN = eep PE = 3 SV = 1 -
 [B2IS18_STRPS] B2INF3 47.94 9 267 128.19 Peptidyl-prolyl cis-trans isomerase Lipid OS = -
Streptococcus pneumoniae (strain anchored CGSP14) GN = ppiA PE = 3 SV = 1 -
 [B2INF3_STRPS] B2IR96 96.59 13 176 127.15 Adenine phosphoribosyltransferase Intracellular
 OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = apt PE = 3 SV = 1 - [B2IR96_STRPS]
 B2IQX1 63.36 12 292 124.75 ATP synthase gamma chain OS = Intracellular *Streptococcus*

pneumoniae (strain CGSP14) GN = atpG PE = 3 SV = 1 - [ATPG_STRPS] B2IQL7 40.41 12 292
 124.49 Phosphate ABC transporter, Lipid phosphate-binding protein, anchored putative OS = -
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_1389 PE = 4 SV = 1 - [B2IQL7_STRPS]
 B2IS49 69.77 10 86 124.47 30S ribosomal protein S17 OS = Intracellular *Streptococcus*
pneumoniae (strain CGSP14) GN = rpsQ PE = 3 SV = 1 - [RS17_STRPS] B2IQV3 35.99 8 289
 123.64 Glycerol uptake facilitator protein, Multi- putative OS = -*Streptococcus* transmembrane
pneumoniae (strain CGSP14) GN = glpF PE = 3 SV = 1 - [B2IQV3_STRPS] B21M98 46.54 14
 535 123.63 CTP synthase OS = *Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN =
 pyrG PE = 3 SV = 1 - [B21M98_STRPS] B2IQ39 47.38 9 344 123.25 Uncharacterized protein OS
 = Secretory *Streptococcus pneumoniae* (strain (released) CGSP14) GN = SPCG_1211 PE = 4 SV =
 1 - (with CS) [B2IQ39_STRPS] B2IP00 65.99 14 397 123.01 Serine protease OS = -*Streptococcus*
N- pneumoniae (strain CGSP14) terminally GN = sphtra PE = 4 SV = 1 - anchored
 [B2IP00_STRPS] (No CS) B2IN41 35.2 16 429 122.13 Histidine--tRNA ligase OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = hisS PE = 3 SV = 1 - [SYH_STRPS] B2IRW4
 40.6 15 436 120.41 GTPase Der OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14)
 GN = der PE = 3 SV = 1 - [DER_STRPS] B2IR68 35.97 13 531 119.86 Uncharacterized protein
 OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1535 PE = 4
 SV = 1 - [B2IR68_STRPS] B2IPR2 27.94 18 841 118.97 DNA gyrase subunit A OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = gyrA PE = 3 SV = 1 - [B2IPR2_STRPS]
 B2IRA1 44.08 17 524 118.46 DEAD-box ATP-dependent RNA Intracellular helicase CshA OS = -
Streptococcus pneumoniae (strain CGSP14) GN = cshA PE = 3 SV = 1 - [B2IRA1_STRPS]
 B2IM46 55.94 11 345 117.68 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain
 terminally CGSP14) GN = SPCG_1931 PE = 4 SV = 1 - anchored [B2IM46_STRPS] (with CS)
 B2ILR7 48.44 16 481 117.48 6-phosphogluconate Intracellular dehydrogenase, decarboxylating
 OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gnd PE = 3 SV = 1 - [B2ILR7_STRPS]
 B2IR57 62.02 19 466 116.97 Cof family protein/peptidyl-prolyl Intracellular cis-trans isomerase,
 cyclophilin type OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1524 PE = 4 SV
 = 1 - [B2IR57_STRPS] B2IND9 76.09 12 230 114.4 Cell division ABC transporter, ATP-
 Intracellular binding protein FtsE OS = *Streptococcus pneumoniae* (strain CGSP14) GN = ftsE PE
 = 4 SV = 1 - [B2IND9_STRPS] B2IPU7 45 13 320 113.45 Ribonucleoside-diphosphate
 Intracellular reductase subunit beta OS = *Streptococcus pneumoniae* (strain CGSP14) GN = nrdF
 PE = 3 SV = 1 - [B2IPU7_STRPS] B2IMS9 43.58 20 553 112.79 Ribonuclease J OS = -
Streptococcus Intracellular *pneumoniae* (strain CGSP14) GN = rnj PE = 3 SV = 1 -
 [B2IMS9_STRPS] B2ISQ9 52.02 15 494 111.9 UPF0371 protein SPCG_0344 OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_0344 PE = 3 SV = 1 - [Y344_STRPS]
 B2IPF4 53.38 14 311 110.86 Adhesion lipoprotein OS = Lipid *Streptococcus pneumoniae* (strain
 anchored CGSP14) GN = Imb PE = 3 SV = 1 - [B2IPF4_STRPS] B2IM45 50.82 12 427 110.79
 UDP-N-acetylglucosamine 1- Intracellular carboxyvinyltransferase OS = *Streptococcus*
pneumoniae (strain CGSP14) GN = murA PE = 3 SV = 1 - [B2IM45_STRPS] B2INN5 58.46 10
 272 109.88 Cell shape-determining protein N- MreC OS = -*Streptococcus pneumoniae* terminally
 (strain CGSP14) GN = mreC PE = 3 anchored SV = 1 - [B2INN5_STRPS] (with CS) B2IM15
 46.84 10 158 109.73 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain
 transmembrane CGSP14) GN = SPCG_1901 PE = 4 SV = 1 - [B2IM15_STRPS] B2IRP9 25.68 14
 740 109.17 GTP pyrophosphokinase OS = Intracellular *Streptococcus pneumoniae* (strain
 CGSP14) GN = reIA PE = 3 SV = 1 - [B2IRP9_STRPS] B2INE1 34.71 11 726 108.73 PTS system,
 IIABC components OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN =
 ptsG PE = 4 SV = 1 - [B2INE1_STRPS] B2IST7 48.44 12 417 108.26 Capsular polysaccharide
 Intracellular biosynthesis protein, putative OS = *Streptococcus pneumoniae* (strain CGSP14) GN =
 SPCG_1812 PE = 3 SV = 1 - [B2IST7_STRPS] B2IP76 71.33 14 286 106.74 Polyamine
 aminopropyltransferase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = speE

PE = 3 SV = 1 - [B2IP76_STRPS] B2IS23 34.49 22 954 105.34 UvrABC system protein A OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = uvrA PE = 3 SV = 1 - [B2IS23_STRPS] B2IS53 52.81 7 89 105.15 30S ribosomal protein S14 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsN PE = 3 SV = 1 - [RS14_STRPS] B2IQN9 49.79 20 486 105.03 Nicotinate Intracellular phosphoribosyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1408 PE = 3 SV = 1 - [B2IQN9_STRPS] B2ISZ6 49.88 13 419 104.34 Sugar ABC transporter, sugar- Lipid binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1871 PE = 4 SV = 1 - [B2ISZ6_STRPS] B2IMA9 47.02 17 487 101.63 Type I restriction-modification Intracellular system, M subunit OS = *Streptococcus pneumoniae* (strain CGSP14) GN = hsdM PE = 4 SV = 1 - [B2IMA9_STRPS] B2IQH2 72.19 13 187 100.73 50S ribosomal protein L10 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplJ PE = 3 SV = 1 - [B2IQH2_STRPS] B2IR61 39.82 14 447 100.57 Asparagine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = asnS PE = 3 SV = 1 - [B2IR61_STRPS] B2IM30 50 15 338 100.22 Transcriptional regulator, putative Secretory OS = -*Streptococcus pneumoniae* (strain (released) CGSP14) GN = lytR PE = 4 SV = 1 - (with CS) [B2IM30_STRPS] B2ISV9 32.29 10 511 99.16 Choline transporter OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1834 PE = 3 SV = 1 - [B2ISV9_STRPS] B2IS51 78.22 12 101 98.15 50S ribosomal protein L24 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplX PE = 3 SV = 1 - [RL24_STRPS] B2IRX0 76.67 11 240 97.84 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1687 PE = 4 SV = 1 - [B2IRX0_STRPS] B2ISX8 46.73 11 336 97.53 Non-canonical purine NTP Intracellular pyrophosphatase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1853 PE = 3 SV = 1 - [B2ISX8_STRPS] B2IN24 45.35 14 505 97.46 4-alpha-glucanotransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = maIM PE = 3 SV = 1 - [B2IN24_STRPS] B2IM61 54.55 12 275 97.4 Purine operon repressor OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = purR PE = 4 SV = 1 - [B2IM61_STRPS] B2ILX0 55.38 16 455 97.1 Acetyl-CoA carboxylase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = accC PE = 4 SV = 1 - [B2ILX0_STRPS] B2IQ19 35.91 15 763 96.38 DNA helicase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pcrA PE = 3 SV = 1 - [B2IQ19_STRPS] B2IPZ6 69.23 10 91 95.95 DNA-binding protein HU OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = hlpA PE = 3 SV = 1 - [B2IPZ6_STRPS] B2INE5 31.57 12 396 95.45 S-adenosylmethionine synthase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = metK PE = 3 SV = 1 - [METK_STRPS] B2IS82 28.53 15 659 94.22 Serine/threonine protein kinase Intracellular/ OS = -*Streptococcus pneumoniae* (strain TMH start CGSP14) GN = pkn2 PE = 3 SV = 1 - AFTER 60 [B2IS82_STRPS] B2IS65 58.68 10 121 93.19 30S ribosomal protein S13 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsM PE = 3 SV = 1 - [RS13_STRPS] B21MI4 35.02 18 731 92.99 Penicillin-binding protein 2A OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = pbp2A PE = 4 SV = 1 - anchored [B21MI4_STRPS] (with CS) B2IRK4 37.05 17 637 92.87 tRNA uridine 5- Intracellular carboxymethylaminomethyl modification enzyme MnmG OS = *Streptococcus pneumoniae* (strain CGSP14) GN = mnmG PE = 3 SV = 1 - [B2IRK4_STRPS] B2ISW0 69.01 11 242 92.71 Choline transporter OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1835 PE = 4 SV = 1 - [B2ISW0_STRPS] B2INP6 39.59 10 341 92.59 Tryptophan--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = trpS PE = 3 SV = 1 - [B2INP6_STRPS] B2INC5 39.5 11 281 92.15 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0692 PE = 4 SV = 1 - [B2INC5_STRPS] B2IP66 36.64 17 715 91.51 Transcriptional regulator, putative Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0885 PE = 4 SV = 1 - [B2IP66_STRPS] B2ISY1 46.58 14 541 91.27 Dextran glucosidase DexS, putative Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = dexS PE = 4 SV = 1 - [B2ISY1_STRPS]

B2ISK7 38.51 11 444 91.26 Amino peptidase C OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pepC PE = 4 SV = 1 - [B2ISK7_STRPS] B2INL6 30.75 8 335 91.2 ABC transporter, substrate-binding Lipid protein, putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2165 PE = 4 SV = 1 - [B2INL6_STRPS] B2IQY9 25.83 11 453 90.37 Chromosomal replication initiator Intracellular protein DnaA OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = dnaA PE = 3 SV = 1 - [DNAA_STRPS] B2IRS8 22.34 13 685 90.12 Penicillin-binding protein 2B OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = pbp2B PE = 4 SV = 1 - anchored [B2IRS8_STRPS] (with CS) B2IS56 53.44 8 131 88.46 50S ribosomal protein L18 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplR PE = 3 SV = 1 - [B2IS56_STRPS] B2IQ12 40.13 9 319 88.41 Ribose-phosphate Intracellular pyrophosphokinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = prs PE = 3 SV = 1 - [B2IQ12_STRPS] B2IS67 52.73 9 311 88.17 DNA-directed RNA polymerase Intracellular subunit alpha OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rpoA PE = 3 SV = 1 - [B2IS67_STRPS] B2IS59 64.38 9 146 88.05 50S ribosomal protein L15 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplO PE = 3 SV = 1 - [RL15_STRPS] B2IQ61 41.52 9 289 87.7 Choline kinase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pck PE = 4 SV = 1 - [B2IQ61_STRPS] B2ISY2 24.4 10 705 87.27 Trehalose PTS system, IIABC Multi- components OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = treP PE = 4 SV = 1 - [B2ISY2_STRPS] B2IMQ3 25.97 12 801 86.8 Phenylalanine--tRNA ligase beta Intracellular subunit OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pheT PE = 3 SV = 1 - [B2IMQ3_STRPS] B2IQ02 60.82 9 97 86.49 50S ribosomal protein L27 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmA PE = 3 SV = 1 - [RL27_STRPS] B2IN58 40.3 11 335 86.4 Uncharacterized protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_2101 PE = 4 SV = 1 - [B2IN58_STRPS] B2IQW4 62.2 10 209 84.4 Amino acid ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1486 PE = 4 SV = 1 - [B2IQW4_STRPS] B2IP74 35.23 13 491 84.24 Lysine decarboxylase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = cad PE = 4 SV = 1 - [B2IP74_STRPS] B2IS70 42.49 11 433 83.11 UPF0210 protein SPCG_0246 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0246 PE = 3 SV = 1 - [B2IS70_STRPS] B2IQ66 64.29 13 280 83.05 LicD2 protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = licD2 PE = 4 SV = 1 - [B2IQ66_STRPS] B2IRK5 34.17 12 559 81.76 Ribonuclease J OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IRK5_STRPS] B2IR84 68.24 12 296 81.42 Nucleotide-binding protein Intracellular SPCG_1551 OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1551 PE = 3 SV = 1 - [Y1551_STRPS] B2IMB5 34.93 15 607 81.24 Chaperone protein DnaK OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = dnaK PE = 3 SV = 1 - [DNAK_STRPS] B2IQ62 58.09 12 346 80.31 Alcohol dehydrogenase, zinc- Intracellular containing OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1234 PE = 4 SV = 1 - [B2IQ62_STRPS] B2IMB9 37.86 11 243 79.94 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0494 PE = 4 SV = 1 - [B2IMB9_STRPS] B2IRD4 56.18 5 89 79.7 30S ribosomal protein S15 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsO PE = 3 SV = 1 - [RS15_STRPS] B2IN68 50.79 8 126 79.14 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_0635 PE = 4 SV = 1 - anchored [B2IN68_STRPS] (with CS) BEIQN0 44.09 3 127 78.99 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1399 PE = 4 SV = 1 anchored [B2IQNO_STRPS] (with CS) B2ILY0 36.58 14 514 78.96 Peptide chain release factor 3 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = prfC PE = 3 SV = 1 - [RF3_STRPS] B2IS54 57.58 8 132 78.42 30S ribosomal protein S8 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsH PE = 3 SV = 1 - [RS8_STRPS] B2IMB6

