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(54) **TEST KIT FOR PLASMA OR SERUM ANTIBODY TITER AGAINST PERIODONTAL DISEASE-CAUSING BACTERIA**

(57) The object of the present invention is to provide: a test kit for an antibody titer or an antibody against a periodontal disease-causing bacterium in a blood sample, which enables the testing on a periodontal disease in patients having a wide scope of immunotypes with high accuracy and can be treated by an automated device at a high speed; a periodontal disease-causing bacterium antigen protein which can be suitably used in the kit; a method for testing an antibody titer or the presence of an antibody in a blood sample using the kit; and a kit for typing strains of *Porphyromonas gingivalis*. The present

invention discloses: a test kit comprising a set of polypeptides respectively having the acid sequences represented by SEQ ID NOs: 1, 3, 9, 15, 19, 31, 41, 43, 63, 65 and 67; a modified polypeptide having the amino acid sequence represented by SEQ ID NO: 67; and a method for determining an antibody titer or the presence of an antibody against a periodontal disease-causing bacterium in a blood sample separated from a human body, comprising bringing the blood sample into contact with the above-mentioned set of polypeptides.

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Description

TECHNICAL FIELD

5 [0001] The present invention relates to: a test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium, more specifically a test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium which is suitable for automated testing using a device and comprises a specific periodontal disease-causing bacterium antigen protein; a modified polypeptide for use in the kit; and a method for measuring an antibody titer against a periodontal disease-causing bacterium in a blood sample.

BACKGROUND OF THE INVENTION

[0002] A periodontal disease is a bacterial infection that is developed through an infection of periodontal tissue with oral bacterium.

15 [0003] The diagnosis of periodontal disease in a dental clinical site is carried out based on overall results of clinical tests such as a clinical condition, a photograph of oral cavity, a radiographic image or a periodontal tissue test. Among these tests, the photograph of oral cavity and the radiographic image exploration visually evaluate the morphological change of periodontal tissue, and periodontal tissue test evaluates by measuring various clinical items such as the state of the formation of plaque, the depth of a periodontal pocket, the presence of bleeding during probing or the degree of tooth mobility. Therefore, these tests require complicated operations and a practitioner must have advanced technique for accurately diagnosing the periodontal disease pathologic condition of patients.

20 [0004] In other words, in some cases, a test result may vary depending on the skill level of practitioners, and therefore different diagnoses may be given to a patient. Further, in the clinical dental tests as mentioned above, in spite of a fact that a periodontal disease is a bacterial infection, the periodontal disease is evaluated not at "a level of infection" with a periodontal disease-causing bacterium but at a level of "the morphological change" of periodontal tissue; in other words, the test is carried out by the practitioner's subjectivity. Therefore, there have been a demand for an objective periodontal disease test method which is reasonable from the bacteriological and immunological viewpoints and in which difference in a test result would not occur depending on the skill level of practitioners.

25 [0005] In these situations, a periodontal disease test system is carried out, in which a serum antibody titer against a periodontal disease-causing bacterium is employed as a measure for the periodontal disease testing (Chieko KUDO, Journal of Okayama Dental Society, vol. 28 (1) (2009), pp. 1-14). In this periodontal disease test system, the state of infection with a periodontal disease-causing bacterium or the severity of a periodontal disease (the state of inflammation) is evaluated by detecting/quantifying "a specific antibody" against the periodontal disease-causing bacterium from a trace amount of blood that is collected from a finger tip of a patient and then is separated. According to this system, it is possible to objectively and uniformly evaluate the disease condition of a periodontal disease by employing an immunological technique.

30 [0006] Further, in this periodontal disease test system, plasma is separated from the blood collected from a finger tip, a sample of the plasma is mailed to an inspection agency, an IgG antibody titer against a periodontal disease-causing bacterium is measured in the inspection agency, the severity of a periodontal disease is evaluated, and then a result of the periodontal disease test is notified to each patient (a test on a plasma antibody titer against a periodontal disease-causing bacterium). Thus, the periodontal disease test can be assisted in general practitioners or at home. Further, since test data are comprehensively collected and analyzed, it becomes possible to correlate the test data with disease conditions using an enormous quantity of data.

35 [0007] Meanwhile, in the periodontal disease test system mentioned above, since a large quantity of samples is handled, it is required to treat the samples automatically and at a high speed.

40 [0008] In the test system, the correlation between test results and a periodontal disease become higher with the increase in the types of antibodies against an antigen to be tested in a (blood) sample, and thus the periodontal disease can be tested with higher accuracy. In addition, in a human suffering from periodontal disease, the type of periodontal disease-causing bacterium antigen to be recognized is varied due to the inter-individual differences in the periodontal disease-causing bacteria and the human. Also in this regard, a periodontal disease can be tested with higher accuracy with the increase in the types of antibodies to be tested.

45 [0009] However, if the antibody titers of a variety of antibodies are to be measured, it is difficult to treat samples at a high speed, which is not suitable for automated test using a device.

50 [0010] The antigen used in the current antibody titer measurements is a solution prepared by disrupting a periodontal disease-causing bacterium such as *Porphyromonas gingivalis*, which is a mixture containing a wide variety of bacterial proteins (including LPSs and membrane lipids). Therefore, it has been difficult to treat a large quantity of samples automatically and at a high speed using a device.

Disclosure of Invention

[0011] The object of the present invention is to provide: a test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium, which can cover a various antigen-antibody reactions occurring due to changing antigenicity of periodontal disease-causing bacterium and a various of immunological reactions of a substance to be tested, which can test periodontal diseases in a wide scope of patients having various immunotypes with high accuracy, and which can be treated by an automated device at a high speed; a periodontal disease-causing bacterium antigen protein which can be suitably used in the kit; and a method for testing a plasma or serum antibody titer against a periodontal disease-causing bacterium in a blood sample, which uses the kit.

[0012] Under these problems, the present inventors have studied on the selection of periodontal disease-causing bacterium proteins, which can cover a various antigen-antibody reactions even when the number of types of the protein is small and therefore can evaluate a periodontal disease with high accuracy. As a result, it is found that a specific combination of periodontal disease-causing bacterium proteins can specifically react with plasma antibody in a blood sample separated from a periodontal disease patient, various antigen-antibody reactions are covered and the blood sample can be tested with high accuracy by selectively using the combination of the bacterial proteins, and the blood sample can be treated using an automated device at a high speed. These findings led to the accomplishment of the present invention.

[0013] That is, the present invention provides:

[1] a test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium, comprising a set of polypeptides having the amino acid sequences represented by SEQ ID NOs: 1, 3, 9, 15, 19, 31, 41, 43, 63, 65 and 67;

[2] the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium according to [1], wherein the test kit further comprises polypeptides having the amino acid sequences represented by SEQ ID NOs: 5 and 37;

[3] the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium according to [1] or [2], wherein the test kit further comprises polypeptides having the amino acid sequences represented by SEQ ID NOs: 23, 35 and 47;

[4] the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium according to any one of [1] to [3], wherein the test kit further comprises a polypeptide having the amino acid sequence represented by SEQ ID NO: 17;

[5] a modified polypeptide having the amino acid sequence represented by SEQ ID NO: 63;

[6] the modified polypeptide according to [5], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 64;

[7] a modified polypeptide having the amino acid sequence represented by SEQ ID NO: 65;

[8] the modified polypeptide according to [7], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 66;

[9] a modified polypeptide having the amino acid sequence represented by SEQ ID NO: 67;

[10] the modified polypeptide according to [9], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 68;

[11] a modified polypeptide having the amino acid sequence represented by SEQ ID NO: 141;

[12] the modified polypeptide according to [11], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 142;

[13] a modified polypeptide having the amino acid sequence represented by SEQ ID NO: 145;

[14] the modified polypeptide according to [13], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 146;

[15] a test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 1, 3, 5, 9, 15, 17, 19, 23, 31, 35, 37, 41, 43, 47, 63, 65, 67, 141, 143, 145, 147, 151, 153 and 155;

[16] the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium according to [15], wherein the polypeptide is a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 3, 5, 15, 19, 31, 41, 141 and 145;

[17] the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium according to [15], wherein the polypeptide is a polypeptide encoded by at least one polynucleotide sequence selected from the group consisting of the polynucleotide sequences represented by SEQ ID NOs: 2, 4, 6, 10, 16, 18, 20, 24, 32, 36, 38, 42, 44, 48, 64, 66, 68, 142, 144, 146, 148, 152, 154 and 156;

[18] a method for measuring an antibody titer against a periodontal disease-causing bacterium in a blood sample

separated from a human body, comprising bringing the blood sample into contact with a periodontal disease-causing bacterium antigen polypeptide, said method being characterized in that the periodontal disease-causing bacterium antigen polypeptide is a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 1, 3, 5, 9, 15, 17, 19, 23, 31, 35, 37, 41, 43, 47, 63, 65,
5 67, 141, 143, 145, 147, 151, 153 and 155;

[19] a method for determining the presence of an antibody against a periodontal disease-causing bacterium in a blood sample separated from a human body, comprising bringing the blood sample into contact with a periodontal disease-causing bacterium antigen polypeptide, said method being characterized in that the periodontal disease-causing bacterium antigen polypeptide is a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 3, 5, 15, 19, 31, 41, 141 and 145;

[20] the method according to [19], wherein the polypeptide is a polypeptide encoded by at least one polynucleotide sequence selected from the group consisting of the polynucleotide sequences represented by SEQ ID NOs: 4, 6, 16, 20, 32, 42, 142 and 146;

[21] a typing kit for strains of *Porphyromonas gingivalis*, comprising a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 151, 153 and 155;

[22] the typing kit according to [21], wherein the polypeptide is a polypeptide encoded by at least one polynucleotide sequence selected from the group consisting of the polynucleotide sequences represented by SEQ ID NOs: 152, 154 and 156;

[23] a polypeptide having the amino acid sequence represented by SEQ ID NO: 151;

[24] the polypeptide according to [23], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 152;

[25] a polypeptide having the amino acid sequence represented by SEQ ID NO: 153;

[26] the polypeptide according to [25], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 154;

[27] a polypeptide having the amino acid sequence represented by SEQ ID NO: 155;

[28] the polypeptide according to [27], comprising an amino acid sequence encoded by the nucleotide sequence represented by SEQ ID NO: 156.

[0014] In a first aspect, the present invention provides a test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium, which comprises a specific antigen protein.

[0015] According to the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium of the present invention, the specific antigen protein is reacted with a small amount of blood separated from a human body and the IgG antibody titer against a periodontal disease-causing bacterium in a blood sample (plasma or serum)
35 is measured, thereby testing the infection with the periodontal disease-causing bacterium of the subject. Generally, the following embodiments are included.

[0016] In one embodiment, the test kit comprises:

- (1) a lancet for cutting the body of the subject to make a small wound to cause slight bleeding;
- (2) a capillary for collecting the blood;
- (3) a bottle in which a solution containing a specific periodontal disease-causing bacterium antigen protein is placed;
- (4) a cylinder for compressing the inside of the bottle to separate plasma from the blood; and
- (5) a cap for hermetically sealing the bottle,

wherein the blood collected using the capillary is mixed with the solution in the bottle to cause an antigen-antibody reaction between the antigen protein in the bottle and an IgG antibody against a periodontal disease-causing bacterium when the IgG antibody is present in the blood sample, and immunoprecipitation is measured, thereby testing the infection with the periodontal disease-causing bacterium.

[0017] In another embodiment, the test kit is used in an ELISA method, in which:

- (1) an antigen protein is immobilized onto a 96-well plate for immobilizing;
- (2) a blood sample (plasma or serum) is added to the 96-well plate to cause an antigen-antibody reaction;
- (3) the 96-well plate is washed, and then an anti-human IgG secondary antibody is added thereto to cause an antigen-antibody reaction; and
- (4) the 96-well plate is washed, and then a light-developing or luminous reaction caused by the presence of the specifically bound anti-human IgG secondary antibody is carried out, thereby detecting a signal thereof.

[0018] In still another embodiment, the test kit is used in an antigen immobilization filter method, in which:

- (1) an antigen protein is immobilized onto a filter (through biotinylation, etc.);
- (2) a blood sample (plasma or serum) is added to the filter to cause an antigen-antibody reaction in the filter;
- (3) the filter is washed, and then an anti-human IgG secondary antibody is added thereto to cause an antigen-antibody reaction;
- (4) the filter is washed, and then a light-developing or luminous reaction caused by the presence of the specifically bound anti-human IgG secondary antibody is carried out, thereby detecting a signal thereof.

[0019] The characteristic feature of this aspect of the present invention resides in the periodontal disease-causing bacterium *Porphyromonas gingivalis* antigen protein contained in the test kit, and the antigen protein specifically reacts with an antibody against a periodontal disease-causing bacterium in a periodontal disease patient and also reacts with antibodies in a wide scope of periodontal disease patients.

[0020] That is, the periodontal disease-causing bacterium antigen protein contained in the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium according to the present invention is a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 1, 3, 5, 9, 15, 17, 19, 23, 31, 35, 37, 41, 43, 47, 141, 143, 145, 147, 151, 153 and 155 shown in the Sequence Listing. When one of these antigen proteins or a combination of two or more of these antigen proteins is used, the antibody titer or the type of an antibody in a periodontal disease patient can be tested and the degree or type of the infection with a periodontal disease-causing bacterium can also be tested. Further, there is inter-individual variability in the periodontal disease-causing bacterium antigens and the immunotypes of individual periodontal disease patients (types of antibodies against periodontal disease-causing bacteria). However, when the antigen proteins of the present invention are used in combination, periodontal disease patients having an extensive immunotypes can be covered. Furthermore, a periodontal disease-causing bacterium strain SU63, which is a risk factor for cardiovascular diseases or cerebrovascular diseases, can be detected.

[0021] Preferably, the periodontal disease-causing bacterium antigen protein to be used in the present invention is a polypeptide encoded by at least one nucleotide sequence selected from the group consisting of nucleotide sequences represented by SEQ ID NOs: 2, 4, 6, 10, 16, 18, 20, 24, 32, 36, 38, 42, 44, 48, 142, 144, 146, 148, 152, 154 and 156 shown in the Sequence Listing.

[0022] More preferably, the periodontal disease-causing bacterium antigen protein contained in the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium of the present invention is a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 3, 5, 15, 19, 31, 41, 63 and 67 shown in the Sequence Listing, and preferably is a polypeptide encoded by at least one polynucleotide sequence selected from the group consisting of the polynucleotide sequences represented by SEQ ID NOs: 4, 6, 16, 20, 32, 42, 64 and 68.

[0023] In a second aspect, the present invention provides a modified polypeptide for use in the above-mentioned test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium.

[0024] During discovering periodontal disease-causing bacterium antigen proteins suitable for the antibody titer test kit, the present inventors find that some of the antigen proteins have a protease activity, in spite of the fact that the antigen proteins can react with antibodies in a wide scope of periodontal disease patients, and therefore often adversely affect the testing due to the self-digestion activity or the decomposing activity on other antigen proteins thereof. Then, the present inventors produced a modified polypeptide in which the protease activity is eliminated while keeping the antigenicity of these antigen proteins by a genetic engineering technique.

[0025] That is, the modified polypeptide for use in the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium of the present invention is a polypeptide having the amino acid sequence represented by SEQ ID NO: 63, 65 or 67, and includes a modified polypeptide produced by deleting a cysteine residue at position-477 or position-488 in a wild-type polypeptide represented by SEQ ID NO: 51 or substituting the cysteine residue by another amino acid residue, preferably an alanine residue (i.e., a polypeptide represented by SEQ ID NO: 63 and 65 respectively) and a modified polypeptide produced by deleting a cysteine residue at position-471 in a wild-type polypeptide represented by SEQ ID NO: 57 or substituting the cysteine residue by another amino acid residue, preferably an alanine residue (i.e., a polypeptide represented by SEQ ID NO: 67). When one of these modified polypeptides or a combination of two or more of these modified polypeptides is used, antibodies against particularly more extensive types of periodontal disease-causing bacteria can be tested.

[0026] In a third aspect, the present invention provides a method for measuring an antibody titer against a periodontal disease-causing bacterium in a blood sample separated from a human body, comprising bringing the blood sample into contact with a periodontal disease-causing bacterium antigen polypeptide, wherein the blood sample to be used in the method is preferably blood, serum or plasma collected from a fingertip capillary or a vein, the periodontal disease-causing bacterium antigen polypeptide is a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 1, 3, 5, 9, 15, 17, 19, 23, 31, 35, 37, 41, 43, 47, 63, 65, 67, 141, 143, 145, 147, 151, 153 and 155. The periodontal disease-causing bacterium antigen polypeptide is preferably

a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 3, 5, 15, 19, 31, 41, 63 and 67.

[0027] The method according to this aspect of the present invention can be suitably carried out using the test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium of the first aspect, preferably the periodontal disease-causing bacterium plasma antibody titer test.

[0028] Further, it also becomes possible to test the degree of progression (severity) of a periodontal disease on the basis of the antibody titer.

[0029] In a fourth aspect, the present invention provides a typing kit for a *Porphyromonas gingivalis* strain. The kit enables the typing of *Porphyromonas gingivalis* strains, particularly strain FDC381 and strain SU63, occurring in a blood sample separated from a human body to examine whether or not any one of the strains is present in the sample. The kit comprises a polypeptide having at least one amino acid sequence selected from the group consisting of the amino acid sequences represented by SEQ ID NOs: 151, 153 and 155, and the polypeptide is preferably encoded by at least one polynucleotide selected from the group consisting of the polynucleotides represented by SEQ ID NOs: 152, 154 and 156.

EFFECT OF THE INVENTION

[0030] According to the present invention, it becomes possible to provide a periodontal disease test kit, which enables the high speeded and highly accurate testing on a periodontal disease. It also becomes possible to provide a periodontal disease test kit, which enables the objective testing on a periodontal disease without depending on the skill level of practitioners.

[0031] Further, when the periodontal disease test kit of the present invention becomes widely used, a periodontal disease can be tested in a unified manner in dental clinics across the country. Further, by making a database of the measurement results obtained in the test, it becomes possible to establish or modify the determination criteria or treatment guidelines for periodontal diseases.

[0032] Furthermore, the periodontal disease test kit of the present invention can detect a risk factor for cardiovascular or cerebrovascular diseases.

BRIEF DESCRIPTION OF DRAWINGS

[0033]

Fig. 1 illustrates antigen-antibody reactions between periodontal disease-causing bacterium antigen proteins and human sera. A: an antigen-antibody reaction with healthy subject sera; and B and C: antigen-antibody reactions with periodontal disease patient sera.

Fig. 2 illustrates a SDS-PAGE electrophoresis pattern of a *Porphyromonas gingivalis* strain FDC381 antigen protein roughly purified using an antibody column. Lane A: an antigen protein roughly purified from a healthy subject serum column; lane B: an antigen protein roughly purified from a patient serum 1 column, lane C: an antigen protein roughly purified from a patient sera 2.

Fig. 3 illustrates antigen-antibody reactions between a roughly purified strain FDC381 antigen protein and sera. Lane A: an antigen protein roughly purified from a healthy subject serum column, lane B: an antigen protein roughly purified from a patient serum 1 column, lane C: an antigen protein roughly purified from a patient serum 2 column.

Fig. 4 illustrates an SDS-PAGE electrophoresis pattern of a *Porphyromonas gingivalis* strain SU63 antigen protein roughly purified using an antibody column. Lane A: an antigen protein roughly purified from a healthy subject serum column, lane B: an antigen protein roughly purified from a patient serum 1 column, lane C: an antigen protein roughly purified from a patient serum 2 column.

Fig. 5 illustrates antigen-antibody reactions between a roughly purified strain SU63 antigen protein and sera. Lane A: an antigen protein roughly purified from a healthy subject serum column, lane B: an antigen protein roughly purified from a patient serum 1 column, lane C: an antigen protein roughly purified from a patient serum 2 column.

Fig. 6 illustrates the results of the Mascot search of antigen proteins.

Fig. 7 illustrates the genetic information on identified antigen proteins and the results of selection thereof.

Fig. 8 illustrates antigen-antibody reactions between sera and synthetic antigen proteins.

Fig. 9 illustrates signal values of the antigen-antibody reactions between sera and synthetic antigen proteins.

Fig. 10 illustrates SDS-PAGE electrophoresis patterns showing the stability of synthetic antigen proteins.

Fig. 11 illustrates signal values of the antigen-antibody reactions between healthy subject sera and synthetic antigen proteins.

Fig. 12 illustrates signal values of the antigen-antibody reactions between diverse patient sera and antigen proteins.

Fig. 13 illustrates the summary of the antigen-antibody reactions between diverse patient sera and antigen proteins.

Fig. 14 illustrates the stability and antigenicity of modified polypeptides.

Fig. 15 illustrates antigen-antibody reactions between sera and synthetic antigen proteins.

Fig. 16 illustrates signal values of the antigen-antibody reactions between sera and synthetic antigen proteins.

Fig. 17 illustrates the summary of the antigen-antibody reactions between diverse sera and antigen proteins.

5 Fig. 18 illustrates signal average values and S/N values of the antigen-antibody reactions between healthy subject sera and patient sera and antigen proteins.

Fig. 19 illustrates ROC curves and AUC values determined for signal values of individual sera against diverse antigen proteins.

Fig. 20 illustrates the antigen-antibody reactions between sera and diverse antigen proteins (strain SU63).

10 Fig. 21 illustrates the summary of the antigen-antibody reactions between sera and antigen proteins (strain SU63).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 **[0034]** The present invention is described more in detail with reference to examples hereinbelow. However, the present invention is not limited by the examples.

[0035] Throughout the whole of the examples, *Porphyromonas gingivalis* strain FDC381 and strain SU63 are used as the periodontal disease-causing bacteria. Strain FDC381 is sold by Summit Pharmaceuticals International Corporation, and strain SU63 is available in the form of a type-II or type-IV fimbriated strain.

20 Production of an antibody column

[0036] When a human is infected with a periodontal bacterium, a variety of antibodies against the periodontal disease-causing bacterium antigen are produced in the human body through an immune response. Antigens recognized by the produced antibodies are varied depending on the difference in immune responses in the individuals.

25 **[0037]** Therefore, it is examined as to what type of antigen among the components in a periodontal disease-causing bacterium antigen preparation solution is targeted by the antibodies, and sera for use in the purification of more antigens are selected on the basis of the results of the examination.

30 Preparation of antigen proteins

[0038] An antigen preparation solution (Institute of Immunology Co., Ltd., 200 μ g protein equivalent), which was prepared by disrupting cells of *Porphyromonas gingivalis* (strain FDC381 and strain SU63) with ultrasonic waves and then subjecting to ultracentrifugation to collect a supernatant fraction, was added with phosphate-buffered saline (PBS) to prepare a solution having a volume of 270 μ l. Trichloroacetic acid was added to the solution, the resultant mixture was allowed to stand in ice and then centrifuged at a low temperature, and then a supernatant was removed therefrom. Ice-cold ethanol was added to a precipitate to wash, the resultant solution was centrifuged again at a low temperature, and then a supernatant was removed therefrom. The above-mentioned procedure was repeated two times. The precipitate was air-dried, and then added with 120 μ l of PBS containing 0.06% of sodium dodecyl sulfate (SDS) to dissolve the precipitate. In this manner, an antigen protein solution for each of the strains was prepared.

40 Quantification of antigen proteins

[0039] Each of prepared standards (concentrations: 1000, 500, 250, 125, 62.5 or 31.25 ng/ μ l) (25 μ l), an antigen protein solution diluted with PBS (25 μ l) as a control was added to each well of a 96-well plate, and then a protein working solution (a mixture of Thermo scientific, Reagent A:B = 50:1) (200 μ l) was added to each well. Subsequently, the reaction solution was stirred with a shaker and then incubated in a constant-temperature-humidity unit at 37°C for 30 minutes. Subsequently, an absorbance at 577 nm was measured using a plate reader (Intermed, NJ2000). In this manner, the collected antigen proteins were quantified.

50 SDS-PAGE electrophoresis

[0040] Each of the quantified *P. gingivalis* bacteria (strain FDC381 and strain SU63) antigen proteins was prepared into a solution having a protein concentration of 400 ng/ μ l using a sample buffer (Invitrogen). The prepared sample was thermally denatured and then subjected to SDS-PAGE electrophoresis (a protein solution: 5 μ l).

55 **[0041]** A gel that had been subjected to SDS-PAGE electrophoresis was subjected to blotting on a polyvinylidene fluoride membrane (a PVDF membrane) using an iBlot dry blotting system (Invitrogen).

[0042] After transferring onto the PVDF membrane, the electrophoresed layout (a molecular weight marker, strain FDC381, strain SU63) was cleaved as one set. A cleaved one set of slits was subjected to Ponceau staining to confirm

the occurrence of blotting of proteins. The remaining slits were placed in a Falcon tube and then immersed in a blocking solution (Tris-buffered saline (abbreviated as "TBS", hereinbelow) containing 3% of skim milk and 0.1% of Tween 20).

Antigen-antibody reaction using serum

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[0043] After the removal of the blocking solution from the blocked slit, a serum reaction solution (a solution prepared by adding 8 μ l of a serum collected from a healthy subject or a periodontal disease patient to 20 ml of TBS containing 3% of skim milk) was added to the slit, and the resultant solution was shaken at room temperature. Subsequently, the slit was washed with TBS containing 0.05% of Tween 20. After washing, a 5000-fold-diluted horseradish peroxidase-conjugated goat anti-human IgG antibody reaction solution (a solution prepared by adding an anti-human secondary antibody (CHEMICON) to TBS containing 3% of skim milk) was added to the slit, and the resultant product was shaken at room temperature. Subsequently, the slit was washed with TBS containing 0.05% of Tween 20. After washing, the slit was immersed in TBS containing 0.61 mg/mL of 4-methoxy-1-naphthol and 0.018% of aqueous hydrogen peroxide, the development of a color was confirmed, and the slit was washed with purified water and then dried.

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[0044] The results of the reactions between the antigen proteins and the human sera are shown in Fig. 1.

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[0045] Almost no signal was observed in the antigen-antibody reaction with the healthy subject sera (Fig. 1A: normal subject sera).

[0046] On the other hand, clear signals were observed in the antigen-antibody reaction with periodontal disease patient sera, and the patterns of the signals were various (Figs. 1B and 1C). The various signals observed in the antigen-antibody reaction with patient sera could be roughly classified into two groups, i.e., signals having clear bands (Fig. 1B) and signals showing wholly spread smears (Fig. 1C). The various antigen-antibody reaction patterns are formed due to the difference in sizes and specificity to the strains.

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[0047] Signals having the below-mentioned sizes were observed specifically strong in many of the patient sera.

46 kDa (antigen proteins of strain FDC381 and strain SU63)

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25 to 37 kDa (antigen proteins of strain FDC381 and strain SU63)

100 to 110 kDa (antigen proteins of strain FDC381 and strain SU63)

57 kDa (an antigen protein of strain SU63)

150 to 250 kDa (an antigen protein of strain SU63)

[0048] Reviewing the results of the antigen-antibody reactions using periodontal disease patient sera, roughly two types of signal patterns (clear bands and wholly spread smears) were observed, and various antigen-antibody reaction patterns were formed depending on the combinations (sizes, types of strains) of the antigens. That is, it was demonstrated that the antibodies produced in persons infected with *P. gingivalis* bacteria were varied and there were cases in which some proteins were recognized as antigen proteins in some sera but were not at all recognized in the other sera. This fact agrees with a report that antibodies contained in varied periodontal disease patient sera utilize a variety of proteins as antigen thereof. Therefore, it was demonstrated that, for the purpose of measuring the infection with *P. gingivalis* bacteria employing antibody titers, various test antigen proteins were needed.

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[0049] Further, it is considered that, when an antigen protein that is common between strain FDC381 and strain SU63 is used in the test, it is difficult so far as to identify the strains. On the other hand, when only an antigen protein specific to a strain is used in the test, if positive results are obtained, it is suspected that the infection with the strain may occur.

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[0050] Although the antigen-antibody reaction patterns observed in patient sera were various, no significant difference in patterns was observed between sera from first-visit patients (FV) and sera from maintenance patients (SPT). Thus, antigen proteins that showed strong signals in patient sera were identified and they were made candidates for the antigens to be used in the test.

[0051] As shown in the results, a group of antigen proteins of 46 kDa, 25 to 37 kDa, 100 to 110 kDa, 57 kDa and 150 to 250 kDa are mentioned as the band showing strong signals against many patient sera. Then, the presence of antigenicity of the group of proteins against sera from various patients was determined. The results are shown in

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Table 1.

