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(54) METHOD FOR PREDICTING THERAPEUTIC EFFECT OF IMMUNOTHERAPY ON CANCER PATIENT, AND GENE SET AND KIT TO BE USED IN THE METHOD

(57) Provided is a gene set which is useful for predicting the therapeutic effect of an immunotherapy on a cancer patient. Also provided is a method for examining whether an immunotherapy is efficacious or not, said method comprising quantifying the expression amount of each of the genes constituting the aforesaid gene set. This examination method is useful for determining a therapeutic strategy for the cancer patient.

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Description

Technical Field

[0001] The present invention relates to a method for predicting therapeutic effect of immunotherapy on a cancer patient and a gene set and a kit for use in the method, etc.

Background Art

[0002] Immunotherapy for various cancers has not yet been the optimum treatment for every patient, although it is effective in some cases. One reason for that is because the immunotherapy is mediated by immunological competence, which differs by individuals, in order to suppress the growth of cancer cells. So far, a method for predicting effect of cancer immunotherapy has not been found. Thus, the efficacy cannot be evaluated until the treatment is actually performed. Although a method for predicting effect of chemotherapy on a breast cancer patient has been known to be performed by measuring a gene expression level, the method is complicated method that requires combining gene expression with other factors, and also, the method is intended for predicting only effect of chemotherapy for breast cancer (Patent Document 1). A method for predicting therapeutic effect of cancer immunotherapy or prognosis of a patient after immunotherapy has been unknown so far.

Patent Document 1
JP-A-2008-536094

Disclosure of the Invention

Problem to be Solved by the Invention

[0003] An object of the present invention is to provide a method for accurately predicting therapeutic effect of immunotherapy on a cancer patient.

Means for Solving the Problem

[0004] The present inventors attempted to predict therapeutic effect of cancer immunotherapy based on the results which had been obtained by conducting peptide vaccine therapy for prostate cancer patients over long years. First, gene expression profiles in prostate cancer patients before or after peptide vaccine therapy were analyzed with DNA microarrays. Subsequently, the patients were classified into a good prognosis group and a poor prognosis group on the basis of a survival time after the treatment, and a gene or a gene group was selected for accurately predicting whether the patient belongs to the good prognosis group or the poor prognosis group based on the expression level before the treatment. Then, it was confirmed that the therapeutic effect of immunotherapy could be predicted from the expression level(s) of the selected gene(s). In this way, the present invention has been completed.

[0005] Accordingly, in one aspect, the present invention provides a method for predicting effect of immunotherapy on a cancer patient, comprising a step of (1) measuring an expression level of each gene included in a gene set in a sample obtained from the cancer patient before or after the immunotherapy, wherein the gene set consists of at least one gene selected from the group of genes shown in Table 1, 19, 34, or 35.

The method for predicting effect of immunotherapy on a cancer patient may further comprise a step of (2) determining prognosis of the patient by discriminant analysis using the expression level.

[0006] In an another aspect, the present invention provides a gene set for predicting effect of immunotherapy on a cancer patient, consisting of at least one gene selected from the group of genes shown in Table 1, 19, 34 or 35, and a biomarker for predicting effect of immunotherapy on a cancer patient, consisting of at least one gene selected from the group of genes shown in Table 1, 19, 34 or 35.

[0007] In a further aspect, the present invention provides a probe and a primer for each gene included in the gene set, and a kit for predicting effect of immunotherapy on a cancer patient, comprising a probe and primers for each gene included in the gene set and/or an antibody specifically recognizing an expression product of each gene included in the gene set.

[0008] In a still further aspect, the present invention provides a method for screening for a cancer patient for whom immunotherapy is effective, comprising the step of (1) measuring the expression level of each gene included in a gene set consisting of at least one gene selected from the group of genes shown in Table 1, 19, 34, or 35 in a sample obtained from the cancer patient before the immunotherapy.

The method for screening for a cancer patient may further comprise a step of

(2) determining prognosis of the patient by discriminant analysis using the expression level.