35.19 13 378 78.27 Chaperone protein DnaJ OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = dnaJ PE = 3 SV = 1 - [B2IMB6_STRPS] B2IQS5 42.11 12 247 78.16 Amino acid ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1447 PE = 4 SV = 1 - [B2IQS5_STRPS] B2IRH0 33.01 12 512 76.84 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus anchored pneumoniae* (strain CGSP14) GN = SPCG_0089 PE = 4 SV = 1 - [B2IRHO_STRPS] B21PW9 50.88 11 283 76.72 Ribosome biogenesis GTPase A OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1141 PE = 3 SV = 1 - [B2IPW9_STRPS] B2IN18 48.09 11 418 76.44 Tyrosine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tyrS PE = 3 SV = 1 - [SYY_STRPS] B2IQV9 33.39 13 572 75.14 Phosphoglucosyltransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pgm PE = 3 SV = 1 - [B2IQV9_STRPS] B2IP77 38.9 9 419 74.94 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0896 PE = 4 SV = 1 - [B2IP77_STRPS] B2IQ63 53.19 8 235 74.22 2-C-methyl-D-erythritol 4- Intracellular phosphate cytidylyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = ispD PE = 3 SV = 1 - [ISPD_STRPS] B2IRQ4 65.34 11 251 74.2 Manganese ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1621 PE = 4 SV = 1 - [B2IRQ4_STRPS] B2IQX3 62.36 8 178 73.32 ATP synthase subunit delta OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = atpH PE = 3 SV = 1 - [ATPD_STRPS] B2INX4 27.48 12 826 73.21 DNA topoisomerase 4 subunit A Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = parC PE = 3 SV = 1 - [B2INX4_STRPS] B2IN76 40.67 12 450 72.88 UDP-N-acetylmuramoylalanine--D- Intracellular glutamate ligase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = murD PE = 3 SV = 1 - [MURD_STRPS] B2IPC3 41.47 10 299 72.75 GTPase Era OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = era PE = 3 SV = 1 - [ERA_STRPS] B2IRX7 40.79 13 353 72.1 Methionine import ATP-binding Intracellular protein MetN OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = metN PE = 3 SV = 1 - [B2IRX7_STRPS] B2INU1 50.65 9 306 70.6 Bifunctional protein FoID OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = foID PE = 3 SV = 1 - [B2INU1_STRPS] B2ILW4 36.11 6 324 69.62 Enoyl-(Acyl-carrier-protein) Intracellular reductase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fabK PE = 4 SV = 1 - [B2ILW4_STRPS] B2IS45 40.35 3 114 69.56 50S ribosomal protein L22 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplV PE = 3 SV = 1 - [RL22_STRPS] B2IPZ1 30.21 13 652 67.59 DNA ligase OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = ligA PE = 3 SV = 1 - [DNLJ_STRPS] B2IMZ3 34.66 12 603 66.96 ABC transporter, ATP- Multi-binding/permease protein OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2040 PE = 4 SV = 1 - [B2IMZ3_STRPS] B2IS63 54.72 6 212 66.76 Adenylate kinase OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = adk PE = 3 SV = 1 - [KAD_STRPS] B2INV5 53.01 6 83 65.46 30S ribosomal protein S20 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsT PE = 3 SV = 1 - [B2INV5_STRPS] B2IRK2 38.07 10 373 65.45 tRNA-specific 2-thiouridylase Intracellular MnmA OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = mnmA PE = 3 SV = 2 - [MNMA_STRPS] B2IRB7 27.22 2 158 65.21 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1584 PE = 4 SV = 1 - anchored [B2IRB7_STRPS] (with CS) B2INE6 56.27 10 311 64.68 Dihydroorotate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pyrD PE = 3 SV = 1 - [B2INE6_STRPS] B2IMB0 27.41 15 777 64.13 Type I restriction-modification Intracellular system, R subunit OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = hsdR PE = 4 SV = 1 - [B2IMBO_STRPS] B2ISQ4 20.27 11 750 63.95 Penicillin-binding protein 2X OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = pbp2X PE = 4 SV = 1 - anchored [B2ISQ4_STRPS] (with CS) B2IPQ3 27.88 12 556 63.73 Formate--tetrahydrofolate ligase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fhs PE = 3 SV = 1 - [FTHS_STRPS] B2IMU7 41.84 5 141 63.38 50S ribosomal

protein L11 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplK PE = 3 SV = 1 - [RL11_STRPS] B2ISY0 38 4 100 62.82 UPF0154 protein SPCG_1855 OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1855 PE = 3 SV = 1 - anchored [B2ISY0_STRPS] (with CS) B2IQP4 29.49 9 434 62.33 Peptidase, U32 family OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1416 PE = 4 SV = 1 - [B2IQP4_STRPS] B2IR79 57.14 11 259 62.31 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1546 PE = 4 SV = 1 - anchored [B2IR79_STRPS] (with CS) B2ILW2 21.6 8 324 62.19 3-oxoacyl-[acyl-carrier-protein] Intracellular synthase 3 OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = fabH PE = 3 SV = 1 - [FABH_STRPS] B2IPZ9 43.28 9 305 61.07 Riboflavin biosynthesis protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = mreA PE = 3 SV = 1 - [B2IPZ9_STRPS] B2IN32 29.98 13 587 60.72 Aspartate--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = aspS PE = 3 SV = 1 - [SYD_STRPS] B2IRR9 42.46 7 179 60.72 Cell division protein SepF OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = sepF PE = 3 SV = 1 - [SEPF_STRPS] B2IN19 28.12 13 690 60.07 Cation-transporting ATPase, E1-E2 Intracellular family OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = ctpC PE = 3 SV = 1 - [B2IN19_STRPS] B2IQY8 36.71 9 444 59.95 UDP-N-acetylmuramate--L-alanine Intracellular ligase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = murC PE = 3 SV = 1 - [MURC_STRPS] B2IS26 33.86 8 189 59.81 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_0204 PE = 4 SV = 1 [B2IS26_STRPS] B2INY7 45.25 8 263 59.27 ABC transporter, ATP-binding Intracellular protein OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0812 PE = 4 SV = 1 - [B2INY7_STRPS] B2ILR6 14.66 6 464 58.78 Mid-cell-anchored protein Z OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = mapZ PE = 3 SV = 1 - anchored [B2ILR6_STRPS] (with CS) B2ILX8 36.68 10 488 58.59 Glutamyl-tRNA(Gln) Intracellular amidotransferase subunit A OS = *Streptococcus pneumoniae* (strain CGSP14) GN = gatA PE = 3 SV = 1 - [GATA_STRPS] B2IMN3 35.26 7 380 58.5 Queuine tRNA-ribosyltransferase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = tgt PE = 3 SV = 1 - [TGT_STRPS] B2IN17 21.09 12 825 58.4 Penicillin-binding protein 1B OS = Intracellular/ *Streptococcus pneumoniae* (strain TMH start CGSP14) GN = pbp1B PE = 4 SV = 1 - AFTER 60 [B2IN17_STRPS] B2IR27 45.45 8 330 57.61 Phosphate acyltransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = plsX PE = 3 SV = 1 - [PLSX_STRPS] B2INF7 73.21 6 112 56.77 ATP cone domain-containing Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0728 PE = 4 SV = 1 - [B2INF7_STRPS] B2IMZ8 20.78 9 563 56.13 Arginine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = argS PE = 3 SV = 1 - [SYR_STRPS] B2ILU5 35.6 10 427 55.96 Trigger factor OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = tig PE = 3 SV = 1 - [TIG_STRPS] B2INK8 56.55 9 336 55.86 tRNA-dihydrouridine synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2157 PE = 3 SV = 1 [B2INK8_STRPS] B2IM90 37.14 11 560 55.51 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0465 PE = 4 SV = 1 - [B2IM90_STRPS] B2IQA4 37.72 12 448 54.94 Glutamate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gdhA PE = 3 SV = 1 - [B2IQA4_STRPS] B2IPPO 30.1 8 495 54.8 Glucose-6-phosphate 1- Intracellular dehydrogenase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = zwf PE = 3 SV = 1 - [B2IPPO_STRPS] B2IPZ5 24.33 13 633 54.29 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1167 PE = 4 SV = 1 - [B2IPZ5_STRPS] B2IS10 23.73 8 649 54.27 DNA mismatch repair protein MutL Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = mutL PE = 3 SV = 1 - [MUTL_STRPS] B2IN96 47.62 10 378 54.14 Lactate oxidase OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = IctO PE = 4 SV = 1 - [B2IN96_STRPS] B2IND6 51.69 6 236 53.89 Branched-chain amino acid ABC Intracellular

transporter, ATP-binding protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0703 PE = 4 SV = 1 - [B2IND6_STRPS] B2IS98 58.85 9 209 53.7 Probable nicotinate-nucleotide Intracellular adenylyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = nadD PE = 3 SV = 1 - [B2IS98_STRPS] B2IPN8 43.01 8 272 53.21 Cof family protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1058 PE = 4 SV = 1 - [B2IPN8_STRPS] B2INN8 51.27 10 275 52.83 Energy-coupling factor transporter N- ATP-binding protein EcfA OS = terminally *Streptococcus pneumoniae* (strain anchored CGSP14) GN = ecfA PE = 3 SV = 1 - (with CS) [B2INN8_STRPS] B2ISR0 23.55 8 535 52.43 Glucan 1,6-alpha-glucosidase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = dexB PE = 4 SV = 1 - [B2ISRO_STRPS] B2IRF6 52.19 8 228 52.22 Potassium uptake protein, Trk N- family OS = *-Streptococcus pneumoniae* terminally (strain CGSP14) GN = trkA PE = 4 SV = 1 - anchored [B2IRF6_STRPS] (with CS) B2IM43 17.16 7 443 51.63 CBS domain protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1928 PE = 4 SV = 1 - [B2IM43_STRPS] B2ILX2 40 6 255 51.58 Acetyl-coenzyme A carboxylase Intracellular carboxyl transferase subunit alpha OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = accA PE = 3 SV = 1 - [ACCA_STRPS] B2ISA0 34.78 10 368 51.32 GTP-binding protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1723 PE = 4 SV = 1 - [B2ISAO_STRPS] B2IPB9 23.19 10 677 50.7 Endo-beta-N-acetylglucosaminidase OS = *-Streptococcus pneumoniae* (strain (released) CGSP14) GN = lytB PE = 4 SV = 1 - (with CS) [B2IPB9_STRPS] B2IQW9 61.87 6 139 50.69 ATP synthase epsilon chain OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = atpC PE = 3 SV = 1 - [ATPE_STRPS] B2IPN9 24.94 7 429 50.34 Signal recognition particle receptor Intracellular FtsY OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = ftsY PE = 3 SV = 1 - [B2IPN9_STRPS] B2IRI7 17.95 9 713 49.57 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0106 PE = 4 SV = 1 - [B2IRI7_STRPS] B2IQH3 24.39 7 488 49.39 Chlorohydrolase OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = trzA PE = 4 SV = 1 - [B2IQH3_STRPS] B2IRD2 21.59 7 264 48.68 Acyltransferase family protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1599 PE = 4 SV = 1 - [B2IRD2_STRPS] B2IR63 31.14 7 395 48.62 Aspartate aminotransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = aspB PE = 3 SV = 1 - [B2IR63_STRPS] B2INK4 23.36 10 608 48.46 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_2153 PE = 4 SV = 1 - anchored [B2INK4_STRPS] (with CS) B2IN18 28.14 7 501 48.06 Zinc ABC transporter, zinc-binding Lipid lipoprotein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2137 PE = 3 SV = 1 - [B2IN18_STRPS] B2IPU4 40.71 7 253 47.82 Lactose phosphotransferase system Intracellular repressor OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = lacR PE = 4 SV = 1 - [B2IPU4_STRPS] B2INN7 41.94 8 279 47.56 Energy-coupling factor transporter N- ATP-binding protein EcfA OS = terminally *Streptococcus pneumoniae* (strain anchored CGSP14) GN = ecfA PE = 3 SV = 1 - (with CS) [B2INN7_STRPS] B2IS50 52.46 7 122 47.46 50S ribosomal protein L14 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplN PE = 3 SV = 1 - [RL14_STRPS] B2IRI6 32.63 3 95 47.46 Bacteriocin, putative OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_0105 PE = 4 SV = 1 - anchored [B2IRI6_STRPS] (with CS) B2IPJ1 50 9 264 47.22 Iron-compound ABC transporter, Intracellular ATP-binding protein OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1013 PE = 4 SV = 1 - [B2IPJ1_STRPS] B2IPR3 40.89 7 247 47.21 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1083 PE = 4 SV = 1 - anchored [B2IPR3_STRPS] (with CS) B2IR24 28.28 9 389 47.15 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0036 PE = 3 SV = 1 - [B2IR24_STRPS] B2ISX7 60.12 8 173 47.03 Phosphoesterase OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1852 PE = 3 SV = 1 - [B2ISX7_STRPS] B2IM86 29.62 8 449 46.75

Potassium uptake protein, Trk N- family OS = *-Streptococcus pneumoniae* terminally (strain CGSP14) GN = trkA PE = 4 SV = 1 - anchored [B2IM86_STRPS] (with CS) B2IPT8 33.13 9 326 46.7 Tagatose 1,6-diphosphate aldolase Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = lacD PE = 3 SV = 1 - [B2IPT8_STRPS] B2ILZ5 39.81 10 540 46.59 60 kDa chaperonin OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = groL PE = 3 SV = 1 - [CH60_STRPS] B2ILX7 34.58 8 480 46.43 Aspartyl/glutamyl-tRNA(Asn/Gln) Intracellular amidotransferase subunit B OS = *Streptococcus pneumoniae* (strain CGSP14) GN = gatB PE = 3 SV = 1 - [GATB_STRPS] B2IMQ9 16.96 8 737 45.59 Polyribonucleotide Intracellular nucleotidyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = pnp PE = 3 SV = 2 - [PNP_STRPS] B2IQN3 45.45 5 66 44.91 30S ribosomal protein S21 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsU PE = 3 SV = 1 - [B2IQN3_STRPS] B2IR90 38.37 2 172 44.68 Non-heme iron-containing ferritin Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1557 PE = 3 SV = 1 - [B2IR90_STRPS] B2IMS2 42.66 7 293 44.59 Fructose-bisphosphate aldolase Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = fba PE = 3 SV = 1 - [B2IMS2_STRPS] B2IRX2 10.53 5 399 44.06 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1689 PE = 4 SV = 1 - [B2IRX2_STRPS] B2ISH7 20.98 10 815 43.34 Formate acetyltransferase, putative Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = pfIF PE = 4 SV = 1 - [B2ISH7_STRPS] B2ISG1 26.84 7 190 43.06 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1779 PE = 4 SV = 1 - [B2ISG1_STRPS] B21MY2 73.21 7 56 42.86 Uncharacterized protein OS = Secretory *Streptococcus pneumoniae* (strain (released) CGSP14) GN = SPCG_0626 PE = 4 SV = 1 - (with CS) [B21MY2_STRPS] B2IM36 15.5 8 671 42.81 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1921 PE = 4 SV = 1 [B2IM36_STRPS] B2IM12 26.96 8 471 42.77 Pneumolysin OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = ply PE = 4 SV = 1 - [B2IM12_STRPS] B2INM4 34.62 6 182 42.71 Ribosomal subunit interface protein Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2173 PE = 4 SV = 1 [B2INM4_STRPS] B2IPW4 27.34 9 567 42.61 Dihydrolipoyl dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = acoL PE = 4 SV = 1 - [B2IPW4_STRPS] B2IQZ9 38.89 6 180 42.56 Hypoxanthine-guanine Intracellular phosphoribosyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = hgt PE = 4 SV = 1 - [B2IQZ9_STRPS] B2IS21 19.23 6 234 42.44 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0199 PE = 4 SV = 1 - [B2IS21_STRPS] B2IPB4 18.18 3 66 42.39 50S ribosomal protein L35 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpml PE = 3 SV = 1 - [RL35_STRPS] B2IM29 23.68 5 418 42.24 Putative competence-damage Intracellular inducible protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = cinA PE = 3 SV = 1 - [CINA_STRPS] B2IR86 66.5 11 197 41.89 Probable GTP-binding protein EngB Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = engB PE = 3 SV = 1 - [ENGB_STRPS] B2IPS2 46.7 4 212 41.29 Uridine kinase OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = udk PE = 3 SV = 1 - [URK_STRPS] B2INP0 22.46 5 276 41.12 Uncharacterized protein OS = Intracellular/ *Streptococcus pneumoniae* (strain TMH start CGSP14) GN = SPCG_2189 PE = 4 SV = 1 - AFTER 60 [B2INP0_STRPS] B2ILX1 42.71 7 288 41.03 Acetyl-coenzyme A carboxylase Intracellular carboxyl transferase subunit beta OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = accD PE = 3 SV = 1 - [B2ILX1_STRPS] B2IMY9 31.48 10 486 40.7 Glutamate--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gltX PE = 3 SV = 1 - [SYE_STRPS] B2ISL4 51.11 9 270 40.64 Cof family protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0299 PE = 4 SV = 1 - [B2ISL4_STRPS] B2IQH7 42.21 5 289 40.55 Homoserine kinase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = thrB PE = 3 SV = 1 - [KHSE_STRPS] B2IS89 36.54 7 208 40.54 Guanylate kinase OS = -