Reactions of patient sera against antigen protein groups									
5		Patient serum No.	Plasma antibody titer		Common antigen			SU63-specific antigen	
			FDC381:	SU63:	46kDa	25-37kDa	100-110kDa	57kDa	150-250kDa
10	FV	7056	7.36	0.1	○				
		7457	6.18	0.6	○	○	○		○
		6991	4.43	0.9				○	
15		7492	3.76	0.59	○		○		○
		6809	3.35	0.9		○	○	○	
		6816	1.89	0.76	○				
		7125	1.89	0.09	○	○	○		○
20		7500	1.87	0.93		○	○		○
		7107	1.8	0.06		○	○		○
		7835	1.64	0.19	○				
25		7350	5.45	10.18	○				
		7523	15.11	9.08		○	○		○
		7524	44.9	7.35		○	○	○	○
30		6921	7.55	5.07	○		○	○	
		6896	4.36	4.37		○	○		○
		6975	7.98	4.31	○	○	○	○	○
		6923	5.2	4.11	○	○	○	○	
35		7393	15.2	4.07	○	○			○
		6926	5.63	3.61	○			○	
	7495	11.38	2.61			○	○		
40	6817	0.11	1.32		○				
	6820	0.68	2.01	○		○		○	
	6828	0.55	1.97						
	6855	0.58	1.03		○	○			
45	6863	-0.32	2.2	○					
	6867	0.93	1.46	○			○		
	6874	0.03	2.83	○		○	○		
	6881	0.66	1.4	○	○	○			
50	6889	0.28	2.23	○	○	○			
	6904	0.88	1.51	○					
	6935	0.63	1.31	○					
55	6968	0.08	1.93	○	○			○	

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(continued)

Reactions of patient sera against antigen protein groups									
		Patient serum No.	Plasma antibody titer		Common antigen			SU63-specific antigen	
			FDC381:	SU63:	46kDa	25-37kDa	100-110kDa	57kDa	150-250kDa
SPT	381 ≥ 1 SU ≥ 1	7082	133.77	39.79	○	○	○		
		6918	30.24	6.71	○	○	○	○	
		6870	13.32	5.85	○	○	○	○	
		6980	13.36	5.52	○	○	○		○
		6872	6.19	4.66	○		○	○	
		7001	3.77	3.32	○	○	○		○
		6802	1.16	3.05	○	○	○		
		7268	23.57	2.87	○	○	○	○	
		7004	1.54	2.31		○	○		○
	381 ≥ 1 SU < 1	6823	4.57	0.44		○	○		
		7234	3.25	0.63	○		○	○	
		7230	3.22	0.87	○		○		
		7381	3.22	0.09	○	○	○	○	
		7210	1.74	0.32	○	○	○	○	
		7253	1.57	-0.19	○				○
7263		1.49	0.07				○		
7135		1.49	0.14	○	○	○			

[0052] As apparent from Table 1, it was demonstrated that, when combinations of these five types of antigen protein groups were used, it became possible to confirm the presence of an antibody against a *P. gingivalis* bacterium in all of the patient sera and the testing on extensive periodontal disease patients could be covered.

Selection of sera to be used in production of immunoaffinity column

[0053] Next, for the purpose of purifying the five types of antigen protein groups using an immunoaffinity column, sera to be used for the production of the column were selected. In this selection, two types of patient serum pools were used for the production of the antibody column with taking the antigen-antibody reaction patterns and the plasma antibody titer measurements in patient sera into consideration. That is, No. 7350, No. 6921 and No. 6870 serum pools, which showed clear bands against target antigens and had high plasma antibody titers, were used for the production of the antibody column for purifying antigen protein groups of 46 kDa (strain FDC381 and strain SU63) and 57 kDa (strain SU63). On the other hand, No. 7107, No. 7523 and No. 6980 serum pools, which showed wholly spread smear-like band patterns but showed strong signals against target antigens and had high plasma antibody titers, were used for the production of the antibody column for purifying antigen protein groups of 25 to 37 kDa (strain FDC381 and strain SU63), 100 to 110 kDa (strain FDC381 and strain SU63) and 150 to 250 kDa (strain SU63). As controls for comparison purposes, serum pools of healthy subjects NAI, TOM and KOB were used in the production of the antibody column for purifying antigen proteins from healthy subjects.

[0054] On the other hand, as antigen proteins to be used in the plasma antibody titer test, five types of antigen protein groups (46 kDa, 25 to 37 kDa, 100 to 110 kDa, 57 kDa, and 150 to 250 kDa) were selected. For the purpose of purifying the five types of antigen protein groups using immunoaffinity columns, three types of columns were produced. That is, column A: a healthy subject serum column (NAI, TOM and KOB serum ligands), column B: a column for purifying a clear band (No. 7350, No. 6921 and No. 6870 serum ligands) and column C: a column for purifying a smear-like band (No.

7107, No. 7523 and No. 6980 serum ligands) were produced.

Purification of antigen proteins using immunoaffinity columns

5 **[0055]** For identifying the selected antigen proteins, it is needed to purify the antigen proteins from antigen preparation solutions. Then, antibody columns were produced using the selected sera and the antigen proteins were purified.

[0056] Immunoaffinity columns were produced in accordance with the method mentioned below based on Masato OKADA and Kaori MIYAZAKI ed., "Experiment note of proteins (second volume)", Yodosha, pp. 131-136 (1990) and Kiyoshi TAKATSU et al., ed., "Antibody experiment manual for study of proteins", Yodosha, pp. 53-61 (2005).

10 **[0057]** A periodontal disease patient serum or a healthy subject serum (1.5 ml) (each serum: 500 μ l \times 3 samples) was added with an antibody binding buffer (a 50-mM tartrate buffer, 3 M NaCl, pH 9.0) (2.5 ml) and sodium chloride (0.26 g), and then mixed, thereby preparing an antibody reaction solution.

[0058] On the other hand, protein G sepharose (GE Healthcare) was added to an Econo-PACK column (BIO-RAD) and washed with ultrapure water and then with an antibody-binding buffer. The whole of the prepared antibody reaction solution was added to the column, the column was hermetically sealed, and then solution was stirred using a rotator. After stirring, the antibody reaction solution was removed, and then the column was washed with an antibody-binding buffer (a 50-mM tartrate buffer, 3 M NaCl, pH 9.0).

15 **[0059]** A cross-linker BS3 (PIERCE) (100 mg) was dissolved in a cross-linker solution (0.2 M triethanolamine-HCl, pH 8.0) (6.8 ml), and the resultant solution was dispensed into three columns (a healthy subject serum column and two patient serum columns) and then stirred at room temperature using a rotator.

[0060] After the removal of the cross-linker solution from the column, the column was washed with a blocking solution (0.2 M ethanolamine-HCl, pH 8.0). The column was hermetically sealed, and then the blocking solution was added to the column and stirred at room temperature using a rotator. Subsequently, the blocking solution was removed, then the column was washed with an elution solution (0.1 M glycine-HCl, pH 2.8) and then with 50 mM Tris-HCl (pH 7.5), and then the column was added with 50 mM Tris-HCl (pH 7.5) and stored (immunoaffinity columns A, B and C).

Preparation of antigen protein samples

30 **[0061]** A solution (270 μ l) was prepared by adding PBS to an antigen preparation solution of *P. gingivalis* bacteria (strain FDC381 and strain SU63) (Institute of Special Immunity Co. Ltd., 200 μ g protein equivalent). Trichloroacetic acid was added to the solution, and the resultant solution was allowed to stand in ice and then centrifuged at a low temperature to remove a supernatant. Ice-cold ethanol was further added to the resultant solution to wash the precipitate, and then the resultant solution was centrifuged again at a low temperature to remove a supernatant. The above-mentioned procedure was repeated three times.

35 **[0062]** After air-drying the precipitate, PBS (200 μ l) containing 0.06% of SDS was added to dissolve the precipitate. Subsequently, the protein solutions for each of the strains were combined. PBS (containing 0.01% of Brij-35 and 0.2% of CHAPS) in the same volume as that of the combined protein solution was added to the combined protein solution to dissolve the precipitate, thereby preparing antigen protein samples for each of the strains.

40 Quantification of antigen proteins

[0063] Each of the prepared standards (concentrations: 1000, 500, 250, 125, 62.5 and 31.25 ng/ μ l) (25 μ l), an antigen protein sample diluted with PBS (25 μ l) and PBS (25 μ l) as a control were added to each well of a 96-well plate, and then a protein working solution (a mixture of Thermo scientific, Reagent A:B = 50:1) (200 μ l) was added to each well. Subsequently, the reaction solution was stirred with a shaker and then incubated in a constant-temperature-humidity unit at 37°C for 30 minutes. Subsequently, an absorbance at 577 nm was measured using a plate reader (Intermed, NJ2000). In this manner, the antigen proteins in the samples were quantified.

Purification of antigen proteins using immunoaffinity columns

50 **[0064]** An antigen protein sample (about 133 μ g/1.5 ml) was applied onto each of PBS-equilibrated immunoaffinity columns (A, B and C) (the buffer composition for the sample: PBS containing 0.03% SDS, 0.005% Brij-35 and 0.2% CHAPS (Dojin Laboratories). The column was stirred using a rotator at room temperature, a flow-through was collected and stored as a sample. A wash buffer (0.005% Brij-35, 0.1% CHAPS, 20 mM Tris-HCl and 500 mM NaCl, pH 7.5) was added to the column, and then the column was stirred using a rotator at room temperature to wash the column. After stirring, a flow-through was collected and stored as a sample. This procedure was repeated three times.

55 **[0065]** After the column was washed with ultrapure water, an elution buffer (0.05% trifluoroacetic acid) (5 ml) was added two times, and an eluted protein solution was collected and lyophilized.

SDS-PAGE electrophoresis

[0066] A portion of the antigen protein sample, a flow-through obtained in each step and the eluted protein was separated and then prepared into a sample buffer containing mercaptoethanol at a final concentration of 5%.

[0067] The prepared sample was thermally denatured and then subjected to SDS-PAGE electrophoresis (10 μ l for CBB staining, 5 μ l for antigen-antibody reaction).

[0068] A gel that had been subjected to SDS-PAGE electrophoresis was washed with distilled water, then immersed in a CBB staining solution, and stirred at room temperature. Subsequently, the gel was washed with distilled water until bands could be observed clearly.

[0069] The gel that had been subjected to SDS-PAGE electrophoresis was also subjected to blotting on a PVDF membrane using an iBlot dry blotting system (Invitrogen).

[0070] After transferring onto the PVDF membrane, a layout was cleaved as one set and then immersed in a blocking solution (TBS containing 3% of skim milk and 0.1% of Tween 20).

Antigen-antibody reactions using sera

[0071] With respect to a slit that had been subjected to blocking, the blocking solution was removed therefrom, then a serum reaction solution (a solution prepared by adding 8 μ l of a serum to 20 ml of TBS containing 3% of skim milk) was added to the slit, and the resultant product was shaken at room temperature. The sets of sera added are as follows.

Set A (healthy subject sera): NAI, TOM, KOB

Set B (patient sera 1): No. 7350, No. 6921

Set C (patient sera 2): No. 7107, No. 7523, No. 6980

[0072] The slit was washed with TBS containing 0.05% of Tween 20, a 5000-fold-diluted horseradish peroxidase-conjugated sheep anti-human secondary antibody reaction solution (a solution prepared by adding a human secondary antibody (CHEMICON) to TBS containing 3% of skim milk) was added thereto, and the resultant product was stirred at room temperature.

[0073] Subsequently, the slit was washed with TBS containing 0.05% of Tween 20, the slit was immersed in a color-developing solution (TBS containing 0.61 mg/ml of 4-methoxy-1-naphthol and 0.018% of aqueous hydrogen peroxide), and the development of color in the slit was confirmed, and then the slit was washed with purified water and dried.

Purification of strain FDC381 antigen protein

[0074] The SDS-PAGE electrophoresis patterns of the antigen proteins purified from the columns are shown in Fig. 2.

[0075] A flow-through obtained after the application of the antigen protein samples onto immunoaffinity columns was confirmed, and any significant difference was not observed between a healthy subject serum column and patient serum columns. However, when purified proteins were observed, proteins purified from the patient serum columns apparently showed stronger signals in CBB staining (Fig. 2, lanes B and C) as compared with a protein purified from the healthy subject serum column (Fig. 2, lane A). Particularly, a band having a size of about 50 kDa showed a significantly strong signal in the patient serum columns. A band having a size larger than 25 kDa also showed a strong signal in the patient sera (Fig. 2, lanes B and C).

Purification of strain SU63 antigen protein

[0076] The SDS-PAGE electrophoresis patterns of the antigen proteins purified from the columns were shown in Fig. 4.

[0077] When proteins purified after the application of the antigen protein samples onto immunoaffinity columns were observed, antigen proteins purified from the patient serum columns apparently showed stronger signals in CBB staining (Fig. 4, lanes B and C) as compared with an antigen protein purified from the healthy subject serum column (Fig. 4, lane A), although it was not so clear than in the case of strain FDC381. A particularly significant difference was observed between a band having a size slightly larger than 25 kDa and a band having a size slightly larger than 50 kDa. A strong signal observed in the purification of strain FDC381 also tended to show a strong signal in the purification of strain SU63 (Fig. 4).

Antigen-antibody reactions of antigen proteins

[0078] The results of the antigen-antibody reactions of antigen proteins of strain FDC381 and strain SU63 which were subjected to SDS-PAGE electrophoresis are shown in Fig. 3 and Fig. 5, respectively.

[0079] Serum set A (healthy subject sera) reacted with proteins each having a high molecular weight but hardly with proteins each having a size of 150 kDa or less among any of the antigen proteins eluted from the healthy subject serum column and the patient serum columns (Figs. 3 and 5).

[0080] On the other hand, serum set B (the patient sera 1; a group of sera used in the production of column B) strongly reacted with the antigen proteins eluted from the patient serum columns (Figs. 3 and 5, lanes B and C of the "patient sera 1"). Note that stronger signals were observed in the proteins eluted from the patient serum column (column B).

[0081] Similar to serum set B, serum set C (the patient sera 2; a group of sera used for the production of column C) also strongly reacted with the proteins eluted from the patient serum columns (Figs. 3 and 5, lanes B and C of the "patient sera 2"). On the other hand, serum set C relatively reacted also with the proteins eluted from the healthy subject serum column (Figs. 3 and 5, lane A of the "patient sera 2"). Note that stronger signals were observed in the proteins eluted from the patient serum column (column C).

[0082] When the proteins eluted from the immunoaffinity columns were observed, the number of the proteins eluted from the patient serum columns was apparently larger as compared with that of the proteins eluted from the healthy subject serum column. It is considered that this is because many antibodies against *P. gingivalis* bacteria were bound to the protein G sepharose in each of the patient serum columns, and a larger number of the antibodies could be bound to the *P. gingivalis* bacterium antigen proteins during purification process using the same to be purified.

[0083] From the above, candidates for the antigen proteins were selected by comparing the proteins purified using the healthy subject serum column with the proteins purified using the patient serum columns.

[0084] As a result of the comparison among the purified antigen proteins, it was found that antigen proteins shown in Table 1 as candidates, each of which contained a common antigen (46 kDa, 25 to 37 kDa, and 100 to 110 kDa), were contained in larger amounts in the proteins purified from the patient serum columns than in the proteins purified from the healthy subject serum column. Therefore, it is possible to select as candidates for the antigen proteins that could be used in the test kit by elucidating the entire constitutions of the purified proteins and comparing both proteins. Then, all of the proteins constituting each of the purified proteins were subjected to mass spectrometry and identified.

Mass spectrometry

[0085] In the same manner as mentioned above, a roughly purified antigen protein was separated by subjecting to SDS-PAGE electrophoresis, all of three lanes of each of bands of a CBB-stained eluted protein (column A, B and C) were cleaved in one lump. The cleaved gel was placed in a Falcon tube, ultrapure water (200 μ l) was added thereto, and the resultant solution was subjected to mass spectrometry as mentioned below.

Preparation of samples for mass spectrometry

[0086] The prepared sample was protein-digested using ProGest (Genomic Solutions) workstation, reduced with dithiothreitol at 60°C, and then cooled to room temperature. Subsequently, the resultant product was alkylated with iodoacetamide. The alkylated product was incubated at 37°C for 4 hours in the presence of trypsin, and formic acid was added to the solution to terminate the reaction. A supernatant obtained after the termination of the reaction was used as a sample for the analysis.

LC/MS/MS

[0087] The prepared sample was subjected to a nano-LC/MS/MS analysis using ThermoFisher LTQ Orbitrap XL.

[0088] A hydrolysis product (30 μ l) was applied onto an ID C12 column (Jupiter Proteo, Phenomenex) vented column having a size of 5 mm \times 75 μ m. The gradient elution was carried out at 300 nl/min on an ID C12 column having a size of 15 cm \times 75 μ m.

[0089] With respect to MS/MS, analysis was carried out using a mass spectrometer that was operated by data-dependent mode, six most abundant ions. The Orbitrap MS scan was carried out at an FWHM resolution of 60000.

[0090] The MS/MS data was searched using a Mascot (www.matrixscience.com) local copy.

[0091] The parameters for the LC/MS/MS search were set as follows.

Type of search: MS/MS ion search

Classification: whole bacteria or whole organism species

Enzyme: trypsin

Default modification: carbamidemethylation

Variable modification: oxidation, acetylation, pyroglutamylation and deamidation

Mass value: monoisotopic

Mass of protein: not limited

Peptide mass tolerance: ± 10 ppm (Orbitrap)
 Fragment mass tolerance: ± 0.5 dalton (LTQ)
 Maximum value of error cutting: 2

5 SCAFFOLD

[0092] A sample was processed in Scaffold algorithm (www.proteomesoftware.com) using a DAT file created by Mascot. LTQ Orbitrap XL data parameter had identified a protein that matches two or more peptides.

[0093] The results are shown in Fig. 6.

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Strain FDC381

[0094] With respect to the analyzed three samples (A to C; A: a healthy subject serum column, B: a patient serum column 1, C: a patient serum column 2), the Mascot search was carried out on the whole bacteria. As a result, 28 types in total of proteins were identified (Fig. 6, left). Among the identified proteins, each of proteins of Nos. 9, 17 and 18 was a part of an IgG antibody. Among the 28 types of proteins, 15 types were identified only in protein groups purified from the patient serum columns (B and C). On the other hand, the other 10 types of proteins were observed also in the protein groups purified from the healthy subject serum column (A), but the number of spectral counts was high in the protein groups purified from the patient serum columns (B and C).

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Strain SU63

[0095] With respect to the analyzed three samples (A to C), the Mascot search was carried out on the whole bacteria. As a result, 28 types in total of proteins were identified (Fig. 6, right). Among the identified proteins, each of proteins of Nos. 8 and 12 was a part of an IgG antibody. Among the 28 types of proteins, 20 types were identified only in protein groups purified from the patient serum columns (B and C). On the other hand, 5 types of proteins were observed also in the protein groups purified from the healthy subject serum column (A), but the number of spectral counts was high in the protein groups purified from the patient serum columns (B and C). The protein of No. 22 was identified only in the healthy subject serum column (A).

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[0096] Reviewing the results obtained this time, in both strain FDC381 and strain SU63, the number of types and the amount together were apparently larger in the protein groups eluted from the patient serum columns than those in the protein groups eluted from the healthy subject serum column. This fact suggests that antigen proteins maintained in antibodies in patient sera were purified by an immunoaffinity column method. Among the identified proteins, proteins that have been already reported as antigens were included. From this fact, it is suggested that the proteins produced by the purification employing the immunoaffinity column method in this time also are highly probably antigen proteins.

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Selection of synthesized proteins

[0097] Proteins that have been observed in the two times of the antigen protein identification were organized, and proteins to be actually synthesized were selected.

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[0098] For the proteins that have been identified, those proteins which had been identified in both strains based on accession numbers were described as the same line, genetic information of the proteins were examined to determine whether or not the function is known and whether or not antigenicity is known, and the proteins were classified (Fig. 7).

[0099] With respect to the matter that whether or not the proteins were specific to patients, a case where the spectrum count of a protein that had been identified from the healthy subject column was apparently high was determined "x", a case where the spectrum count of a protein that had been identified from the healthy subject column and the spectrum count of a protein that had been identified from the patient column were almost the same was determined " Δ ", and a case where the spectrum count of a protein that had been identified from the patient column was apparently high was determined "o" (primary selection). In addition, for the purpose of determining whether or not proteins were specific to *P. gingivalis* bacteria, proteins having high homology were examined on the basis of nucleotide sequences. A protein having slight homology with other bacterial species was determined " Δ ", a protein having high homology with other bacterial species was determined " \times ", and a protein having low homology and being specific to *P. gingivalis* bacteria was determined "o" (secondary selection).

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[0100] Proteins that had been identified by the two times of mass spectrometry were organized. As a result, 37 types in total of proteins were identified as candidate antigen proteins. As a result of the overlapping of amino acid sequences, the primary selection and the secondary selection, proteins that fulfilled all of the requirements were 13 types in total of proteins, i.e., proteins of Nos. 3, 4, 6, 9, 10, 15, 16, 19, 24, 26, 37, 32 and 37.

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[0101] It was thought to select patient-specific and *P. gingivalis*-bacteria-specific proteins through the primary selection

and the secondary selection. However, the selection of candidate proteins in this stage might cause the loss of available antigen proteins. For example, in the case of a protein that is not selected because of its non-patient-specificity, if the protein has a satisfactorily higher antibody titer against patients than that against healthy subjects, the protein is a protein that can be used as a test antigen. Therefore, it was considered that the loss of candidate antigen proteins could be better prevented when antigenicity of actually synthesized proteins was evaluated.

[0102] Then, protein synthesis was carried out using, as candidates, 31 types in total of proteins, other than protein Nos. 1, 17, 18, 25, 30 and 31 of which the amino acid sequences were overlapped, among 37 types in total of proteins that had been identified by mass spectrometry.

Protein synthesis and evaluation of antigenicity of synthesized proteins

[0103] For the purpose of evaluating whether or not the 31 types of candidate proteins actually showed antigenicity, proteins were synthesized and the antigenicity of the synthesized proteins was subsequently evaluated using healthy subject sera and patient sera.

[0104] Genetic information on each of the proteins of interest was obtained from a database (antigen protein information), desired genes were amplified from genomic DNA of a *P. gingivalis* bacterium strain using the synthesized primer pairs represented by SEQ ID NOs: 71 to 132 in the Sequence Listing and cloned into plasmid DNA (a pDONR vector) using a Gateway system (Invitrogen).

[0105] Subsequently, the pDONR vector DNA into which each of the genes had been cloned was treated with a restriction enzyme and then ligated to a protein expression vector (CellFree Sciences Co., Ltd.: a pEu vector) that had been treated with the same restriction enzyme. A ligation product was introduced into a cell of *Escherichia coli* (*E. coli*) by transformation. Subsequently, a clone having the gene introduced thereto was selected.

[0106] Plasmid DNA was collected from the selected clone and then subjected to sequence analysis. With respect to a clone in which any significant mutation was not recognized from the results of the sequence analysis, a large amount of a plasmid was prepared, a protein was synthesized using a wheat germ cell-free protein synthesis system, and the resultant protein was purified using a GST tag.

[0107] It was tried to carry out in vitro protein synthesis with respect to all of 31 types of genes. However, with respect to No. 33, the cloning into the protein expression vector was not achieved; and with respect to No. 5, the protein synthesis was not achieved or the amount of a synthesized protein was extremely small. Therefore, antigenicity was evaluated on the remaining 29 types of proteins. For the evaluation of antigenicity with respect to the synthesized proteins, dot blot analysis was carried out.

Dot blotting

[0108] 29 types of antigen proteins were subjected to dot blotting. The amount of each of the antigen proteins was adjusted to 50 ng, four sets of dot blot were produced for each of the antigen proteins. With respect to the proteins of Nos. 32 and 35, it was impossible to quantify the proteins and therefore a solution of the synthesized protein (4 μ l) was subjected to dot blotting. After the dot blotting, the dot blot was immersed in a blocking solution (a TBS solution containing skim milk (3%) and Tween 20 (0.1%)).

[0109] As a primary antibody, a portion (8 μ l) of a healthy subject serum pool prepared by mixing sera from normal subjects NAI, TOM and KOB (3 μ l for each) together, a portion (8 μ l) of a patient serum pool 1 prepared by mixing sera from periodontal disease patients Nos. 7350 and 6921 (4 μ l for each) together, and a portion (8 μ l) of a patient serum pool 2 prepared by mixing sera from periodontal disease patients Nos. 7107, 7523 and 6980 (3 μ l for each) together were used. Each of the portions was added to TBS (20 ml) containing 3% of skim milk. The slit of the dot blot was added to each of the three kinds of antibody solutions, thereby carrying out an antigen-antibody reaction.

[0110] Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, a 5000-fold-diluted horseradish peroxidase-conjugated goat anti-human IgG antibody reaction solution (a solution prepared by adding an anti-human secondary antibody (CHEMICON) to TBS containing skim milk (3%)) was added to the resultant mixture and then shaken at room temperature. Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, the slit was immersed in TBS containing 4-methoxy-1-naphthol (0.61 mg/ml) and hydrogen peroxide (0.018%), the occurrence of development of color was confirmed, and then the slit was washed with purified water and dried.

[0111] The layouts of the synthesized proteins that had been subjected to dot blotting and the results of the dot blot analysis are shown in Fig. 8.

[0112] As a result of the dot blot analysis, in the reaction with the healthy subject serum pools, development of color on spots was recognized in Nos. 2, 6, 10, 26, 29, 32 and 35. While in the reaction with the patient serum pool 1, development of color on spots was observed in Nos. 2, 3, 4, 6, 9, 10, 11, 13, 19, 21, 22, 24, 26, 28, 32 and 35. In the reaction with the patient serum pool 2, development of color on spots was observed in Nos. 2, 3, 4, 6, 10, 11, 19, 24, 26, 32 and 35.

[0113] When confirmed with visual observations, in each of the spots, the development of color in the patient serum pools was stronger than that in the healthy subject serum pools. In the patient serum pools, development of color on spots, which was not observed in the healthy subject serum pools, was confirmed.

5 Detection of signal values

[0114] Subsequently, the results of the dot blot analysis were quantified in the following manner.

10 1. Using ImageQuant LAS4000 (GE Healthcare), an image of the membrane was taken. After adjusting the focus, the image was taken under the following conditions.
Conditions for image capture

15 Exposure Type: Precision
Exposure Time: 1/100 sec.
Sensitivity/Resolution: Standard

20 2. Using ImageQuant TL (GE Healthcare), the dot signal values in the image taken were determined.
Conditions for signal value capture
In an Array analysis mode, a signal value was digitized in conjunction with the spot size and placement of the dots.
As for the setting of background, a part adjacent to the spot was set.
3. After digitizing, the signal values for individual spots were summarized in a table. A signal value that is the largest value among the signal values of spots on which any antigen protein was not arranged (blank), was employed as a reference value, and a signal value that was smaller than the reference value was deemed as a noise. The quantified signal values are shown in Fig. 9.

25 **[0115]** As a result of the digitization of the signals of the spots, almost the same results as those obtained by the confirmation with visual observations were obtained. Note that No. 28 in patient serum pool 1 had so low signal value as to be determined as a noise. On the other hand, with respect to No. 34, although almost no spot was confirmed with visual observations, a signal value was detected. With respect to No. 19 in the patient serum pool 2, a signal value was so small as to be determined as a noise.

30 **[0116]** Taken these results together, among the 29 types of synthesized proteins, those proteins reacted only with the patient sera without reacting with the healthy subject sera were 10 types of proteins, i.e., proteins of Nos. 3, 4, 9, 11, 13, 19, 21, 22, 24 and 28. On the other hand, those proteins showed higher color development in the patient sera than in the healthy subject sera were 16 types of proteins, i.e., proteins of Nos. 2, 3, 4, 6, 9, 10, 11, 13, 19, 21, 22, 24, 26, 28, 32 and 35.

35 **[0117]** These results demonstrated that the proteins of Nos. 2, 3, 4, 6, 9, 10, 11, 13, 19, 21, 22, 24, 26, 28, 32 and 35 were suitable as the antigen proteins to be used in the antibody titer test kit of the present invention.

40 Stability of synthesized proteins

[0118] For the purpose of confirming as to whether or not the synthesized antigen proteins could be actually used in the antibody titer test kit, SDS-PAGE was carried out to examine the states of the synthesized proteins.

45 **[0119]** A sample was prepared so that an antigen protein was contained at a concentration of 50 ng/10 μ l sample buffer (+DTT). With respect to the proteins of Nos. 5, 32 and 35, there was no quantified value and therefore a sample was prepared by mixing a solution of the synthesized protein (4 μ l) with a sample buffer (+DTT) (6 μ l).

[0120] The prepared sample was thermally denatured, and the sample (10 μ l in total) was applied to carry out SDS-PAGE electrophoresis. Subsequently, a gel that had been subjected to electrophoresis was washed with distilled water, then stained with CBB, and then washed with distilled water until bands could be observed clearly. The results are shown in Fig. 10.

50 **[0121]** With respect to proteins other than the proteins of Nos. 5, 32 and 35, it was confirmed that proteins having desired sizes were synthesized. On the other hand, with respect to the protein of No. 5, no band was observed; and with respect to the proteins of Nos. 32 and 35, multiple bands were observed.