In a still further aspect, the present invention provides a method for predicting effect of immunotherapy on a cancer patient, comprising a step of measuring an expression level of IL-6 protein in blood obtained from the cancer patient before or after the immunotherapy.
The level of IL-6 protein in blood can also serve as a biomarker for predicting effect of immunotherapy on a cancer patient.

Effects of the Invention

[0009] According to the present invention, effect of immunotherapy on a patient can be predicted by determination of gene expression profiles of the cancer patient before the start of immunotherapy. The present invention enables prediction of patients for whom immunotherapy is not effective (poor prognosis group), and the present invention provides useful information for choosing a treatment method for cancer patients.

Brief Description of the Drawings

[0010]

Figure 1 shows the distribution of 16968 genes in t-test. The ordinate represents the level of significance between two groups, and the abscissa represents the logarithm of the gene expression ratio between two groups. n = 40 (20 short-lived individuals and 20 long-lived individuals);
Figure 2 shows the distribution of 16968 genes in Wilcoxon test. The ordinate represents the level of significance between two groups, and the abscissa represents the gene expression ratio between two groups. n = 40 (20 short-lived individuals and 20 long-lived individuals);
Figure 3 shows the distribution of 13 genes in t-test. The ordinate represents the level of significance between two groups, and the abscissa represents the logarithm of the gene expression ratio between two groups. n = 40 (20 short-lived individuals and 20 long-lived individuals);
Figure 4 shows the distribution of 13 genes in Wilcoxon test. The ordinate represents the level of significance between two groups, and the abscissa represents the gene expression ratio between two groups. n = 40 (20 short-lived individuals and 20 long-lived individuals);
Figure 5 shows genes advantageous for discrimination. The ordinate represents the frequencies of the genes for use in gene sets that offers a discrimination rate of 80% or more;
Figure 6 shows the difference in gene expression in peripheral blood mononuclear cells (PBMCs) between a long-lived group and a short-lived group before vaccination (A) or after vaccination (B);
Figure 7 shows results of determining the gene expression levels of DEFA1 (A), DEFA4 (B), CEACAM8 (C) and MPO (D) in the peripheral blood mononuclear cells (PBMCs) of a long-lived group (Long) and a short-lived group (Short) after vaccination by real-time PCR. The expression level of each gene was determined with GAPDH as an internal standard; and
Figure 8 shows results of determining the level of IL-6 protein in the plasmas of patients in a long-lived group (Long) and a short-lived group (Short). Mann-Whitney test was used as a statistical test.

Best Mode for Carrying Out the Invention

[0011] In one aspect, the present invention provides a method for predicting effect of immunotherapy on a cancer patient, comprising measuring the expression levels of one or more genes in a sample obtained from the cancer patient. For the above-described prediction method, a gene set which is available for accurate prediction of whether a cancer patient after given immunotherapy belongs to a good prognosis group or a poor prognosis group based on the expression level is utilized. For the above-described prediction method, an expression level of at least one gene selected from the group of genes shown in Table 1, 19, 34, or 35 is utilized. The above-described prediction method can be used as a method for predicting a patient for whom immunotherapy is not expected to be effective; a patient for whom immunotherapy is expected to be effective; or a patient who is resistant to immunotherapy, and determining whether immunotherapy is applicable or not.
[0012] A gene set, which is used for the above-described prediction method provided as one aspect of the present invention, can be selected arbitrarily from 54 genes shown in Table 1, 100 genes shown in Table 19, 36 genes shown in Table 34, 19 genes shown in Table 35 and/or IL-6 gene without being limited by the number and the kind of the gene.
[0013] Preferably, the gene set for use in the above-described prediction method provided as one aspect of the present invention consists of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 genes selected from 13 genes shown in Table 2. The gene set preferably comprises 4 genes: LOC653600, TNFRSF19, P4HA1, and SONE1. Specific examples of the gene set

include a gene set consisting of LOC653600, TNFRSF19, P4HA1 and SYNE1; a gene set consisting of LOC653600, TNFRSF19, G3BP2, ZNF83, C6orf222, ZBTB20, P4HA1, GP1BA, HLA-A29.1, SYNE1 and NAP1L1; and gene sets represented by No. 1 of Table 10, Nos. 1 to 18 of Table 11, Nos. 1 to 55 of Table 12, Nos. 1 to 71 of Table 13, Nos. 1 to 63 of Table 14, Nos. 1 to 45 of Table 15, Nos. 1 to 22 of Table 16 or Nos. 1 to 7 of Table 17.