Streptococcus pneumoniae (strain CGSP14) GN = gmk PE = 3 SV = 1 - [B2IS89_STRPS] B2IRQ3 18.92 7 650 40.16 Endopeptidase O OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = pepO PE = 4 SV = 1 - [B2IRQ3_STRPS] B2IR80 26.03 6 292 39.76 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1547 PE = 4 SV = 1 - [B2IR80_STRPS] B2INZ2 20.82 6 413 39.4 D-alanyl-D-alanine N- carboxypeptidase OS = *-Streptococcus terminally pneumoniae* (strain CGSP14) anchored GN = dacA PE = 3 SV = 1 - (with CS) [B2INZ2_STRPS] B2IR93 45.95 6 259 39.26 Triosephosphate isomerase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tpiA PE = 3 SV = 1 - [B2IR93_STRPS] B2IP56 34.48 8 290 39.23 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_0875 PE = 4 SV = 1 - [B2IP56_STRPS] B2IPZ7 46.59 6 279 39.17 DegV family protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1169 PE = 4 SV = 1 - [B2IPZ7_STRPS] B2INK5 19.52 8 502 39.03 Glycerol kinase OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = glpK PE = 3 SV = 1 - [GLPK_STRPS] B2INM2 32.67 7 150 38.84 50S ribosomal protein L9 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplI PE = 3 SV = 1 - [RL9_STRPS] B2IPH6 21.17 4 359 38.78 Peptide chain release factor 1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = prfA PE = 3 SV = 1 - [RF1_STRPS] B2INGO 44.21 3 95 38.52 30S ribosomal protein S16 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsP PE = 3 SV = 1 - [RS16_STRPS] B2IQZ0 33.07 7 378 38.34 DNA polymerase III subunit beta Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = dnaN PE = 4 SV = 1 - [B2IQZO_STRPS] B2IR11 29.29 8 420 37.79 DNA repair protein radA OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = radA PE = 3 SV = 1 - [B2IR11_STRPS] B2ISKO 7.86 8 1463 37.3 DNA polymerase III PolC-type OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = polC PE = 3 SV = 1 - [B2ISKO_STRPS] B2IMD8 40.15 8 264 37.04 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0513 PE = 4 SV = 1 - [B2IMD8_STRPS] B2IP06 33.66 7 303 36.91 Tagatose-6-phosphate kinase OS Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = fruB PE = 3 SV = 1 - [B2IP06_STRPS] B2IPT6 46.2 5 171 36.86 Galactose-6-phosphate isomerase Intracellular subunit LacB OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = lacB PE = 3 SV = 1 - [LACB_STRPS] B2IMT1 28.33 7 406 36.74 Beta-lactam resistance factor OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = murM PE = 4 SV = 1 - [B2IMT1_STRPS] B2IN52 24.87 8 567 36.72 Dihydroxy-acid dehydratase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ilvD PE = 3 SV = 1 - [ILVD_STRPS] B2IRH4 23.13 5 320 36.59 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_0093 PE = 4 SV = 1 - anchored [B2IRH4_STRPS] (No CS) B2IME1 27.51 7 378 36.3 Transcription Intracellular termination/antitermination protein NusA OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = nusA PE = 3 SV = 1 - [B2IME1_STRPS] B2ISX2 51.67 9 240 36.23 Pseudouridine synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rluB PE = 3 SV = 1 - [B2ISX2_STRPS] B2IN61 77.55 6 49 35.89 50S ribosomal protein L33 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmG PE = 3 SV = 1 - [RL33_STRPS] B2IQ98 60.64 4 94 35.66 50S ribosomal protein L31 type B Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = rpmE PE = 3 SV = 1 - [B2IQ98_STRPS] B2IPY5 32.19 7 379 35.53 Glycogen biosynthesis protein GlgD Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = glgD PE = 4 SV = 1 - [B2IPY5_STRPS] B2IRA3 21.23 4 438 35.35 Pyridine nucleotide-disulfide Intracellular oxidoreductase OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = merA PE = 3 SV = 1 - [B2IRA3_STRPS] B2IR65 48.8 6 209 35.25 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1532 PE = 4 SV = 1 - [B2IR65_STRPS] B2INN1 36.99 7 346 34.94 Elongation factor Ts OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tsf PE = 3 SV = 1 - [EFTS_STRPS] B2IRV5 19.83 3 343 34.85 Phospho-2-dehydro-3- Intracellular

deoxyheptanate aldolase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = aroF PE = 3 SV = 1 - [B2IRV5_STRPS] B2IN60 53.33 4 60 34.75 50S ribosomal protein L32 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmF PE = 3 SV = 1 - [RL32_STRPS] B2IQ13 32.08 6 371 34.73 Aminotransferase, class-V OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1185 PE = 3 SV = 1 - [B2IQ13_STRPS] B2INQ1 36.11 5 180 34.11 Transcriptional regulator, TetR Intracellular family OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2200 PE = 4 SV = 1 - [B2INQ1_STRPS] B2IP08 15.91 7 767 33.57 SpoE family protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = ftsK PE = 3 SV = 1 - [B2IP08_STRPS] B2IRW8 41.24 6 177 33.56 Transcriptional repressor NrdR OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = nrdR PE = 3 SV = 1 - [B2IRW8_STRPS] B2ISL9 57.61 6 184 33.49 GTP cyclohydrolase 1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = foIE PE = 3 SV = 1 - [GCH1_STRPS] B2IQL2 25.35 4 217 32.6 Phosphate-specific transport Intracellular system accessory protein PhoU OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1384 PE = 3 SV = 1 - [B2IQL2_STRPS] B2ILW1 39.58 4 144 32.47 Transcriptional regulator, MarR Intracellular family OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0414 PE = 4 SV = 1 - [B2ILW1_STRPS] B2IS33 15.25 5 518 32.44 Competence-induced protein Ccs4 Multi- OS = -*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0211 PE = 4 SV = 1 - [B2IS33_STRPS] B2INX7 16.53 4 236 32.43 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0802 PE = 4 SV = 1 - [B2INX7_STRPS] B2INM3 14.16 7 657 32.29 DHH subfamily 1 protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_2172 PE = 4 SV = 1 - [B2INM3_STRPS] B2INY8 22.62 6 420 32.18 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0813 PE = 4 SV = 1 - [B2INY8_STRPS] B2IN02 20.77 5 443 32.03 Sensor histidine kinase PnpS OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_2049 PE = 4 SV = 1 - [B2IN02_STRPS] B2IMR8 13.73 5 459 31.87 Transmembrane protein Vexp3 OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0562 PE = 3 SV = 1 - [B2IMR8_STRPS] B2IP11 34.65 7 404 31.83 Probable tRNA sulfurtransferase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = thiI PE = 3 SV = 1 - [THII_STRPS] B2INZ1 26.38 8 470 31.82 FeS assembly protein SufB OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0816 PE = 4 SV = 1 - [B2INZ1_STRPS] B2IQ37 41.27 5 252 31.78 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1209 PE = 4 SV = 1 - [B2IQ37_STRPS] B2IN78 31.83 6 399 31.49 Cell division protein DivIB OS = Intracellular/ *Streptococcus pneumoniae* (strain TMH start CGSP14) GN = divIB PE = 3 SV = 1 - AFTER 60 [B2IN78_STRPS] B2IS71 21.69 5 249 31.41 Phosphoglycerate mutase family Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gpmB PE = 4 SV = 1 - [B2IS71_STRPS] B2IQJ2 23.71 5 388 31.39 Chorismate synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = aroC PE = 3 SV = 1 - [AROC_STRPS] B2IST9 13.89 7 583 31.29 ABC transporter, ATP- Multi- binding/permease protein OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1814 PE = 4 SV = 1 - [B2IST9_STRPS] B2IPQ2 32.05 6 234 30.92 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1072 PE = 4 SV = 1 - [B2IPQ2_STRPS] B2ISM0 18.89 3 270 30.69 7,8-dihydroneopterin aldolase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = suID PE = 3 SV = 1 - [B2ISMO_STRPS] B2IMH1 16.74 6 472 30.67 Cof family protein OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1962 PE = 4 SV = 1 - [B2IMH1_STRPS] B2ISV2 17.09 5 392 30.34 Galactokinase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = galK PE = 3 SV = 1 - [B2ISV2_STRPS] B2IR20 12.6 8 889 30.19 DNA polymerase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = polA PE = 3 SV = 1 - [B2IR20_STRPS] B2IR50 26.4 6 481 30 UDP-N-acetylmuramoyl-L-alanyl-D-

Intracellular glutamate--L-lysine ligase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = murE PE = 3 SV = 1 - [B2IR50_STRPS] B2ISI6 20.93 6 258 29.94 Isoprenyl transferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = uppS PE = 3 SV = 1 - [B2IS16_STRPS] B2IMQ1 31.47 7 375 29.85 Phenylalanine--tRNA ligase alpha Intracellular subunit OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pheS PE = 3 SV = 1 - [B2IMQ1_STRPS] B2ISS1 20.39 6 407 29.77 Tryptophan synthase beta chain Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = trpB PE = 3 SV = 1 - [TRPB_STRPS] B2IMZ0 22.05 5 449 29.65 Glucose-6-phosphate isomerase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pgi PE = 3 SV = 1 - [G6PI_STRPS] B2IRD1 13.75 4 778 29.59 Cation-transporting ATPase, E1-E2 Multi- family OS = -*Streptococcus pneumoniae* transmembrane (strain CGSP14) GN = ctpE PE = 3 SV = 1 - [B2IRD1_STRPS] B2IQ17 51.09 5 229 29.49 Glutamine amidotransferase, class I Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1189 PE = 4 SV = 1 - [B2IQ17_STRPS] B2IPV5 30.5 5 200 29.44 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1127 PE = 4 SV = 1 - [B2IPV5_STRPS] B2ILW5 27.78 6 306 29.34 Malonyl CoA-acyl carrier protein Intracellular transacylase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fabD PE = 3 SV = 1 - [B2ILW5_STRPS] B2IQ16 29.48 6 424 29.26 Psr protein OS = -*Streptococcus pneumoniae* (strain CGSP14) TMH start GN = SPCG_1358 PE = 4 SV = 1 - AFTER 60 [B2IQ16_STRPS] B2IPB3 40.51 5 195 28.99 Translation initiation factor IF-3 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = infC PE = 3 SV = 1 - [B2IPB3_STRPS] B2IM63 18.52 7 432 28.92 Competence-induced protein Ccs50 N- OS = -*Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1944 PE = 4 SV = 1 - anchored [B2IM63_STRPS] (No CS) B2IP99 44.53 6 247 28.66 Uridylate kinase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pyrH PE = 3 SV = 1 - [B2IP99_STRPS] B2IPF5 10.79 5 834 28.65 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_0977 PE = 4 SV = 1 - anchored [B2IPF5_STRPS] (No CS) B2IM26 26.1 6 318 28.61 Autolysin OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = lytA PE = 4 SV = 1 - [B2IM26_STRPS] B2INF6 43.1 5 239 28.52 tRNA (guanine-N(1)-)- Intracellular methyltransferase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = trmD PE = 3 SV = 1 - [TRMD_STRPS] B2ISI0 15.25 6 833 28.41 Leucine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = leuS PE = 3 SV = 1 - [SYL_STRPS] B2IQH8 20.5 6 439 28.08 Homoserine dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = hom PE = 3 SV = 1 - [B2IQH8_STRPS] B2INT8 57.89 3 76 27.91 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0763 PE = 4 SV = 1 - [B2INT8_STRPS] B2IMD9 15.64 2 211 27.64 tRNA (guanine-N(7)-)- Intracellular methyltransferase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = trmB PE = 3 SV = 1 - [TRMB_STRPS] B2IQS2 26.88 4 253 27.63 SpoU rRNA methylase family Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = trmH PE = 3 SV = 1 - [B2IQS2_STRPS] B2IR73 16.69 5 623 27.4 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1540 PE = 4 SV = 1 - [B2IR73_STRPS] B2IPP4 10.12 6 662 27.29 UvrABC system protein B OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = uvrB PE = 3 SV = 1 - [UVRB_STRPS] B2IQK6 26.91 4 301 27.27 UDP-N- Intracellular acetylenolpyruvoylglucosamine reductase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = murB PE = 3 SV = 1 - [MURB_STRPS] B2IP98 25.45 6 444 27.26 Methylenetetrahydrofolate--tRNA- Intracellular (uracil-5-)-methyltransferase TrmFO OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = trmFO PE = 3 SV = 2 - [TRMFO_STRPS] B2IQK5 19.48 5 385 27.06 Spermidine/putrescine import ATP- Intracellular binding protein PotA OS = *Streptococcus pneumoniae* (strain CGSP14) GN = potA PE = 3 SV = 1 - [B2IQK5_STRPS] B2INN3 30.61 7 392 26.86 Secreted 45 kd protein OS = Secretory *Streptococcus pneumoniae* (strain (released) CGSP14) GN = SPCG_2182 PE = 4 SV = 1 - (with

CS) [B2INN3_STRPS] B2IN20 40.78 4 103 26.71 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2067 PE = 4 SV = 1 - [B2IN20_STRPS] B2IPN6 8.14 6 1179 26.7 Chromosome partition protein Smc Intracellular OS = *Streptococcus pneumoniae* (strain CGSP14) GN = smc PE = 3 SV = 1 - [B2IPN6_STRPS] B2IRW9 52.07 5 121 26.67 Transcriptional regulator, GntR Intracellular family OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1686 PE = 4 SV = 1 - [B2IRW9_STRPS] B2IS84 13.47 2 438 26.55 Ribosomal RNA small subunit Intracellular methyltransferase B OS = *Streptococcus pneumoniae* (strain CGSP14) GN = sunL PE = 3 SV = 1 - [B2IS84_STRPS] B2IMX9 19.88 7 332 26.49 Pneumococcal surface protein, Lipid putative OS = *Streptococcus anchored pneumoniae* (strain CGSP14) GN = SPCG_0623 PE = 4 SV = 1 - [B2IMX9_STRPS] B2IP15 8.69 4 541 26.36 RNA methyltransferase, TrmA Intracellular family OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1007 PE = 3 SV = 1 - [B2IPI5_STRPS] B2IPS8 15.14 7 555 25.77 DNA repair protein RecN OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = recN PE = 3 SV = 1 - [B2IPS8_STRPS] B2INY5 17.06 6 551 25.65 DNA polymerase III subunits Intracellular gamma and tau OS = *Streptococcus pneumoniae* (strain CGSP14) GN = dnaX PE = 4 SV = 1 - [B2INY5_STRPS] B2ISU0 10.1 4 594 25.61 ABC transporter, ATP- Multi- binding/permease protein OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1815 PE = 4 SV = 1 - [B2ISU0_STRPS] B2INU6 24.53 6 424 25.32 Phosphopentomutase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = deoB PE = 3 SV = 1 - [B2INU6_STRPS] B2IM77 17.44 2 281 25.26 Undecaprenyl-diphosphatase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = uppP PE = 3 SV = 1 - [UPPP_STRPS] B2IS76 21.11 5 398 25.26 Hydroxymethylglutaryl-CoA Intracellular synthase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = mvaS PE = 4 SV = 1 - [B2IS76_STRPS] B2IPD9 20.3 4 266 24.91 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_0961 PE = 4 SV = 1 - anchored [B2IPD9_STRPS] (No CS) B2IP88 14.52 6 420 24.84 Gamma-glutamyl phosphate Intracellular reductase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = proA PE = 3 SV = 1 - [PROA_STRPS] B2IQU2 18.36 4 305 24.77 Glycine--tRNA ligase alpha subunit Intracellular OS = *Streptococcus pneumoniae* (strain CGSP14) GN = glyQ PE = 3 SV = 1 - [SYGA_STRPS] B2INW0 25 5 220 24.67 Deoxyribose-phosphate aldolase Intracellular OS = *Streptococcus pneumoniae* (strain CGSP14) GN = deoC PE = 3 SV = 1 - [DEOC_STRPS] B2IRB6 28.25 6 223 24.63 Cytidylate kinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = cmk PE = 3 SV = 1 - [KCY_STRPS] B2IP93 24.91 5 289 24.4 Ribosomal RNA small subunit Intracellular methyltransferase I OS = *Streptococcus pneumoniae* (strain CGSP14) GN = rsml PE = 3 SV = 1 - [B2IP93_STRPS] B2IPN4 29.88 5 328 24.28 GMP reductase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = guaC PE = 3 SV = 1 - [GUAC_STRPS] B2IN17 24.9 5 257 24.1 Fucose operon repressor, putative Intracellular OS = *Streptococcus pneumoniae* (strain CGSP14) GN = fcsR PE = 4 SV = 1 - [B2IN17_STRPS] B21MP1 31.07 5 206 24.09 Hydrolase, haloacid dehalogenase- Intracellular like family OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2031 PE = 4 SV = 1 - [B2IMP1_STRPS] B2IRV6 17.78 3 343 23.78 Phospho-2-dehydro-3- Intracellular deoxyheptonate aldolase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = aroG PE = 3 SV = 1 - [B2IRV6_STRPS] B2IQ68 27.02 5 359 23.7 Carbamoyl-phosphate synthase Intracellular small chain OS = *Streptococcus pneumoniae* (strain CGSP14) GN = carA PE = 3 SV = 1 - [B2IQ68_STRPS] B2IMY1 17.2 3 279 23.45 Thymidylate synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = thyA PE = 3 SV = 1 - [B2IMY1_STRPS] B2IM44 17.88 3 274 23.39 DNA-entry nuclease OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = endA PE = 4 SV = 1 - anchored [B2IM44_STRPS] (No CS) B2IR18 22.54 3 213 23.34 Amino acid ABC transporter, ATP- Intracellular binding protein, putative OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0107 PE = 4 SV = 1 - [B2IR18_STRPS] B2IRQ1 5.51 2 653 23.17 PTS system IIABC components OS = Multi- *Streptococcus pneumoniae* (strain transmembrane