55 **[0122]** With respect to No. 5, although so far as the synthesis of messenger RNA could be confirmed, the synthesis of a protein could not be confirmed. Therefore, it was assumed that the protein was very instable or was difficult to be synthesized.

[0123] On the other hand, the proteins of Nos. 32 and 35 are known as proteases, and therefore it was considered that synthesized proteins also had a protease activity and was self-digested. If some of the antigen proteins have a protease activity, the decomposition of the other antigen proteins contained in the antibody titer test kit of the present

invention may occur, and therefore the stability of the proteins may be deteriorated and the proteins cannot be used for the testing.

[0124] As mentioned above, it was found that the protein of No. 5 was difficult to be synthesized and therefore could not be used, and the proteins of Nos. 32 and 35 had a protease activity and therefore could not be used without any modification.

Antigen-antibody reactions with patient sera

[0125] As apparent from Table 1, it is considered that proteins which can be utilized as antigens by the antibodies are different among the individual sera. Then, for the purpose of selecting antigen proteins having high reactivity with many patient sera, antigen-antibody reactions of proteins in which antigenicity was observed with varied patient sera were examined.

[0126] In this experiment, 16 types of antigen proteins showed stronger color development in the patient sera than in the healthy subject sera were subjected to dot blotting. The amount of a protein subjected to dot blotting was 50 ng. With respect to Nos. 32 and 35, the protein concentration was unknown, and therefore 4 μ l of a synthesized protein solution was applied. Each of the antigen proteins was reacted with each of the healthy subject sera to set a reference value.

Detection of signal values

[0127]

1. Using ImageQuant LAS4000 (GE Healthcare), an image of the membrane was taken. After adjusting the focus, the image was taken under the following conditions.

Conditions for image capture

Exposure Type: Precision
 Exposure Time: 1/100 sec.
 Sensitivity/Resolution: Standard

2. Next, using ImageQuant TL (GE Healthcare), the dot signal values in the image taken were determined.

Conditions for signal value capture

In an Array analysis mode, the spot size of the dots was fit, the spots were arranged in the layout of 3 columns x 8 rows for the layout of 2 columns x 8 rows (2 x 8), and the signal values were digitized.

3. After the digitization, from the signal value of each spot, a column located at the center was determined as a background that was closed to the spots, and the differences therefrom were organized as the signal value of each spot in a table. A signal value of a spot that could not be confirmed with visual observations was treated as being undetectable.

4. As the healthy subject reference, signal values for NAI, TOM and KOB were determined. Among these values, signal values of spots which could be confirmed with visual observations were compared and the largest signal value among the three samples was employed as a healthy subject serum reference (Fig. 11). As for a spot for which the color development could not be confirmed in any sample, the reference value was set as "0". A spot which showed a higher signal value than the signal value was marked with a round stamp and organized (Figs. 12 and 13).

[0128] As a result, it was confirmed that the number of color-developed spots was apparently larger in the patient sera as compared with those in healthy subject sera and their signal values were also higher as compared with the developed colors in the healthy subject sera. As expected, there were proteins that could not be used as antigens in some patients.

[0129] Here, it was demonstrated that periodontal diseases could be determined in all of the patients by using the antigen protein Nos. 32 or 35, whose reactions with all of the patient antibodies were recognized. With taking the changing antigenicity of periodontal disease-causing bacteria and various immunoresponses of subject to be tested into consideration, the test on periodontal diseases in a wide scope of periodontal disease-causing bacteria and subjects to be tested can be achieved using a properly selected combination of at least two among the selected proteins.

[0130] A color-developing signal value reflects an antibody titer against periodontal disease-causing bacteria proteins in a plasma or serum from a patient. Therefore, the degree of progression (severity) of a periodontal disease can be tested on the basis of the signal values obtained. In this case also, the test on a wide scope of periodontal disease-causing bacteria and the degree of progression of periodontal disease based on subjects to be tested can also be

achieved using a properly selected one or a combination of at least two among the selected proteins.

Signal value comparison between healthy subject sera and patient sera

5 **[0131]** Antigen proteins which exhibited higher signal values against patient sera as compared with the healthy subject reference were organized, and it was found that the antigen proteins of Nos. 32 and 35 showed higher signal values against all of the sera collected from 23 persons investigated as compared with the reference, and therefore were particularly suitable as antigen proteins to be used in the antibody titer test kit of the present invention (Figs. 12 and 13).
10 In addition, the antigen proteins of Nos. 2, 3 and 26 showed higher signal values against sera from 19 persons, 16 persons and 12 persons, respectively, among 23 persons investigated as compared with the reference, and therefore it was found that the rates of covering the subjects to be tested were relatively high (Figs. 12 and 13).

[0132] As mentioned above, both the signal values and cover rates of the antigen proteins of Nos. 32 and 35 were high and therefore it was found that these antigen proteins were suitable as antigens to be used in the antibody titer test kit. However, these antigen proteins have a protease activity and therefore cannot be used as the test antigen without
15 modifications.

[0133] On the other hand, for using proteins other than Nos. 32 and 35 as antigens for the antibody titer test kit, it is needed to combine at least two of the antigen proteins. For example, it was confirmed that, when the antigen proteins of Nos. 2 and 3 were used in combination, the cover rate could be increased to 100%.

[0134] As mentioned above, the antigen proteins of Nos. 32 and 35 had excellent properties. However, the antigen
20 proteins are proteases and therefore cannot be used as test antigens.

[0135] Therefore, modified polypeptides in which a protease activity was eliminated while keeping the antigenicity of these antigen proteins, were produced.

[0136] The amino acid sequences for the proteins of Nos. 32 and 35 were analyzed using a Genetyx homology search tool, and two cysteine residues of which occurrence had been confirmed in the proteins, were substituted by an alanine
25 residue in the following manner. The amino acid sequences for two modified polypeptides (Nos. 32A and 32B) produced for No. 32 are respectively shown in SEQ ID NOs: 63 and 65, and the polynucleotide sequences encoding the modified polypeptides are respectively shown in SEQ ID NOs: 64 and 66. The amino acid sequences for two modified polypeptides (Nos. 35A and 35B) produced for No. 35 are respectively shown in SEQ ID NOs: 67 and 69, and the polynucleotide
30 sequences encoding the modified polypeptides are respectively shown in SEQ ID NOs: 68 and 70.

Production of protein expression plasmid

Primer synthesis

35 **[0137]** The below-mentioned primer pairs represented by the SEQ ID NOs in the Sequence Listing, each of which contains a mutation-introduced site, were synthesized.

Modified polypeptide No. 32A

40 Forward primer: SEQ ID NO: 133
Reverse primer: SEQ ID NO: 134
Modified polypeptide No. 32B
Forward primer: SEQ ID NO: 135
Reverse primer: SEQ ID NO: 136

45 Modified polypeptide No. 35A

Forward primer: SEQ ID NO: 137
Reverse primer: SEQ ID NO: 138

50 Modified polypeptide No. 35B

Forward primer: SEQ ID NO: 139
Reverse primer: SEQ ID NO: 140
55 Phosphorylation of primers: T4 Polynucleotide Kinase (Toyobo Co., Ltd.)

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Composition of reaction solution for preparation of 20 μ l

[0138]

5	Synthetic primer (50 μ M)	14 μ l
	10 \times Protruding End Kinase Buffer	2 μ l
	10 mM ATP	2 μ l
	T4 Polynucleotide Kinase (5 to 20 U/ μ l)	2 μ l

10

Reaction composition

[0139] After retaining at 37°C for 60 min and then at 95°C for 5 min, 50 μ l of DW was added (10 pmol/ μ l primer DNA).
Inverse PCR: Prime STAR MAX (Takara)

15

Composition of reaction solution for reparation of 50 μ l

[0140]

20	Takara PrimeSTAR MAX Premix (2 \times)	25 μ l
	Forward primer (10 pmol/ μ l)	1.5 μ l
	Reverse primer (10 pmol/ μ l)	1.5 μ l

25

Template DNA (plasmid DNA of No. 32 or 35 antigen protein:

[0141]

	10 ng/ μ l)	1 μ l
30	Sterilized water	21 μ l

Reaction conditions

[0142] After treating at 98°C for 30 sec, a cycle of 98°C for 10sec, 55°C for 5 sec and 72°C for 50 sec was repeated 30 times.

[0143] A PCR product was purified using a QIAGEN kit, and then the purified product was treated with DpnI to decompose Template DNA (plasmid DNA).

[0144] The PCR product was purified in the composition shown below using a QIAGEN kit.

40

DpnI treatment

[0145]

45	Purified PCR product	30 μ l
	NEB4	5 μ l
	BSA	5 μ l
	DpnI (20 unit/ μ L)	0.5 μ l
	Sterilized water	9.5 μ l
50	37°C, for 1 hour	

[0146] Purification of restriction enzyme treatment product: a DNA product was purified by carrying out PCI treatment.

Ligation

55

[0147]

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Purified DNA 5 μ l
Ligation Mighty Mix 5 μ l
16°C, for 1 hour

5

[0148] A ligation product was introduced into a cell of *Escherichia coli* (*E. coli*) by transformation.

[0149] Plasmid DNA in which the introduction of a gene had been confirmed was subjected to sequence analysis.

10 **[0150]** A plasmid into which a desired mutation had been introduced was prepared in a large amount, and a protein was synthesized using a wheat germ cell-free protein synthesis system and then purified using a GST tag.

Confirmation of synthesized proteins

SDS-PAGE

15

[0151] A synthesized protein solution (4 μ l) was mixed with a sample buffer (+DTT) (6 μ l) to prepare a sample. The prepared sample was thermally denatured, and a total portion of the sample (10 μ l) was applied and subjected to SDS-PAGE electrophoresis.

20

CBB staining

[0152] A gel that had been subjected to SDS-PAGE was washed with distilled water and then immersed in a CBB staining solution, and then the solution was stirred.

[0153] The gel was washed with distilled water until bands could be observed clearly.

25

Dot blotting

30 **[0154]** Four types of modified polypeptides produced (Nos. 32A, 32B, 35A and 35B) were subjected to dot blotting. Each of the modified polypeptides to be dot-blotted was applied at a volume of 4 μ l at an antigen protein concentration of 12.5 ng/ μ l so that 50 ng of the antigen protein could be applied. Three sets of dot blotting were carried out. The dot blots were immersed in TBS containing 5% of skim milk overnight to cause blocking, and then reacted with serum solutions as mentioned below at room temperature.

Serum solutions (primary antibody)

35

[0155]

A: a solution prepared by mixing a portion (8 μ l) of a mixture of sera from NAI, TOM and KOB (3 μ l for each) with TBS containing 3% of skim milk (20ml).

40 B: a solution prepared by mixing a portion (8 μ l) of a mixture of sera of Nos. 7350 and 6921 (4 μ l for each) with TBS containing 3% skim milk (20 ml).

C: a solution prepared by mixing a portion (8 μ l) of a mixture of sera of Nos. 7107, 7523 and 6980 (3 μ l for each) with TBS containing 3% skim milk (20 ml), 140 mL 1 \times TBS.

45 **[0156]** Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, a 5000-fold-diluted horseradish peroxidase-conjugated goat anti-human IgG antibody reaction solution (a solution prepared by adding anti-human secondary antibody (CHEMICON) to TBS containing skim milk (3%)) (20 ml) was added to the slit and the resultant product was shaken at room temperature. Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, the slit was immersed in TBS containing 4-methoxy-1-naphthol (0.61 mg/ml) and hydrogen peroxide (0.018%), the occurrence of development of color was confirmed, and then the slit was washed with purified water and dried.

50

[0157] The results are shown in Fig. 14.

55 **[0158]** With respect to No. 32, the protease activity was inhibited in both of the modified polypeptides. On the other hand, with respect to No. 35, the protease activity was not inhibited completely in the modified polypeptide of No. 35B but the protease activity was inhibited in the modified polypeptide of No. 35A. In addition, the antigenicity was examined, and it was confirmed that antigenicity was maintained in all of the modified polypeptides.

Test methods

Dot blot analysis

5 **[0159]** In the same manner as the experiment mentioned above, each of 16 types of antigen proteins was dot-blotting against each of sera from 10 healthy subjects and sera from 20 periodontal disease patients (with respect to the antigen proteins of No. 32 and No. 35, produced modified polypeptides of No. 32A and No. 35A were used). The amount of a protein subjected to the dot blotting was 50 ng (with respect to the antigen protein of No. 4, the protein concentration was low and therefore 37 ng was applied, and the volume of a protein solution became insufficient during the test and therefore the blotting was not carried out against serum Nos. H9, H10, P10 and P20).

10 **[0160]** After the dot blotting, the dots were immersed in a blocking solution (a TBS solution containing skim milk (5%)).

[0161] As a primary antibody solution, a solution prepared by mixing each of the healthy subject sera (H1 to H10) or each of the periodontal disease patient sera (P1 to P20) (8 μ L) with TBS (20 mL) containing 3% of skim milk was used. The blocked slit was immersed in the solution and an antigen-antibody reaction was carried out at room temperature for 2 hours.

15 **[0162]** Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, a 5000-fold-diluted horseradish peroxidase-conjugated goat anti-human IgG antibody reaction solution (a solution prepared by adding an anti-human secondary antibody (MILLIPORE) to TBS containing skim milk (3%)) (20 mL) was added to the slit and an antigen-antibody reaction was carried out at room temperature for 1 hour.

20 **[0163]** Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, the slit was immersed in TBS containing 4-methoxy-1-naphthol (0.61 mg/ml) and hydrogen peroxide (0.018%), the occurrence of development of color was confirmed, and then the slit was washed with purified water and dried. The results are shown in Fig. 15.

Detection of signal values

25 **[0164]**

1. Using ImageQuant LAS4000 (GE Healthcare), an image of the membrane was taken. After adjusting the focus, the image was taken under the following conditions.

30 Conditions for image capture

Exposure Type: Precision

Exposure Time: 1/100 sec.

Sensitivity/Resolution: Standard

35 2. Using ImageQuant TL (GE Healthcare), the dot signal values in the image taken were determined.
Conditions for signal value capture

40 **[0165]** In an Array analysis mode, a round-shaped cursor was moved so as to surround the whole area of the spots of the dots wherein the spots of the dots were arranged in the layout of 2 columns \times 8 rows, and the signal values were digitized.

[0166] The background was set in a Spot Edge Average mode, so that signal values of the spots could be reflected against the background surrounding the spots.

45 Analysis of signal values

[0167]

50 1. With respect to signal values of each serum against individual antigen proteins, signal average values for a healthy subject serum group and a patient serum group were calculated (Figs. 16 and 17). As a result, it was found that the antigen proteins of Nos. 13, 21, 22 and 28 were not reacted with any of the sera or reacted at a low reaction rate. Subsequently, a Signal/Noise ratio was determined employing the signal value of the patient serum group as a Signal value and the signal value of the healthy subject serum group as a Noise value. In this determination, when a healthy subject serum group had a signal average value of 0, the calculation of a calculated value was impossible and therefore the healthy subject serum group was determined "Noise: 0" (Fig. 18). As a result, it was demonstrated that, with respect to the antigen proteins of Nos. 2, 6, 10 and 26, the signal average values were high but the S/N ratios were low, and therefore the antigen proteins were not suitable for the test of a periodontal disease in a wide scope of patients having various immunotypes. On the other hand, with respect to the antigen proteins of Nos. 3,

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4, 9, 11, 19, 24, 32A and 35A, the S/N ratios were high and therefore it was considered that the antigen proteins had high specificity to the patient sera.

2. With respect to signal values of each serum against the individual antigen proteins, an ROC (Receiver Operating Characteristic) curve and an area under the ROC curve (Area under the curve, AUC) were determined using statistical software Excel 2010 (Fig. 19).

[0168] The ROC curve was a graph showing as to how the positive prevalence (sensitivity) and the false positive prevalence (1 - degree of specificity) are changed when a boundary value (a cut-off value) is altered. Given the ideal test, a state in which each of the sensitivity and degree of specificity is 1.0 (i.e., a state in which the value of each of the sensitivity and degree of specificity is positioned at an upper left end point) is the ideal state. Therefore, it is considered that high diagnostic-predictive performance of the test can be achieved when the ROC curve graph is shifted so as to become close to the upper left end. Thus, by measuring the area under the curve ROC, it is possible to determine the predictive-diagnostic performance of the test.

[0169] Generally, the predictive/diagnostic capability on the basis of AUC values can be determined as follows.

AUC 0.9 to 1.0: High accuracy

AUC 0.7 to 0.9: Moderate accuracy

AUC 0.5 to 0.7: Low accuracy

[0170] From the results shown in Fig. 19, it was found that the antigen proteins of Nos. 3, 4, 9, 11, 19, 24, 32A and 35A had such diagnostic/predictive capability of AUC 0.6 or more.

Production of high-expression antigen proteins Test methods

Synthesis of proteins of Nos. 32N, 32C, 35N and 35C

[0171] A desired gene sequence was amplified so that the size of the nucleotide become half of the original sequence on the basis of each of the nucleotide sequences for Nos. 32A and No. 35A using each of the synthesized primer pairs shown in the primer list below.

Production of protein-expressing plasmid

Primer synthesis

[0172] The primer pairs represented by SEQ ID NOs shown in the Sequence Listing were synthesized.

Modified polypeptide No. 32N

Forward primer: SEQ ID NO: 157

Reverse primer: SEQ ID NO: 158

Modified polypeptide No. 32C

Forward primer: SEQ ID NO: 159

Reverse primer: SEQ ID NO: 160

Modified polypeptide No. 35N

Forward primer: SEQ ID NO: 161

Reverse primer: SEQ ID NO: 162

Modified polypeptide No. 35C

Forward primer: SEQ ID NO: 163

Reverse primer: SEQ ID NO: 164

[0173] Subsequently, the amplified DNA product was treated with a restriction enzyme and then ligated to a protein expression vector (CellFree Sciences Co., Ltd.; a pEu vector) that had been treated with the same restriction enzyme, so as to be matched in reading frame with each other. A ligation product was introduced into a cell of *Escherichia coli* (*E. coli*) by transformation. Subsequently, a clone having the gene introduced thereinto was selected.

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5 [0174] Plasmid DNA was collected from the selected clone and then subjected to sequence analysis. The amino acid sequences for two modified polypeptides (Nos. 32N and 32C) produced for No. 32A are shown in SEQ ID NOs: 141 and 143, and the polynucleotide sequences respectively encoding the modified polypeptides are shown in SEQ ID NOs: 142 and 144. The amino acid sequences for two modified polypeptides (Nos. 35N and 35C) produced for No. 35A are shown in SEQ ID NOs: 145 and 147, and the polynucleotide sequences respectively encoding the modified polypeptides are shown in SEQ ID NOs: 146 and 148.

[0175] With respect to a clone in which any significant mutation was not recognized from the results of the sequence analysis, a large amount of a plasmid was prepared, a protein was synthesized using a wheat germ cell-free protein synthesis system, and the resultant protein was purified using a GST tag.

10 [0176] The synthesis of 4 types of proteins, i.e., the N-terminal and the C-terminal of No. 32A and the N-terminal and the C-terminal of No. 35A, were successfully achieved, and these proteins were subjected to dot blot analysis.

[0177] As a result, it was demonstrated that these modified peptides could react with the patient serum groups even when the lengths thereof were half of the original sequences thereof, and it was confirmed that the antigenicity of these modified peptides was maintained.

15 Antigen proteins capable of recognizing *Porphyromonas gingivalis* strain SU63

Test methods

20 Confirmation of homology among strains

[0178] The search was carried out on the basis of amino acid sequences for each of the proteins of strain FDC381 using a blastp of BLAST (Basic Local Alignment Search Tool) in NCBI site.

25 [0179] From the results of the search, the homology among proteins in *Porphyromonas gingivalis* bacterium strains W83, ATCC33277 and TDC60, which have been registered on a database, was confirmed.

Cloning of gene derived from strain SU63 and synthesis of proteins

30 [0180] The genetic information on the selected proteins was reviewed from a database, and a desired gene was amplified from genomic DNA derived from *P. gingivalis* bacterium strain SU63 using each of the primer pairs represented by SEQ ID NOs shown in the following Sequence Listing.

Strain Su63: No. 15 antigen protein (No. 15Su)

Forward primer: SEQ ID NO: 165

35 Reverse primer: SEQ ID NO: 166

Strain Su63: No. 16 antigen protein (No. 16Su)

Forward primer: SEQ ID NO: 167

Reverse primer: SEQ ID NO: 168

Strain Su63: No. 34 antigen protein (No. 34Su)

40 Forward primer: SEQ ID NO: 169

Reverse primer: SEQ ID NO: 170

Strain Su63: No. 37 antigen protein (No. 37Su)

Forward primer: SEQ ID NO: 171

45 Reverse primer: SEQ ID NO: 172

[0181] Subsequently, the amplified DNA product was treated with a restriction enzyme and then ligated to a protein expression vector (CellFree Sciences Co., Ltd.; a pEu vector) that had been treated with the same restriction enzyme. A ligation product was introduced into a cell of *Escherichia coli* (*E. coli*) by transformation. Subsequently, a clone having the gene introduced therein was selected.

50 [0182] Plasmid DNA was collected from the selected clone and then subjected to sequence analysis. The amino acid sequences for the antigen proteins of No. 15Su, No. 16Su, No. 34Su and No. 37Su are respectively shown in SEQ ID NOs: 149, 151, 153 and 155, and the polynucleotide sequences encoding the antigen proteins are respectively shown in SEQ ID NOs: 150, 152, 154 and 156. As a result, it was demonstrated that the sequence for the polynucleotide encoding the antigen protein of No. 15Su was different by one nucleotide from corresponding polynucleotide sequence in strain W83, the sequence for the polynucleotide encoding the antigen protein of No. 16Su had high homology with the corresponding polynucleotide sequences in strains TDC60 and ATCC33277, and the sequence for the polynucleotide encoding the antigen protein of No. 34Su had high homology with the polynucleotide sequence of strain ragA (strain A011/9).

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[0183] With respect to a clone in which any significant mutation was not recognized from the results of the sequence analysis, a large amount of a plasmid was prepared, a protein was synthesized using a wheat germ cell-free protein synthesis system, and the resultant protein was purified using a GST tag.

[0184] As a result, four types of proteins derived from strain SU63, i.e., proteins of Nos. 15, 16, 34 and 37, were successfully synthesized, and the proteins were subjected to dot blot analysis.

Dot blot analysis

[0185] Eight types of antigen proteins (strain FDC381: Nos. 15, 16, 34 and 37, strain SU63: Nos. 15Su, 16Su, 34Su and 37Su) were dot-blotted. The amount of a protein to be dot-blotted was 50 ng.

[0186] After dot blotting, the dot was immersed in a blocking solution (a TBS solution containing skim milk (5%)).

[0187] As a primary antibody solution, a solution prepared by mixing each of the healthy subject sera (H1 to H10) or each of the periodontal disease patient sera (P1 to P20) (4 μ L) with TBS (10 mL) containing 3% of skim milk was used. The blocked slit was immersed in the solution and an antigen-antibody reaction was carried out at room temperature for 2 hours.

[0188] Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, a 5000-fold-diluted horseradish peroxidase-conjugated goat anti-human IgG antibody reaction solution (a solution prepared by adding an anti-human secondary antibody (MILLIPORE) to TBS containing skim milk (3%)) (10 mL) was added to the slit and an antigen-antibody reaction was carried out at room temperature for 1 hour.

[0189] Subsequently, the slit was washed with TBS containing Tween 20 (0.05%). After washing, the slit was immersed in TBS containing 4-methoxy-1-naphthol (0.61 mg/ml) and hydrogen peroxide (0.018%), the occurrence of development of color was confirmed, and then the slit was washed with purified water and dried. The results are shown in Figs. 24 and 25.

[0190] As apparent from these figures, the antigen protein of No. 15 was an antigen protein from both strains FDC381 and SU63 and showed antigenicity against the patient serum P19. On the other hand, the antigen proteins of No. 16, 34 and 37, either one of the antigen proteins of strain FDC381 and strain SU63, had antigenicity against either one of the healthy subject sera and the patient sera.

[0191] Consequently, it was suggested that the antigen proteins of Nos. 16, 34 and 37 enables the discrimination and recognition between the infection with strain FDC381 and the infection with strain SU63.

Results of test

[0192] The homology between strains was examined, and the homology between the proteins from strain FDC381 and the protein on the database is as follows. It was confirmed that the homology between strains was low.

No.	strain W83	strain ATCC33277	strain TDC60
15	387/387 (100%)	202/398 (51%)	213/402 (53%)
16	553/553 (100%)	242/566 (43%)	243/566 (43%)
34	1015/1016 (99%)	737/1039 (71%)	719/1022 (70%)
37	500/500 (100%)	249/511 (49%)	249/511 (49%)

[0193] A sequence analysis was carried out and it was demonstrated that the homology between each protein derived from strain FDC381 and proteins derived from strain SU63 were as follows.

No. 15: 99.7%

No. 16: 41.4%

No. 34: 65.2%

No. 37: 47.3%

INDUSTRIAL APPLICABILITY

[0194] The test kit for a plasma or serum antibody titer against a periodontal disease-causing bacterium of the present invention can be suitably used in a test system for a plasma or serum antibody titer against a periodontal disease-causing bacterium which treats a large quantity of samples automatically and at a high speed.

Sequence Listing Free Text

[0195]

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- SEQ ID NO: 1 is an amino acid sequence for a conserved hypothetical protein having a zinc-carboxypeptidase domain.
- SEQ ID NO: 2 is a nucleotide sequence encoding a conserved hypothetical protein having a zinc-carboxypeptidase domain.
- 5 SEQ ID NO: 3 is an amino acid sequence for a hypothetical protein PG1881.
SEQ ID NO: 4 is a nucleotide sequence encoding a hypothetical protein PG1881.
SEQ ID NO: 5 is an amino acid sequence for a hypothetical protein PGN_0291.
SEQ ID NO: 6 is a nucleotide sequence encoding a hypothetical protein PGN_0291.
SEQ ID NO: 7 is an amino acid sequence for a hypothetical protein PG0491.
- 10 SEQ ID NO: 8 is a nucleotide sequence encoding a hypothetical protein PG0491.
SEQ ID NO: 9 is an amino acid sequence for a hypothetical protein PGN_1611.
SEQ ID NO: 10 is a nucleotide sequence encoding a hypothetical protein PGN_1611.
SEQ ID NO: 11 is an amino acid sequence for a hypothetical protein PGN_0477.
SEQ ID NO: 12 is a nucleotide sequence encoding a hypothetical protein PGN_0477.
- 15 SEQ ID NO: 13 is an amino acid sequence for a hypothetical protein PGN_0860.
SEQ ID NO: 14 is a nucleotide sequence encoding a hypothetical protein PGN_0860.
SEQ ID NO: 15 is an amino acid sequence for a 53 kDa major outer membrane protein.
SEQ ID NO: 16 is a nucleotide sequence encoding a 53 kDa major outer membrane protein.
SEQ ID NO: 17 is an amino acid sequence for a 35 kDa hemin-binding protein.
- 20 SEQ ID NO: 18 is a nucleotide sequence encoding a 35 kDa hemin-binding protein.
SEQ ID NO: 19 is an amino acid sequence for a heme-binding protein FetB.
SEQ ID NO: 20 is a nucleotide sequence encoding a heme-binding protein FetB.
SEQ ID NO: 21 is an amino acid sequence for an NAD-dependent glutamate dehydrogenase.
SEQ ID NO: 22 is a nucleotide sequence encoding an NAD-dependent glutamate dehydrogenase.
- 25 SEQ ID NO: 23 is an amino acid sequence for a phosphoserine aminotransferase.
SEQ ID NO: 24 is a nucleotide sequence encoding a phosphoserine aminotransferase.
SEQ ID NO: 25 is an amino acid sequence for a TonB-binding receptor Tlr.
SEQ ID NO: 26 is a nucleotide sequence encoding a TonB-binding receptor Tlr.
SEQ ID NO: 27 is an amino acid sequence for fimbrillin (strain FDC381).
- 30 SEQ ID NO: 28 is a nucleotide sequence encoding fimbrillin.
SEQ ID NO: 29 is an amino acid sequence for a trace component FimE (strain FDC381).
SEQ ID NO: 30 is a nucleotide sequence encoding a trace component FimE.
SEQ ID NO: 31 is an amino acid sequence for HmuY'.
SEQ ID NO: 32 is a nucleotide sequence encoding HmuY'.
- 35 SEQ ID NO: 33 is an amino acid sequence for an M24 family peptidase.
SEQ ID NO: 34 is a nucleotide sequence encoding an M24 family peptidase.
SEQ ID NO: 35 is an amino acid sequence for glyceraldehyde-3-phosphate dehydrogenase type-1.
SEQ ID NO: 36 is a nucleotide sequence encoding glyceraldehyde-3-phosphate dehydrogenase type-1.
SEQ ID NO: 37 is an amino acid sequence for ferritin.
- 40 SEQ ID NO: 38 is a nucleotide sequence encoding ferritin.
SEQ ID NO: 39 is an amino acid sequence for a serine hydroxymethyl transferase.
SEQ ID NO: 40 is a nucleotide sequence encoding a serine hydroxymethyl transferase.
SEQ ID NO: 41 is an amino acid sequence for an outer membrane lipoprotein Omp28.
SEQ ID NO: 42 is a nucleotide sequence encoding an outer membrane lipoprotein Omp28.
- 45 SEQ ID NO: 43 is an amino acid sequence for a promising lysyl endopeptidase precursor.
SEQ ID NO: 44 is a nucleotide sequence encoding a promising lysyl endopeptidase precursor.
SEQ ID NO: 45 is an amino acid sequence for a quinone family NAD (P) dehydrogenase.
SEQ ID NO: 46 is a nucleotide sequence encoding a quinone family NAD (P) dehydrogenase.
SEQ ID NO: 47 is an amino acid sequence for a DNA-binding protein from a starved cell Dps.
- 50 SEQ ID NO: 48 is a nucleotide sequence encoding a DNA-binding protein from a starved cell Dps.
SEQ ID NO: 49 is an amino acid sequence for an immunoresponsive 42 kDa antigen PG33.
SEQ ID NO: 50 is a nucleotide sequence encoding an immunoresponsive 42 kDa antigen PG33.
SEQ ID NO: 51 is an amino acid sequence for Lys-gingipain.
SEQ ID NO: 52 is a nucleotide sequence encoding Lys-gingipain.
- 55 SEQ ID NO: 53 is an amino acid sequence for a peptidyl-arginine deiminase.
SEQ ID NO: 54 is a nucleotide sequence encoding a peptidyl-arginine deiminase.
SEQ ID NO: 55 is an amino acid sequence for a ragA protein (strain FDC381).
SEQ ID NO: 56 is a nucleotide sequence encoding a ragA protein.