[0014] In another preferable embodiment, the gene set for use in the above-described prediction method provided as one aspect of the present invention consists of 1 to 29 genes (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 genes), selected from 29 genes shown in Table 20. Specific examples of the gene set include gene sets shown in Tables 23 to 33. Those skilled in the art can appropriately select even a gene set comprising 12 or more genes according to the description of Examples.

[0015] In another preferable embodiment, the gene set for use in the above-described prediction method provided as one aspect of the present invention consists of at least one gene selected from genes shown in Table 34. The gene set preferably comprises 4 genes: DEFA1, DEFA4, CEACAM8 and MPO. Specific examples of the gene set include a gene set consisting of DEFA1, DEFA4, CEACAM8 and MPO, and a gene set consisting of at least one gene selected from the group consisting of DEFA1, DEAF3, DEFA4, ELA2, CSTG, CAMP, MPO, MUMP9 and CEACAM8.

[0016] In another preferable embodiment, the gene set for use in the above-described prediction method provided as one aspect of the present invention consists of at least one gene selected from genes shown in Table 35 (PRKAR1A, LRRN3, PCDH17, TTN, LAIR2, RNASE3, CEACAM6, AZU1, HIST1H4C, PGLYRP1, CEACAM8, LCN2, MPO, CAMP, DEFA1, DEFA3, CTSG, DEFA4 and ELA2). The gene set preferably comprises 4 genes: LRRN3, PCDH17, HIST1H9C and PGLYRP1. Specific examples of the gene set include a gene set consisting of LRRN3, PCDH17, HIST1H9C and PGLYRP1, and a gene set consisting of at least one gene selected from the group consisting of LRRN3, PCDH17, HIST1H4C and PGLYRP1.

In another preferable embodiment, the gene set for use in the above-described prediction method provided as one aspect of the present invention consists of IL-6 gene or comprises IL-6 gene.

In another preferable embodiment, examples of the gene set for use in the above-described prediction method provided as one aspect of the present invention include a gene set consisting of at least one gene selected from the group consisting of LOC653600, TNFRSF19, P4HA1, SYNE1, DEFA1, DEFA4, CEACAM8, MPO, LRRN3, PCDH17, HIST1H4C and PGLYRP1, and a gene set comprising at least one gene selected from the group consisting of LOC653600, TNFRSF19, P4HA1, SYNE1, DEFA1, DEFA4, CEACAM8, MPO, LRRN3, PCDH17, HIST1H4C and PGLYRP1.

[0017] In the present specification, a cancer patient is not particularly limited as long as the cancer patient is a human. Examples thereof include patients with prostate cancer, pancreatic cancer, breast cancer, liver cancer or the like. The prostate cancer may be progressive recurrent prostate cancer.

[0018] In the present specification, an immunotherapy means a method for treating cancer by activating immune response to tumor antigen proteins in the cancer patient. Examples of immunotherapy include peptide vaccine therapy using tumor antigen peptides; adoptive immunotherapy using lymphocytes such as cytotoxic T cells or natural killer cells; DNA vaccine therapy which involves introducing viral vectors expressing tumor antigen proteins or tumor antigen peptides into organisms; and dendritic cell vaccine therapy which involves administering dendritic cells displaying tumor antigen peptides. One preferable example of the immunotherapy, includes peptide vaccine therapy.

[0019] An expression level of each gene can be measured by a conventional method. Examples of the method for measuring the expression level include DNA microarray, DNA chip, PCR (including real-time PCR) and Northern blot methods. One preferable example of the method for measuring the expression level includes a DNA microarray method.