CGSP14) GN = scrA PE = 4 SV = 1 - [B2IQR1_STRPS] B2IQR5 19.92 4 246 23.16 CppA protein OS = -*Streptococcus Intracellular pneumoniae* (strain CGSP14) GN = cppA PE = 4 SV = 1 - [B2IQR5_STRPS] B2ILR4 22.22 2 117 23.07 Cell cycle protein GpsB OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gpsB PE = 3 SV = 1 - [B2ILR4_STRPS] B2IQN2 20.25 4 316 22.86 HPr kinase/phosphorylase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = hprK PE = 3 SV = 1 - [B2IQN2_STRPS] B2IMA5 25 6 448 22.78 Glutamine synthetase, type I OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = glnA PE = 3 SV = 1 - [B2IMA5_STRPS] B2IQ75 33.87 4 186 22.38 LemA protein OS = - *Streptococcus N- pneumoniae* (strain CGSP14) terminally GN = lemA PE = 4 SV = 1 - anchored [B2IQ75_STRPS] (No CS) B2INX5 19.41 5 340 21.91 Branched-chain-amino-acid Intracellular aminotransferase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = ilvE PE = 3 SV = 1 - [B2INX5_STRPS] B2IMY7 43.48 4 207 21.8 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0630 PE = 4 SV = 1 - [B2IMY7_STRPS] B2IPY6 14.21 4 380 21.63 Glucose-1-phosphate Intracellular adenyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = glgC PE = 3 SV = 1 - [GLGC_STRPS] B2IM69 20.91 4 416 21.42 L-threonine dehydratase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ilvA PE = 3 SV = 1 - [B2IM69_STRPS] B2INA8 35.36 5 263 21.29 Phosphomethylpyrimidine kinase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = thiD PE = 4 SV = 1 - [B2INA8_STRPS] B2ISK9 13.48 3 267 21.26 PTS system, mannose-specific IIC Multi- component OS = -*Streptococcus transmembrane(Lipid pneumoniae* (strain CGSP14) modified GN = manM PE = 4 SV = 1 - N-termini) [B2ISK9_STRPS] B2IQ32 12.93 3 441 21.17 Glycosyl transferase, group 1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1204 PE = 4 SV = 1 - [B2IQ32_STRPS] B21M58 8.12 3 308 20.91 Membrane protein insertase YidC Multi- OS = - *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = yidC PE = 3 SV = 1 - [B21M58_STRPS] B2INR5 26.54 4 309 20.79 Oxidoreductase, aldo/keto Intracellular reductase family OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0740 PE = 4 SV = 1 - [B2INR5_STRPS] B2INC9 36.22 3 196 20.35 ATP-dependent Clp protease Intracellular proteolytic subunit OS = CGSP14) GN = clpP PE = 3 SV = 1 - [CLPP_STRPS] B2IQ35 25.75 4 369 20.25 RNA polymerase sigma factor SigA Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rpoD PE = 3 SV = 1 - [B2IQ35_STRPS] B21MR7 20 3 215 20.15 ABC transporter, ATP-binding Intracellular protein Vexp2 OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0561 PE = 4 SV = 1 - [B21MR7_STRPS]

TABLE-US-00006 TABLE 6 Proteins identified in MP from serotype 3. The accession numbers refer to SwissProt on the date of filing. Accession # Coverage # Peptides #AAs Score Description Localization

B2IQV2 90.84 45 404 2064.28 Elongation factor Tu OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tuf PE = 3 SV = 1 - [B2IQV2_STRPS] B2IRT9 69.68 49 442 1423.4 Sugar ABC transporter, sugar- Lipid binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1656 PE = 4 SV = 1 - [B2IRT9_STRPS] B2INY1 82.25 60 400 1396.37 30S ribosomal protein S1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsA PE = 4 SV = 1 - [B2INY1_STRPS] B2ISJ9 86.15 57 693 1352.29 Elongation factor G OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = fusA PE = 3 SV = 1 - [EFG_STRPS] B2IPX8 86.64 41 434 1214.93 Enolase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = eno PE = 3 SV = 1 - [ENO_STRPS] B2INB2 75.63 49 591 1080.64 Pyruvate oxidase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = spxB PE = 3 SV = 1 - [B2INB2_STRPS] B2IN25 76.44 47 450 1068.79 Maltose/maltodextrin ABC Lipid transporter, maltose/maltodextrin- anchored binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2072 PE = 4 SV = 1 - [B2IN25_STRPS] B2INK4 76.15 51 608 829.85 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_2153 PE = 4 anchored SV = 1 - [B2INK4_STRPS] (No CS)

B2ISK8 52.48 17 303 715.49 PTS system, mannose-specific IID Multi- component OS = -
Streptococcus transmembrane pneumoniae (strain CGSP14) GN = manN PE = 4 SV = 1 -
 [B2ISK8_STRPS] B2IR98 86.7 33 376 633.37 Sugar ABC transporter, ATP-binding Intracellular
 protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1565 PE = 3 SV = 1 -
 [B2IR98_STRPS] B2IMI7 75.21 29 359 616.73 Glyceraldehyde-3-phosphate Intracellular
 dehydrogenase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gapA PE = 3 SV = 1 -
 [B2IMI7_STRPS] B2ISL0 55.12 20 332 608.4 PTS system, mannose-specific IIAB Intracellular
 components OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = manL PE = 4 SV = 1 -
 [B2ISL0_STRPS] B2IRH0 78.91 39 512 573.11 ABC transporter, substrate-binding Lipid protein
 OS = -*Streptococcus anchored pneumoniae* (strain CGSP14) GN = SPCG_0089 PE = 4 SV = 1 -
 [B2IRH0_STRPS] B2IS43 74.01 23 277 570.24 50S ribosomal protein L2 OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = rpIB PE = 3 SV = 1 - [RL2_STRPS] B2INK5
 70.12 34 502 534.8 Glycerol kinase OS = -*Streptococcus Intracellular pneumoniae* (strain
 CGSP14) GN = glpK PE = 3 SV = 1 - [GLPK_STRPS] B2IRV7 73.6 55 837 530.96 Protein
 translocase subunit SecA Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN =
 secA PE = 3 SV = 1 - [B2IRV7_STRPS] B2INW2 65.51 28 374 519.89 Lipoprotein OS = -
Streptococcus Lipid pneumoniae (strain CGSP14) anchored GN = SPCG_0787 PE = 4 SV = 1 -
 [B2INW2_STRPS] B2IPD4 58.15 28 313 480.11 Foldase protein PrsA OS = Lipid *Streptococcus*
pneumoniae (strain anchored CGSP14) GN = prsA PE = 3 SV = 1 - [PRSA_STRPS] B2IR00 59.97
 38 652 462.58 ATP-dependent zinc Multi- metalloprotease FtsH OS = transmembrane
Streptococcus pneumoniae (strain CGSP14) GN = ftsH PE = 3 SV = 1 - [B2IR00_STRPS] B2IM39
 54.12 57 1225 460.95 DNA-directed RNA polymerase Intracellular subunit beta' OS = -
Streptococcus pneumoniae (strain CGSP14) GN = rpoC PE = 3 SV = 1 - [RPOC_STRPS] B2IM81
 63.18 37 774 432.81 Formate acetyltransferase OS = Intracellular *Streptococcus pneumoniae*
 (strain CGSP14) GN = pfl PE = 4 SV = 1 - [B2IM81_STRPS] B2IM49 72.73 25 330 394.5
 Aspartate--ammonia ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN =
 asnA PE = 3 SV = 1 - [ASNA_STRPS] B2IN96 69.05 25 378 394.18 Lactate oxidase OS = -
Streptococcus Intracellular pneumoniae (strain CGSP14) GN = IctO PE = 4 SV = 1 -
 [B2IN96_STRPS] B2IPT8 93.56 27 326 382.46 Tagatose 1,6-diphosphate aldolase Intracellular OS
 = -*Streptococcus pneumoniae* (strain CGSP14) GN = lacD PE = 3 SV = 1 - [B2IPT8_STRPS]
 B2INN2 73.36 26 259 380.63 30S ribosomal protein S2 OS = Intracellular *Streptococcus*
pneumoniae (strain CGSP14) GN = rpsB PE = 3 SV = 2 - [RS2_STRPS] B2IQX0 86.32 28 468
 375.02 ATP synthase subunit beta OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14)
 GN = atpD PE = 3 SV = 1 - [ATPB_STRPS] B2IMA2 85.18 25 398 374.17 Phosphoglycerate
 kinase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pgk PE = 3 SV = 1 -
 [PGK_STRPS] B2IS46 81.11 26 217 368.3 30S ribosomal protein S3 OS = Intracellular
Streptococcus pneumoniae (strain CGSP14) GN = rpsC PE = 3 SV = 1 - [RS3_STRPS] B2ISZ0
 64.49 31 659 336.33 Oligopeptide ABC transporter, Lipid oligopeptide-binding protein AmiA
 anchored OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = amiA PE = 4 SV = 1 -
 [B2ISZ0_STRPS] B2ISG3 77.23 21 202 332.38 General stress protein 24, putative Intracellular OS
 = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1781 PE = 4 SV = 1 -
 [B2ISG3_STRPS] B21ML8 69.46 25 406 332.33 Acetate kinase OS = -*Streptococcus Intracellular*
pneumoniae (strain CGSP14) GN = ackA PE = 3 SV = 1 - [B21ML8_STRPS] B2IP54 71.66 27 501
 328.6 Pyruvate kinase OS = -*Streptococcus Intracellular pneumoniae* (strain CGSP14) GN =
 SPCG_0873 PE = 3 SV = 1 - [B2IP54_STRPS] B2IN10 57.69 12 338 324.09 Glycerol-3-
 phosphate Intracellular dehydrogenase [NAD(P)+] OS = *Streptococcus pneumoniae* (strain
 CGSP14) GN = gpsA PE = 3 SV = 1 - [GPDA_STRPS] B2IRR1 66.09 12 230 318.07 2,3-
 bisphosphoglycerate- Intracellular dependent phosphoglycerate mutase OS = -*Streptococcus*
pneumoniae (strain CGSP14) GN = gpmA PE = 3 SV = 1 - [GPMA_STRPS] B2IRI6 36.84 5 95
 310.42 Bacteriocin, putative OS = N- terminally *Streptococcus pneumoniae* (strain anchored

CGSP14) GN = SPCG_0105 PE = 4 (with CS) SV = 1 - [B2IRI6_STRPS] B2IQY1 57.35 26 551 302.6 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain TMH CGSP14) GN = SPCG_1503 PE = 4 SV = start 1 - [B2IQY1_STRPS] AFTER 60 B2ISJ7 67.88 14 137 301.47 30S ribosomal protein S12 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsL PE = 3 SV = 1 - [RS12_STRPS] B2IQX2 38.92 21 501 285.3 ATP synthase subunit alpha OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = atpA PE = 3 SV = 1 - [ATPA_STRPS] B2IP53 50.15 16 335 269.91 ATP-dependent 6- Intracellular phosphofructokinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = pfkA PE = 3 SV = 1 - [PFKA_STRPS] B2IR47 51.69 27 652 263.77 Oligopeptide ABC transporter, Lipid oligopeptide-binding protein AliB anchored OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = aliB PE = 4 SV = 1 - [B2IR47_STRPS] B2ISZ6 50.36 17 419 260.6 Sugar ABC transporter, sugar- Lipid binding protein OS = -*Streptococcus anchored pneumoniae* (strain CGSP14) GN = SPCG_1871 PE = 4 SV = 1 - [B2ISZ6_STRPS] B2IMB5 52.72 24 607 254.75 Chaperone protein Dnak OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = dnaK PE = 3 SV = 1 - [DNAK_STRPS] B2INT1 42.12 26 584 253.89 Septation ring formation regulator N- EzrA OS = - *Streptococcus pneumoniae* terminally (strain CGSP14) GN = ezrA PE = 3 SV = anchored 1 - [B2INT1_STRPS] (No CS) B2IM12 66.03 26 471 237.78 Pneumolysin OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = ply PE = 4 SV = 1 - [B2IM12_STRPS] B2IS47 55.47 11 137 228.66 50S ribosomal protein L16 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIP PE = 3 SV = 1 - [RL16_STRPS] B2IRM9 68.48 18 276 218.21 ABC transporter, substrate-binding Lipid protein OS = -*Streptococcus anchored pneumoniae* (strain CGSP14) GN = SPCG_0152 PE = 4 SV = 1 - [B2IRM9_STRPS] B2IRG4 64.53 23 203 217.88 30S ribosomal protein S4 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsD PE = 3 SV = 1 - [RS4_STRPS] B2IS57 86.59 19 164 213.33 30S ribosomal protein S5 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsE PE = 3 SV = 1 - [B2IS57_STRPS] B2IM40 44.9 29.1 4 824 208.41 DNA-directed RNA polymerase Intracellular subunit beta OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rpoB PE = 3 SV = 1 - [B2IM40_STRPS] B2ISM3 87.16 20 148 207.33 50S ribosomal protein L13 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIM PE = 3 SV = 1 - [B2ISM3_STRPS] B2IQ88 76.52 13 115 207.04 50S ribosomal protein L19 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIS PE = 3 SV = 1 - [RL19_STRPS] B2ISD1 80.77 12 104 201.22 Thioredoxin OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = trxA PE = 3 SV = 1 - [B2ISD1_STRPS] B2IS40 62.98 15 208 197.25 50S ribosomal protein L3 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIC PE = 3 SV = 1 - [RL3_STRPS] B2IMJ9 41.57 28 890 195.17 Aldehyde-alcohol dehydrogenase Intracellular OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = adhE PE = 3 SV = 1 - [B2IMJ9_STRPS] B2IPW4 45.86 24 567 191.95 Dihydrolipoyl dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = acoL PE = 4 SV = 1 - [B2IPW4_STRPS] B2IP07 28.31 14 650 190.79 PTS system, fructose specific IIABC Multi- components OS = -*Streptococcus* transmembrane *pneumoniae* (strain CGSP14) GN = fruA PE = 4 SV = 1 - [B2IP07_STRPS] B2IS55 84.27 13 178 180.96 50S ribosomal protein L6 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIF PE = 3 SV = 1 - [RL6_STRPS] B2IME4 33.44 20 930 180.14 Translation initiation factor IF-2 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = infB PE = 3 SV = 1 - [IF2_STRPS] B2ISW0 53.72 12 242 178.66 Choline transporter OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1835 PE = 4 SV = 1 - [B2ISW0_STRPS] B2IS66 43.31 8 127 176.98 30S ribosomal protein S11 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsK PE = 3 SV = 1 - [RS11_STRPS] B2IPR1 43.98 13 332 173.61 L-lactate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = Idh PE = 3 SV = 1 - [B2IPR1_STRPS] B2IRS1 44.15 15 419 169.63 Cell division protein FtsZ OS = Intracellular *Streptococcus pneumoniae*

(strain CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS1_STRPS] B2IPP2 36.89 22 721 166.14 Amino acid ABC transporter, amino Multi- acid-binding protein/permease transmembrane protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1062 PE = 3 SV = 1 - [B2IPP2_STRPS] B2ILZ5 43.89 21 540 165.77 60 kDa chaperonin OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = groL PE = 3 SV = 1 - [CH60_STRPS] B2IS41 65.22 16 207 162.59 50S ribosomal protein L4 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpID PE = 3 SV = 1 - [RL4_STRPS] B2ILW6 75.31 15 243 162.56 3-ketoacyl-(Acyl-carrier-protein) Intracellular reductase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fabG PE = 4 SV = 1 - [B2ILW6_STRPS] B2ISY9 34.54 14 498 161.94 Oligopeptide ABC transporter, Multi- permease protein Amic OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = amiC PE = 3 SV = 1 - [B2ISY9_STRPS] B2ISY6 58.31 18 331 161.56 Oligopeptide ABC transporter, ATP- Intracellular binding protein AmiF OS = *Streptococcus pneumoniae* (strain CGSP14) GN = amiF PE = 3 SV = 1 - [B2ISY6_STRPS] B2IS52 77.22 13 180 159.07 50S ribosomal protein L5 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplE PE = 3 SV = 1 - [RL5_STRPS] B2ILW7 50.24 10 414 153.03 3-oxoacyl-[acyl-carrier-protein] Intracellular synthase 2 OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fabF PE = 3 SV = 1 - [B2ILW7_STRPS] B2ISY7 46.76 16 355 151.21 Oligopeptide ABC transporter, ATP- Intracellular binding protein AmiE OS = *Streptococcus pneumoniae* (strain CGSP14) GN = amiE PE = 3 SV = 1 - [B2ISY7_STRPS] B2ILU5 34.89 13 427 150.71 Trigger factor OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = tig PE = 3 SV = 1 - [TIG_STRPS] B2IMU8 55.46 15 229 148.33 50S ribosomal protein L1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIA PE = 3 SV = 1 - [RL1_STRPS] B2IS44 56.99 10 93 148.01 30S ribosomal protein S19 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsS PE = 3 SV = 1 - [RS19_STRPS] B2IQT6 47.49 18 459 146.83 NADH oxidase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = nox PE = 4 SV = 1 - [B2IQT6_STRPS] B2INP5 40.65 15 492 145.57 Inosine-5'-monophosphate Intracellular dehydrogenase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = imdH PE = 3 SV = 1 - [B2INP5_STRPS] B2INF2 51.27 20 513 140.36 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0719 PE = 4 SV = 1 - [B2INF2_STRPS] B2IRS2 50.33 12 457 140.22 Cell division protein FtsA OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ftsZ PE = 3 SV = 1 - [B2IRS2_STRPS] B2IPY5 51.98 15 379 138.63 Glycogen biosynthesis protein GlgD Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glgD PE = 4 SV = 1 - [B2IPY5_STRPS] B2IQH2 47.59 12 187 137.62 50S ribosomal protein L10 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIJ PE = 3 SV = 1 - [B2IQH2_STRPS] B2IPM7 29.64 14 577 137.31 Phosphoenolpyruvate-protein Intracellular phosphotransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1121 PE = 3 SV = 1 - [B2IPM7_STRPS] B2ISJ8 57.69 11 156 133.5 30S ribosomal protein S7 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsG PE = 3 SV = 1 - [RS7_STRPS] B2IS54 74.24 10 132 133.28 30S ribosomal protein S8 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsH PE = 3 SV = 1 - [RS8_STRPS] B2INZ1 46.38 17 470 132.61 FeS assembly protein SufB OS = Intracellular *Streptococcus pneumoniae* (strain lar CGSP14) GN = SPCG_0816 PE = 4 SV = 1 - [B2INZ1_STRPS] B2IS53 62.92 10 89 131.91 30S ribosomal protein S14 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsN PE = 3 SV = 1 - [RS14_STRPS] B2IS59 63.7 9 146 131.45 50S ribosomal protein L15 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIO PE = 3 SV = 1 - [RL15_STRPS] B2INH5 50.5 17 299 127.85 SPFH domain/Band 7 family OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_2124 PE = 4 SV = anchored 1 - [B2INH5_STRPS] (No CS) B2IRE3 73.42 12 158 127.54 PTS system, IIB component OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0062 PE = 4 SV = 1 - [B2IRE3_STRPS] B2IN11 64.88 14 299 125.53 UTP--glucose-1-