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SEQ ID NO: 57 is an amino acid sequence for an arginine-specific cysteine proteinase RgpA.
SEQ ID NO: 58 is a nucleotide sequence encoding an arginine-specific cysteine proteinase RgpA.
SEQ ID NO: 59 is an amino acid sequence for an outer membrane protein 41 precursor.
SEQ ID NO: 60 is a nucleotide sequence encoding an outer membrane protein 41 precursor.
5 SEQ ID NO: 61 is an amino acid sequence for a lipoprotein RagB (strain FDC381).
SEQ ID NO: 62 is a nucleotide sequence encoding a lipoprotein RagB.
SEQ ID NO: 63 is an amino acid sequence for a mutation-introduced Lys-gingipain.
SEQ ID NO: 64 is a nucleotide sequence encoding a mutation-introduced Lys-gingipain.
10 SEQ ID NO: 65 is an amino acid sequence for a mutation-introduced Lys-gingipain.
SEQ ID NO: 66 is a nucleotide sequence encoding a mutation-introduced Lys-gingipain.
SEQ ID NO: 67 is an amino acid sequence for a mutation-introduced arginine-specific cysteine proteinase RgpA.
SEQ ID NO: 68 is a nucleotide sequence encoding a mutation-introduced arginine-specific cysteine proteinase RgpA.
SEQ ID NO: 69 is an amino acid sequence for a mutation-introduced arginine-specific cysteine proteinase RgpA.
15 SEQ ID NO: 70 is a nucleotide sequence encoding a mutation-introduced arginine-specific cysteine proteinase RgpA.
SEQ ID NO: 71 is a forward primer used in the PCR amplification of a polynucleotide encoding a conserved hypothetical protein having a zinc-carboxypeptidase domain.
SEQ ID NO: 72 is a reverse primer used in the PCR amplification of a polynucleotide encoding a conserved hypothetical protein having a zinc-carboxypeptidase domain.
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SEQ ID NO: 77 is a forward primer used in the PCR amplification of a polynucleotide encoding a hypothetical protein PG0491.
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SEQ ID NO: 83 is a forward primer used in the PCR amplification of a polynucleotide encoding a hypothetical protein PGN_0860.
40 SEQ ID NO: 84 is a reverse primer used in the PCR amplification of a polynucleotide encoding a hypothetical protein PGN_0860.
SEQ ID NO: 85 is a forward primer used in the PCR amplification of a polynucleotide encoding a 53 kDa major outer membrane protein.
45 SEQ ID NO: 86 is a reverse primer used in the PCR amplification of a polynucleotide encoding a 53 kDa major outer membrane protein.
SEQ ID NO: 87 is a forward primer used in the PCR amplification of a polynucleotide encoding a 35 kDa heme-binding protein.
SEQ ID NO: 88 is a reverse primer used in the PCR amplification of a polynucleotide encoding a 35 kDa heme-binding protein.
50 SEQ ID NO: 89 is a forward primer used in the PCR amplification of a polynucleotide encoding a heme-binding protein FetB.
SEQ ID NO: 90 is a reverse primer used in the PCR amplification of a polynucleotide encoding a heme-binding protein FetB.
55 SEQ ID NO: 91 is a forward primer used in the PCR amplification of a polynucleotide encoding an NAD-dependent glutamate dehydrogenase.
SEQ ID NO: 92 is a reverse primer used in the PCR amplification of a polynucleotide encoding an NAD-dependent glutamate dehydrogenase.

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SEQ ID NO: 93 is a forward primer used in the PCR amplification of a polynucleotide encoding a phosphoserine aminotransferase.

SEQ ID NO: 94 is a reverse primer used in the PCR amplification of a polynucleotide encoding a phosphoserine aminotransferase.

5 SEQ ID NO: 95 is a forward primer used in the PCR amplification of a polynucleotide encoding a TonB-binding receptor Tlr.

SEQ ID NO: 96 is a reverse primer used in the PCR amplification of a polynucleotide encoding a TonB-binding receptor Tlr.

SEQ ID NO: 97 is a forward primer used in the PCR amplification of a polynucleotide encoding fimbrillin.

10 SEQ ID NO: 98 is a reverse primer used in the PCR amplification of a polynucleotide encoding fimbrillin.

SEQ ID NO: 99 is a forward primer used in the PCR amplification of a polynucleotide encoding a trace component FimE.

SEQ ID NO: 100 is a reverse primer used in the PCR amplification of a polynucleotide encoding a trace component FimE.

15 SEQ ID NO: 101 is a forward primer used in the PCR amplification of a polynucleotide encoding HmuY'.

SEQ ID NO: 102 is a reverse primer used in the PCR amplification of a polynucleotide encoding HmuY'.

SEQ ID NO: 103 is a forward primer used in the PCR amplification of a polynucleotide encoding an M24 family peptidase.

20 SEQ ID NO: 104 is a reverse primer used in the PCR amplification of a polynucleotide encoding an M24 family peptidase.

SEQ ID NO: 105 is a forward primer used in the PCR amplification of a polynucleotide encoding glyceraldehyde-3-phosphate dehydrogenase type-1.

SEQ ID NO: 106 is a reverse primer used in the PCR amplification of a polynucleotide encoding glyceraldehyde-3-phosphate dehydrogenase type-1.

25 SEQ ID NO: 107 is a forward primer used in the PCR amplification of a polynucleotide encoding ferritin.

SEQ ID NO: 108 is a reverse primer used in the PCR amplification of a polynucleotide encoding ferritin.

SEQ ID NO: 109 is a forward primer used in the PCR amplification of a polynucleotide encoding a serine hydroxymethyl transferase.

30 SEQ ID NO: 110 is a reverse primer used in the PCR amplification of a polynucleotide encoding a serine hydroxymethyl transferase.

SEQ ID NO: 111 is a forward primer used in the PCR amplification of a polynucleotide encoding an outer membrane lipoprotein Omp28.

SEQ ID NO: 112 is a reverse primer used in the PCR amplification of a polynucleotide encoding an outer membrane lipoprotein Omp28.

35 SEQ ID NO: 113 is a forward primer used in the PCR amplification of a polynucleotide encoding a promising lysyl endopeptidase precursor.

SEQ ID NO: 114 is a reverse primer used in the PCR amplification of a polynucleotide encoding a promising lysyl endopeptidase precursor.

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SEQ ID NO: 117 is a forward primer used in the PCR amplification of a polynucleotide encoding a DNA-binding protein from a starved cell Dps.

45 SEQ ID NO: 118 is a reverse primer used in the PCR amplification of a polynucleotide encoding a DNA-binding protein from a starved cell Dps.

SEQ ID NO: 119 is a forward primer used in the PCR amplification of a polynucleotide encoding an immunoresponsive 42 kDa antigen PG33.

50 SEQ ID NO: 120 is a reverse primer used in the PCR amplification of a polynucleotide encoding an immunoresponsive 42 kDa antigen PG33.

SEQ ID NO: 121 is a forward primer used in the PCR amplification of a polynucleotide encoding Lys-gingipain.

SEQ ID NO: 122 is a reverse primer used in the PCR amplification of a polynucleotide encoding Lys-gingipain.

SEQ ID NO: 123 is a forward primer used in the PCR amplification of a polynucleotide encoding a peptidyl-arginine deiminase.

55 SEQ ID NO: 124 is a reverse primer used in the PCR amplification of a polynucleotide encoding a peptidyl-arginine deiminase.

SEQ ID NO: 125 is a forward primer used in the PCR amplification of a polynucleotide encoding a ragA protein.

SEQ ID NO: 126 is a reverse primer used in the PCR amplification of a polynucleotide encoding a ragA protein.

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SEQ ID NO: 127 is a forward primer used in the PCR amplification of a polynucleotide encoding an arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 128 is a reverse primer used in the PCR amplification of a polynucleotide encoding an arginine-specific cysteine proteinase RgpA.

5 SEQ ID NO: 129 is a forward primer used in the PCR amplification of a polynucleotide encoding an outer membrane protein 41 precursor.

SEQ ID NO: 130 is a reverse primer used in the PCR amplification of a polynucleotide encoding an outer membrane protein 41 precursor.

10 SEQ ID NO: 131 is a forward primer used in the PCR amplification of a polynucleotide encoding a lipoprotein RagB.

SEQ ID NO: 132 is a reverse primer used in the PCR amplification of a polynucleotide encoding a lipoprotein RagB.

SEQ ID NO: 133 is a forward primer used in the PCR amplification of a polynucleotide encoding mutation-introduced Lys-gingipain.

SEQ ID NO: 134 is a reverse primer used in the PCR amplification of a polynucleotide encoding mutation-introduced Lys-gingipain.

15 SEQ ID NO: 135 is a forward primer used in the PCR amplification of a polynucleotide encoding mutation-introduced Lys-gingipain.

SEQ ID NO: 136 is a reverse primer used in the PCR amplification of a polynucleotide encoding mutation-introduced Lys-gingipain.

20 SEQ ID NO: 137 is a forward primer used in the PCR amplification of a polynucleotide encoding a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 138 is a reverse primer used in the PCR amplification of a polynucleotide encoding a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 139 is a forward primer used in the PCR amplification of a polynucleotide encoding a mutation-introduced arginine-specific cysteine proteinase RgpA.

25 SEQ ID NO: 140 is a reverse primer used in the PCR amplification of a polynucleotide encoding a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 141 is an amino acid sequence for about half of the N-terminal side of a mutation-introduced Lys-gingipain.

SEQ ID NO: 142 is a nucleotide sequence encoding about half of the N-terminal side of a mutation-introduced Lys-gingipain.

30 SEQ ID NO: 143 is an amino acid sequence for about half of the C-terminal side of a mutation-introduced Lys-gingipain.

SEQ ID NO: 144 is a nucleotide sequence encoding about half of the C-terminal side of a mutation-introduced Lys-gingipain.

35 SEQ ID NO: 145 is an amino acid sequence for about half of the N-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 146 is a nucleotide sequence encoding about half of the N-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 147 is an amino acid sequence for about half of the C-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

40 SEQ ID NO: 148 is a nucleotide sequence encoding about half of the C-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 149 is an amino acid sequence for fimbrillin (strain SU63).

SEQ ID NO: 150 is a nucleotide sequence encoding fimbrillin (strain SU63).

45 SEQ ID NO: 151 is an amino acid sequence for a trace component FimE (strain SU63).

SEQ ID NO: 152 is a nucleotide sequence encoding a trace component FimE (strain SU63).

SEQ ID NO: 153 is an amino acid sequence for a ragA protein(strain SU63).

SEQ ID NO: 154 is a nucleotide sequence encoding a ragA (strain SU63).

SEQ ID NO: 155 is an amino acid sequence for a lipoprotein RagB (strain SU63).

50 SEQ ID NO: 156 is a nucleotide sequence encoding a lipoprotein RagB (strain SU63).

SEQ ID NO: 157 is a forward primer used in the PCR amplification of a polynucleotide encoding about half of the N-terminal side of a mutation-introduced Lys-gingipain.

SEQ ID NO: 158 is a reverse primer used in the PCR amplification of a polynucleotide encoding about half of the N-terminal side of a mutation-introduced Lys-gingipain.

55 SEQ ID NO: 159 is a forward primer used in the PCR amplification of a polynucleotide encoding about half of the C-terminal side of a mutation-introduced Lys-gingipain.

SEQ ID NO: 160 is a reverse primer used in the PCR amplification of a polynucleotide encoding about half of the C-terminal side of a mutation-introduced Lys-gingipain.

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SEQ ID NO: 161 is a forward primer used in the PCR amplification of a polynucleotide encoding about half of the N-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 162 is a reverse primer used in the PCR amplification of a polynucleotide encoding about half of the N-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

5 SEQ ID NO: 163 is a forward primer used in the PCR amplification of a polynucleotide encoding about half of the C-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

SEQ ID NO: 164 is a reverse primer used in the PCR amplification of a polynucleotide encoding about half of the C-terminal side of a mutation-introduced arginine-specific cysteine proteinase RgpA.

10 SEQ ID NO: 165 is a forward primer used in the PCR amplification of a polynucleotide encoding fimbrillin of strain SU63.

SEQ ID NO: 166 is a reverse primer used in the PCR amplification of a polynucleotide encoding fimbrillin of strain SU63.

SEQ ID NO: 167 is a forward primer used in the PCR amplification of a polynucleotide encoding a trace component FimE of strain SU63.

15 SEQ ID NO: 168 is a reverse primer used in the PCR amplification of a polynucleotide encoding a trace component FimE of strain SU63.

SEQ ID NO: 169 is a forward primer used in the PCR amplification of a polynucleotide encoding a ragA protein of strain SU63.

20 SEQ ID NO: 170 is a reverse primer used in the PCR amplification of a polynucleotide encoding a ragA protein of strain SU63.

SEQ ID NO: 171 is a forward primer used in the PCR amplification of a polynucleotide encoding a lipoprotein RagB of strain SU63.

25 SEQ ID NO: 172 is a reverse primer used in the PCR amplification of a polynucleotide encoding a lipoprotein RagB of strain SU63.

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 5 Phe Leu Cys Gln Lys Ile Arg Ala Leu Thr Pro Ile Trp Gly Thr His
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Ile Lys Ile Glu Ala Glu Asp Gly Tyr Ala Asn Asp Ile Trp Ala Asp
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Leu Asn Gly Asn Gly Lys Tyr Asp Ser Gly Glu Arg Leu Asp Ser Gly
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Glu Phe Arg Asp Val Glu Phe Arg Gln Thr Lys Ala Ile Val Tyr Gly
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Ala Thr Phe Ile Asp Ile Ser Asn Cys Thr Gly Leu Thr Ala Phe Asp
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Gly Asp Asn Gly Leu Thr Ala Leu Asp Leu Ser Ala Asn Thr Leu Leu
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Glu Glu Leu Val Tyr Ser Asn Asn Glu Val Thr Thr Ile Asn Leu Ser
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30 Phe Thr Gly Ile Gly Tyr Val Gly Ser Trp Gly Lys Ala Asn Glu Asp
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40 Arg Ser Phe Arg Ile Ala Gly Gly Glu Gln Lys Phe Ser Val Ala Asp
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50 Gly Trp Ile Ala Gln Leu Pro Gly Gly Tyr Gly Phe Glu Ser Ile Phe
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Lys Tyr Lys Gln Asn Gly Glu Tyr Gln Ile Phe Gly Pro Ser Ser Thr
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55 Ala Phe Asp Leu Lys Glu Thr Gly His Val Trp Asn Tyr Asp Phe Gly

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5 Glu Lys Thr Val Tyr Ala Ile Val Asn Ile Thr Pro Lys Val Glu Ala
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15 Ala Ala Tyr Ala Ala Phe Ser Asp Ala Gly Ser Glu Ile Ala Thr Leu
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20 Val Asn Ser Gln Asp Gln Met Ile Met Ser Gly Lys Pro Val Val Gln
165 170 175

25 Thr Ile Leu Ala Asn Val Ser Ala Ala Asn Ala Ser Val Gln Asn Lys
180 185 190

30 Val Pro Ile Ile Val Lys Arg Ala Ala Ile Arg Ala Ser Met Thr Ile
195 200 205

35 Thr Gln Gln Pro Val Asn Gly Ala Tyr Glu Ile Lys Ala Leu Arg Pro
210 215 220

40 Gly Asn Val Glu Val Gly Ile Ala Thr Val Ser Asp Leu Lys Trp Ala
225 230 235 240

45 Val Ala Gln Tyr Glu Lys Lys Tyr Tyr Leu Gln Gln Lys Asp Asp Ala
245 250 255

50 Leu Ser Pro Ala Ala Ser Phe Val Pro Ala Ser Thr Asn Asp Tyr Asn
260 265 270

55 Gly Ala Asn Gly Ala Met Lys Tyr Tyr Asp Tyr Ser Gln Leu Ala Asn
275 280 285

60 Arg Ile Thr Val His Gln Leu Asn Gly Ala Tyr Ser Ala Ala Asp Val
290 295 300

65 Pro Asn Ala Pro Tyr Lys Tyr Val Ser Gly Thr Thr His Ala Asp Asn
305 310 315 320

70 Asp Tyr Arg Lys Gly Asn Thr Thr Tyr Ile Leu Val Lys Gly Lys Leu
325 330 335

75 Lys Pro Val Ala Thr Met Trp Ala Asp Gly Glu Gln Ala Thr Tyr Gln
340 345 350

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Glu Gly Gly Asp Leu Phe Leu Gly Leu Val Thr Gly Lys Phe Tyr Ala
 355 360 365

5 Asn Glu Ala Asn Ala Asn Ala Ala Asn Pro Ala Ser Gly Gly Ala Gly
 370 375 380

10 Asn Pro Arg Val Val Thr Tyr Lys Ala Ala Ala Val Tyr Tyr Tyr Ala
 385 390 395 400

15 Trp Leu Asn Pro Asn Thr Leu Asp Pro Thr Thr Trp Thr Met Ser Pro
 405 410 415

Ala Arg Arg Asn Asn Ile Tyr Asn Val Asn Ile Ser Lys Phe Arg Asn
 420 425 430

20 Ile Gly Leu Ser Gly Asn Pro Phe Val Pro Thr Asp Pro Asp Pro Asn
 435 440 445

25 Asn Pro Asp Thr Pro Asp Asn Pro Asp Thr Pro Asp Pro Glu Asp Pro
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Asp Thr Pro Asn Pro Glu Glu Pro Leu Pro Val Gln Lys Thr Tyr Met
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30 Val Val Asp Val Thr Val Thr Pro Trp Thr Leu His Asn Tyr Asp Ile
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35 Glu Phe

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 <212> DNA
 <213> Porphyromonas\201@gingivalis

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 gagtatggtg gtgtagacaa aatcaacgac ttgactgttt atggtgtcgg tgatggcaag 240
 50 attgatgtga gaaaactttc tacagctgat ctgcaagtta atcagggagc ctctactact 300
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 55 gttgcatatg aagcagctta cgctgccttt tctgatgccg gcagtgagat tgctacgttg 480

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 5 gcaatacgtg catcaatgac tattactcag cagcccgtga atgggtgctta tgaatcaag 660
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 35 <213> Porphyromonas\201@gingivalis

<400> 17

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45 Phe Lys Lys Asn Val Val Leu Glu Val Phe Thr Ala Glu Trp Cys Gly
 35 40 45

50 Tyr Cys Pro Gly Gly Lys Glu Arg Ile Ala Lys Ala Ile Glu Met Leu
 50 55 60

Asp Asp Glu Tyr Lys Glu Arg Val Phe Gln Thr Phe Val His Tyr Asn
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55 Asp Gly Ile Ser Lys Lys Trp Pro Arg Val Gly Gln Leu Phe Ile Ala

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10	Arg	Met	Glu	Lys	Lys	Gly	Glu	Asn	Leu	Ser	Ile	Gly	Ala	Pro	Ile	Ala
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25	Thr	Ala	Thr	Phe	Thr	Gly	Lys	Val	Asp	Ala	Asp	Leu	Ile	Gly	Lys	Pro
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30	Leu	Met	Leu	Thr	Ala	Tyr	Val	Leu	Lys	Asn	Asn	Met	Lys	Pro	Ile	Asn
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45	Asp	Gly	Ser	Phe	Thr	Ile	Lys	Lys	Glu	Phe	Lys	Leu	Asp	Gly	Phe	Glu
	225					230					235					240
50	Ile	Lys	Asp	Thr	Asp	Val	Leu	Ala	Phe	Val	His	His	Pro	Met	Ser	Asn
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55	Ala	Glu	Asn	His	Ser	Ile	Ile	Asn	Ala	Gly	Gln	Glu	Ser	Leu	Asp	Lys
				260					265					270		
60	Ala	Glu	Pro	Thr	Ala	Thr	Glu	Gln	Ile	Val	Ala	Thr	Pro	Ser	Val	Lys
			275					280					285			
65	Ala	Tyr	Val	Gln	Asn	Gly	Lys	Ile	Val	Val	Glu	Glu	Glu	Tyr	Ser	Lys
		290					295					300				
70	Met	Glu	Val	Phe	Asn	Ala	Thr	Gly	Gln	Leu	Val	Lys	Asn	Glu	Ser	Leu
	305					310					315					320
75	Val	Pro	Gly	Val	Tyr	Val	Val	Arg	Ile	Thr	Ala	Asn	Gly	Val	Met	Tyr
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Phe Leu Lys Val Leu Val Pro
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<212> DNA
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15 attgaaatgt tggatgatga atataaggag cgtgtttttc agacatttgt tcattataat 240
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20 ctttcaatag gtgctccaat agcaattaaa aataagatta tgaaggttt tggatgatgg 420
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25 gcatatgtat tgaaaaaaca tatgaagcct attaatccgc aaaatggagc tggggatgga 600
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55 Leu Leu Val Thr Phe Gly Ser Ser Tyr Lys Ala Pro Arg Glu Thr Tyr

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15	Gly	Ile	Tyr	Ile	Asp	Ala	Pro	Asp	Glu	Ala	Leu	Glu	Lys	Leu	Ala	Arg
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20	Leu	Gly	Tyr	Lys	Lys	Ile	Asn	Val	Gln	Ser	Leu	His	Val	Ile	Pro	Gly
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25	Arg	Glu	Tyr	Asp	Glu	Met	Ile	Asp	Phe	Val	Asn	Lys	Phe	Lys	Ala	Ala
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35	Glu	Asp	Met	Arg	Glu	Val	Ala	Glu	Ile	Leu	His	Lys	Arg	Phe	Gln	Gln
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40	Thr	Ile	Glu	Lys	Gly	Glu	Ala	Ile	Val	Phe	Met	Gly	His	Gly	Thr	Glu
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45	His	Ala	Ala	Asn	Asp	Arg	Tyr	Ala	Arg	Ile	Asn	Lys	Ile	Met	Lys	Asn
				180					185					190		
50	Tyr	Ser	Lys	Phe	Met	Ile	Val	Gly	Thr	Val	Glu	Ser	Asp	Pro	Ser	Ile
			195					200					205			
55	Asn	Asp	Val	Ile	Ala	Glu	Leu	Lys	Glu	Thr	Gly	Ala	Thr	Ala	Val	Thr
		210					215					220				
60	Met	Met	Pro	Leu	Met	Ser	Val	Ala	Gly	Asp	His	Ala	Thr	Asn	Asp	Met
	225					230					235					240
65	Ala	Gly	Asp	Glu	Asp	Asp	Ser	Trp	Lys	Thr	Leu	Leu	Thr	Asn	Ala	Gly
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70	Tyr	Thr	Val	Ser	Ile	Asp	Lys	Leu	Asp	Asn	Gly	Asn	Phe	Ser	Ala	Leu
				260					265					270		
75	Gly	Asp	Ile	Glu	Glu	Ile	Arg	Asn	Ile	Trp	Leu	Lys	His	Met	Lys	Ala
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Thr Ser Ala Arg
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tataaagctc cacgcgaaac ctatgcgaag attgagaaga cttttgccgc agcttatccc 180
15 gatcaaagga taagctggac atacacgtct tctattatcc gaaagaaact ggctcagcag 240
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20 tttgtcaata agttaaaggc agcacatagt gatattactg tgaaggtagg ggctccgctt 420
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25 acgatagaga aagtggaagc tattgtattc atgggacacg gcaccgagca tgctgccaat 540
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accgtcgagt ccgatccctc tatcaatgat gttattgccg aactgaaaga aaccggtgcc 660
30 acggccgtaa caatgatgcc gctgatgagt gtggcaggcg accatgctac gaatgatatg 720
gccggagatg aggacgatag ctggaagacg ttgctgacca atgccggcta cacagtttct 780
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35 atctggctca agcatatgaa agccacctct gctcgtctaa 879

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45 Lys Asn Gln Glu Ile Met Thr Met Leu Glu Ala Lys His Pro Gly Glu
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Ser Glu Phe Leu Gln Ala Val Lys Glu Val Leu Leu Ser Val Glu Glu
20 25 30

50 Val Tyr Asn Gln His Pro Glu Phe Glu Lys Asn Gly Ile Ile Glu Arg
35 40 45

55 Ile Val Glu Pro Asp Arg Val Phe Thr Phe Arg Val Pro Trp Val Asp
50 55 60

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Asp Gln Gly Lys Val Gln Val Asn Ile Gly Tyr Arg Val Gln Phe Asn
 65 70 75 80

5 Asn Ala Ile Gly Pro Tyr Lys Gly Gly Ile Arg Phe His Pro Ser Val
 85 90 95

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

Ala Leu Thr Thr Leu Pro Met Gly Gly Gly Lys Gly Ala Asp Phe
 115 120 125

15 Ser Pro Lys Gly Lys Ser Glu Ala Glu Ile Met Arg Phe Cys Gln Ser
 130 135 140

20 Phe Met Thr Glu Leu Trp Arg Asn Ile Gly Pro Asp Thr Asp Ile Pro
 145 150 155 160

Ala Gly Asp Ile Gly Val Gly Gly Arg Glu Val Gly Tyr Met Phe Gly
 165 170 175

25 Met Tyr Lys Lys Leu Ala Arg Glu His Thr Gly Thr Leu Thr Gly Lys
 180 185 190

30 Gly Phe Glu Phe Gly Gly Ser Arg Leu Arg Pro Glu Ser Thr Gly Phe
 195 200 205

35 Gly Ala Val Tyr Phe Val Gln Asn Met Cys Lys Gln Asn Gly Val Asp
 210 215 220

Tyr Lys Gly Lys Thr Leu Ala Ile Ser Gly Phe Gly Asn Val Ala Trp
 225 230 235 240

40 Gly Val Ala Gln Lys Ala Thr Glu Leu Gly Ile Lys Val Val Thr Ile
 245 250 255

45 Ser Gly Pro Asp Gly Tyr Val Tyr Asp Pro Asp Gly Ile Asn Thr Pro
 260 265 270

50 Glu Lys Phe Arg Cys Met Leu Asp Leu Arg Asp Ser Gly Asn Asp Val
 275 280 285

Val Ser Asp Tyr Val Lys Arg Phe Pro Asn Ala Gln Phe Phe Pro Gly
 290 295 300

55 Lys Lys Pro Trp Glu Gln Lys Val Asp Phe Ala Met Pro Cys Ala Thr
 305 310 315 320

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Gln Asn Glu Met Asn Leu Glu Asp Ala Lys Thr Leu His Lys Asn Gly
 325 330 335

5 Val Thr Leu Val Ala Glu Thr Ser Asn Met Gly Cys Thr Ala Glu Ala
 340 345 350

10 Ser Glu Tyr Tyr Val Ala Asn Lys Met Leu Phe Ala Pro Gly Lys Ala
 355 360 365

15 Val Asn Ala Gly Gly Val Ser Cys Ser Gly Leu Glu Met Thr Gln Asn
 370 375 380

Ala Met His Leu Val Trp Thr Asn Glu Glu Val Asp Lys Trp Leu His
 385 390 395 400

20 Gln Ile Met Gln Asp Ile His Glu Gln Cys Val Thr Tyr Gly Lys Asp
 405 410 415

25 Gly Asn Tyr Ile Asp Tyr Val Lys Gly Ala Asn Ile Ala Gly Phe Met
 420 425 430

Lys Val Ala Lys Ala Met Val Ala Gln Gly Val Cys
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 40 gaaaagaacg gtatcatcga gcgtatcgta gagccggatc gtgtattcac attccgtgta 180
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 45 ctgaagttcc tcggtttcga acagatgttc aagaatgcac tcaactactct ccccatgggt 360
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 ctgcgtcccg aatctaccgg tttcgggtgct gtttacttcg tacagaacat gtgtaagcaa 660
 55 aacggtgtag actacaaggg caaaactctt gctatctccg gattcggtaa cgttgcttgg 720