[0020] A DNA microarray method is performed using a microarray comprising a probe for a gene to be measured. One example of the available microarray includes HumanWG-6 v3.0 Expression BeadChip manufactured by Illumina, Inc. Alternatively, a probe for a gene to be measured may be synthesized and immobilized on an appropriate substrate such as slide glass to prepare a desired microarray. The method for preparing a microarray is well known in the art. The analysis of microarray data is also well known and can be performed with reference to, for example, "Microarrays for an Integrative Genomics" (translated by Yujin Hoshida, published by Springer-Verlag Tokyo, Inc.).

[0021] A sample of human patient for use in measurement of a gene expression level is not limited, and, for example, peripheral blood obtained from a patient can be used. Myeloid dendritic cells (MDCs), granalocytic MDSCs, peripheral blood mononuclear cells (PBMCs), granulocytes or erythrocytes in the peripheral blood may be used for the measurement of the gene expression level. Also, the patient-derived sample may be a sample obtained before immunotherapy, a sample obtained after immunotherapy, or samples obtained before and after immunotherapy. The above-described prediction method provided as one aspect of the present invention may be carried out using the sample obtained before immunotherapy in order to predict whether immunotherapy is not expected to be effective for the patient or immunotherapy is expected to be effective for the patient, and determine whether immunotherapy is applicable or not. Alternatively, the above-described prediction method provided as one aspect of the present invention may be carried out using the sample obtained after immunotherapy in order to predict whether or not after the sample is obtained, immunotherapy is expected to be effective for the patient, and determine whether further immunotherapy is applicable or not.

[0022] A measurement of a gene expression level using a DNA microarray method is, for example, described as follows. Firstly, total RNA is extracted from the peripheral blood of a patient and purified. Subsequently, biotinylated cRNA is synthesized using Illumina TotalPrep RNA Amplification Kit (manufactured by Life Technologies Corp. (Ambion)) or the like. This biotinylated cRNA is hybridized to a microarray and then reacted with Cy3-labeled streptavidin. The microarray after the reaction is scanned with a specific scanner. The Cy3 fluorescence of each spot can be quantified using specific software such as BeadStudio to obtain an expression level of each gene.

[0023] When a gene expression level is measured by PCR, for example, cDNA can be prepared from mRNA in a sample and used as a template in PCR to determine the gene expression level in the sample. For determination of a gene expression level by PCR, real-time PCR may be used. Primers for use in PCR can be designed appropriately by those skilled in the art to be capable of specifically hybridizing to a gene of interest. Also, for the real-time PCR, a probe that specifically hybridizes to a gene of interest and is bound with a fluorescent dye to allow determination of a PCR product is used. The probe can be designed appropriately by those skilled in the art. The real-time PCR may be performed using a fluorescent dye such as SYBR (registered trademark) Green.

[0024] Alternatively, an expression level of each gene may be measured by measuring an expression level of a protein which is an expression product of the gene. A protein localized in a cell membrane or a cytoplasm can be measured by flow cytometry using an antibody labeled with a fluorescent dye. Alternatively, an enzyme antibody method (ELISA), a Western blot method, or the like may be used.

[0025] The method for predicting effect of immunotherapy on a cancer patient provided as one aspect of the present invention comprises measuring an expression level of one or more genes in a sample obtained from the cancer patient and may further comprise conducting discriminant analysis using the measured expression level. As a result of the discriminant analysis, prognosis of the patient, such as whether the effect of immunotherapy would be observed, can be determined. The discriminant analysis can be carried out, for example, as described in Examples. Specifically, genes for use in the determination are selected, and discriminant functions based on known data (training data) are obtained. Then, the expression levels of the genes in a patient as a subject are applied thereto to calculate the probability of 1 (long life) or 0 (short life) for the patient. The prognosis of the patient is determined according to results in which the probability exceeds 50% (long life (good prognosis) or short life (poor prognosis)). When the probability of long life (good prognosis) exceeds 50%, the patient is predicted to respond to the immunotherapy. By contrast, when the probability of short life (poor prognosis) exceeds 50%, the patient is predicted not to respond to the immunotherapy.