phosphate Secretory uridylyltransferase OS = (released) *Streptococcus pneumoniae* (strain (with CS) CGSP14) GN = galU PE = 3 SV = 1 - [B2IN11_STRPS] B2IS19 43.76 23 617 125.33 Proline-tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = proS PE = 3 SV = 1 - [SYP_STRPS] B2IPB5 60.5 12 119 125.17 50S ribosomal protein L20 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIT PE = 3 SV = 1 - [RL20_STRPS] B2IMN9 40.47 11 425 125.01 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2034 PE = 4 SV = 1 - [B2IMN9_STRPS] B2IS90 47.86 20 537 123.3 Ribonuclease Y OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = rny PE = 3 SV = 1 - [B2IS90_STRPS] B2IPB4 36.36 6 66 122.81 50S ribosomal protein L35 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpml PE = 3 SV = 1 - [RL35_STRPS] B2IQW2 62.61 5 115 121.34 Bacterocin transport accessory Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = bta PE = 4 SV = 1 - [B2IQW2_STRPS] B2INS1 30.9 21 848 120 Aminopeptidase N OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pepN PE = 4 SV = 1 - [B2INS1_STRPS] B2IPP1 53.66 9 246 116.21 Amino acid ABC transporter, ATP- Intracellular binding protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1061 PE = 4 SV = 1 - [B2IPP1_STRPS] B2IMN0 34.94 8 352 116.08 Alcohol dehydrogenase, zinc- Intracellular containing OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = adh PE = 3 SV = 1 - [B2IMN0_STRPS] B2IRN0 53.52 14 284 114.19 Lipoprotein OS = -*Streptococcus* Lipid *pneumoniae* (strain CGSP14) anchored GN = SPCG_0153 PE = 3 SV = 1 - [B2IRN0_STRPS] B2IQZ2 48.66 14 374 110.45 Ribosome-binding ATPase YchF OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ychF PE = 3 SV = 1 - [B2IQZ2_STRPS] B2IQ10 41.57 13 344 105.33 Uncharacterized protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1352 PE = 4 SV = 1 - [B2IQ10_STRPS] B2IS49 60.47 7 86 105.08 30S ribosomal protein S17 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsQ PE = 3 SV = 1 - [RS17_STRPS] B2IR07 35.97 15 442 104.04 Adenylosuccinate synthetase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = purA PE = 3 SV = 1 - [B2IR07_STRPS] B2IMU7 42.55 6 141 103.8 50S ribosomal protein L11 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIK PE = 3 SV = 1 - [RL11_STRPS] B2ISV9 21.53 9 511 103.52 Choline transporter OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1834 PE = 3 SV = 1 - [B2ISV9_STRPS] B2IQV9 40.56 16 572 103.11 Phosphoglucosmutase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = pgm PE = 3 SV = 1 - [B2IQV9_STRPS] B2IR17 52.51 12 339 101.9 Ribose-phosphate Intracellular pyrophosphokinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = prsA PE = 3 SV = 1 - [B2IR17_STRPS] B2IRQ3 37.38 18 650 101.35 Endopeptidase O OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = pepO PE = 4 SV = 1 - [B2IRQ3_STRPS] B2IS63 54.72 6 212 100 Adenylate kinase OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = adk PE = 3 SV = 1 - [KAD_STRPS] B2IMK3 38.15 13 658 99.61 Transketolase OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = tktA PE = 3 SV = 1 - [B2IMK3_STRPS] B2IMS2 55.97 12 293 98.56 Fructose-bisphosphate aldolase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = fba PE = 3 SV = 1 - [B2IMS2_STRPS] B2IMH3 55.78 12 346 96.72 Catabolite control protein A OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = ccpA PE = 4 SV = 1 - [B2IMH3_STRPS] B2IPY8 48.31 15 474 96.35 Glyceraldehyde-3-phosphate Intracellular dehydrogenase, NADP-dependent OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gapN PE = 3 SV = 1 - [B2IPY8_STRPS] B2ILY5 30.81 10 555 96.12 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0438 PE = 4 SV = 1 - [B2ILY5_STRPS] B2IS42 95.92 11 98 95.91 50S ribosomal protein L23 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIW PE = 3 SV = 1 - [RL23_STRPS] B2IM28 43.56 13 388 95.69 Protein RecA OS = -*Streptococcus* Intracellular *pneumoniae* (strain CGSP14) GN = recA PE = 3 SV = 1 - [RECA_STRPS] B2IRR6 27.44 10 266 95.52 Cell division protein

DivIVA OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1633 PE = 4 SV = 1 - [B2IRR6_STRPS] B2IS68 64.06 8 128 93.87 50S ribosomal protein L17 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplQ PE = 3 SV = 1 - [RL17_STRPS] B2IPR2 22.47 13 841 93.61 DNA gyrase subunit A OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gyrA PE = 3 SV = 1 - [B2IPR2_STRPS] B2IM73 29.94 12 521 93.46 Amino acid ABC transporter, amino Multi- acid-binding protein/permease transmembrane protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glnH PE = 3 SV = 1 - [B2IM73_STRPS] B2INV5 59.04 7 83 93.23 30S ribosomal protein S20 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsT PE = 3 SV = 1 - [B2INV5_STRPS] B2IRH9 39.61 21 616 93.09 Capsular polysaccharide Multi- biosynthesis protein, putative OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0098 PE = 4 SV = 1 - [B2IRH9_STRPS] B2INY7 55.13 9 263 92.69 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0812 PE = 4 SV = 1 - [B2INY7_STRPS] B2INW3 45.21 14 511 92.64 Sugar ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0788 PE = 4 SV = 1 - [B2INW3_STRPS] B2IQU4 57.5 9 280 89.47 Oxidoreductase, aldo/keto Intracellular reductase family OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1466 PE = 4 SV = 1 - [B2IQU4_STRPS] B2ILR7 36.8 13 481 87.34 6-phosphogluconate Intracellular dehydrogenase, decarboxylating OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gnd PE = 3 SV = 1 - [B2ILR7_STRPS] B2IRW4 23.85 14 436 87.08 GTPase Der OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = der PE = 3 SV = 1 - [DER_STRPS] B2IQW3 46.76 10 278 86.59 Amino acid ABC transporter, amino Lipid acid-binding protein OS = anchored *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1485 PE = 4 SV = 1 - [B2IQW3_STRPS] B2IPI7 33.14 8 341 85.78 Iron-compound ABC transporter, Lipid iron compound-binding protein OS = anchored *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1009 PE = 4 SV = 1 - [B2IPI7_STRPS] B2IS65 58.68 12 121 85.63 30S ribosomal protein S13 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsM PE = 3 SV = 1 - [RS13_STRPS] B2ISM4 59.23 8 130 85.44 30S ribosomal protein S9 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsI PE = 3 SV = 1 - [RS9_STRPS] B2INT7 37.9 17 752 85.35 ATP-dependent Clp protease, ATP- Intracellular binding subunit ClpE OS = *Streptococcus pneumoniae* (strain CGSP14) GN = clpE PE = 3 SV = 1 - [B2INT7_STRPS] B2IMU6 41.18 8 238 85.34 Uncharacterized protein OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_0590 PE = 4 SV = 1 - [B2IMU6_STRPS] B2IQR1 39.42 15 520 84.59 GMP synthase [glutamine- Intracellular hydrolyzing] OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = guaA PE = 3 SV = 1 - [GUAA_STRPS] B2IPT6 68.42 8 171 84.01 Galactose-6-phosphate isomerase Intracellular subunit LacB OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = lacB PE = 3 SV = 1 - [LACB_STRPS] B2INL3 30.25 17 810 83.74 ATP-dependent Clp protease, ATP- Intracellular binding subunit OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2162 PE = 3 SV = 1 - [B2INL3_STRPS] B2IS51 78.22 10 101 83.43 50S ribosomal protein L24 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplX PE = 3 SV = 1 - [RL24_STRPS] B2INN1 42.77 10 346 83.3 Elongation factor Ts OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tsf PE = 3 SV = 1 - [EFTS_STRPS] B2IS56 67.18 10 131 82.77 50S ribosomal protein L18 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplR PE = 3 SV = 1 - [B2IS56_STRPS] B2ISX8 27.68 8 336 82.74 Non-canonical purine NTP Intracellular pyrophosphatase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1853 PE = 3 SV = 1 - [B2ISX8_STRPS] B2INT0 24.07 12 648 81.85 DNA gyrase subunit B OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gyrB PE = 3 SV = 1 - [B2INT0_STRPS] B2IN71 31.13 15 620 81.8 Elongation factor Tu family protein Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0638 PE = 4 SV = 1 - [B2IN71_STRPS] B2IPU8 19.19 9 719 79.81 Ribonucleoside-diphosphate Intracellular

reductase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = nrdE PE = 3 SV = 1 - [B2IPU8_STRPS] B2INM4 49.45 9 182 79.8 Ribosomal subunit interface protein Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2173 PE = 4 SV = 1 - [B2INM4_STRPS] B2ISV2 28.57 7 392 79.49 Galactokinase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = galK PE = 3 SV = 1 - [B2ISV2_STRPS] B2IMZO 45.21 9 449 77.7 Glucose-6-phosphate isomerase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pgi PE = 3 SV = 1 - [G6PI_STRPS] B2IRD4 61.8 6 89 77.46 30S ribosomal protein S15 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsO PE = 3 SV = 1 - [RS15_STRPS] B2IR65 69.38 11 209 76.49 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1532 PE = 4 SV = 1 - [B2IR65_STRPS] B2IR90 43.02 4 172 76.3 Non-heme iron-containing ferritin Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1557 PE = 3 SV = 1 - [B2IR90_STRPS] B2IPY6 28.16 9 380 75.91 Glucose-1-phosphate Intracellular adenylyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = glgC PE = 3 SV = 1 - [GLGC_STRPS] B2IN68 38.1 7 126 75.25 Uncharacterized protein OS = N-terminally *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_0635 PE = 4 SV = (No CS) 1 - [B2IN68_STRPS] B2IR60 97.92 8 96 74.99 30S ribosomal protein S6 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsF PE = 3 SV = 1 - [RS6_STRPS] B2IQ02 48.45 7 97 74.43 50S ribosomal protein L27 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmA PE = 3 SV = 1 - [RL27_STRPS] B2IM41 40.05 10 377 74.15 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1926 PE = 3 SV = 1 - [B2IM41_STRPS] B2IR71 16.41 12 914 73.99 Cation-transporting ATPase, E1-E2 Multi- family OS = -*Streptococcus pneumoniae* transmembrane (strain CGSP14) GN = pacL PE = 3 SV = 1 - [B2IR71_STRPS] B2IQV3 35.99 6 289 72.94 Glycerol uptake facilitator protein, Multi- putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glpF PE = 3 SV = 1 - [B2IQV3_STRPS] B2ILW5 35.95 9 306 72.85 Malonyl CoA-acyl carrier protein Intracellular transacylase OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = fabD PE = 3 SV = 1 - [B2ILW5_STRPS] B2IRQ6 42.81 10 313 71.49 Manganese ABC transporter, Lipid manganese-binding adhesion anchored liprotein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1623 PE = 3 SV = 1 - [B2IRQ6_STRPS] B2IQX4 60.98 9 164 71.29 ATP synthase subunit b OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = atpF PE = 3 SV = 1 - anchored [ATPF_STRPS] (No CS) B2IPT0 26.59 10 628 71.03 Elongation factor 4 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = lepA PE = 3 SV = 1 - [B2IPT0_STRPS] B2ISJ1 27.74 11 602 69.46 Glutamine--fructose-6-phosphate Intracellular aminotransferase [isomerizing] OS = *Streptococcus pneumoniae* (strain CGSP14) GN = glmS PE = 3 SV = 1 - [B2ISJ1_STRPS] B2IQU1 23.75 13 678 68.47 Glycine--tRNA ligase beta subunit Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glyS PE = 3 SV = 1 - [SYGB_STRPS] B2INH0 41.42 11 338 68.3 Ornithine carbamoyltransferase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = arcB PE = 3 SV = 1 - [OTC_STRPS] B2IQL4 61.42 12 267 68.04 Phosphate import ATP-binding Intracellular protein PstB OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL4_STRPS] B2ING9 37.9 10 409 65.94 Arginine deiminase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = arcA PE = 3 SV = 1 - [ARCA_STRPS] B2IR87 27.8 9 410 65.69 ATP-dependent Clp protease ATP- Intracellular binding subunit ClpX OS = *Streptococcus pneumoniae* (strain CGSP14) GN = clpX PE = 3 SV = 1 - [CLPX_STRPS] B2IPY4 19.29 7 477 64.75 Glycogen synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = glgA PE = 3 SV = 1 - [GLGA_STRPS] B2ISY0 36 4 100 63.86 UPF0154 protein SPCG_1855 OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1855 PE = 3 SV = anchored 1 - [B2ISY0_STRPS] (No CS) B2IQL1 28.04 6 271 63.33 Amino acid ABC transporter, amino Lipid acid-binding protein OS = anchored *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1383 PE = 4 SV = 1 - [B2IQL1_STRPS]

B2IN60 55 5 60 32.27 50S ribosomal protein L32 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmF PE = 3 SV = 1 - [RL32_STRPS] B2IQK0 22.48 15 872 63.2 Alanine -- tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = alaS PE = 3 SV = 1 - [SYA_STRPS] B2INE4 30.43 8 447 62.91 DEAD-box ATP-dependent RNA Intracellular helicase CshB OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = rheB PE = 3 SV = 1 - [B2INE4_STRPS] B2IS50 59.84 8 122 62.57 50S ribosomal protein L14 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIN PE = 3 SV = 1 - [RL14_STRPS] B2IND2 34.2 8 386 62.51 Branched-chain amino acid ABC Lipid transporter, amino acid-binding anchored protein OS = -*Streptococcus pneumoniae* strain CGSP14) GN = SPCG_0699 PE = 4 SV = 1 - [B2IND2_STRPS] B2IRC1 40.12 8 339 61.9 UDP-glucose 4-epimerase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = galE PE = 4 SV = 1 - [B2IRC1_STRPS] B2IR96 77.27 7 176 61.33 Adenine phosphoribosyltransferase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = apt PE = 3 SV = 1 - [B2IR96_STRPS] B2IM46 42.61 9 345 61.11 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1931 PE = 4 SV = anchored 1 - [B2IM46_STRPS] (No CS) B21MG8 28.22 8 404 61 Aspartate aminotransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = aspC PE = 4 SV = 1 - [B21MG8_STRPS] B2IQN0 59.06 5 127 60.7 Uncharacterized protein OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1399 PE = 4 SV = anchored 1 - [B2IQN0_STRPS] (No CS) B2IMQ9 22.52 11 737 60.06 Polyribonucleotide Intracellular nucleotidyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = pnp PE = 3 SV = 2 - [PNP_STRPS] B2IQ40 24.39 14 898 59.69 Phosphoenolpyruvate carboxylase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = ppc PE = 3 SV = 1 - [B2IQ40_STRPS] B2IND9 60.43 10 230 59.42 Cell division ABC transporter, ATP- Intracellular binding protein FtsE OS = *Streptococcus pneumoniae* (strain CGSP14) GN = ftsE PE = 4 SV = 1 - [B2IND9_STRPS] B2IQU5 27.92 10 480 59.4 Peptidoglycan N-acetylglucosamine N- deacetylase A OS = -*Streptococcus terminally pneumoniae* (strain CGSP14) anchored GN = pgdA PE = 4 SV = 1 - (No CS) [B2IQU5_STRPS] B2IQ23 39.06 8 425 59.39 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1195 PE = 4 SV = 1 - [B2IQ23_STRPS] B2INH1 22.54 6 315 59 Carbamate kinase OS = -*Streptococcus* Secretory *pneumoniae* (strain CGSP14) (released) GN = arcC PE = 3 SV = 1 - (with CS) [B2INH1_STRPS] B2IRI7 20.76 11 713 58.73 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0106 PE = 4 SV = 1 - [B2IRI7_STRPS] B2IMP1 37.86 6 206 56.51 Hydrolase, haloacid dehalogenase- Intracellular like family OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2031 PE = 4 SV = 1 - [B2IMP1_STRPS] B2IQH6 45.83 13 312 56.05 Bifunctional methionine sulfoxide N- reductase A/B protein OS = terminally *Streptococcus pneumoniae* (strain anchored CGSP14) GN = msrA PE = 3 SV = 1 - (No CS) [B2IQH6_STRPS] B2IMB6 28.84 8 378 56.02 Chaperone protein DnaJ OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = dnaJ PE = 3 SV = 1 - [B2IMB6_STRPS] B2IS18 32.46 7 419 55.7 Zinc metalloprotease OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = eep PE = 3 SV = 1 - [B2IS18_STRPS] B2IR93 26.64 4 259 55.23 Triosephosphate isomerase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tpiA PE = 3 SV = 1 - [B2IR93_STRPS] B2ILX0 37.58 11 455 54.82 Acetyl-CoA carboxylase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = accC PE = 4 SV = 1 - [B2ILX0_STRPS] B2IS39 65.69 6 102 54.76 30S ribosomal protein S10 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsJ PE = 3 SV = 1 - [RS10_STRPS] B2IQZ9 53.89 9 180 54.16 Hypoxanthine-guanine Intracellular phosphoribosyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = hgt PE = 4 SV = 1 - [B2IQZ9_STRPS] B2IMZ6 14.35 10 857 54.16 DNA mismatch repair protein MutS Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = hexA PE = 3 SV = 1 - [B2IMZ6_STRPS] B2IR84 36.49 7 296 53.72 Nucleotide-binding protein Intracellular SPCG_1551 OS = -*Streptococcus pneumoniae*