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 25 <213> Porphyromonas\201@gingivalis

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40 Ser Val Leu Glu Val Ser His Arg Asp Lys Glu Phe Asp Ala Val Met
 35 40 45

45 Leu Glu Ala Arg Asn Leu Phe Lys Glu Leu Leu Asp Val Pro Glu Gly
 50 55 60

50 Tyr Glu Val Leu Phe Leu Gly Gly Gly Ala Ser Leu Gln Phe Tyr Gln
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55 Val Pro Leu Asn Leu Leu Lys Lys Lys Ala Ala Phe Ile Asn Thr Gly
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60 Thr Trp Ala Thr Asn Ala Ile Lys Gln Ala Lys Ile Met Thr Gln Val
 100 105 110

65 Tyr Gly Gly Glu Val Glu Val Leu Ala Ser Ser Glu Asp Lys Asn Phe
 115 120 125

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Ser Tyr Ile Pro Lys Asp Phe Val Ile Pro Glu Asp Val Asp Tyr Phe
 130 135 140

5 His Phe Thr Thr Asn Asn Thr Ile Tyr Gly Thr Glu Ile Arg Lys Asp
 145 150 155 160

Phe Asp Thr Lys Thr Arg Leu Val Ala Asp Met Ser Ser Asp Ile Phe
 10 165 170 175

Ser Arg Pro Ile Asp Val Ser Lys Tyr Asp Leu Ile Tyr Gly Gly Ala
 15 180 185 190

Gln Lys Asn Ile Gly Pro Ala Gly Ala Thr Phe Val Leu Val Lys Thr
 195 200 205

20 Asp Val Leu Gly Gln Val Asp Arg Pro Leu Pro Asp Met Leu Asn Tyr
 210 215 220

Gln Ile His Ile Lys Lys Asp Ser Met Phe Asn Thr Pro Pro Val Phe
 225 230 235 240

25 Pro Val Tyr Val Ala Leu Gln Thr Met Lys Trp Tyr Lys Glu Leu Gly
 245 250 255

Gly Val Lys Val Leu Glu Lys Met Asn Leu Asp Lys Ala Ala Leu Ile
 30 260 265 270

Tyr Asp Ala Ile Asp Ser Ser Lys Ile Phe Arg Gly Thr Val Asn Pro
 35 275 280 285

Glu Asp Arg Ser Ile Met Asn Ala Cys Phe Val Met Lys Asp Glu Tyr
 290 295 300

40 Lys Glu Leu Glu Lys Glu Phe Ala Thr Phe Ala Ala Ser Arg Gly Met
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Val Gly Ile Lys Gly His Arg Ser Val Gly Gly Phe Arg Ala Ser Leu
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<213> Porphyromonas\201@gingivalis

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<212> PRT

<213> Porphyromonas\201@gingivalis

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 50 Leu Gln Thr Val Thr Val Tyr Ser Thr Arg Thr Ala Val Pro Leu Lys
 35 40 45
 55 Lys Ile Pro Ala Lys Met Glu Leu Ile Ser Ser Arg Asn Ile Lys Gln
 50 55 60

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

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55 Ser Val His Gly Arg Phe Asn Gly Thr Val Glu Val Lys Asp Gly Gln

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20	Gly	Ala	Lys 115	Tyr	Val	Val	Met	Ser 120	Ala	Pro	Ser	Lys	Asp 125	Asp	Thr	Pro	
25	Met	Phe 130	Val	Cys	Gly	Val	Asn 135	Thr	Asp	Lys	Tyr	Val 140	Lys	Gly	Thr	Lys	
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70	Leu	Gly	Tyr 275	Thr	Asp	Glu	Glu	Val 280	Val	Ser	Ser	Asp	Phe 285	Ile	Gly	Glu	
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Gln Val Gly Tyr Asn Gly Phe Ala His Trp Leu Lys Lys Gln Ser Leu
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5 Glu Glu Met Glu His Ala Tyr Asp Met Met Asp Tyr Leu Leu Lys Arg
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10 Gly Gly Glu Val Lys Ile Glu Ala Ile Asp Ala Val Pro Gln Lys Phe
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15 Gly Ser Val Leu Glu Val Phe Gln Gln Val Tyr Glu His Glu Cys Lys
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20 Val Thr Glu Met Ile Glu Ala Val Val Arg Ala Ala Ser Glu Ala Gly
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25 Asp Met Ala Ser Gln Asp Phe Phe Trp Lys Tyr Ile Arg Glu Gln Val
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 15 Gly Tyr Pro Gly Lys Arg Tyr Tyr Gly Gly Cys Glu Val Val Asp Gln
 20 Ser Glu Gln Ile Ala Ile Asp Arg Ile Lys Gln Leu Tyr Gly Ala Glu
 25 Trp Ala Asn Val Gln Pro His Ser Gly Ala Gln Ala Asn Met Ala Val
 30 Leu Leu Ala Cys Leu Glu Ala Gly Asp Thr Phe Met Gly Leu Asn Leu
 35 Glu His Gly Gly His Leu Ser His Gly Ser Leu Val Asn Ser Ser Gly
 40 Ile Leu Tyr Arg Pro Ile Gly Tyr Asn Leu Ser Glu Glu Thr Gly Met
 45 Val Asp Tyr Asp His Met Glu Lys Met Ala Ile Glu His Lys Pro Lys
 50 Leu Ile Ile Gly Gly Gly Ser Ala Tyr Ser Arg Glu Trp Asp Tyr Lys
 55 Arg Met Arg Glu Ile Ala Asp Lys Val Gly Ala Leu Leu Met Ile Asp
 60 Met Ala His Pro Ala Gly Leu Ile Ala Ala Gly Leu Leu Glu Asn Pro
 65 Val Lys Tyr Ala His Ile Val Thr Ser Thr Thr His Lys Thr Leu Arg
 70 Gly Pro Arg Gly Gly Ile Ile Leu Met Gly Lys Asp Phe Asp Asn Pro
 75 Trp Gly Lys Lys Thr Pro Lys Gly Glu Ile Lys Lys Met Ser Ala Leu

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Leu Asp Ser Ala Val Phe Pro Gly Val Gln Gly Gly Pro Leu Glu His
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 5 Val Ile Ala Ala Lys Ala Val Ala Phe Gly Glu Ala Leu Asp Pro Ser
 275 280 285
 10 Phe Lys Glu Tyr Gln Thr Gln Val Lys Lys Asn Ala Ala Val Leu Ala
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 15 Gln Ala Phe Met Asp Lys Gly Tyr Lys Val Ile Ser Gly Gly Thr Asp
 305 310 315 320
 20 Asn His Ser Met Leu Ile Asp Leu Arg Pro Lys Phe Pro Glu Leu Thr
 325 330 335
 25 Gly Lys Val Ala Glu Lys Ala Leu Val Ala Ala Asp Ile Thr Val Asn
 340 345 350
 30 Lys Asn Met Val Pro Phe Asp Ser Arg Ser Ala Phe Gln Thr Ser Gly
 355 360 365
 35 Phe Arg Val Gly Thr Pro Ala Ile Thr Thr Arg Gly Val Lys Glu Asp
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 40 Lys Met Gly Tyr Ile Val Glu Leu Ile Asp Arg Val Leu Ser Ala Pro
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45 Thr Asn Val Thr Pro Asp Asn Pro Asp Asp Asn Pro Ser Glu Ile Asp
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50 Ile Thr Gln Thr His Thr Glu Lys Tyr Val Leu Ala Glu Glu Phe Thr
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Gly Gln Lys Cys Leu Asn Cys Pro Lys Gly His Arg Lys Leu Ala Ala
 65 70 75 80

55 Leu Lys Glu Gln Tyr Gly Lys Arg Leu Thr Val Val Gly Ile His Ala
 85 90 95

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Gly Pro Gly Ser Leu Val Pro Pro Leu Phe Arg Thr Glu Ala Gly Asp
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5 Ala Tyr Tyr Ser Lys Phe Ala Asn Asn Thr Pro Leu Pro Ala Leu Met
115 120 125

10 Val Ser Arg Lys Lys Phe Gly Ser Ser Tyr Val Tyr Asp Lys Ser Tyr
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15 Lys Thr Trp Asp Val Pro Ile Ala Glu Gln Met Glu Gln Lys Ala Lys
145 150 155 160

Ile Asn Ile Phe Ala Val Ala Glu Tyr Thr Asp Thr Gln Lys Ile Lys
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20 Val Thr Val Lys Gly Lys Ile Leu Glu Gly Asn Thr Leu Pro Lys Ser
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25 Met Val Gln Val Tyr Leu Leu Glu Asp Lys Leu Ile Ala Pro Gln Val
195 200 205

30 Asp Gly Asn Thr Thr Val Glu Asn Tyr Glu His Asn His Val Leu Arg
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Gly Ala Val Asn Gly Ile Trp Gly Glu Glu Phe Val Asn Leu Lys Asp
225 230 235 240

35 Tyr Leu Tyr Thr Tyr Ala Val Glu Pro Leu Ser Gly Met Ser Phe Val
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 35 40 45

45 Asp Ala Met Thr Ile Arg Leu Thr Pro Asp Phe Asn Pro Glu Asp Leu
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50 Ile Ala Gln Ser Arg Trp Gln Ser Gln Arg Asp Gly Arg Pro Val Arg
 65 70 75 80

55 Ile Gly Gln Val Ile Pro Val Asp Val Asp Phe Ala Ser Lys Ala Ser
 85 90 95

60 His Ile Ser Ser Ile Gly Asp Val Asp Val Tyr Arg Leu Gln Phe Lys
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65 Leu Glu Gly Ala Lys Ala Ile Thr Leu Tyr Tyr Asp Ala Phe Asn Ile

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Asp Ala Met Lys Ile Ser Ile Leu Lys Lys Thr Pro Ala Leu Asn Thr
 370 375 380

5 Trp Ile Ser Ser Ser Gly Ser Gly Gly Thr Asp Asp His Phe Tyr Phe
 385 390 395 400

10 Lys Tyr Asp Gln Gly Gly Thr Glu Gly Gly Ser Ser Gly Ser Ser Leu
 405 410 415

Phe Asn Gln Asn Lys His Val Val Gly Thr Leu Thr Gly Gly Ala Gly
 420 425 430

15 Asn Cys Gly Gly Thr Glu Phe Tyr Gly Arg Leu Asn Ser His Trp Asn
 435 440 445

20 Glu Tyr Ala Ser Asp Gly Asn Thr Ser Arg Met Asp Ile Tyr Leu Asp
 450 455 460

25 Pro Gln Asn Asn Gly Gln Thr Thr Ile Leu Asn Gly Thr Tyr Arg Asp
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Gly Tyr Lys Pro Leu Pro Ser Val Pro Arg Leu Leu Leu Gln Ser Thr
 485 490 495

30 Gly Asp Gln Val Glu Leu Asn Trp Thr Ala Val Pro Ala Asp Gln Tyr
 500 505 510

35 Pro Ser Ser Tyr Gln Val Glu Tyr His Ile Phe Arg Asn Gly Lys Glu
 515 520 525

Ile Ala Thr Thr Lys Glu Leu Ser Tyr Ser Asp Ala Ile Asp Glu Ser
 530 535 540

40 Ile Ile Gly Ser Gly Ile Ile Arg Tyr Glu Val Ser Ala Arg Phe Ile
 545 550 555 560

45 Tyr Pro Ser Pro Leu Asp Gly Val Glu Ser Tyr Lys Asp Thr Asp Lys
 565 570 575

Thr Ser Ala Asp Leu Ala Ile Gly Asp Ile Gln Thr Lys Leu Lys Pro
 580 585 590

50 Asp Val Thr Pro Leu Pro Gly Gly Gly Val Ser Leu Ser Trp Lys Val
 595 600 605

55 Pro Phe Leu Ser Gln Leu Val Ser Arg Phe Gly Glu Ser Pro Asn Pro
 610 615 620

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	Val	Phe	Lys	Thr	Phe	Glu	Val	Pro	Tyr	Val	Ser	Ala	Ala	Ala	Ala	Gln
	625					630					635					640
5	Thr	Pro	Asn	Pro	Pro	Val	Gly	Val	Val	Ile	Ala	Asp	Lys	Phe	Met	Ala
					645					650					655	
10	Gly	Thr	Tyr	Pro	Glu	Lys	Ala	Ala	Ile	Ala	Ala	Val	Tyr	Val	Met	Pro
				660					665					670		
15	Ser	Ala	Pro	Asp	Ser	Thr	Phe	His	Leu	Phe	Leu	Lys	Ser	Asn	Thr	Asn
			675					680					685			
20	Arg	Arg	Leu	Gln	Lys	Val	Thr	Thr	Pro	Ser	Asp	Trp	Gln	Ala	Gly	Thr
		690					695					700				
25	Trp	Leu	Arg	Ile	Asn	Leu	Asp	Lys	Pro	Phe	Pro	Val	Asn	Asn	Asp	His
	705					710					715					720
30	Met	Leu	Phe	Ala	Gly	Ile	Arg	Met	Pro	Asn	Lys	Tyr	Lys	Leu	Asn	Arg
					725					730					735	
35	Ala	Ile	Arg	Tyr	Val	Arg	Asn	Pro	Asp	Asn	Leu	Phe	Ser	Ile	Thr	Gly
				740					745					750		
40	Lys	Lys	Ile	Ser	Tyr	Asn	Asn	Gly	Val	Ser	Phe	Glu	Gly	Tyr	Gly	Ile
			755					760					765			
45	Pro	Ser	Leu	Leu	Gly	Tyr	Met	Ala	Ile	Lys	Tyr	Leu	Val	Val	Asn	Thr
		770					775						780			
50	Asp	Ala	Pro	Lys	Ile	Asp	Met	Ser	Leu	Val	Gln	Glu	Pro	Tyr	Ala	Lys
	785					790					795					800
55	Gly	Thr	Asn	Val	Ala	Pro	Phe	Pro	Glu	Leu	Val	Gly	Ile	Tyr	Val	Tyr
					805					810					815	
60	Lys	Asn	Gly	Thr	Phe	Ile	Gly	Thr	Gln	Asp	Pro	Ser	Val	Thr	Thr	Tyr
				820					825					830		
65	Ser	Val	Ser	Asp	Gly	Thr	Glu	Ser	Asp	Glu	Tyr	Glu	Ile	Lys	Leu	Val
			835					840					845			
70	Tyr	Lys	Gly	Ser	Gly	Ile	Ser	Asn	Gly	Val	Ala	Gln	Ile	Glu	Asn	Asn
		850					855					860				
75	Asn	Ala	Val	Val	Ala	Tyr	Pro	Ser	Val	Val	Thr	Asp	Arg	Phe	Ser	Ile
	865					870					875					880

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Lys Asn Ala His Met Val His Ala Ala Ala Leu Tyr Ser Leu Asp Gly
 885 890 895

5 Lys Gln Val Arg Ser Trp Asn Asn Leu Arg Asn Gly Val Thr Phe Ser
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10 Val Gln Gly Leu Thr Ala Gly Thr Tyr Met Leu Val Met Gln Thr Ala
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15 Asn Gly Pro Val Ser Gln Lys Ile Val Lys Gln
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 25 tccgccggaa cgcattcatt cgacgatgca atgactatcc gccttactcc ggatttcaat 180
 ccggaagacc tgatcgcaaca gagccgttgg caatcgcaaa gagatggccg gcccgctccgg 240
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 ctttattacg atgcattcaa tattccggag ggcggacgcc tctatatcta taccoccgac 420
 catgaaattg tgttgggagc atatacgaac gccactcatc gccgcaacgg agcttttgcc 480
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 ttgcctgaca tcaagatctc cgggtcgggt tatatattcg acaaagtcgg cggacgcccc 600
 40 gtaacggata accattacgg gatcgggtgag gacgattccg attcggattg cgagatcaac 660
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 atggtaaaag gacagtatat ctcaatgtgc tcaggcaacc tgctcaataa tacgaaagga 780
 45 gactttactc cgctgatcat ttctgccgga cactgtgctt ccataacaac caatttcgggt 840
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 gccggtattc atcatccggc cggagatgcc atgaagattt ccatacctaaa gaagactccg 1140
 55 gctctgaata catggatctc ctccagtggt tccggagggga ctgacgatca cttctatttc 1200

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 55 <400> 45

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 5 Ile Asn Lys Ala Trp Ala Lys Ala Ile Glu Gly Ala Ala Thr Ile His
 20 25 30
 His Leu Tyr Glu Gln Tyr Pro Asn Gly Gln Ile Asp Leu Ala His Glu
 10 35 40 45
 Gln Ala Leu Leu Glu Ala His Asp Arg Ile Val Phe Gln Phe Pro Leu
 50 55 60
 15 Tyr Trp Tyr Ala Ala Pro Tyr Leu Leu Lys Lys Trp Met Asp Glu Val
 65 70 75 80
 20 Phe Thr Glu Gly Trp Ala Tyr Gly Ala Gly Gly Asp Lys Met Glu Gly
 85 90 95
 Lys Glu Ile Cys Ala Ala Val Ser Cys Gly Ser Pro Lys Ser Ala Phe
 100 105 110
 25 Ala Glu Gly Ala Gln Gln Cys His Thr Leu Arg Ser Tyr Leu Asn Val
 115 120 125
 30 Phe Asp Gly Ile Ala Ala Phe Leu Arg Ala Arg Phe Thr Gly Tyr His
 130 135 140
 Ala Cys Tyr Asp Ser Tyr Asn Pro Arg Leu Pro Glu Met Leu Pro Ala
 145 150 155 160
 35 Asn Cys Glu Ala Tyr Leu Arg Phe Ile Lys Gly Glu
 165 170
 40 <210> 46
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 45 <400> 46
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 ggacaaatcg atctagcaca tgaacaagcc ctgctggagg ctcatgaccg catcgtcttc 180
 50 caattcccc tctattggta tgcagctccc tatctgctga agaagtggat ggacgaggtc 240
 tttactgagg gctgggccta tgggtccggt ggagacaaga tggagggtaa agaaatctgt 300
 gcagcagtct cctgcggatc acccaaatca gcttttgccg aaggagcaca gcaatgccac 360
 55 acgctgcgaa gctacttgaa tgtattcgac gggatagctg ctttctgcg cgctcgattc 420

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 <213> Porphyromonas\201@gingivalis

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15
 Val Val Lys Gly Leu Ser Gly Leu Leu Ala Asp Leu Gln Val Tyr Tyr
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20
 Ser Asn Leu Arg Gly Phe His Trp Asn Ile Arg Gly Ala Glu Phe Phe
 35 40 45

25
 Val Leu His Glu Gln Tyr Glu Lys Met Tyr Asp Asp Leu Ala Gly Lys
 50 55 60

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

30
 Asn Arg Phe Ser Glu Tyr Leu Lys Val Ala Glu Val Lys Glu Glu His
 85 90 95

35
 Glu Leu Val Cys Ala Ala Ser Thr Leu Lys Asn Val Thr Asp Thr Leu
 100 105 110

Gln Ile Ile Met Ala Lys Glu Arg Ala Ile Ala Glu Val Ala Gly Glu
 115 120 125

40
 Ala Gly Asp Glu Val Thr Val Asp Leu Met Ile Gly Phe Leu Ser Glu
 130 135 140

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ttgtccggtt tgttgccga cctccaagtg tattactcca accttcgagg gttccactgg 120

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 5 aaccgcttca gcgagtacct gaaagtagca gaagtgaagg aagagcacga actcgtttgc 300
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 gccatcgag aagttgccgg tgaggcaggc gatgaggtaa cgggtggattt gatgatcggg 420
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 20 25 30
 25 Thr Ala Phe Gln Arg Asp Lys Ala Ser Asp His Trp Phe Ile Asp Ile
 35 40 45
 30 Ala Gly Gly Ala Gly Met Ala Leu Ser Gly Trp Asn Asn Asp Val Asp
 50 55 60
 35 Phe Val Asp Arg Leu Ser Ile Val Pro Thr Phe Gly Ile Gly Lys Trp
 65 70 75 80
 His Glu Pro Tyr Phe Gly Thr Arg Leu Gln Phe Thr Gly Phe Asp Ile
 85 90 95
 40 Tyr Gly Phe Pro Gln Gly Ser Lys Glu Arg Asn His Asn Tyr Phe Gly
 100 105 110
 45 Asn Ala His Leu Asp Phe Met Phe Asp Leu Thr Asn Tyr Phe Gly Val
 115 120 125
 Tyr Arg Pro Asn Arg Val Phe His Ile Ile Pro Trp Ala Gly Ile Gly
 130 135 140
 50 Phe Gly Tyr Lys Phe His Ser Glu Asn Ala Asn Gly Glu Lys Val Gly
 145 150 155 160
 55 Ser Lys Asp Asp Met Thr Gly Thr Val Asn Val Gly Leu Met Leu Lys
 165 170 175

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Phe Arg Leu Ser Arg Val Val Asp Phe Asn Ile Glu Gly Gln Ala Phe
180 185 190

5 Ala Gly Lys Met Asn Phe Ile Gly Thr Lys Arg Gly Lys Ala Asp Phe
195 200 205

10 Pro Val Met Ala Thr Ala Gly Leu Thr Phe Asn Leu Gly Lys Thr Glu
210 215 220

Trp Thr Glu Ile Val Pro Met Asp Tyr Ala Leu Val Asn Asp Leu Asn
225 230 235 240

15 Asn Gln Ile Asn Ser Leu Arg Gly Gln Val Glu Glu Leu Ser Arg Arg
245 250 255

20 Pro Val Ser Cys Pro Glu Cys Pro Glu Pro Thr Gln Pro Thr Val Thr
260 265 270

Arg Val Val Val Asp Asn Val Val Tyr Phe Arg Ile Asn Ser Ala Lys
275 280 285

25 Ile Asp Arg Asn Gln Glu Ile Asn Val Tyr Asn Thr Ala Glu Tyr Ala
290 295 300

30 Lys Thr Asn Asn Ala Pro Ile Lys Val Val Gly Tyr Ala Asp Glu Lys
305 310 315 320

35 Thr Gly Thr Ala Ala Tyr Asn Met Lys Leu Ser Glu Arg Arg Ala Lys
325 330 335

Ala Val Ala Lys Met Leu Glu Lys Tyr Gly Val Ser Ala Asp Arg Ile
340 345 350

40 Thr Ile Glu Trp Lys Gly Ser Ser Glu Gln Ile Tyr Glu Glu Asn Ala
355 360 365

45 Trp Asn Arg Ile Val Val Met Thr Ala Ala Glu
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50 <211> 1140
<212> DNA
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caagaagcta ctacacagaa caaagcaggg atgcacaccg cattccaacg tgataaggcc 120

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5 catgagcctt atttcggtac tcgtctccaa ttcacaggat tcgacatcta tggattcccg 300
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10 gcaggtatag gatttggtta taaattccat agcgaaaacg ccaatggtga aaaagtagga 480
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35 <213> Porphyromonas\201@gingivalis

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20 25 30

Thr Cys Thr Asn Asn Ser Phe Lys Gln Phe Asp Ala Ser Phe Ser Phe
35 40 45

Asn Glu Val Glu Leu Thr Lys Val Glu Thr Lys Gly Gly Thr Phe Ala
50 55 60

Ser Val Ser Ile Pro Gly Ala Phe Pro Thr Gly Glu Val Gly Ser Pro
65 70 75 80

Glu Val Pro Ala Val Arg Lys Leu Ile Ala Val Pro Val Gly Ala Thr

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

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Gly Asp Tyr Phe Pro Glu Met Tyr Thr Phe Arg Met Ser Ala Ser Ser
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 5 Pro Glu Glu Leu Thr Asn Ile Ile Asp Lys Val Leu Met Tyr Glu Lys
 355 360 365
 10 Ala Thr Met Pro Asp Lys Ser Tyr Leu Glu Lys Val Leu Leu Ile Ala
 370 375 380
 15 Gly Ala Asp Tyr Ser Trp Asn Ser Gln Val Gly Gln Pro Thr Ile Lys
 385 390 395 400
 20 Tyr Gly Met Gln Tyr Tyr Tyr Asn Gln Glu His Gly Tyr Thr Asp Val
 405 410 415
 25 Tyr Asn Tyr Leu Lys Ala Pro Tyr Thr Gly Cys Tyr Ser His Leu Asn
 420 425 430
 30 Thr Gly Val Ser Phe Ala Asn Tyr Thr Ala His Gly Ser Glu Thr Ala
 435 440 445
 35 Trp Ala Asp Pro Leu Leu Thr Thr Ser Gln Leu Lys Ala Leu Thr Asn
 450 455 460
 40 Lys Asp Lys Tyr Phe Leu Ala Ile Gly Asn Cys Cys Ile Thr Ala Gln
 465 470 475 480
 45 Phe Asp Tyr Val Gln Pro Cys Phe Gly Glu Val Ile Thr Arg Val Lys
 485 490 495
 50 Glu Lys Gly Ala Tyr Ala Tyr Ile Gly Ser Ser Pro Asn Ser Tyr Trp
 500 505 510
 55 Gly Glu Asp Tyr Tyr Trp Ser Val Gly Ala Asn Ala Val Phe Gly Val
 515 520 525
 60 Gln Pro Thr Phe Glu Gly Thr Ser Met Gly Ser Tyr Asp Ala Thr Phe
 530 535 540
 65 Leu Glu Asp Ser Tyr Asn Thr Val Asn Ser Ile Met Trp Ala Gly Asn
 545 550 555 560
 70 Leu Ala Ala Thr His Ala Gly Asn Ile Gly Asn Ile Thr His Ile Gly
 565 570 575
 75 Ala His Tyr Tyr Trp Glu Ala Tyr His Val Leu Gly Asp Gly Ser Val
 580 585 590

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Met Pro Tyr Arg Ala Met Pro Lys Thr Asn Thr Tyr Thr Leu Pro Ala
595 600 605

5 Ser Leu Pro Gln Asn Gln Ala Ser Tyr Ser Ile Gln Ala Ser Ala Gly
610 615 620

10 Ser Tyr Val Ala Ile Ser Lys Asp Gly Val Leu Tyr Gly Thr Gly Val
625 630 635 640

15 Ala Asn Ala Ser Gly Val Ala Thr Val Ser Met Thr Lys Gln Ile Thr
645 650 655

20 Glu Asn Gly Asn Tyr Asp Val Val Ile Thr Arg Ser Asn Tyr Leu Pro
660 665 670

25 Val Ile Lys Gln Ile Gln Val Gly Glu Pro Ser Pro Tyr Gln Pro Val
675 680 685

30 Ser Asn Leu Thr Ala Thr Thr Gln Gly Gln Lys Val Thr Leu Lys Trp
690 695 700

35 Glu Ala Pro Ser Ala Lys Lys Ala Glu Gly Ser Arg Glu Val Lys Arg
705 710 715 720

40 Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro Ala Asn Asp Val Arg
725 730 735

45 Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp Asn Val Trp Gly Asp
740 745 750

50 Asn Thr Gly Tyr Gln Phe Leu Leu Asp Ala Asp His Asn Thr Phe Gly
755 760 765

55 Ser Val Ile Pro Ala Thr Gly Pro Leu Phe Thr Gly Thr Ala Ser Ser
770 775 780

60 Asn Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile Pro Ala Asn Ala Asp
785 790 795 800

65 Pro Val Val Thr Thr Gln Asn Ile Ile Val Thr Gly Gln Gly Glu Val
805 810 815

70 Val Ile Pro Gly Gly Val Tyr Asp Tyr Cys Ile Thr Asn Pro Glu Pro
820 825 830

75 Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly Gly Asn Gln Pro Ala
835 840 845