(strain CGSP14) GN = SPCG_1551 PE = 3 SV = 1 - [Y1551_STRPS] B2ISX0 31.78 8 321 53.7 Iron-compound ABC transporter, Lipid iron-compound-binding protein anchored OS = -
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_1845 PE = 4 SV = 1 - [B2ISX0_STRPS] B2ILX6 56.45 6 186 52.99 Elongation factor P OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = efp PE = 3 SV = 1 - [EFP_STRPS] B2IMZ8 23.45 9 563 52.53 Arginine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = argS PE = 3 SV = 1 - [SYR_STRPS] B2INY8 20.48 9 420 52.28 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0813 PE = 4 SV = 1 - [B2INY8_STRPS] B2IRA3 23.52 7 438 52.2 Pyridine nucleotide-disulfide Intracellular oxidoreductase OS = -
Streptococcus pneumoniae (strain CGSP14) GN = merA PE = 3 SV = 1 - [B2IRA3_STRPS] B2IPW2 16.67 6 330 51.71 Acetoin dehydrogenase, E1 Intracellular component, beta subunit, putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = acoB PE = 4 SV = 1 - [B2IPW2_STRPS] B2IRU1 40.52 9 232 51.05 Putative N-acetylmannosamine-6- Intracellular phosphate 2-epimerase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = nanE PE = 3 SV = 1 - [B2IRU1_STRPS] B2ISY2 5.67 4 705 50.76 Trehalose PTS system, IIABC Multi- components OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = treP PE = 4 SV = 1 - [B2ISY2_STRPS] B2IRX3 29.29 10 297 50.56 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1690 PE = 4 SV = 1 - [B2IRX3_STRPS] B2IQY9 24.72 10 453 50.5 Chromosomal replication initiator Intracellular protein DnaA OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = dnaA PE = 3 SV = 1 - [DNAA_STRPS] B2IMI1 36.61 4 183 50.13 Transcription Intracellular termination/antitermination protein NusG OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = nusG PE = 3 SV = 1 - [B2IMI1_STRPS] B2ING0 66.32 5 95 49.96 30S ribosomal protein S16 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsP PE = 3 SV = 1 - [RS16_STRPS] B2INE1 11.85 6 726 48.69 PTS system, IIABC components OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = ptsG PE = 4 SV = 1 - [B2INE1_STRPS] B2IMN6 33 9 494 48.52 Threonine synthase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = thrC PE = 4 SV = 1 - [B2IMN6_STRPS] B2ILR1 12.8 8 719 47.81 Penicillin-binding protein 1A OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = pbp1A PE = 4 SV = 1 - anchored [B2ILR1_STRPS] (No CS) B2IR57 22.53 8 466 47.14 Cof family protein/peptidyl-prolyl Intracellular cis-trans isomerase, cyclophilin type OS = -
Streptococcus pneumoniae (strain CGSP14) GN = SPCG_1524 PE = 4 SV = 1 - [B2IR57_STRPS] B2IRQ1 10.57 5 653 46.37 PTS system IIABC components OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = scrA PE = 4 SV = 1 - [B2IRQ1_STRPS] B2IRE5 23.91 5 276 46.36 PTS system, IID component OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_0064 PE = 4 SV = 1 - [B2IRE5_STRPS] B2IRX0 47.08 8 240 46.21 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1687 PE = 4 SV = 1 - [B2IRX0_STRPS] B2IR19 40.73 7 302 45.81 Amino acid ABC transporter, Lipid periplasmic amino acid-binding anchored protein, putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0108 PE = 4 SV = 1 - [B2IR19_STRPS] B2IQL3 29.76 6 252 45.8 Phosphate import ATP-binding Intracellular protein PstB OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pstB PE = 3 SV = 1 - [B2IQL3_STRPS] B2IQ98 60.64 5 94 45.67 50S ribosomal protein L31 type B Intracellular OS = -
Streptococcus pneumoniae (strain CGSP14) GN = rpmE PE = 3 SV = 1 - [B2IQ98_STRPS] B2IRE7 15.8 6 386 44.99 Sugar isomerase domain protein Intracellular AgaS OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = agaS PE = 4 SV = 1 - [B2IRE7_STRPS] B2IMZ5 21.99 9 564 44.92 ABC transporter, ATP- Multi- binding/permease protein OS = transmembrane *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2042 PE = 4 SV = 1 - [B2IMZ5_STRPS] B2IRQ7 56.4 7 172 44.31 Probable thiol peroxidase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = tpx PE = 3 SV = 1 - [B2IRQ7_STRPS] B2ILX8 33.61 8 488 43.22 Glutamyl-

tRNA(Gln) Intracellular amidotransferase subunit A OS = *Streptococcus pneumoniae* (strain CGSP14) GN = gatA PE = 3 SV = 1 - [GATA_STRPS] B2IM45 29.04 8 427 43.07 UDP-N-acetylglucosamine 1- Intracellular carboxyvinyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = murA PE = 3 SV = 1 - [B2IM45_STRPS] B2IM60 25 6 416 43.06 Diaminopimelate decarboxylase Intracellular OS = *Streptococcus pneumoniae* (strain CGSP14) GN = lysA PE = 3 SV = 1 - [B2IM60_STRPS] B2IRA1 21.95 9 524 42.94 DEAD-box ATP-dependent RNA Intracellular helicase CshA OS = *Streptococcus pneumoniae* (strain CGSP14) GN = cshA PE = 3 SV = 1 - [B2IRA1_STRPS] B2IR78 17.33 6 450 42.72 Phosphoglucosamine mutase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = glmM PE = 3 SV = 1 - [GLMM_STRPS] B2IST7 22.06 7 417 42.6 Capsular polysaccharide Intracellular biosynthesis protein, putative OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1812 PE = 3 SV = 1 - [B2IST7_STRPS] B2INT8 57.89 2 76 41.48 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0763 PE = 4 SV = 1 - [B2INT8_STRPS] B2IQ61 25.95 6 289 41.38 Choline kinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = pck PE = 4 SV = 1 - [B2IQ61_STRPS] B2ILR6 14.66 5 464 41.32 Mid-cell-anchored protein Z OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = mapZ PE = 3 SV = 1 - [B2ILR6_STRPS] B2ILR4 29.91 3 117 40.9 Cell cycle protein GpsB OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gpsB PE = 3 SV = 1 - [B2ILR4_STRPS] B2IQ25 28.88 8 419 40.5 UDP-N-acetylglucosamine 1- Intracellular carboxyvinyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = murZ PE = 3 SV = 1 - [B2IQ25_STRPS] B2IQH8 27.11 8 439 40.48 Homoserine dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = hom PE = 3 SV = 1 - [B2IQH8_STRPS] B2IN58 28.96 6 335 40.08 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_2101 PE = 4 SV = 1 - [B2IN58_STRPS] B2IRK5 20.21 7 559 40.01 Ribonuclease J OS = *Streptococcus pneumoniae* (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IRK5_STRPS] B2INR2 26.51 10 679 39.94 Methionine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = metG PE = 3 SV = 1 - [B2INR2_STRPS] B2IQ29 30.5 8 436 39.93 GTPase Obg OS = *Streptococcus pneumoniae* (strain CGSP14) GN = obg PE = 3 SV = 1 - [OBG_STRPS] B2IMY2 73.21 7 56 39.82 Uncharacterized protein OS = Secretory *Streptococcus pneumoniae* (strain (released) CGSP14) GN = SPCG_0626 PE = 4 SV = (with CS) 1 - [B2IMY2_STRPS] B2IPW3 33.6 8 375 39.59 Dihydrolipoamide acetyltransferase Intracellular OS = *Streptococcus pneumoniae* (strain CGSP14) GN = acoC PE = 3 SV = 1 - [B2IPW3_STRPS] B2IR18 38.5 6 213 39.57 Amino acid ABC transporter, ATP- Intracellular binding protein, putative OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0107 PE = 4 SV = 1 - [B2IR18_STRPS] B2ISR0 13.08 6 535 39.53 Glucan 1,6-alpha-glucosidase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = dexB PE = 4 SV = 1 - [B2ISR0_STRPS] B2IM72 56.91 6 246 39.3 Amino acid ABC transporter, ATP- Intracellular binding protein OS = *Streptococcus pneumoniae* (strain CGSP14) GN = glnQ PE = 4 SV = 1 - [B2IM72_STRPS] B2IS45 48.25 3 114 39.27 50S ribosomal protein L22 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpIV PE = 3 SV = 1 - [RL22_STRPS] B2IM26 26.1 5 318 39.16 Autolysin OS = *Streptococcus pneumoniae* (strain CGSP14) GN = lytA PE = 4 SV = 1 - [B2IM26_STRPS] B2IM71 46.48 4 71 38.4 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0446 PE = 4 SV = 1 - [B2IM71_STRPS] B2INE0 30.09 8 329 38.01 Cell division protein FtsX OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = ftsX PE = 3 SV = 1 - [B2INE0_STRPS] B2IQ19 15.99 8 763 37.52 DNA helicase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = pcrA PE = 3 SV = 1 - [B2IQ19_STRPS] B2IRN7 21.52 6 660 37.25 Threonine--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = thrS PE = 3 SV = 1 - [B2IRN7_STRPS] B2IS67 24.44 7 311 36.89 DNA-directed RNA polymerase Intracellular subunit alpha OS = *Streptococcus pneumoniae* (strain CGSP14) GN

= rpoA PE = 3 SV = 1 - [B2IS67_STRPS] B2IQA4 21.65 8 448 36.16 Glutamate dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gdhA PE = 3 SV = 1 - [B2IQA4_STRPS] B2INE5 20.96 6 396 36.08 S-adenosylmethionine synthase Intracellular OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = metK PE = 3 SV = 1 - [METK_STRPS] B2IQN3 51.52 4 66 35.91 30S ribosomal protein S21 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpsU PE = 3 SV = 1 - [B2IQN3_STRPS] B2IRK4 18.21 8 637 35.62 tRNA uridine 5- Intracellular carboxymethylaminomethyl modification enzyme MnmG OS = *Streptococcus pneumoniae* (strain CGSP14) GN = mnmG PE = 3 SV = 1 - [B2IRK4_STRPS] B2IR76 14.89 4 282 35.33 DegV family protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1543 PE = 4 SV = 1 - [B2IR76_STRPS] B2IRT0 25.85 4 294 35.15 ROK family protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1647 PE = 4 SV = 1 - [B2IRT0_STRPS] B2IR21 58.62 7 145 35.06 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0033 PE = 4 SV = 1 - [B2IR21_STRPS] B2IPF4 25.4 8 311 35.02 Adhesion lipoprotein OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = Imb PE = 3 SV = 1 - [B2IPF4_STRPS] B2ILV9 26.21 6 454 34.9 Aspartokinase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = lysC PE = 3 SV = 1 - [B2ILV9_STRPS] B2IPP0 17.37 7 495 34.88 Glucose-6-phosphate 1- Intracellular dehydrogenase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = zwf PE = 3 SV = 1 - [B2IPP0_STRPS] B2IRU0 8.43 5 510 34.78 PTS system, IIBC components OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1657 PE = 4 SV = 1 - [B2IRU0_STRPS] B2IQX1 34.93 7 292 34.34 ATP synthase gamma chain OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = atpG PE = 3 SV = 1 - [ATPG_STRPS] B2ISZ2 16.94 5 490 34.05 Sucrose phosphorylase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1867 PE = 4 SV = 1 - [B2ISZ2_STRPS] B2IP00 28.21 6 397 33.73 Serine protease OS = -*Streptococcus pneumoniae* (strain CGSP14) terminally GN = sphtra PE = 4 SV = 1 - anchored [B2IP00_STRPS] (No CS) B2INW0 31.82 5 220 33.64 Deoxyribose-phosphate aldolase Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = deoC PE = 3 SV = 1 - [DEOC_STRPS] B2IM98 15.7 6 535 33.16 CTP synthase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pyrG PE = 3 SV = 1 - [B2IM98_STRPS] B2IMU0 11.8 4 466 33.1 Dipeptidase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = pepV PE = 4 SV = 1 - [B2IMU0_STRPS] B2IPU4 37.15 5 253 33.02 Lactose phosphotransferase system Intracellular repressor OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = lacR PE = 4 SV = 1 - [B2IPU4_STRPS] B2IRR9 38.55 5 179 32.57 Cell division protein SepF OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = sepF PE = 3 SV = 1 - [SEPF_STRPS] B2IQJ5 27.56 5 225 32.43 3-dehydroquinate dehydratase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = aroD PE = 3 SV = 1 - [AROD_STRPS] B2INC8 38.43 7 216 32.37 Uracil phosphoribosyltransferase Intracellular OS = - *Streptococcus pneumoniae* (strain CGSP14) GN = upp PE = 3 SV = 1 - [B2INC8_STRPS] B2INU6 33.73 8 424 32.09 Phosphopentomutase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = deoB PE = 3 SV = 1 - [B2INU6_STRPS] B2IME1 24.87 6 378 32.06 Transcription Intracellular termination/antitermination protein NusA OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = nusA PE = 3 SV = 1 - [B2IME1_STRPS] B2INN5 22.79 4 272 31.93 Cell shape-determining protein N- MreC OS = -*Streptococcus pneumoniae* terminally (strain CGSP14) GN = mreC PE = 3 anchored SV = 1 - [B2INN5_STRPS] (No CS) B2IN76 12.22 6 450 31.55 UDP-N-acetylmuramoylalanine--D- Intracellular glutamate ligase OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = murD PE = 3 SV = 1 - [MURD_STRPS] B2INF3 16.1 4 267 31.17 Peptidyl-prolyl cis-trans isomerase Lipid OS = -*Streptococcus pneumoniae* (strain anchored CGSP14) GN = ppiA PE = 3 SV = 1 - [B2INF3_STRPS] B2IQ22 31.82 6 286 30.97 Methionine aminopeptidase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = map PE = 1 SV = 1 - [B2IQ22_STRPS] B2IS48 70.59 5 68 30.8 50S ribosomal protein L29 OS = Intracellular

Streptococcus pneumoniae (strain CGSP14) GN = rpmC PE = 3 SV = 1 - [RL29_STRPS] B2IS10
 19.57 6 649 30.36 DNA mismatch repair protein MutL Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = mutL PE = 3 SV = 1 - [MUTL_STRPS] B2IM44 29.93 6 274
 30.16 DNA-entry nuclease OS = N- *Streptococcus pneumoniae* (strain terminally CGSP14) GN = endA PE = 4 SV = 1 - anchored [B2IM44_STRPS] (No CS) B2INF7 54.46 5 112 29.66 ATP cone domain-containing Intracellular protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0728 PE = 4 SV = 1 - [B2INF7_STRPS] B2IS34 13.3 7 737 29.66 Anaerobic ribonucleoside Intracellular triphosphate reductase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0212 PE = 4 SV = 1 - [B2IS34_STRPS] B2IN20 40.78 3 103 29.58 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2067 PE = 4 SV = 1 - [B2IN20_STRPS] B2IMB7 38.95 4 95 29.51 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0492 PE = 4 SV = 1 - [B2IMB7_STRPS] B2IMY9 20.58 6 486 29.3 Glutamate--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = gltX PE = 3 SV = 1 - [SYE_STRPS] B2IQ75 46.77 5 186 29.27 LemA protein OS = *-Streptococcus pneumoniae* (strain CGSP14) terminally GN = lemA PE = 4 SV = 1 - anchored [B2IQ75_STRPS] (No CS) B2IPN9 20.75 6 429 29.19 Signal recognition particle receptor Intracellular FtsY OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = ftsY PE = 3 SV = 1 - [B2IPN9_STRPS] B2INM2 45.33 7 150 28.94 50S ribosomal protein L9 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rplI PE = 3 SV = 1 - [RL9_STRPS] B2IR80 20.89 5 292 28.84 Uncharacterized protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1547 PE = 4 SV = 1 - [B2IR80_STRPS] B2IPW9 32.16 8 283 28.64 Ribosome biogenesis GTPase A OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1141 PE = 3 SV = 1 - [B2IPW9_STRPS] B2IN61 75.51 4 49 28.27 50S ribosomal protein L33 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmG PE = 3 SV = 1 - [RL33_STRPS] B2IM15 25.95 5 158 28.26 Uncharacterized protein OS = Multi- *Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1901 PE = 4 SV = 1 - [B2IM15_STRPS] B2IPZ5 13.9 7 633 28.19 ABC transporter, ATP-binding Intracellular protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1167 PE = 4 SV = 1 - [B2IPZ5_STRPS] B2IMA9 20.94 6 487 28.12 Type I restriction-modification Intracellular system, M subunit OS = *Streptococcus pneumoniae* (strain CGSP14) GN = hsdM PE = 4 SV = 1 - [B2IMA9_STRPS] B2IQ67 11.63 7 1058 28.04 Carbamoyl-phosphate synthase Intracellular large chain OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = carB PE = 3 SV = 1 - [CARB_STRPS] B2IMV8 16.5 4 491 28.03 PTS system, IIC component, Multi-putative OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0602 PE = 4 SV = 1 - [B2IMV8_STRPS] B2INP6 15.54 4 341 28 Tryptophan--tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = trpS PE = 3 SV = 1 - [B2INP6_STRPS] B2INU9 26.02 4 269 27.83 Purine nucleoside phosphorylase Intracellular OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = pnp PE = 3 SV = 1 - [B2INU9_STRPS] B2IMS9 20.8 7 553 27.26 Ribonuclease J OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = rnj PE = 3 SV = 1 - [B2IMS9_STRPS] B2ILY3 41.94 4 62 27.17 50S ribosomal protein L28 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = rpmB PE = 3 SV = 1 - [RL28_STRPS] B2IRW8 34.46 5 177 26.92 Transcriptional repressor NrdR OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = nrdR PE = 3 SV = 1 - [B2IRW8_STRPS] B2IMH1 20.97 7 472 26.92 Cof family protein OS = *-Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1962 PE = 4 SV = 1 - [B2IMH1_STRPS] B21PM 6 13.79 2 87 26.87 Phosphocarrier protein HPr OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1120 PE = 4 SV = 1 [B2IPM6_STRPS] B2IRB7 27.22 2 158 26.84 Uncharacterized protein OS = N- terminally *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_1584 PE = 4 SV = (No CS) 1 - [B2IRB7_STRPS] B2ILW4 23.46 5 324 26.69 Enoyl-(Acyl-carrier-protein) Intracellular reductase OS = *-Streptococcus pneumoniae*

(strain CGSP14) GN = fabK PE = 4 SV = 1 - [B2ILW4_STRPS] B2IN41 16.32 7 429 26.52 Histidine -- tRNA ligase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = hisS PE = 3 SV = 1 - [SYH_STRPS] B2IQU2 16.07 3 305 26.2 Glycine -- tRNA ligase alpha subunit Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = glyQ PE = 3 SV = 1 - [SYGA_STRPS] B2IPW1 23.91 4 322 26.19 Acetoin dehydrogenase, E1 Intracellular component, alpha subunit, putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = acoA PE = 4 SV = 1 - [B2IPW1_STRPS] B2ISG1 25.79 5 190 26.04 Uncharacterized protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1779 PE = 4 SV = 1 - [B2ISG1_STRPS] B2IPZ7 19.35 4 279 25.42 DegV family protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1169 PE = 4 SV = 1 - [B2IPZ7_STRPS] B2ISK9 13.48 4 267 25.25 PTS system, mannose-specific IIC Multi- component OS = -*Streptococcus pneumoniae* (strain CGSP14) (Lipid GN = manM PE = 4 SV = 1 - modified [B2ISK9_STRPS] N-termini) B2IS70 20.32 5 433 25.16 UPF0210 protein SPCG_0246 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0246 PE = 3 SV = 1 - [B2IS70_STRPS] B2INL6 15.22 4 335 24.97 ABC transporter, substrate-binding Lipid protein, putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2165 PE = 4 SV = 1 - [B2INL6_STRPS] B2IPF2 46.6 4 191 24.92 Thioredoxin family protein OS = Lipid *Streptococcus pneumoniae* (strain anchored CGSP14) GN = SPCG_0974 PE = 4 SV = 1 - [B2IPF2_STRPS] B2INJ2 24.59 7 427 24.82 DltD protein OS = -*Streptococcus pneumoniae* (strain CGSP14) terminally GN = dltD PE = 4 SV = 1 - anchored [B2INJ2_STRPS] (No CS) B2IPH6 13.65 3 359 24.36 Peptide chain release factor 1 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = prfA PE = 3 SV = 1 - [RF1_STRPS] B2ILY0 20.43 4 514 24.35 Peptide chain release factor 3 OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = prfC PE = 3 SV = 1 - [RF3_STRPS] B2ILV5 20.88 4 182 24.04 Alkyl hydroperoxide reductase Intracellular AhpD OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = mip PE = 3 SV = 1 - [B2ILV5_STRPS] B2IRX2 21.8 4 399 23.82 Uncharacterized protein OS = Multi-*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = SPCG_1689 PE = 4 SV = 1 - [B2IRX2_STRPS] B2IM58 11.36 4 308 23.79 Membrane protein insertase YidC Multi- OS = -*Streptococcus pneumoniae* (strain transmembrane CGSP14) GN = yidC PE = 3 SV = 1 - [B2IM58_STRPS] B2IR27 23.03 4 330 23.79 Phosphate acyltransferase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = plsX PE = 3 SV = 1 - [PLSX_STRPS] B2IQL7 22.95 4 292 23.74 Phosphate ABC transporter, Lipid phosphate-binding protein, anchored putative OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1389 PE = 4 SV = 1 - [B2IQL7_STRPS] B2IR86 41.62 7 197 23.54 Probable GTP-binding protein EngB Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = engB PE = 3 SV = 1 - [ENGB_STRPS] B2IP77 21 5 419 23.53 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0896 PE = 4 SV = 1 - [B2IP77_STRPS] B2ISL2 23.6 5 339 23.5 Alcohol dehydrogenase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = adhP PE = 3 SV = 1 - [B2ISL2_STRPS] B2IPR3 21.05 5 247 23.35 Uncharacterized protein OS = N-*Streptococcus pneumoniae* (strain terminally CGSP14) GN = SPCG_1083 PE = 4 SV = anchored 1 - [B2IPR3_STRPS] (with CS) B2IR97 25 6 252 23.12 Methyltransferase, putative OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1564 PE = 4 SV = 1 - [B2IR97_STRPS] B2IS98 24.4 4 209 22.81 Probable nicotinate-nucleotide Intracellular adenyllyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = nadD PE = 3 SV = 1 - [B2IS98_STRPS] B2IQS3 22.11 4 303 22.58 Thioredoxin reductase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = trxB PE = 3 SV = 1 - [B2IQS3_STRPS] B2IPW5 31.61 6 329 22.56 Lipoate-protein ligase, putative OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1137 PE = 4 SV = 1 - [B2IPW5_STRPS] B2IMB4 30.77 5 182 22.24 Protein GrpE OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = grpE PE = 3 SV = 1 - [B2IMB4_STRPS] B2ILY4 43.8 3 121 22.23 Uncharacterized protein

OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = asp23 PE = 4 SV = 1 - [B2ILY4_STRPS] B2IPH3 18.37 7 479 22.18 tRNA modification GTPase MnmE Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = thdF PE = 3 SV = 1 - [B2IPH3_STRPS] B2INP7 17.59 6 540 22.16 ABC transporter, ATP-binding Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_2196 PE = 4 SV = 1 - [B2INP7_STRPS] B2IP74 14.66 5 491 21.9 Lysine decarboxylase OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = cad PE = 4 SV = 1 - [B2IP74_STRPS] B2ISG4 52.24 2 67 21.88 Uncharacterized protein OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1782 PE = 3 SV = 1 - [B2ISG4_STRPS] B2ISY1 17.74 7 541 21.79 Dextran glucosidase DexS, putative Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = dexS PE = 4 SV = 1 - [B2ISY1_STRPS] B2IMA5 15.4 5 448 21.67 Glutamine synthetase, type I OS = Intracellular *Streptococcus pneumoniae* (strain CGSP14) GN = glnA PE = 3 SV = 1 - [B2IMA5_STRPS] B2IN77 24.72 6 352 21.61 UDP-N-acetylglucosamine -- N- Intracellular acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N- acetylglucosamine transferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = murG PE = 3 SV = 1 - [MURG_STRPS] B2IQ12 25.39 5 319 21.48 Ribose-phosphate Intracellular pyrophosphokinase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = prs PE = 3 SV = 1 - [B2IQ12_STRPS] B2ISL4 16.3 4 270 21.32 Cof family protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_0299 PE = 4 SV = 1 - [B2ISL4_STRPS] B2IQ78 13.38 5 523 21.2 Signal recognition particle protein Intracellular OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = ffh PE = 3 SV = 1 - [B2IQ78_STRPS] B2IS71 21.69 5 249 21.06 Phosphoglycerate mutase family Intracellular protein OS = -*Streptococcus pneumoniae* (strain CGSP14) GN = gpmB PE = 4 SV = 1 - [B2IS71_STRPS] B2IM30 15.38 5 338 20.64 Transcriptional regulator, putative Secretory OS = -*Streptococcus pneumoniae* (released (strain CGSP14) GN = lytR PE = 4 SV = (with CS) 1 - [B2IM30_STRPS] B2IQN9 10.91 4 486 20.01 Nicotinate Intracellular phosphoribosyltransferase OS = *Streptococcus pneumoniae* (strain CGSP14) GN = SPCG_1408 PE = 3 SV = 1 - [B2IQN9_STRPS]

Example 2: Microparticles (MPs) Confer Serotype-Independent Protective Immunity

[0188] For each immunization experiment male C57BL/6 wild-type mice ca 5 weeks old were used. Before immunization, mice were anesthetized by inhalation of isofluorane (Abbott) and then intranasally administered with 50 µl/mouse of microparticles (MP) combined with the adjuvant aluminium hydroxide (Sigma Aldrich, 10 mg/ml in PBS) or the adjuvant alone for the control groups. Immunization was repeated again after two weeks from the first immunization following the same conditions described above. After 4 weeks of immunization, mice were infected by intranasal administration of 50 µl/mouse of 5×10⁶ CFU, for *S. pneumoniae* type 1 infection experiments (FIG. 7), or 10⁶ CFU, for *S. pneumoniae* type 3 infection experiments (FIG. 11). [0189] All mice were anesthetized by inhalation of isofluorane prior to challenge with bacteria. After the infection, clinical symptoms of the mice were monitored multiple times per day (in accordance with the ethical permit). Blood samples (5 µl/mouse) were taken every day of infection and level of bacteremia was assessed by plating serial dilutions of blood samples onto blood-agar plates. Mice that reached humane end-points were anesthetized by inhalation of isofluorane and intranasal administered with 100 µl/mouse of the fluorescent marker Bacterisense 645 (Perkin Elmer) 30 minutes before the sacrifice. Prior to sacrifice, mice were anesthetized again (isofluorane inhalation). After sacrifice, mice were placed in the IVIS Spectrum Imaging System to detect the fluorescent signal of the bacterial infection. After the IVIS imaging, lungs and spleens were collected for further analysis. Bacterial amount in the lungs was assessed by CFU count of bacteria after plating serial dilutions of lung homogenates onto blood-agar plates.

[0190] Intranasal immunization with microparticles (MP) from a serotype 4 *S. pneumoniae* increased survival after a challenge with serotype 1 *S. pneumoniae* (FIG. 7A). The increase in survival was significant also in comparison to immunization with MV.sub.L. The immunization greatly reduced the bacterial load in the lungs of the mice surviving the challenge with serotype 1

S. pneumoniae (FIG. 7B). Furthermore, mice immunized with serotype 3 MP and then challenged with serotype 3 bacteria showed 100% protection in the pneumonia model, higher than immunization with the currently available vaccine PCV13, which only conferred a protection of 75% in the model. Moreover, protection against pneumococcal infection was absent in mice lacking B cells, suggesting the involvement of an adaptive immune response with anti-pneumococcal antibodies (FIG. 11).

Example 3: Serotype-Independent Binding and Opsonophagocytic Activity of Antibodies Raised Against Pneumococcal Membrane Particles

[0191] Antibodies raised against the inventive MP from serotype 4 as antigen were reactive against *Streptococcus pneumoniae* of other serotypes, such as 1, 6B and 3 (FIG. 9). In other words, the MP were able to elicit production of serotype-independent antibodies, much more so than MV.sub.L (FIG. 9B).

[0192] The production of anti-MP antibodies in mice was more efficient (in particular at time point 2 weeks) when the antigen was administered together with an adjuvant. However, satisfactory results were obtainable even without an adjuvant (FIG. 8). Moreover, antibodies in sera were able to bind both the encapsulated T4 (TIGR4) and the unencapsulated isogenic mutant T4R to the same extent, indicating that the antibody response was capsule-independent.

[0193] Importantly, the anti-MP antibodies showed opsonophagocytic activity, increasing adhesion of bacteria and killing of internalized bacteria by RAW murine macrophages (FIG. 10).

Materials and Methods

[0194] Bacterial strains and growth conditions. In this study six strains of *Streptococcus pneumoniae* were used: TIGR4 or T4 (serotype 4) (22), its isogenic mutants lacking the cytotoxin pneumolysin (T4 Δ ply) (23), the autolysin LytA (T4 Δ lytA) (3), or the capsule (T4R) (24), ATCC 6301 (serotype 1) and 1-33 (serotype 3). Bacteria were grown in C+Y medium pH 7.9-8.0 (for serotype 4) or THY medium (for serotype 1 and 3) at 37° C. A spectrophotometer (Genesys 20, Thermo Spectronic) was used to follow the growth by monitoring the optical density (OD) at 600 nm.

[0195] Isolation and purification of membrane vesicles (MV) and microparticles (MP). For isolation of MV.sub.L from a liquid culture, pneumococcal strains (TIGR4, T4 Δ ply or T4 Δ lytA) were grown at 37° C. in C+Y medium, pH 7.9-8.0 until OD.sub.600 nm=0.9. The culture was then centrifuged (17,000×g for 30 minutes at 4° C.) to remove bacterial cells from the supernatant. The cell-free supernatant was filtered through a 0.22 μ m filter (Sarstedt) and centrifuged (120,000×g for 2 hours at 4° C.) to sediment the vesicles. Pellets were washed twice in phosphate-buffered saline (PBS) and resuspended in PBS.

[0196] For isolation of microparticles (MP) from bacteria grown on plates, the pneumococcal strains were streaked on blood agar plates and incubated overnight at 37° C. with 5% CO.sub.2. Bacteria were harvested from plates, resuspended in PBS and MP were pelleted following the same procedure as mentioned above.

[0197] Crude MV.sub.L/MP preparations were further purified by density gradient centrifugation using Optiprep™ Density Gradient Medium (Sigma). Pelleted particle fractions were adjusted to 50% (w/v) Optiprep™ in a total volume of 2 ml and overlaid with one fraction of 30% (w/v) Optiprep™ (9 ml) followed by a fraction of 5% (w/v) Optiprep™ (3 ml). Gradients were centrifuged at 250,000×g for 3 hours at 4° C. and the first 4 ml on top, containing the particles, were collected. After 3 washes with PBS (250,000×g for 2 hours at 4° C.), pellets were recovered in PBS and stored at -80° C.

[0198] Electron microscopy. To visualize MV.sub.L and MP on bacteria, *S. pneumoniae* T4R was grown in C+Y medium until OD.sub.600=0.4 at 37° C. Bacteria were harvested by centrifugation for 10 min at 4,000×g, 4° C. and pellets were suspended in 100 μ l PBS. Glow discharged carbon coated grids (Oxford Instruments, UK) were incubated for 1 min with a drop of bacterial solution or purified MV.sub.L/MP preparation and negatively stained with 2% uranyl acetate in water (7

times for 10 sec). Specimens were examined on a FEI CM120 microscope operated at 80 kV. Images were collected with a side mounted camera MegaView III (Olympus Soft Imaging solutions).

[0199] Atomic force microscopy. 5 µl of MP samples isolated from *S. pneumoniae* were placed onto freshly cleaved mica (Goodfellow Cambridge Ltd., Cambridge, United Kingdom). The specimens on the mica were blot dried and placed into a desiccator for at least 2 h. Imaging was performed on a Nanoscope IIIa (Digital Instruments, Santa Barbara) Atomic Force Microscope using Tapping Mode with standard silicon cantilevers oscillating at resonant frequency (270 to 305 kHz). Images were collected at a scan rate of 0.8-1.5 Hz, depending on sample number and the size of the scan. The final images were fitted in both axes and presented in a surface plot of the height mode.