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Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys Lys Tyr Thr Phe Thr
 850 855 860
 5 Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp Met Glu Val Glu Asp
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5 Asn Asn Pro Tyr Asp Ser Thr Pro Leu Gly Glu Arg Arg Glu Arg Ala
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10 Tyr Arg Asp Val Ser Lys Ser Phe Thr Asn Thr Ala Glu Tyr Lys Phe
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20 Ser Asp Lys Leu Met Leu Leu Ser Gln Gly Lys Thr Gly Asn Ser Leu
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Ser Leu Pro Glu His Arg Val Ala Glu Tyr Ala Tyr Leu Ser Phe Phe
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25 Ser Arg Phe Asn Tyr Gly Phe Asp Lys Trp Met Tyr Ile Asp Phe Ser
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30 Val Arg Asn Asp Gln Ser Ser Arg Phe Gly Ser Asn Asn Arg Ser Ala
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40 Thr Thr Gly Asn Ser Glu Ile Gly Asn Tyr Asn His Gln Ala Leu Val
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Ala Gly Asn Pro Asp Leu Ser Trp Glu Lys Gln Ser Gln Phe Asn Phe
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55 Phe Tyr Val Arg Thr Thr Asn Asp Met Leu Ile Asp Val Pro Met Pro
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 5 Asn Thr Gly Val Asp Leu Ser Leu Lys Gly Thr Ile Tyr Gln Asn Lys
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 10 Asp Trp Asn Val Tyr Ala Ser Ala Asn Phe Asn Tyr Asn Arg Gln Glu
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 50 Phe Thr Glu Asn Gly Gly Gly Leu Met Gln Leu Asn Lys Asp Lys Met
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 75 Ala Arg Asn Leu Leu Thr Val Thr Lys Tyr Lys Gly Phe Asp Pro Glu
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 15 Asp Asn Leu Lys Phe Thr Glu Val Gln Thr Pro Lys Gly Met Ala Gln
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 25 Thr Leu Pro Ile Leu Ser Arg Ser Leu Ala Val Ser Asp Thr Arg Glu
 30 Met Lys Val Glu Val Val Ser Ser Lys Phe Ile Glu Lys Lys Asn Val
 35 Leu Ile Ala Pro Ser Lys Gly Met Ile Met Arg Asn Glu Asp Pro Lys
 40 Lys Ile Pro Tyr Val Tyr Gly Lys Ser Tyr Ser Gln Asn Lys Phe Phe
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 55 Lys Thr Leu Arg Ile Tyr Thr Glu Ile Thr Val Ala Val Ser Glu Thr
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 65 Phe Glu Asp Thr Tyr Lys Arg Met Phe Met Asn Tyr Glu Pro Gly Arg
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 75 Ala Lys Lys Tyr Glu Gly Asp Ile Lys Asp Phe Val Asp Trp Lys Asn
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5 Pro Val Thr Ala Asn Ala Ile Gln Gln Phe Val Lys Gln Glu Tyr Glu
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10 Lys Glu Gly Asn Asp Leu Thr Tyr Val Leu Leu Ile Gly Asp His Lys
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15 Asp Ile Pro Ala Lys Ile Thr Pro Gly Ile Lys Ser Asp Gln Val Tyr
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Gly Gln Ile Val Gly Asn Asp His Tyr Asn Glu Val Phe Ile Gly Arg
 325 330 335

20 Phe Ser Cys Glu Ser Lys Glu Asp Leu Lys Thr Gln Ile Asp Arg Thr
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Ile His Tyr Glu Arg Asn Ile Thr Thr Glu Asp Lys Trp Leu Gly Gln
 355 360 365

25 Ala Leu Cys Ile Ala Ser Ala Glu Gly Gly Pro Ser Ala Asp Asn Gly
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30 Glu Ser Asp Ile Gln His Glu Asn Val Ile Ala Asn Leu Leu Thr Gln
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Tyr Gly Tyr Thr Lys Ile Ile Lys Cys Tyr Asp Pro Gly Val Thr Pro
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35 Lys Asn Ile Ile Asp Ala Phe Asn Gly Gly Ile Ser Leu Ala Asn Tyr
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40 Thr Gly His Gly Ser Glu Thr Ala Trp Gly Thr Ser His Phe Gly Thr
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45 Thr His Val Lys Gln Leu Thr Asn Ser Asn Gln Leu Pro Phe Ile Phe
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55 Val Ala Ile Ile Ala Ser Thr Ile Asn Gln Ser Trp Ala Ser Pro Met
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 10 Met Val Glu Lys Tyr Lys Lys Asp Gly Glu Lys Met Leu Asp Thr Trp
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 70 Gln Ile Leu Leu Asp Ala Asp His Asp Gln Tyr Gly Gln Val Ile Pro
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25	Glu	Ala	Gly	Lys	Lys	Tyr	His	Phe	Leu	Met	Lys	Lys	Met	Gly	Ser	Gly
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EP 3 139 167 A1

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10 Thr Leu Gln Gly Gly Val Ala Ala Gln Phe Leu Asn Asp Asn Asn Asn
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15 Lys Asp Leu Met Asp Arg Leu Gly Ala Ile Gly Ser Leu Ser Val Gly
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20 Gln Ala His Thr Phe Leu Gly Lys Asn Gly Glu Gln Glu Ile Asn Thr
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25 Asn Phe Gly Ala Ala His Phe Asp Phe Met Phe Asp Val Val Asn Tyr
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 40 Ala Pro Phe Val Asn Trp Asp Leu Gly Ile Leu Asn Asp His Gly Arg
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 45 Ala Asp Glu Asp Glu Val Ser Gly Ile Ala Gly Tyr Tyr Phe Val Tyr
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 Asn Arg Leu Asn Gln Gln Ala Asn Ala Phe Val Asn Asn Thr Glu Ala
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 55 Lys Ser Phe Leu Ala Glu Gly Lys Val Leu Gln Ala Leu Ala Ile Trp
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 5 Ser Gly Ala Lys Asp Leu Gly Val Ile Leu Leu Lys Glu Tyr Asn Pro
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 30 Met Val Val Asp Lys Tyr Pro Leu Ile Gly Ala Ala Asp Ala Ser Glu
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20 Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro Ala Asn Asp Val Arg
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Pro Val Val Arg Val Lys Ser Phe Thr Glu Gln Val Tyr Ser Leu Asn
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<220>
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 <400> 110
 5 ggggaccact ttgtacaaga aagctgggctc actagtttac caagcaaaga gaggataatc 60
 ggc 63

 <210> 111
 10 <211> 61
 <212> DNA
 <213> Artificial Sequence

 <220>
 15 <223> Description of Artificial Sequence: Synthetic DNA

 <400> 111
 ggggacaagt ttgtacaaaa aagcaggctc acccgggaaa aagctatttc tctcgcctcac 60
 g 61
 20

 <210> 112
 <211> 62
 <212> DNA
 25 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic DNA

 <400> 112
 30 ggggaccact ttgtacaaga aagctgggctc actagtttat ttgccatcgg attgcggatt 60
 ga 62

 <210> 113
 35 <211> 62
 <212> DNA
 <213> Artificial Sequence

 <220>
 40 <223> Description of Artificial Sequence: Synthetic DNA

 <400> 113
 ggggacaagt ttgtacaaaa aagcaggctc acccgggaac aaatattaca aatcactttt 60
 gc 62
 45

 <210> 114
 <211> 64
 <212> DNA
 50 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic DNA

 <400> 114
 55 ggggaccact ttgtacaaga aagctgggctc actagtctac tgcttcacga tcttttgct 60
 caca 64

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<210> 115
 <211> 59
 <212> DNA
 <213> Artificial Sequence
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 <220>
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 10 ggggacaagt ttgtacaaaa aagcaggctc acccgggaaa aaaacgctcg taatagtcg 59
 <210> 116
 <211> 58
 <212> DNA
 15 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: Synthetic DNA
 <400> 116
 20 ggggaccact ttgtacaaga aagctgggctc actagttcat tctcctttga taaagcgg 58
 <210> 117
 <211> 61
 <212> DNA
 25 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Synthetic DNA
 30 <400> 117
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 t 61
 35
 <210> 118
 <211> 61
 <212> DNA
 <213> Artificial Sequence
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 <220>
 <223> Description of Artificial Sequence: Synthetic DNA
 <400> 118
 45 ggggaccact ttgtacaaga aagctgggctc actagtttac ttggtagcgt aggcagacag 60
 c 61
 <210> 119
 <211> 63
 <212> DNA
 50 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: Synthetic DNA
 55 <400> 119

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ggggacaagt ttgtacaaaa aagcaggctc actcgagaaa gctaaatctt tattattagc 60
 act 63
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 <210> 120
 <211> 62
 <212> DNA
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 <223> Description of Artificial Sequence: Synthetic DNA
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 15
 aa 62
 <210> 121
 <211> 63
 <212> DNA
 <213> Artificial Sequence
 20
 <220>
 <223> Description of Artificial Sequence: Synthetic DNA
 25
 <400> 121
 ggggacaagt ttgtacaaaa aagcaggctc actcgagagg aaattattat tgctgatcgc 60
 ggc 63
 30
 <210> 122
 <211> 61
 <212> DNA
 <213> Artificial Sequence
 35
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 <400> 122
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 g 61
 <210> 123
 <211> 59
 <212> DNA
 <213> Artificial Sequence
 45
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 50
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 ggggacaagt ttgtacaaaa aagcaggctc actcgagaaa aagcttttac aggctaaag 59
 55
 <210> 124
 <211> 65
 <212> DNA
 <213> Artificial Sequence

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<220>
 <223> Description of Artificial Sequence: Synthetic DNA

<400> 124
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 tcacg 65

<210> 125
 <211> 63
 <212> DNA
 <213> Artificial Sequence

<220>
 15 <223> Description of Artificial Sequence: Synthetic DNA

<400> 125
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 20 ttg 63

<210> 126
 <211> 59
 <212> DNA
 25 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic DNA

<400> 126
 30 ggggaccact ttgtacaaga aagctgggtc gcgggccgctt agaaagaaat ctgaatacc 59

<210> 127
 <211> 61
 <212> DNA
 35 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic DNA

<400> 127
 40 ggggacaagt ttgtacaaaa aagcaggctc actcgagttg aacaagtttg tttcgattgc 60
 t 61
 45

<210> 128
 <211> 61
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic DNA

<400> 128
 55 ggggaccact ttgtacaaga aagctgggtc actagtttac tttacagcga gtttctctac 60
 g 61

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<210> 129
 <211> 61
 <212> DNA
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 <220>
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 10 ggggacaagt ttgtacaaaa aagcaggctc actcgagaag gtaaagtact taatgctcac 60
 a 61
 <210> 130
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 <212> DNA
 <213> Artificial Sequence
 15
 <220>
 <223> Description of Artificial Sequence: Synthetic DNA
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 <400> 130
 ggggaccact ttgtacaaga aagctgggtc actagtttac ttggagcgaa cgattacaac 60
 acg 63
 25
 <210> 131
 <211> 68
 <212> DNA
 <213> Artificial Sequence
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 <220>
 <223> Description of Artificial Sequence: Synthetic DNA
 <400> 131
 35 ggggacaagt ttgtacaaaa aagcaggctc actcgagaaa aaaataattt attggggtgc 60
 gacagttt 68
 <210> 132
 <211> 69
 <212> DNA
 <213> Artificial Sequence
 40
 <220>
 <223> Description of Artificial Sequence: Synthetic DNA
 45
 <400> 132
 ggggaccact ttgtacaaga aagctgggtc actagtttat atcggccagt tctttattaa 60
 ctgcggatt 69
 50
 <210> 133
 <211> 20
 <212> DNA
 <213> Artificial Sequence
 55
 <220>

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5
10
15
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25
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45
50
55

<223> Description of Artificial Sequence: Synthetic DNA

<400> 133
attacagctc aattcgatta 20

<210> 134
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 134
agcgcagttg ccaatagcta 20

<210> 135
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 135
ccttcggaga ggtaataact 20

<210> 136
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 136
caggctgtac ataatcgaat 20

<210> 137
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 137
gtgaatggcg atttcctatt 20

<210> 138
<211> 20
<212> DNA
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<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 138

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agcagctacg tcgaaaataa 20

5 <210> 139
 <211> 20
 <212> DNA
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<220>
 10 <223> Description of Artificial Sequence: Synthetic DNA

<400> 139
 ctttcgcaga agcattgatg 20

15 <210> 140
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 20 <223> Description of Artificial Sequence: Synthetic DNA

<400> 140
 caggcatgct gaataggaaa 20

25 <210> 141
 <211> 860
 <212> PRT
 <213> Porphyromonas\201@gingivalis

30 <400> 141

Arg Lys Leu Leu Leu Leu Ile Ala Ala Ser Leu Leu Gly Val Gly Leu
 1 5 10 15

35 Tyr Ala Gln Ser Ala Lys Ile Lys Leu Asp Ala Pro Thr Thr Arg Thr
 20 25 30

40 Thr Cys Thr Asn Asn Ser Phe Lys Gln Phe Asp Ala Ser Phe Ser Phe
 35 40 45

Asn Glu Val Glu Leu Thr Lys Val Glu Thr Lys Gly Gly Thr Phe Ala
 50 55 60

45 Ser Val Ser Ile Pro Gly Ala Phe Pro Thr Gly Glu Val Gly Ser Pro
 65 70 75 80

50 Glu Val Pro Ala Val Arg Lys Leu Ile Ala Val Pro Val Gly Ala Thr
 85 90 95

Pro Val Val Arg Val Lys Ser Phe Thr Glu Gln Val Tyr Ser Leu Asn
 100 105 110

55 Gln Tyr Gly Ser Glu Lys Leu Met Pro His Gln Pro Ser Met Ser Lys

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5	Ser	Asp	Asp	Pro	Glu	Lys	Val	Pro	Phe	Val	Tyr	Asn	Ala	Ala	Ala	Tyr	
		130					135					140					
10	Ala	Arg	Lys	Gly	Phe	Val	Gly	Gln	Glu	Leu	Thr	Gln	Val	Glu	Met	Leu	
	145					150					155					160	
15	Gly	Thr	Met	Arg	Gly	Val	Arg	Ile	Ala	Ala	Leu	Thr	Ile	Asn	Pro	Val	
					165					170					175		
20	Gln	Tyr	Asp	Val	Val	Ala	Asn	Gln	Leu	Lys	Val	Arg	Asn	Asn	Ile	Glu	
				180					185					190			
25	Ile	Glu	Val	Ser	Phe	Gln	Gly	Ala	Asp	Glu	Val	Ala	Thr	Gln	Arg	Leu	
			195					200					205				
30	Tyr	Asp	Ala	Ser	Phe	Ser	Pro	Tyr	Phe	Glu	Thr	Ala	Tyr	Lys	Gln	Leu	
		210					215					220					
35	Phe	Asn	Arg	Asp	Val	Tyr	Thr	Asp	His	Gly	Asp	Leu	Tyr	Asn	Thr	Pro	
	225					230					235					240	
40	Val	Arg	Met	Leu	Val	Val	Ala	Gly	Ala	Lys	Phe	Lys	Glu	Ala	Leu	Lys	
					245					250					255		
45	Pro	Trp	Leu	Thr	Trp	Lys	Ala	Gln	Lys	Gly	Phe	Tyr	Leu	Asp	Val	His	
				260					265					270			
50	Tyr	Thr	Asp	Glu	Ala	Glu	Val	Gly	Thr	Thr	Asn	Ala	Ser	Ile	Lys	Ala	
			275					280					285				
55	Phe	Ile	His	Lys	Lys	Tyr	Asn	Asp	Gly	Leu	Ala	Ala	Ser	Ala	Ala	Pro	
	290						295					300					
60	Val	Phe	Leu	Ala	Leu	Val	Gly	Asp	Thr	Asp	Val	Ile	Ser	Gly	Glu	Lys	
	305					310					315					320	
65	Gly	Lys	Lys	Thr	Lys	Lys	Val	Thr	Asp	Leu	Tyr	Tyr	Ser	Ala	Val	Asp	
					325					330					335		
70	Gly	Asp	Tyr	Phe	Pro	Glu	Met	Tyr	Thr	Phe	Arg	Met	Ser	Ala	Ser	Ser	
				340					345					350			
75	Pro	Glu	Glu	Leu	Thr	Asn	Ile	Ile	Asp	Lys	Val	Leu	Met	Tyr	Glu	Lys	
			355					360					365				

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Ala Thr Met Pro Asp Lys Ser Tyr Leu Glu Lys Val Leu Leu Ile Ala
 370 375 380

5 Gly Ala Asp Tyr Ser Trp Asn Ser Gln Val Gly Gln Pro Thr Ile Lys
 385 390 400

10 Tyr Gly Met Gln Tyr Tyr Tyr Asn Gln Glu His Gly Tyr Thr Asp Val
 405 410 415

Tyr Asn Tyr Leu Lys Ala Pro Tyr Thr Gly Cys Tyr Ser His Leu Asn
 420 425 430

15 Thr Gly Val Ser Phe Ala Asn Tyr Thr Ala His Gly Ser Glu Thr Ala
 435 440 445

20 Trp Ala Asp Pro Leu Leu Thr Thr Ser Gln Leu Lys Ala Leu Thr Asn
 450 455 460

Lys Asp Lys Tyr Phe Leu Ala Ile Gly Asn Cys Ala Ile Thr Ala Gln
 465 470 475 480

25 Phe Asp Tyr Val Gln Pro Cys Phe Gly Glu Val Ile Thr Arg Val Lys
 485 490 495

30 Glu Lys Gly Ala Tyr Ala Tyr Ile Gly Ser Ser Pro Asn Ser Tyr Trp
 500 505 510

Gly Glu Asp Tyr Tyr Trp Ser Val Gly Ala Asn Ala Val Phe Gly Val
 515 520 525

35 Gln Pro Thr Phe Glu Gly Thr Ser Met Gly Ser Tyr Asp Ala Thr Phe
 530 535 540

40 Leu Glu Asp Ser Tyr Asn Thr Val Asn Ser Ile Met Trp Ala Gly Asn
 545 550 555 560

45 Leu Ala Ala Thr His Ala Gly Asn Ile Gly Asn Ile Thr His Ile Gly
 565 570 575

Ala His Tyr Tyr Trp Glu Ala Tyr His Val Leu Gly Asp Gly Ser Val
 580 585 590

50 Met Pro Tyr Arg Ala Met Pro Lys Thr Asn Thr Tyr Thr Leu Pro Ala
 595 600 605

55 Ser Leu Pro Gln Asn Gln Ala Ser Tyr Ser Ile Gln Ala Ser Ala Gly
 610 615 620

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Ser Tyr Val Ala Ile Ser Lys Asp Gly Val Leu Tyr Gly Thr Gly Val
 625 630 635 640
 5 Ala Asn Ala Ser Gly Val Ala Thr Val Ser Met Thr Lys Gln Ile Thr
 645 650 655
 10 Glu Asn Gly Asn Tyr Asp Val Val Ile Thr Arg Ser Asn Tyr Leu Pro
 660 665 670
 Val Ile Lys Gln Ile Gln Val Gly Glu Pro Ser Pro Tyr Gln Pro Val
 675 680 685
 15 Ser Asn Leu Thr Ala Thr Thr Gln Gly Gln Lys Val Thr Leu Lys Trp
 690 695 700
 20 Glu Ala Pro Ser Ala Lys Lys Ala Glu Gly Ser Arg Glu Val Lys Arg
 705 710 715 720
 25 Ile Gly Asp Gly Leu Phe Val Thr Ile Glu Pro Ala Asn Asp Val Arg
 725 730 735
 Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp Asn Val Trp Gly Asp
 740 745 750
 30 Asn Thr Gly Tyr Gln Phe Leu Leu Asp Ala Asp His Asn Thr Phe Gly
 755 760 765
 35 Ser Val Ile Pro Ala Thr Gly Pro Leu Phe Thr Gly Thr Ala Ser Ser
 770 775 780
 40 Asn Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile Pro Ala Asn Ala Asp
 785 790 795 800
 Pro Val Val Thr Thr Gln Asn Ile Ile Val Thr Gly Gln Gly Glu Val
 805 810 815
 45 Val Ile Pro Gly Gly Val Tyr Asp Tyr Cys Ile Thr Asn Pro Glu Pro
 820 825 830
 50 Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly Gly Asn Gln Pro Ala
 835 840 845
 Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys Lys
 850 855 860
 55 <210> 142
 <211> 2583

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<212> DNA
 <213> Porphyromonas\201@gingivalis

<400> 142

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 gccaaagatta agcttgatgc tccgactact cgaacgacat gtacgaacaa tagcttcaag 120
 cagttcgatg caagcttttc gttcaatgaa gtcgagctga caaagggtga gaccaaaggt 180
 10 ggtactttcg cctcagtgtc aattccgggt gcattcccga ccggtgaggt tggttctccc 240
 gaagtgccag cagttaggaa gttgattgct gtgcctgtcg gagccacacc tgttgttcgc 300
 gtgaaaagtt ttaccgagca agtttactct ctgaaccaat acggttccga aaaactcatg 360
 15 ccacatcaac cctctatgag caagagtgat gatcccgaaa aggttccctt cgtttacaat 420
 gctgctgctt atgcacgcaa aggttttgtc ggacaagaac tgaccaagt agaaatgttg 480
 gggacaatgc gtggtgttcg cattgcagct cttaccatta atcctgttca gtatgatgtg 540
 20 gttgcaaac aattgaaggt tagaaacaac atcgaaattg aagtaagctt tcaaggagct 600
 gatgaagtag ctacacaacg tttgtatgat gcttctttta gcccttattt cgaaacagct 660
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 25 gttcgtatgc ttggtgttc aggtgcaaaa ttcaaagaag ctctcaagcc ttggctcact 780
 tggaaggctc aaaagggctt ctatctggat gtgcattaca cagacgaagc tgaagtagga 840
 acgacaaacg cctctatcaa ggcatattt cacaagaaat acaatgatgg attggcagct 900
 30 agtgctgctc cggctcttctt ggctttgggt ggtgacactg acgttattag cggagaaaaa 960
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 35 cctgaaatgt atactttccg tatgtctgct tcttcccag aagaactgac gaacatcatt 1080
 gataaggtat tgatgtatga aaaggctact atgccagata agagttattt ggagaaagtt 1140
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 40 tacggtatgc agtactacta caaccaagag catggttata ccgacgtgta caactatctc 1260
 aaagcccctt atacaggttg ctacagtcac ttgaataaccg gagtcagctt tgcaaactat 1320
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 45 gcactcacta ataaggacaa atacttctta gctattggca actgcgctat tacagctcaa 1440
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 tatgcctata tcggttcacg tccaaattct tattggggcg aggactacta ttggagtgtg 1560
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 gatgctacat tcttgaggga ttcgtacaac acagtgaatt ctattatgtg ggcaggtaat 1680
 cttgccgcta ctcatgctgg aatatcggc aatattacc atattggtgc tcattactat 1740
 55 tgggaagctt atcatgtcct tggcgatggc tcggttatgc cttatcgtgc aatgcctaag 1800

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5 accaataactt atacgcttcc tgcctctttg cctcagaatc aggcttctta tagcattcag 1860
 gcttctgccg gttcttacgt agctatcttct aaagatggag ttttgtatgg aacaggtggt 1920
 10 gctaatagcca gcggtgttgc gactgtgagt atgactaagc agattacgga aatggtaat 1980
 tatgatgtag ttatcactcg ctctaattat ctctctgtga tcaagcaaat tcaggtaggt 2040
 gagcctagcc cctaccagcc cgtttccaac ttgacagcta caacgcaggg tcagaaagta 2100
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 aaggttgtgc ttgcggcaga caacgtatgg ggagacaata cgggttacca gttcttgttg 2280
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 20 cctgttgta ctacacagaa tattatcggt acaggacagg gtgaagttgt aatccccggt 2460
 ggtgtttacg actattgcat tacgaaccgg gaacctgcat ccggaaagat gtggatcgca 2520
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 25 taa 2583

<210> 143
 <211> 871
 <212> PRT
 30 <213> Porphyromonas\201@gingivalis
 <400> 143

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

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Asn Pro Asn Pro Asn Pro Asn Pro Gly Thr Thr Leu Ser Glu Ser Phe
 115 120 125
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 Glu Asn Gly Ile Pro Ala Ser Trp Lys Thr Ile Asp Ala Asp Gly Asp
 130 135 140
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 Gly His Gly Trp Lys Pro Gly Asn Ala Pro Gly Ile Ala Gly Tyr Asn
 145 150 155 160
 15
 Ser Asn Gly Cys Val Tyr Ser Glu Ser Phe Gly Leu Gly Gly Ile Gly
 165 170 175
 20
 Val Leu Thr Pro Asp Asn Tyr Leu Ile Thr Pro Ala Leu Asp Leu Pro
 180 185 190
 25
 Asn Gly Gly Lys Leu Thr Phe Trp Val Cys Ala Gln Asp Ala Asn Tyr
 195 200 205
 30
 Ala Ser Glu His Tyr Ala Val Tyr Ala Ser Ser Thr Gly Asn Asp Ala
 210 215 220
 35
 Ser Asn Phe Thr Asn Ala Leu Leu Glu Glu Thr Ile Thr Ala Lys Gly
 225 230 235 240
 40
 Val Arg Ser Pro Lys Ala Ile Arg Gly Arg Ile Gln Gly Thr Trp Arg
 245 250 255
 45
 Gln Lys Thr Val Asp Leu Pro Ala Gly Thr Lys Tyr Val Ala Phe Arg
 260 265 270
 50
 His Phe Gln Ser Thr Asp Met Phe Tyr Ile Asp Leu Asp Glu Val Glu
 275 280 285
 55
 Ile Lys Ala Asn Gly Lys Arg Ala Asp Phe Thr Glu Thr Phe Glu Ser
 290 295 300
 Ser Thr His Gly Glu Ala Pro Ala Glu Trp Thr Thr Ile Asp Ala Asp
 305 310 315 320
 Gly Asp Gly Gln Gly Trp Leu Cys Leu Ser Ser Gly Gln Leu Asp Trp
 325 330 335
 Leu Thr Ala His Gly Gly Ser Asn Val Val Ser Ser Phe Ser Trp Asn
 340 345 350
 Gly Met Ala Leu Asn Pro Asp Asn Tyr Leu Ile Ser Lys Asp Val Thr

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		355				360						365				
5	Gly	Ala	Thr	Lys	Val	Lys	Tyr	Tyr	Tyr	Ala	Val	Asn	Asp	Gly	Phe	Pro
		370					375					380				
10	Gly	Asp	His	Tyr	Ala	Val	Met	Ile	Ser	Lys	Thr	Gly	Thr	Asn	Ala	Gly
		385				390					395					400
15	Asp	Phe	Thr	Val	Val	Phe	Glu	Glu	Thr	Pro	Asn	Gly	Ile	Asn	Lys	Gly
					405					410					415	
20	Gly	Ala	Arg	Phe	Gly	Leu	Ser	Thr	Glu	Ala	Asn	Gly	Ala	Lys	Pro	Gln
				420					425					430		
25	Ser	Val	Trp	Ile	Glu	Arg	Thr	Val	Asp	Leu	Pro	Ala	Gly	Thr	Lys	Tyr
			435					440					445			
30	Val	Ala	Phe	Arg	His	Tyr	Asn	Cys	Ser	Asp	Leu	Asn	Tyr	Ile	Leu	Leu
		450					455					460				
35	Asp	Asp	Ile	Gln	Phe	Thr	Met	Gly	Gly	Ser	Pro	Thr	Pro	Thr	Asp	Tyr
		465				470					475					480
40	Thr	Tyr	Thr	Val	Tyr	Arg	Asp	Gly	Thr	Lys	Ile	Lys	Glu	Gly	Leu	Thr
					485					490					495	
45	Glu	Thr	Thr	Phe	Glu	Glu	Asp	Gly	Val	Ala	Thr	Gly	Asn	His	Glu	Tyr
				500					505					510		
50	Cys	Val	Glu	Val	Lys	Tyr	Thr	Ala	Gly	Val	Ser	Pro	Lys	Lys	Cys	Val
			515					520					525			
55	Asn	Val	Thr	Val	Asn	Ser	Thr	Gln	Phe	Asn	Pro	Val	Gln	Asn	Leu	Thr
		530					535					540				
60	Ala	Glu	Gln	Ala	Pro	Asn	Ser	Met	Asp	Ala	Ile	Leu	Lys	Trp	Asn	Ala
		545				550					555					560
65	Pro	Ala	Ser	Lys	Arg	Ala	Glu	Val	Leu	Asn	Glu	Asp	Phe	Glu	Asn	Gly
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70	Ile	Pro	Ala	Ser	Trp	Lys	Thr	Ile	Asp	Ala	Asp	Gly	Asp	Gly	Asn	Asn
				580					585					590		
75	Trp	Thr	Thr	Thr	Pro	Pro	Pro	Gly	Gly	Ser	Ser	Phe	Ala	Gly	His	Asn
				595				600					605			

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Ser Ala Ile Cys Val Ser Ser Ala Ser Tyr Ile Asn Phe Glu Gly Pro
 610 615 620
 5 Gln Asn Pro Asp Asn Tyr Leu Val Thr Pro Glu Leu Ser Leu Pro Gly
 625 630 635 640
 Gly Gly Thr Leu Thr Phe Trp Val Cys Ala Gln Asp Ala Asn Tyr Ala
 10 645 650 655
 Ser Glu His Tyr Ala Val Tyr Ala Ser Ser Thr Gly Asn Asp Ala Ser
 15 660 665 670
 Asn Phe Ala Asn Ala Leu Leu Glu Glu Val Leu Thr Ala Lys Thr Val
 20 675 680 685
 Val Thr Ala Pro Glu Ala Ile Arg Gly Thr Arg Ala Gln Gly Thr Trp
 25 690 695 700
 Tyr Gln Lys Thr Val Gln Leu Pro Ala Gly Thr Lys Tyr Val Ala Phe
 30 705 710 715 720
 Arg His Phe Gly Cys Thr Asp Phe Phe Trp Ile Asn Leu Asp Asp Val
 35 725 730 735
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Lys Pro Tyr Glu Asp Met Ala Thr Thr Val Gln Tyr Arg Asp Gly Leu
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Tyr Ser Val Leu Arg Gly Ala Glu Asn Ala Gly Arg Tyr Thr Leu Ser
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Glu Tyr Met Ser Asp Met Tyr Cys Val Met Gln Gly Asp Gly Gly His
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Ala Thr Pro Tyr Val Thr Trp Thr Ile Pro Arg Ile Glu Thr Ala Asp
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His Ala Ser Asn Tyr Tyr Phe Gly Phe Asn Arg Leu Ile Gln Gln Ala
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5 Lys Thr Glu Val Asp Lys Thr Asn Ala Gln Ile Tyr Leu Ala Glu Ala
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Lys Thr Leu Gln Ala Leu Ala Leu Phe Arg Leu Met Glu Arg Phe Ala
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 15 165 170 175

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 180 185 190

20 Gln Thr Glu Thr Tyr Ser Tyr Ile Met Ser Leu Leu Asp Glu Ala Ile
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Ser Val Leu Pro Glu Thr Asn Ala Asn Asn Met Tyr Val Ser Arg Asp
 25 210 215 220

Tyr Ala Leu Gly Leu Arg Ala Arg Val His Met Ala Met Asp Asn Tyr
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Ile Ser Ala Ala Asn Ser Asp Glu Phe Glu Glu Ala Tyr Arg Lys Met
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10 Leu Ser Lys Ser Arg Gly Ala Glu Val Ser Gly Ala Asp Tyr Met Gln
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15 Ile Leu Lys Asp Glu Arg Thr Arg Glu Met Ile Gly Glu Gly Ser Arg
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20 Leu Asn Asp Met Ile Arg Trp Asn Met Asp Leu Val Val Ser Pro Val
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Gln Ala Val Leu His Lys Ile Ala Val Pro Thr Ile Leu Gln Thr Asp
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25 Asp Pro Thr Arg Val Pro Ala Gly Phe Tyr Ala Phe Thr Trp Glu Ile
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 tattactttg gttttaatcg gttaattcag caagccaatg cttttgtcgg aaatgttaag 360
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Claims

- 50 1. A polypeptide having the amino acid sequence represented by SEQ ID NO: 151.
2. A nucleic acid molecule encoding a polypeptide according to claim 1, wherein the nucleic acid has the sequence of SEQ ID NO: 152.
- 55 3. A test kit for a plasma or serum antibody titer against *Porphyromonas gingivalis*, comprising a polypeptide having the amino acid sequence represented by SEQ ID NO: 151.
4. A method for measuring an antibody titer against *Porphyromonas gingivalis* in a blood sample separated from a

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human body, comprising bringing the blood sample into contact with a *Porphyromonas gingivalis* antigen polypeptide, said method being **characterized in that** the *Porphyromonas gingivalis* antigen polypeptide is a polypeptide having the amino acid sequence represented by SEQ ID NO: 151.