[0200] SDS-PAGE and western blotting. The total amount of proteins in purified MV.sub.L or MP, lysates of A549 cells or dendritic cells stimulated with MV.sub.L or MP were quantified with Pierce™ BCA Protein Assay Kit (Life Technologies). Samples containing equal amounts of total protein were resolved by SDS-PAGE using 4-12% Bis-Tris gels (Life Technologies) and transferred to PVDF membranes. Membranes were then blocked with 5% skim milk in PBS containing 0.1% Tween-20 and incubated with antibodies as indicated. For detection of pneumolysin a mouse monoclonal antibody (Abcam, final dilution 1:500) was used. Polyclonal GAPDH (1:2,000) and LytA (1:2,000) (3) antisera raised in rabbits were used. Rabbit polyclonal antibodies against PspC (1:1,000), RrgB (1:1,000), PsaA (1:25,000), PhtD (1:25,000) and mouse polyclonal antibodies against SrtA (1:500) were kindly provided by Novartis Vaccines and Diagnostics. As loading control for A549 cells and dendritic cell lysates, a mouse monoclonal β-actin antibody (Santa Cruz) was used as primary antibody. Anti-mouse IgG or anti-rabbit IgG conjugated to horseradish peroxidase (GE Healthcare) were used as secondary antibodies (final dilution 1:10,000). Blots were developed with Amersham™ ECL Plus Western blotting detection system (GE Healthcare Life Sciences), using a ChemiDoc™ XRS+ (Bio-Rad Laboratories).

[0201] Tandem mass spectrometry. Proteins in MV.sub.L or MP samples were reduced, alkylated and in-solution digested by trypsin according to a standard operating procedure. Thereafter the samples were purified by Pierce C18 Spin Columns (Thermo Scientific), dried and resolved in 0.1% formic acid. The resulting peptides were separated in reversed-phase on a C18-column and electrosprayed on-line to a Q Exactive Plus mass spectrometer (Thermo Finnigan). Tandem mass spectrometry was performed applying HCD.

[0202] Database searches were made using the Sequest algorithm towards a FASTA database including proteins from *Streptococcus pneumoniae* TIGR4. The search criteria for protein identification were set to at least two matching peptides of 95% confidence level per protein. Only proteins with a Sequest score above 20 were considered for analysis, to avoid the possibility of false positives.

[0203] Subcellular localizations of proteins were predicted using the algorithm of website Locate P located at the following URL: www.cmbi.ru.nl/locatep-db/cgi-bin/locatepdb.py (11).

[0204] Hemolysis assay. Purified MV.sub.L or MP were incubated in 96-well plates with blood from buffy coats (diluted 1:50 in PBS containing 1 mM dithiothreitol (DTT)) for 1 hour at 37° C. After 50 minutes 1% Triton X-100 in PBS was added to the positive control wells in order to lyse all the erythrocytes. Plates were then spun at 400×g for 15 minutes at 4° C.; supernatant was transferred to an optical plate and the optical density was measured at 540 nm.

[0205] A549 cell toxicity assay. A549 lung epithelial cells were grown and maintained at 37° C., with 5% CO.sub.2 in RPMI medium (Gibco) supplemented with 10% (v/v) Fetal bovine serum (FBS) (HyClone). To assess MV.sub.L or MP associated cytotoxic effects, 0.6×10^{sup.6} A549 cells were seeded in 6-well plates and incubated overnight at 37° C. Cells were then washed with PBS and incubated for 24 hours with medium containing MV.sub.L or MP at indicated concentrations. Washed cells were labelled with Fixable Viability Dye eFluor® 780 (1:50,000, eBioscience) for 30

minutes at 4° C. in the dark, in presence or absence of 0.02% NP40 (Sigma) as positive control, and fixed with 4% paraformaldehyde (PFA) for 30 minutes at room temperature. Next, cells were gently scraped into PBS containing 1% FBS and analyzed in a Gallios™ Flow Cytometer (Beckman Coulter).

[0206] A549 cell uptake assay. To assess MV.sub.L and MP uptake by A549 cells via immunoblotting, 0.6×10^6 A549 cells were seeded in 6-well plates and incubated overnight at 37° C. Cells were then washed with PBS and incubated for 24 hours with medium containing the particles at indicated concentrations. After washing, cells were lysed in RIPA buffer containing 1× protease inhibitor cocktail (Roche). Cell lysates were analyzed by SDS-PAGE and western blotting.

[0207] Immunofluorescence microscopy. A549 cells (6.25×10^4) were seeded in 24-well plates with coverslips on the bottom of the wells, and incubated at 37° C. overnight. Cells were then washed, fixed with 4% PFA for 30 minutes and permeabilized with 1% Triton X-100 in PBS for 5 minutes. MV.sub.L or MP were detected with mouse monoclonal anti-Ply (1:200) and polyclonal rabbit antibodies to LytA (1:200). Respective antigens were visualized with Alexa Fluor® 488-conjugated goat-anti mouse IgG antibody (Life Technologies) and Alexa Fluor® 350-conjugated goat-anti rabbit IgG (Life Technologies) (1:1,000).

[0208] Actin cytoskeleton was stained with Alexa Fluor® 594 Phalloidin (Life Technologies, 1:40 dilution) for 1 hour and coverslips were mounted with Vectashield (Vector Laboratories, Inc.) on microscope slides. Images were acquired with a DeltaVision microscope equipped with a 60x-objective. Quick projection images of approximately 20 z-stacks were taken. Orthogonal views were used to visualize the cell monolayer from the x, y, and z axes.

[0209] After growth, pneumococci (serotypes 1, 3 and 6B) have been stained using the sera from the immunized mice as primary antibody (dilution 1:100 in PBS 1% BSA) and Alexa Fluor 488 goat anti mouse (dilution 1:500 in PBS 1% BSA) as secondary antibody.

[0210] Imaging has been performed with high-resolution Delta Vision Elite System using 100×objective (1000× total magnification). FITC Laser intensity 50%, exposure time 0,025 ms (FIGS. 13-15).

Quantification of the Signal Detected on the Bacteria after Immunofluorescence Staining

[0211] Using the functions Image>Adjust>Threshold and Analyze>Measure of ImageJ, the area covered by the bacteria and the area covered by the signal detected on the bacteria after staining with sera were selected, defined and measured. The final signal ratio was calculated by dividing the area of the signal (detected using the sera) by the total area of the bacteria.

[0212] Isolation and differentiation of human monocyte-derived dendritic cells. Dendritic cells were isolated using RosetteSep™ Human Monocyte Enrichment Cocktail (Stemcell Technologies) according to the manufacturer's instructions. In brief, blood from buffy coats from healthy donors was incubated for 20 minutes with RosetteSep™ Human Monocyte Enrichment Cocktail (Stemcell Technologies), layered on top of Ficoll-Paque™ Plus (GE Healthcare) and centrifuged at 1200×g for 20 minutes without acceleration or brake. The monocyte containing layer was recovered, cells washed 7 times with PBS and passed through a 100 µm cell strainer. Monocytes were then differentiated for 6 days in RPMI containing 10% FBS supplemented with 37.5 ng/ml of Granulocyte macrophage colony-stimulating factor (GM-CSF) (Peprotech) and 37.5 ng/ml of Interleukin 4 (IL-4) (Peprotech), changing the medium after 4 days. For experiments, cells were resuspended in RPMI containing 10% FBS.

[0213] Toxicity and apoptosis assay of dendritic cells. 6×10^5 cells were seeded in 96-well plates and incubated with RPMI containing 10% FBS and MV.sub.L or MP at indicated concentrations, or with T4R in a multiplicity of infection (MOI) of 20, as positive control, for 24 hours. Gentamicin (100 µg/ml, Sigma) was added after 1 hour of incubation to stop bacteria from growing in samples stimulated with T4R. Before staining, cells were washed once in PBS and once in Annexin V Binding Buffer (BD Pharmingen). Staining was performed with Fixable Viability Dye eFluor® 780 (1:50,000, eBioscience) and FITC Annexin V (1:20, BD Pharmingen) for 30

minutes at 4° C., followed by two washes with Annexin buffer. Labelled cells were fixed in 4% PFA for 30 minutes, resuspended in PBS containing 1% FBS and analyzed in a Gallios™ Flow Cytometer (Beckman Coulter).

[0214] Activation assay of dendritic cells. 6×10⁵ cells were seeded in 96-well plates and incubated in RPMI containing 10% FBS with MV.sub.L or MP at indicated concentrations, or with 1 µg/ml lipopolysaccharide (LPS) (Sigma) as positive control, for 24 hours. Cells were stained with Phycoerythrin (PE) Mouse Anti-Human CD86 (BD Pharmingen) and PE-Cy™5 Mouse Anti-Human HLA-DR (BD Pharmingen) for 20 minutes at 4° C., washed twice with PBS and resuspended in PBS 1% FBS. Labelled cells were analyzed in a Gallios™ Flow Cytometer (Beckman Coulter).

[0215] Uptake assay with dendritic cells. 10⁵ cells were seeded in 96-well plates and incubated with RPMI containing 10% FBS, in presence or absence of the inhibitors cytochalasin D (0.5 µg/ml, Sigma) and wortmannin (0.5 µg/ml, Sigma) (C/W), or methyl-β-cyclodextrin (MβCD) (10 µM, Sigma), for 30 minutes. Cells were then incubated with particles at indicated concentration for 1 hour and lysed in RIPA buffer containing 1× protease inhibitor cocktail (Roche). Cell lysates were analyzed by SDS-PAGE and western blotting.

[0216] Quantification of cytokines. Different cytokines were assessed (IL-6, IL-8, IL-10 and TNF) in cell-free supernatants of 10⁵ dendritic cells by Enzyme-Linked Immunosorbent Assay (ELISA), using commercially available BD OptEIA™ kits from BD Biosciences. Cells were incubated with particles at indicated concentrations, or with T4R or LPS (as previously described), for 24 hours.

[0217] Opsonophagocytosis assay with RAW cells. RAW 264.7 murine macrophages were grown and maintained at 37° C., with 5% CO.sub.2 in RPMI medium (Gibco) supplemented with 10% (v/v) Fetal bovine serum (FBS) (HyClone). To assess the opsonophagocytosis activity of antibodies in immunized mice sera 2×10⁵ RAW 264.7 cells were seeded in 24-well plates and incubated overnight at 37° C. Serotype 1 bacteria were incubated for 30 minutes at 37° C. with 5% CO.sub.2 with 20% serum from mice immunized with MP+adjuvant, or with adjuvant alone as negative control. RAW cells were then washed with PBS and incubated for 1.5 hours with 2.5×10⁷/well of pre-treated bacteria. Cells were washed three times with PBS to remove unattached bacteria. To measure total uptake of bacteria, cells were incubated with a 50/50 solution of 2% saponin (Sigma) and trypsin-EDTA (Gibco) for 15 minutes at 37° C., to lyse eukaryotic cells, and total bacteria were plated for enumeration. To evaluate phagocytosis 300 µg/ml of Gentamicin (Sigma) and 0.12 mg/ml of Penicillin G (Sigma) were added to separate wells and incubated 15 minutes at 37° C., to kill extracellular bacteria. Cells were then washed three times with PBS and incubated with a 50/50 solution of 2% saponin and trypsin-EDTA for 15 minutes at 37° C. to lyse eukaryotic cells. To evaluate killing of bacteria inside macrophages separate wells were treated with antibiotics (as for phagocytosis), washed three times with PBS and then incubated for 1 hour at 37° C. with medium. Cells were then washed three times with PBS and incubated with a 50/50 solution of 2% saponin and trypsin-EDTA for 15 minutes at 37° C. to lyse eukaryotic cells.

[0218] Mouse IgG ELISA assay. To detect MV.sub.L- and MP-specific mouse IgG in sera of immunized mice optical plates (Sarstedt) were coated with 1 µg/ml of MP in 0.1 M Sodium Carbonate buffer pH 9.5, overnight at 4° C. Wells were then washed three times with PBS containing 0.05% Tween-20 and incubated with PBS with 10% FBS, for 1 hours at room temperature. After three washes wells were incubated with mice sera diluted 1:500 in PBS with 10% FBS, for 2 hours at room temperature. Wells were then washed three times and incubated with anti-mouse IgG-HRP (GE Healthcare) diluted 1:500 in PBS 10% FBS, for 2 hours at room temperature. After three washes wells were then incubated with TMB substrate (BD Bioscience) for 10 minutes and the reaction was stopped with 1 M H.sub.3PO.sub.4. Absorbance at 450 nm was then measured with a plate reader.

[0219] To detect pneumo-specific mouse IgG in sera of immunized mice, bacteria were grown on

blood agar plates overnight at 37° C., resuspended in PBS and heat-inactivated for 2 hours at 60° C. After diluting the bacteria to OD_{sub}600 0.6, optical plates (Sarstedt) were coated with 100 µl of bacteria in 0.1 M Sodium Carbonate buffer pH 9.5, overnight at 4° C. Wells were then washed three times with PBS and incubated with PBS with 2.5% skim milk, for 2 hours at room temperature. After three washes wells were incubated with mice sera diluted 1:500 in PBS, for 1 hour at room temperature. Wells were then washed three times and incubated with anti-mouse IgG-HRP (GE Healthcare) diluted 1:500 in PBS, for 1 hour at room temperature. After three washes well were then incubated with TMB substrate (BD Bioscience) for 10 minutes and the reaction was stopped with 1 M H_{sub}3PO_{sub}4. Absorbance at 450 nm was then measured with a plate reader. [0220] Statistical analysis. For multiple comparisons the nonparametric ANOVA test was used to assess the presence of the differences between the groups, then the Dunn's test was used to make pairwise comparisons; for two groups comparison the non-parametric two tailed Wilcoxon's rank sum test (also known as Mann-Whitney test) was used. Statistically significant data was defined as * P<0.05, ** P<0.01, *** P<0.001, ****=P<0.0001.

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Claims

1. A method for inducing protective immunity against *Streptococcus pneumoniae* in a subject, comprising administering to the subject a composition comprising a *Streptococcus pneumoniae* membrane vesicle microparticle (MP), wherein said MP comprises: i. the protein pneumolysin (Ply) at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; ii. the protein autolysin (LytA) at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; iii. the protein Pneumococcal surface protein C (PspC) at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP; or iv. the protein pilus-1 backbone protein (RrgB) at the level of ≥ 0.020 $\mu\text{g}/\text{mg}$ total protein in the MP.
2. The method according to claim 1, wherein the immunity is protective against a condition selected from pneumococcal sinusitis, pneumococcal otitis, pneumococcal pneumonia, and invasive pneumococcal disease including but not limited to pneumococcal sepsis and pneumococcal meningitis.
3. The method according to claim 1, wherein the composition is a liquid and comprises MPs in an amount of 1 $\mu\text{g}/\text{ml}$.
4. The method according to claim 1, wherein said MP comprises: i. the protein pneumolysin (Ply) at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; ii. the protein autolysin (LytA) at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP; iii. the protein Pneumococcal surface protein C (PspC) at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP; and iv. the protein pilus-1 backbone protein (RrgB) at the level of ≥ 0.020 $\mu\text{g}/\text{mg}$ total protein in the MP.
5. The method according to claim 1, wherein the MP is 5-300 nm in diameter.
6. The method according to claim 1, wherein the MP is 10-125 nm in diameter.
7. The method according to claim 1, wherein the composition elicits antibodies against *Streptococcus pneumoniae* serotype 3 when administered to a mammalian host.
8. The method according to claim 1, wherein the composition elicits serotype independent antibodies against *Streptococcus pneumoniae* when administered to a mammalian host.
9. The method according to claim 1, wherein the composition is administered intranasally.
10. The method according to claim 1, wherein the composition further comprises an adjuvant.
11. The method according to claim 10, wherein the adjuvant comprises aluminium hydroxide.
12. The method according to claim 2, wherein the immunity is protective against invasive

pneumococcal disease.

- 13.** The method according to claim 1, wherein the MP comprises a capsular polysaccharide of a capsular serotype of *Streptococcus pneumoniae* at a level of ≥ 0.001 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 14.** The method according to claim 1, wherein said MP comprises the protein Ply at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 15.** The method according to claim 1, wherein said MP comprises the protein LytA at the level of ≥ 0.070 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 16.** The method according to claim 1, wherein said MP comprises the protein PspC at the level of ≥ 0.130 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 17.** The method according to claim 1, wherein said MP comprises the protein RrgB at the level of ≥ 0.020 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 18.** The method according to claim 1, wherein said MP comprises the protein Ply at the level of ≥ 0.35 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 19.** The method according to claim 1, wherein said MP comprises the protein LytA at the level of ≥ 0.20 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 20.** The method according to claim 1, wherein said MP comprises the protein PspC at the level of > 0.3 $\mu\text{g}/\text{mg}$ total protein in the MP.
 - 21.** The method according to claim 1, wherein said MP comprises the protein RrgB at the level of ≥ 0.028 $\mu\text{g}/\text{mg}$ total protein in the MP.
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