- 5 5. Use of a polypeptide according to claim 1 for testing a plasma or serum antibody titer against *Porphyromonas gingivalis*.
6. Use of a polypeptide according to claim 1 for typing strains of *Porphyromonas gingivalis*.

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Fig.1

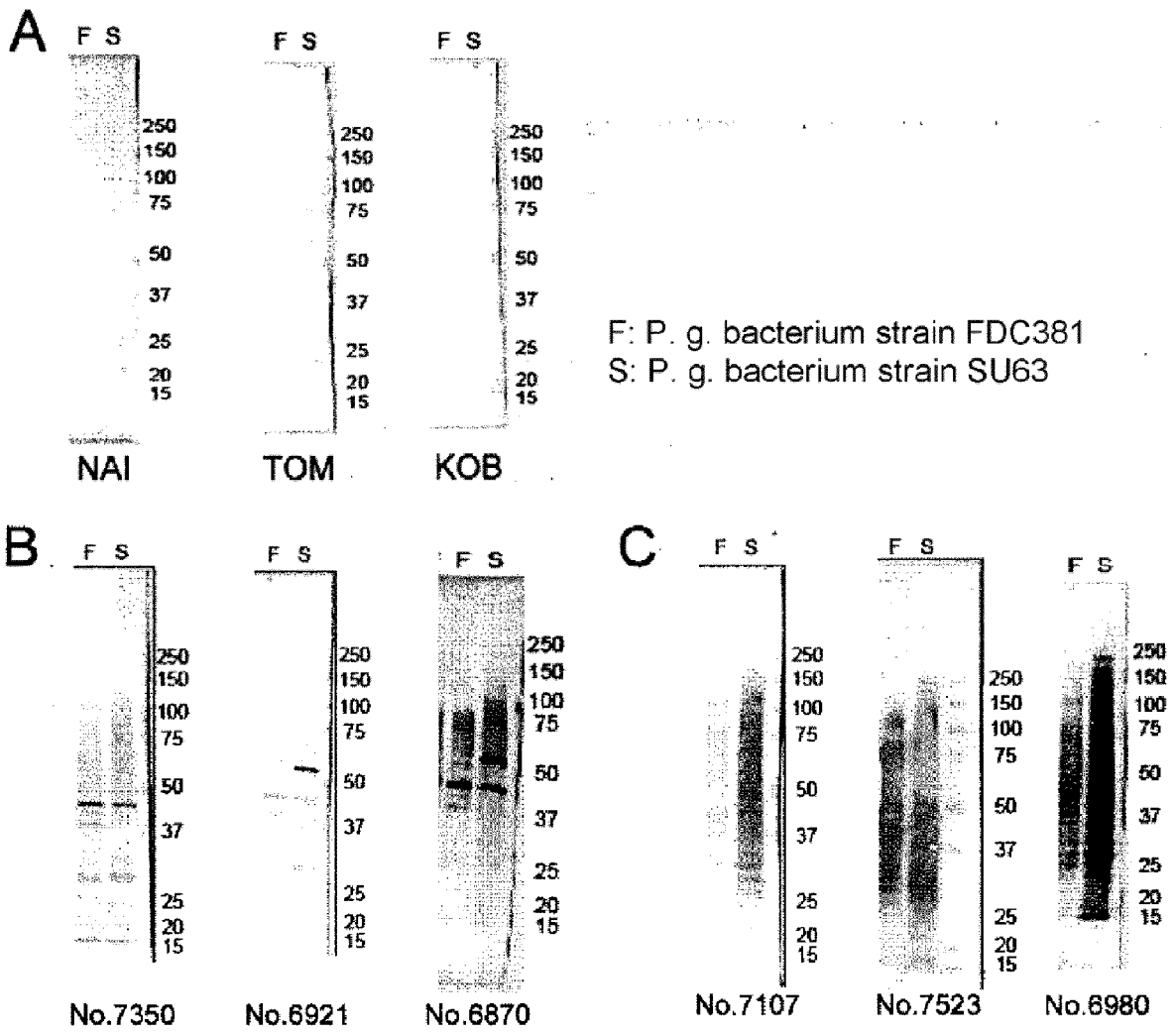
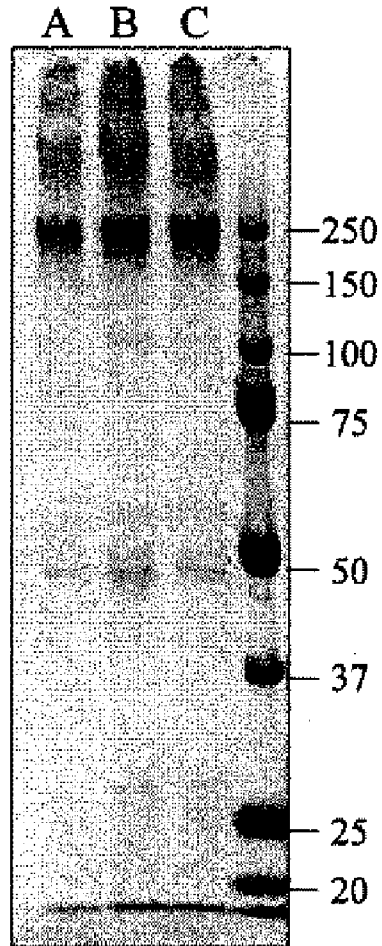


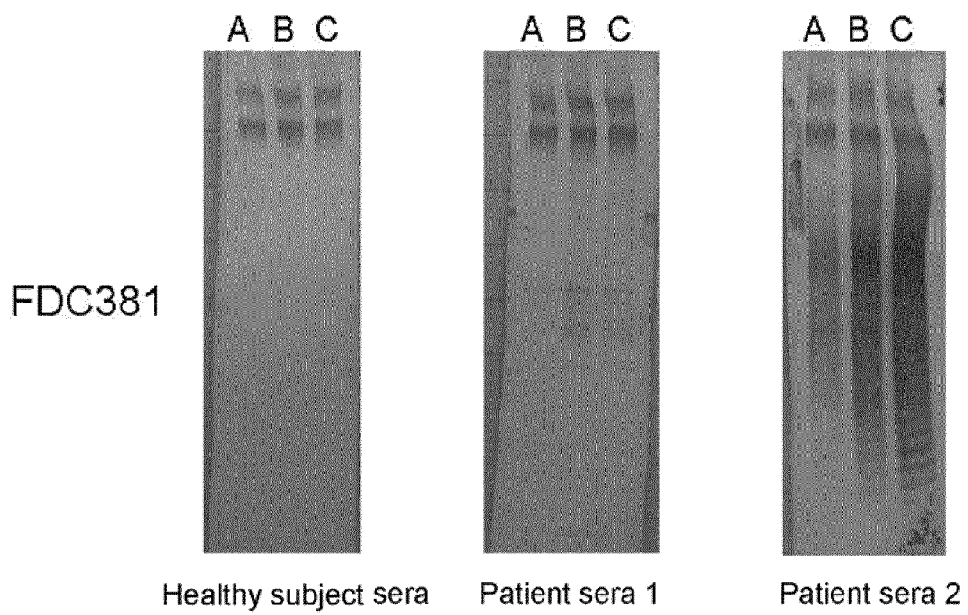
Fig.2



(FDC381)

Lane A: antigen purified from healthy subject sera
Lane B: antigen purified from patient sera 1
Lane C: antigen purified from patient sera 2

Fig.3

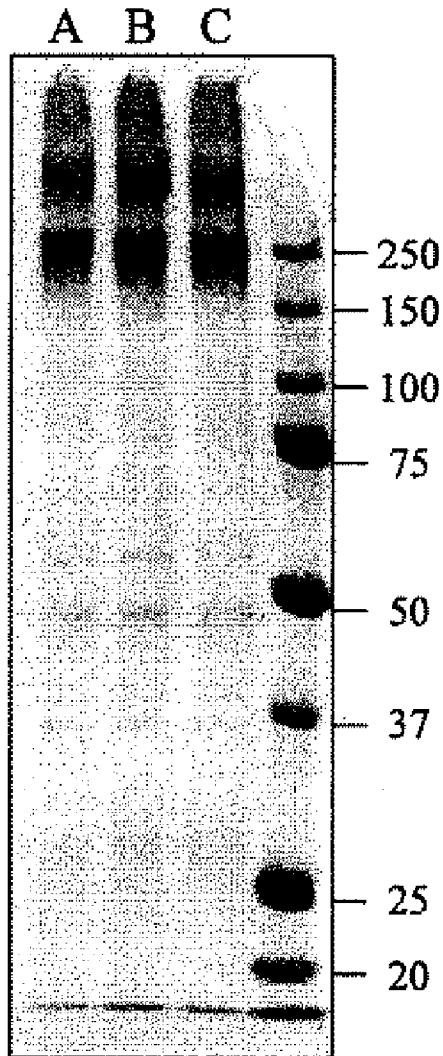


Lane A: antigen purified from healthy subject sera

Lane B: antigen purified from patient sera 1

Lane C: antigen purified from patient sera 2

Fig.4



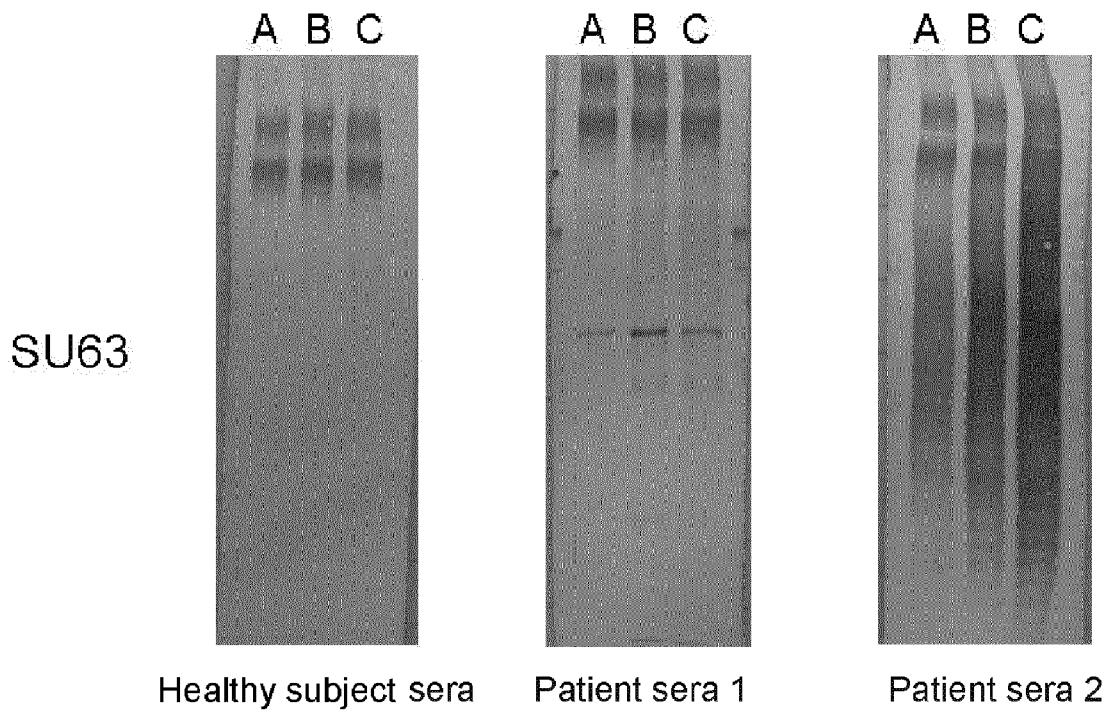
(SU63)

Lane A: antigen purified from healthy subject sera

Lane B: antigen purified from patient sera 1

Lane C: antigen purified from patient sera 2

Fig.5



Lane A: antigen purified from healthy subject sera

Lane B: antigen purified from patient sera 1

Lane C: antigen purified from patient sera 2

Fig.6

		RelyOn 072 - RelyOn BioS		RelyOn Nozee-Re					
#	Visib?	Startr?	MS/MS View: Identified Proteins (28)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	40513A RAW (F04098)	40514A RAW (F04300)	40513A RAW (F04399)
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	glutamate dehydrogenase [Por... gi 34540940	39789A RAW (F04098)	49 kDa				
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	protease precursor [Porphyrom... gi 1813998	39889A RAW (F04070)	186 kDa	*			
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ragA protein [Porphyromonas gi... gi 3454004	39889A RAW (F04089)	112 kDa	*			
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	53kDa major outer membrane p... gi 5832527		54 kDa				
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	phosphoserine aminotransferas... gi 34540980		40 kDa				
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	heme-binding protein FetB [Pa... gi 1889945		33 kDa				
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Lys-zincipain gi 1314751		187 kDa	*			
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	RagA2 [Porphyromonas gingival... gi 1652426		116 kDa	*			
9	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Chain A, Crystal Structure Of ... gi 1521491		23 kDa				
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	glyoxaldehyde 3-phosphate de... gi 34541701		36 kDa				
11	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	HmuY [Porphyromonas gingival... gi 183922		24 kDa				
12	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	M24 family peptidase [Porphy... gi 34540922		67 kDa				
13	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	serine hydroxymethyltransferas... gi 34539916		47 kDa				
14	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	conserved hypothetical protein ... gi 1889941		92 kDa				
15	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	outer membrane lipoprotein Om... gi 34541744		37 kDa				
16	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	peptidylarginine deiminase [Por... gi 3454110		82 kDa				
17	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Chain H, 1.8a Structure Of The... gi 5542161		24 kDa	*			
18	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Chain C, Crystal Structure Of ... gi 1012065		12 kDa				
19	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	porphyrin gi 1314328		186 kDa	*			
20	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	TenB-linked receptor Tlr [Por... gi 1889945		79 kDa				
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22	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	putative lipoprotein [Porphyrom... gi 34540040		240 kDa				
23	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	RagB2 [Porphyromonas xingival... gi 1652427		56 kDa				
24	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	probable lysyl endopeptidase pr... gi 1889952		103 kDa				
25	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	quonone family NAD(P)H dehydr... gi 34541436		29 kDa				
26	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	35 kDa heme binding protein L... gi 1889945		38 kDa				
27	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	DNA-binding protein from star... gi 1889959		18 kDa				
28	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	lipoprotein RagB [Porphyromen... gi 34540043		56 kDa				

Strain FDC381

Strain SU63

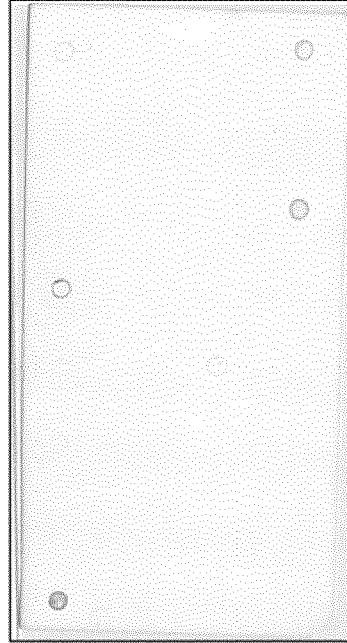
Fig.7

No.	Identified Proteins	Accession#	MW	Strain SU63			Strain FDC381			Primary-selected patient-specific candidates for synthesis	Secondary-selected p.g.-specific candidates for synthesis
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Function: unknown, antigenicity: unknown											
1	putative lipoprotein [Porphyromonas gingivalis]	gi 345400	240 kDa	0	21	13	0	0	0	2	0
2	conserved hypothetical protein w [Porphyromonas gingivalis]	gi 188994	52 kDa	2	10	10	0	2	4	0	0
3	hypothetical protein PG1881 [Porphyromonas gingivalis]	gi 345414	53 kDa	0	6	3				0	0
4	hypothetical protein PGN_0291 [Porphyromonas gingivalis]	gi 188994	134 kDa	0	6	6				0	0
5	hypothetical protein PG0491 [Porphyromonas gingivalis]	gi 345403	80 kDa	0	4	0				0	0
6	hypothetical protein PGN_1511 [Porphyromonas gingivalis]	gi 188995	53 kDa	0	2	5				0	0
7	hypothetical protein PGN_0477 [Porphyromonas gingivalis]	gi 188994	61 kDa	0	2	0				0	0
8	hypothetical protein PGN_0860 [Porphyromonas gingivalis]	gi 188994	39 kDa	0	0	2				0	0
Function: known, antigenicity: unknown											
9	53kDa major outer membrane protein [Porphyromonas gingivalis]	gi 583252	54 kDa	0	28	13	2	12	8	0	0
10	35 kDa hemin binding protein [Porphyromonas gingivalis]	gi 188994	38 kDa	0	7	5	0	0	2	0	0
11	heme-binding protein Fcb [Porphyromonas gingivalis]	gi 188994	33 kDa	0	6	2	0	5	9	0	0
12	MAD-dependent glutamate dehydrogenase [Porphyromonas gingivalis]	gi 150842	49 kDa	0	4	0	11	16	26	0	0
13	phosphoserine aminotransferase [Porphyromonas gingivalis]	gi 345409	40 kDa	0	2	2	3	4	11	0	0
14	TonB-linked receptor Tir [Porphyromonas gingivalis]	gi 188994	79 kDa	0	0	2	0	0	4	0	0
15	fimbria [Porphyromonas gingivalis]	gi 345417	41 kDa	0	4	0				0	0
16	minor component FimE [Porphyromonas gingivalis]	gi 188994	61 kDa	0	3	0				0	0
17	hemagglutinin protein HagA [Porphyromonas gingivalis]	gi 188995	283 kDa	3	0	0				0	0
18	protease precursor [Porphyromonas gingivalis]	gi 181399	186 kDa	0	2	0	6	15	29	0	0
19	HmuY [Porphyromonas gingivalis]	gi 119392	24 kDa	0	2	0	0	4	4	0	0
20	M24 family peptidase [Porphyromonas gingivalis]	gi 345409	67 kDa	0	0	0	0	3	3	0	0
21	glyceraldehyde 3-phosphate dehydrogenase [Porphyromonas gingivalis]	gi 345417	36 kDa	0	2	2	2	2	5	0	0
22	ferritin [Porphyromonas gingivalis]	gi 345409	19 kDa	0	0	2	0	0	0	0	0
23	serine hydroxymethyltransferase [Porphyromonas gingivalis]	gi 345399	47 kDa	0	2	0	2	0	4	0	0
24	outer membrane lipoprotein OmpA [Porphyromonas gingivalis]	gi 345417	32 kDa	0	0	0	0	0	6	0	0
25	porphyrin [Porphyromonas gingivalis]	gi 131432	188 kDa	0	0	0	0	0	22	0	0
26	probable lysyl endopeptidase precursor [Porphyromonas gingivalis]	gi 188995	103 kDa	0	0	0	0	0	3	0	0
27	quinone family NAD(P)H dehydrogenase [Porphyromonas gingivalis]	gi 345414	20 kDa	0	0	0	0	0	2	0	0
28	DNA-binding protein from starved cells [Porphyromonas gingivalis]	gi 188995	18 kDa	0	0	0	0	0	2	0	0
Function: unknown, antigenicity: known											
29	immunoreactive 42 kDa antigen P [Porphyromonas gingivalis]	gi 345404	42 kDa	0	3	2				0	0
Function: known, antigenicity: known											
30	RagA2 [Porphyromonas gingivalis]	gi 1616524	116 kDa	23	27	30	3	4	7	0	0
31	RagB2 [Porphyromonas gingivalis]	gi 1616524	56 kDa	5	14	12	0	0	2	0	0
32	Lys-gingipain [Porphyromonas gingivalis]	gi 131475	187 kDa	0	13	13	0	8	23	0	0
33	peptidylarginine deiminase [Porphyromonas gingivalis]	gi 345411	62 kDa	2	9	10	0	0	4	0	0
34	RagA protein [Porphyromonas gingivalis]	gi 345400	112 kDa	0	4	6	6	12	15	0	0
35	arginine-specific cysteine proteinase [Porphyromonas gingivalis]	gi 188995	185 kDa	25	37	34				0	0
36	outer membrane protein 41 precursor [Porphyromonas gingivalis]	gi 188994	43 kDa	0	2	2				0	0
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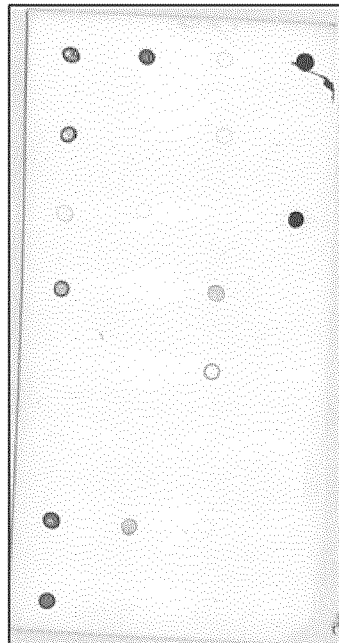
Fig.8

Layout

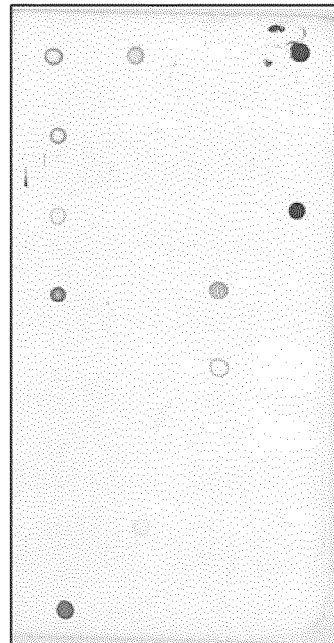
	A	B	C	D
1	2	11	21	32
2	3	12	22	34
3	4	13	23	35
4	6	14	24	36
5	7	15	26	37
6	8	16	27	
7	9	19	28	
8	10	20	29	



Healthy subject serum pool



Patient serum pool 1



Patient serum pool 2

Fig.9

Antigen No.	Healthy subject serum pool	Patient serum pool 1	Patient serum pool 2
2	258581	10299784	4544221
3	0	7938453	3591925
4	0	533560	1030417
6	2586477	8030066	8508309
7	0	0	0
8	0	0	0
9	noize	12972685	0
10	7803423	12163942	13135284
11	0	11823066	4149132
12	0	0	noize
13	0	133558	noize
14	0	0	noize
15	0	0	noize
16	0	0	0
19	0	3750092	noize
20	noize	noize	noize
21	0	331257	noize
22	0	416198	noize
23	0	noize	noize
24	0	3308269	6252161
26	431110	2392354	1561578
27	0	0	0
28	noize	noize	noize
29	120564	0	noize
32	2539358	20813860	21344255
34	0	57190	noize
35	3778184	16754505	15833022
36	noize	noize	noize
37	noize	noize	noize
Blank 1	6471	40767	231273
Blank 2	214	2544	568794
Blank 3	118823	56711	803725

(Note) Antigens having signal values equal to or lower than blank 3 were treated as noises

Fig.10

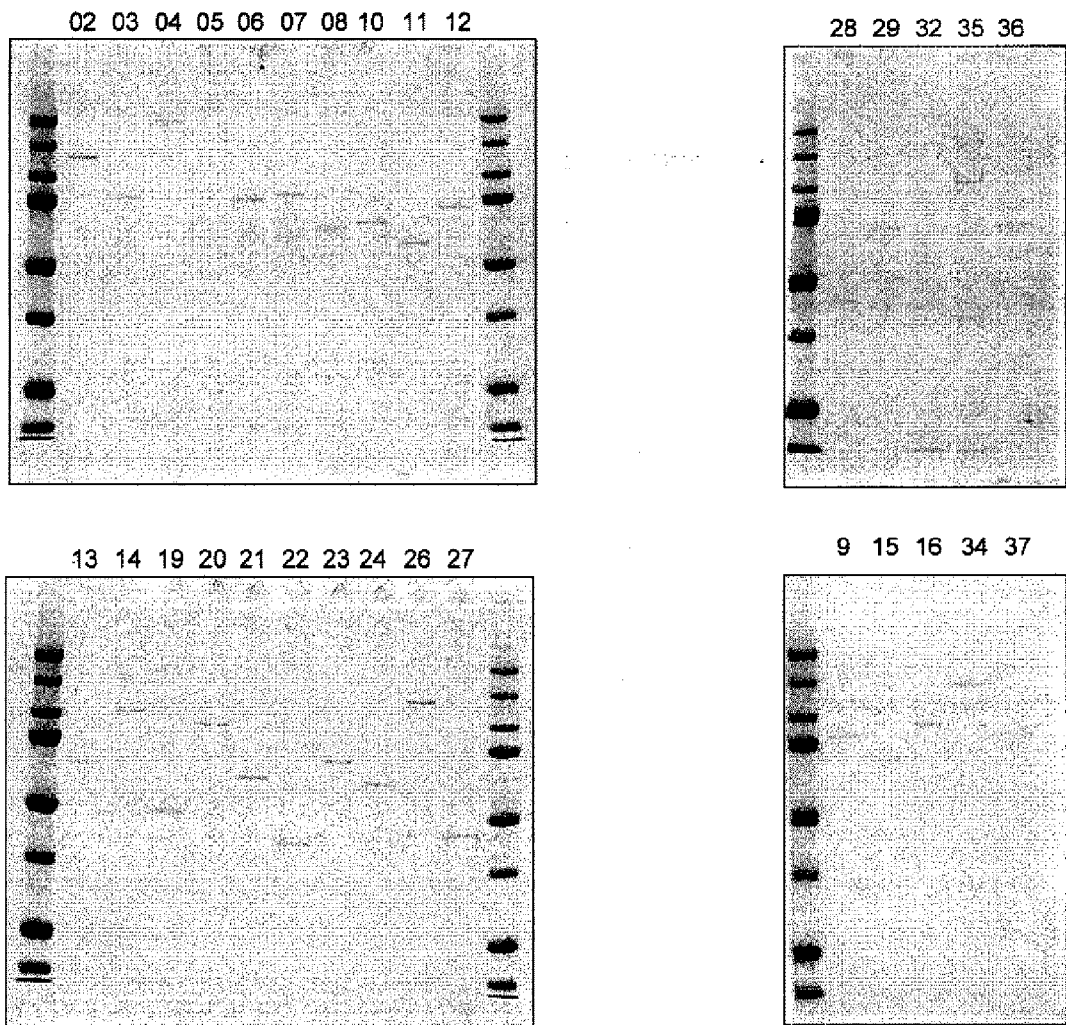


Fig.11

	Antigen protein No.	Healthy subject sera			Healthy subject reference
		TOM	NAI	KOB	
	2	1598447	n.d.	2085186	2085186
	3	n.d.	n.d.	n.d.	0
	4	n.d.	n.d.	n.d.	0
	6	5738276	6763839	7678131	7678131
	9	n.d.	n.d.	n.d.	0
	10	4314709	n.d.	10899925	10899925
	11	n.d.	n.d.	n.d.	0
	13	n.d.	n.d.	n.d.	0
	19	n.d.	n.d.	n.d.	0
	21	n.d.	n.d.	n.d.	0
	22	n.d.	n.d.	n.d.	0
	24	n.d.	n.d.	n.d.	0
	26	867630	n.d.	70695	867630
	28	n.d.	n.d.	n.d.	0
	32	2060812	n.d.	2503064	2503064
	35	1207265	2214521	4781758	4781758

Fig. 12

Antigen protein No.	Patient sera														
	6809	6816	6823	6896	6918	6921	6923	6926	6975	6980	6991	7056			
2	5803638	34058	1831604	11420150	8725092	21783541	4619959	1122471	7637207	3846670	6366756	13327426			
3	4213786	1052796	2673715	1786686	2667842	1717493	n.d.	4076342	n.d.	3063362	755382	n.d.			
4	n.d.	n.d.	4275779	774743	n.d.	260566	n.d.	445621	484018	n.d.	1468594	n.d.			
6	9822269	n.d.	3339197	5638192	9315389	13563288	2638447	n.d.	8730205	1660360	5769628	12045846			
9	2138181	530806	n.d.	n.d.	8130699	19108792	2475879	18839709	n.d.	n.d.	4945931	n.d.			
10	3011852	3196144	51497	4567676	5466625	12568914	8659082	3539280	7551358	4304725	1185998	10191477			
11	n.d.	1698642	n.d.	1784310	n.d.	15880529	n.d.	n.d.	n.d.	4819952	631311	n.d.			
13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1992580	n.d.	n.d.	n.d.	n.d.	n.d.			
19	n.d.	2835786	n.d.	n.d.	843581	n.d.	n.d.	n.d.	n.d.	1057635	n.d.	n.d.			
21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
24	n.d.	n.d.	n.d.	n.d.	400189	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.			
26	n.d.	3100160	n.d.	956146	1811202	n.d.	623094	n.d.	7148056	689655	1040848	1214323			
28	n.d.	n.d.	n.d.	781088	n.d.	n.d.	n.d.	2124685	n.d.	n.d.	n.d.	2654894			
32	10050844	31073871	15677565	24806162	27580055	37993686	21951328	13493555	24471576	33809621	18913419	18459270			
35	16400320	30769150	22164743	26989140	32034025	39202988	28612158	25685815	27395794	34695924	24907585	19496370			

Antigen protein No.	Patient sera														
	7107	7125	7350	7395	7457	7492	7495	7500	7523	7524	7835				
2	7135308	6492410	8247603	3679263	3307117	5189959	6648274	10273473	n.d.	2760055	7201491				
3	1156814	5296326	9692008	n.d.	1984077	n.d.	527102	n.d.	3708897	2113402	n.d.				
4	136479	2753433	2796850	n.d.	n.d.	n.d.	n.d.	n.d.	4009799	n.d.	n.d.				
6	13004770	4107684	5643361	9015055	3946328	1826855.8	2965608	5731338	3647004	n.d.	5566052				
9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9834044	1950829	n.d.	16886309	n.d.				
10	12724626	8993618	8154442	6181998	10541814	335299.48	8538616	12731218	5609949	17302265	8010635				
11	5872259	3720359	28845908	n.d.	n.d.	n.d.	n.d.	2628677	n.d.	n.d.	n.d.				
13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
19	1432198	2206455	4725876	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7109750	n.d.				
21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.				
24	9692976	4159059	4521232	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1074568	n.d.				
26	372510	-36479	1222952	n.d.	n.d.	n.d.	2919715	1769221	n.d.	2666362	n.d.				
28	n.d.	n.d.	n.d.	n.d.	1963418	n.d.	2505485	1727201	1356828	3635711	n.d.				
32	24188732	25021544	35044983	6232588	28278169	26049378	22508836	9803250	20720662	25697472	10567896				
35	33620714	29575885	37979539	11704948	31550088	25661283	22786143	13741482	22551414	26216464	16145927				

Fig.13

		Antigen protein No.															
		2	3	4	6	9	10	11	13	19	21	22	24	26	28	32	35
Patients	6809	○	○			○										○	○
	6816		○			○		○						○		○	○
	6823		○	○												○	○
	6896	○	○	○				○					○			○	○
	6918	○	○		○	○				○			○	○		○	○
	6921	○	○	○	○	○	○	○								○	○
	6923	○				○				○			○			○	○
	6926		○	○		○								○		○	○
	6975	○		○	○									○		○	○
	6980	○	○					○		○						○	○
	6991	○	○	○		○		○						○		○	○
	7056	○			○								○	○		○	○
	7107	○	○	○	○		○	○		○			○			○	○
	7125	○	○	○				○		○			○			○	○
	7350	○	○	○				○		○			○	○		○	○
	7395	○			○											○	○
	7457	○	○											○		○	○
	7492	○														○	○
	7495	○	○			○							○	○		○	○
	7500	○				○	○	○					○	○		○	○
7523		○	○										○		○	○	
7524	○	○			○	○			○		○	○	○		○	○	
7835	○														○	○	

Fig.14

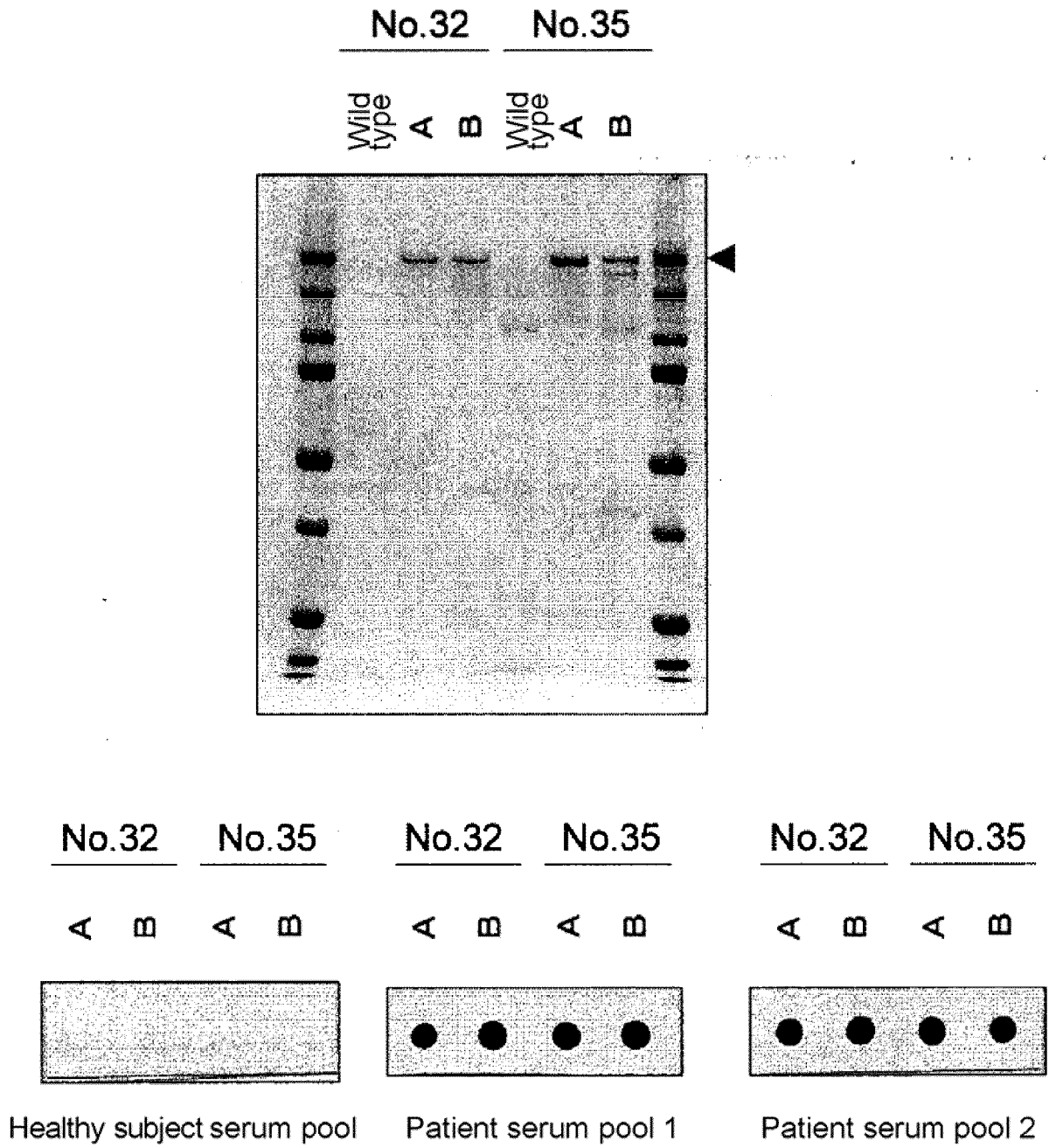
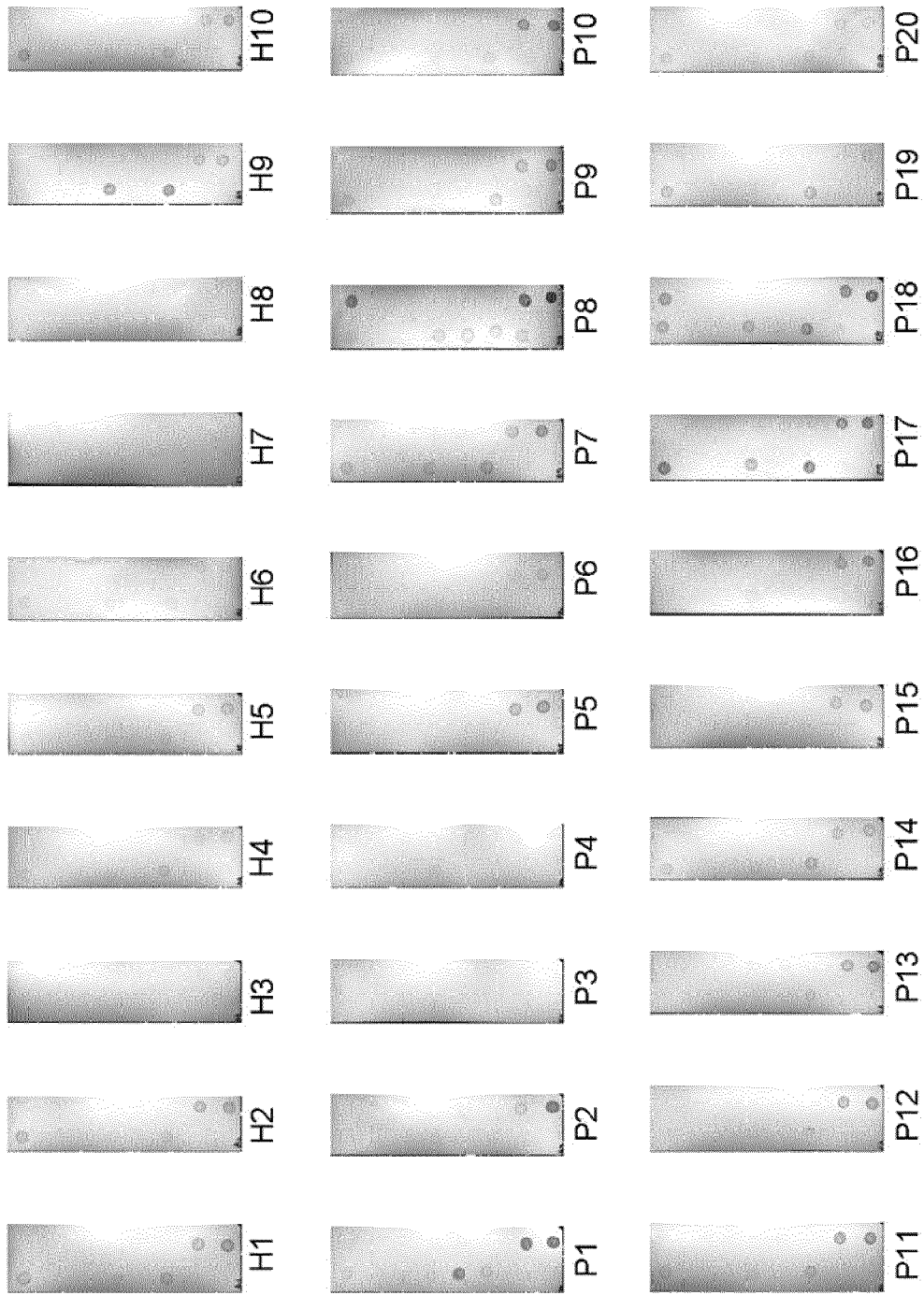


Fig.15



2	19
3	21
4	22
6	24
9	26
10	28
11	32A
13	35A

Layout view

Fig.16

Serum No.	Antigen No.																			
	2	3	4	6	9	10	11	13	19	21	22	24	26	28	32A	35A				
H1	5654109	0	0	1381433	0	4515749	0	0	117043	0	0	0	189483	0	6367032	11798073				
H2	7208773	0	0	7447116	0	2644644	0	0	0	0	0	0	572129	0	8191840	8490813				
H3	0	0	0	478138	0	417326	0	0	0	0	0	0	358337	0	1347285	1244723				
H4	2389618	0	0	1236766	0	3617334	0	0	0	0	0	0	0	0	1476959	2239358				
H5	885872	0	0	996337	0	1843154	0	0	0	0	0	0	0	0	4601450	5098186				
H6	3177780	0	0	1906100	0	1956331	0	0	0	0	0	0	0	0	1919412	1734553				
H7	0	0	0	334140	0	0	0	0	0	0	0	0	0	0	0	0				
H8	188496	0	0	505927	0	1568083	0	0	0	0	0	0	440992	67383	259018	266808				
H9	1391316	0	0	14444905	0	17585332	0	0	0	0	0	0	1063896	0	5076288	7148812				
H10	8325690	0	0	1742290	0	5214255	0	0	155293	0	0	0	807559	0	4951639	8324489				
P1	2100735	492763	129941	1874985	13946742	2939217	1023	0	910676	30133	0	236165	444335	0	20473257	23790316				
P2	520878	421042	0	858542	0	809653	0	0	0	0	0	303320	260798	0	4248022	20782396				
P3	0	0	0	41654	0	0	0	0	0	0	0	0	0	0	730462	646517				
P4	0	0	0	1655204	0	0	0	0	0	0	132241	0	0	0	774381	1141823				
P5	0	0	0	125371	0	0	0	0	0	0	0	402299	0	0	8556587	12433742				
P6	0	0	0	760653	116173	0	0	0	54753	0	0	0	0	0	2632130	5102330				
P7	6324287	0	0	5727508	0	8301958	369154	0	0	0	0	418574	727651	0	8654785	16150790				
P8	3047617	0	0	5501148	5758030	6049493	7015822	0	19070325	0	0	825206	0	0	23401904	24713418				
P9	4149839	0	0	304774	0	7270566	0	0	1592	0	0	0	0	0	9146531	11542314				
P10	1462739	252940	0	865484	0	2918947	0	0	0	0	0	0	0	0	11480440	13529758				
P11	3270706	0	0	1882807	0	4084577	69175	0	227608	0	0	230281	266678	0	12921785	16177136				
P12	644249	0	0	417911	0	2494042	0	0	0	0	0	0	0	0	10550115	13825087				
P13	264425	257478	4712	575860	0	2795891	340783	0	0	0	0	1336425	1122389	0	9244550	13546232				
P14	3835136	0	0	1776591	0	5934493	0	0	0	0	0	0	0	0	5772340	5713361				
P15	1417	0	0	227025	0	238700	39426	0	0	0	0	284636	356886	0	4905648	8373424				
P16	337451	0	301375	1123415	0	682491	660068	0	0	0	0	0	0	0	7655953	11996144				
P17	19458298	224392	0	10971484	0	20892789	832291	0	0	0	0	473064	1235810	0	14333172	19856112				
P18	9926484	1293374	241915	8109836	1193607	12625957	1912661	0	13017556	0	0	349732	374868	0	18439438	27460913				
P19	7159271	0	0	803503	53955	8134039	0	0	0	0	0	0	745666	0	1869678	3228540				
P20	4413581	0	0	1481315	0	2330308	0	0	0	0	0	0	0	0	3404305	3420444				

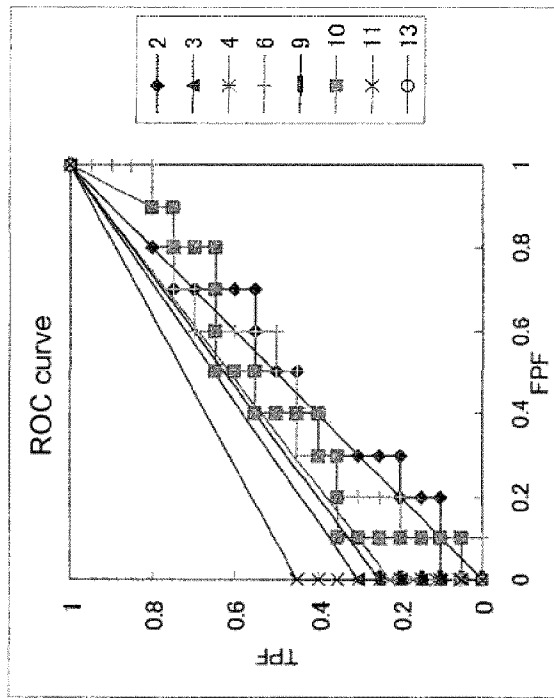
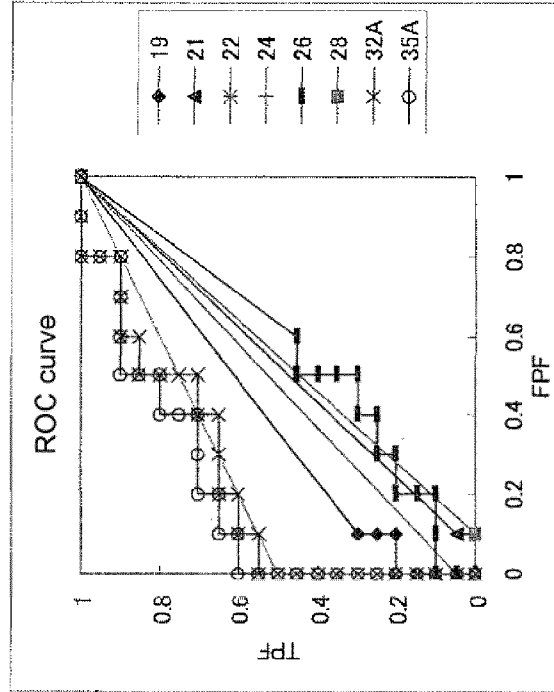
Fig.17

	Serum No.	Antigen No.															
		2	3	4	6	9	10	11	13	19	21	22	24	26	28	32A	35A
Healthy subject serum group	H1	○			○		○				○			○		○	○
	H2	○			○		○							○		○	○
	H3				○		○							○		○	○
	H4	○			○		○									○	○
	H5	○			○		○									○	○
	H6	○			○		○									○	○
	H7				○												
	H8	○			○		○							○	○	○	○
	H9	○		—	○		○							○		○	○
	H10	○		—	○		○				○			○		○	○
Patient serum group	P1	○	○	○	○	○	○	○		○	○		○	○		○	○
	P2	○	○		○		○						○	○		○	○
	P3				○											○	○
	P4				○							○				○	○
	P5				○								○			○	○
	P6				○	○					○					○	○
	P7	○			○		○	○					○	○		○	○
	P8	○			○	○	○	○			○		○			○	○
	P9	○			○		○				○					○	○
	P10	○	○	—	○		○									○	○
	P11	○			○		○	○			○		○	○		○	○
	P12	○			○		○									○	○
	P13	○	○	○	○		○	○					○	○		○	○
	P14	○			○		○									○	○
	P15	○			○		○	○					○	○		○	○
	P16	○		○	○		○	○								○	○
	P17	○	○		○		○	○					○	○		○	○
	P18	○	○	○	○	○	○	○			○		○	○		○	○
	P19	○			○	○	○							○		○	○
	P20	○		—	○		○									○	○

Fig.18

Antigen No.	Signal average value		Signal/Noise ratio	
	Healthy subject G (Noise)	Patient G (Signal)		
2	2922163	3345752	1.14	
3	0	147099	Noise : 0	☆
4	0	37664	Noise : 0	☆
6	2377035	2254253	0.95	
9	0	1053325	Noise : 0	☆
10	3936021	4424951	1.12	
11	0	562020	Noise : 0	☆
13	0	0	Noise : 0	
19	15529	1664125	107.16	☆
21	11704	1507	0.13	
22	0	6612	Noise : 0	
24	0	242985	Noise : 0	☆
26	343240	276752	0.81	
28	6738	0	0.00	
32A	3422092	8959774	2.62	☆
35A	4634482	12671575	2.73	☆

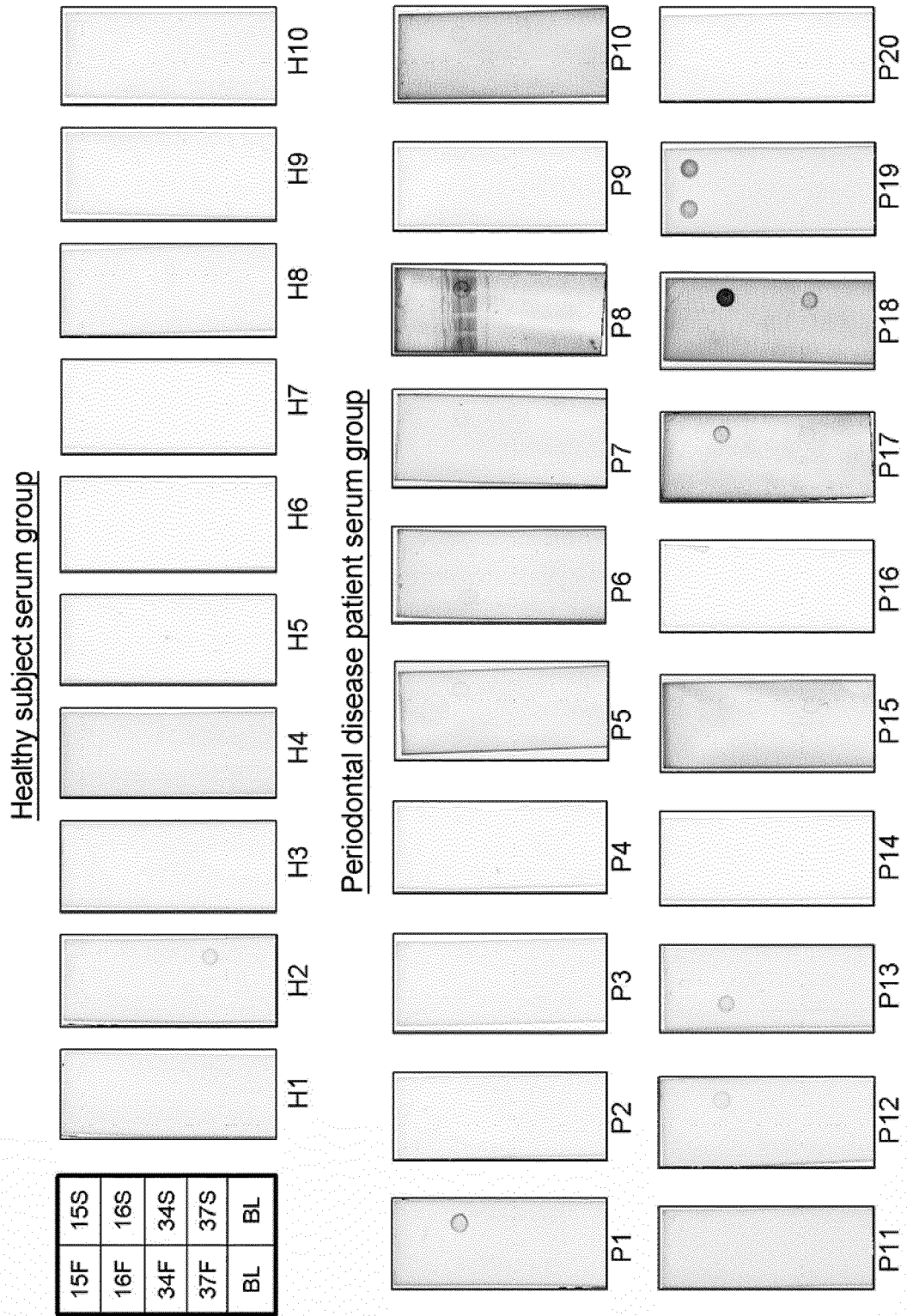
Fig.19



Antigen No.	2	3	4	6	9	10	11	13
AUC	0.490	0.650	0.611	0.495	0.625	0.530	0.725	0.500

Antigen No.	19	21	22	24	26	28	32A	35A
AUC	0.605	0.473	0.525	0.750	0.430	0.450	0.780	0.815

Fig.20





EUROPEAN SEARCH REPORT

Application Number
EP 16 19 0674

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			TECHNICAL FIELDS SEARCHED (IPC)
			G01N C07K
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 10 November 2016	Examiner Van Bohemen, Charles
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EP 16 19 0674

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10-11-2016

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专利名称(译)	针对牙周致病细菌的血浆或血清抗体滴度的检测试剂盒		
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摘要(译)

本发明的目的是提供：用于抗体滴度的测试试剂盒或针对血液样品中的牙周致病细菌的抗体，其能够对具有高免疫型范围的患者牙周病进行测试。精度可以通过自动化设备高速处理；一种牙周致病细菌抗原蛋白，可适用于该试剂盒；使用该试剂盒测试血液样品中抗体滴度或抗体的存在的方法；和用于分类牙龈卟啉单胞菌菌株的试剂盒。本发明公开了一种测试试剂盒，其包含一组多肽，所述多肽分别具有SEQ ID NO：1,3,9,15,19,31,41,43,63,65和67所示的酸序列；修饰的多肽，其具有SEQ ID NO：67所示的氨基酸序列；以及确定抗体滴度或在从人体分离的血液样品中存在针对牙周病细菌的抗体的方法，包括使血液样品与上述多肽组接触。

Table 1.
Reactions of patient sera against antigen protein groups

	Patient serum No.	Plasma antibody titer		Common antigen			SUB3-specific antigen	
		FDC381:	SUB3:	46kDa	25-37kDa	100-110kDa	57kDa	150-250kDa
381 ≥ 1 SU < 1	7056	7.36	0.1	○				
	7457	6.18	0.6	○	○	○		○
	6991	4.43	0.9				○	
	7492	3.76	0.59	○				○
	6809	3.35	0.9		○	○	○	
	6816	1.89	0.76	○				
	7125	1.89	0.09	○	○	○		○
	7500	1.87	0.93		○	○	○	○
	7107	1.8	0.06		○	○		○
	7835	1.64	0.19	○				
	7350	5.45	10.18	○				
	7523	15.11	9.08		○	○		○
	7524	44.9	7.35		○	○	○	○
	6921	7.55	5.07				○	○
	6896	4.36	4.37				○	○
381 ≥ 1 SU ≥ 1	6975	7.98	4.31	○	○	○	○	○
	6923	5.2	4.11	○	○	○	○	○
	7393	15.2	4.07	○	○			○
	6926	5.63	3.61	○			○	
	7495	11.38	2.61				○	○
	6817	0.11	1.32		○			
	6820	0.68	2.01	○		○		○
	6828	0.55	1.97					
	6855	0.58	1.03		○	○		
	6863	-0.32	2.2	○				
	6867	0.93	1.46	○				○
	6874	0.03	2.83	○			○	○
	6881	0.66	1.4	○	○	○		
	6889	0.28	2.23	○	○	○		
	6904	0.88	1.51	○				
6935	0.63	1.31	○					
6968	0.08	1.93	○	○			○	