[0026] The discriminant analysis can be conducted using statistical analysis software SAS (SAS Institute Japan Ltd.), statistical analysis software JMP (SAS Institute Japan Ltd.) or the like. The number of training data is not particularly limited. Those skilled in the art can appropriately determine the number of training data that achieves prediction.

[0027] Alternatively, a standard expression level (standard values of expression levels in long-lived or short-lived individuals) of genes for use in determination may be preliminarily determined for each of long-lived (good prognosis) and short-lived (poor prognosis) individuals in a sufficient number of cases (for example, 100 or 1000 cases). The standard value may be compared with the expression level of the gene in a patient as a subject to determine the prognosis of the patient. For example, when expression levels of DEFA1, DEFA4, CEACAM8 and MPO genes are higher than the standard values of the long-lived group, the patient is a patient for whom immunotherapy cannot be expected to be effective and is determined not to have good prognosis. The standard values of respective expression levels of DEFA1, DEFA4, CEACAM8 and MPO genes can be selected, for example, as shown in Figure 7.

[0028] In Examples described below, genes were selected on the basis of a survival time of 480 days, and the accuracy of the determination was confirmed on the basis of a survival time of 480 days. However, 40 prostate cancer patients of Examples were only patients with a survival time of 900 days or longer or a survival time of 300 days or shorter. Therefore, the same gene set as that of the present invention can be selected even on the basis of any of survival times of 301 to 899 days. Accordingly, the number of days on which the definition of long life or short life is based may be any number of days within the survival time range of 301 to 899 days and is not limited to 480 days.

[0029] The above-described method for predicting effect of immunotherapy on a cancer patient provided as one aspect of the present invention can be used as a method for predicting whether immunotherapy is not expected to be effective for a patient, or whether immunotherapy is expected to be effective for a patient, and determine whether immunotherapy is applicable or not. Accordingly, the above-described method for predicting effect of immunotherapy on a cancer patient may be a method for screening for a cancer patient predicted to respond to immunotherapy.

In addition, a physician can determine a therapeutic strategy for a cancer patient on the basis of results obtained by the above-described method for predicting effect of immunotherapy on a cancer patient. Accordingly, in one aspect, the present invention provides a method for diagnosing or treating cancer, comprising performing the above-described method for predicting effect of immunotherapy on a cancer patient.

[0030] In one aspect, the present invention also provides a gene set that is used for predicting effect of immunotherapy on a cancer patient. The gene set is the same as the gene set for use in the above-described method for predicting effect of immunotherapy on a cancer patient provided as one aspect of the present invention and can be used for preparing a probe for a DNA. microarray, a primer for PCR, or the like that is used in the prediction method of the present invention.

[0031] In one aspect, the present invention also provides a biomarker for predicting effect of immunotherapy on a cancer patient. In the present specification, the biomarker can serve as an index for predicting effect of immunotherapy. The biomarker may be a gene or may be a protein expressed therefrom. The gene set for use in the above-described method for predicting effect of immunotherapy on a cancer patient provided as one aspect of the present invention can be used as the biomarker. For example, the biomarker may be at least one gene selected from the group of genes shown in Table 1, 19, 34 or 35; a gene set comprising LOC653600, TNFRSF19, P4HA1 and SONE1; a gene set comprising DEFA1, DEFA4, CEACAM8 and MPO; or a gene set comprising LRRN3, PCDH17, HIST1H4C and PGLYRP1. When DEFA1, DEFA4, CEACAM8 and MPO are used as biomarkers, the higher expression levels of these genes than the standard values of the long-lived group can serve as an index for predicting the patient not to respond to the immunotherapy.

Alternatively, a protein which is expression product of the gene set for use in the above-described method for predicting effect of immunotherapy on a cancer patient provided as one aspect of the present invention may be used as a biomarker for predicting effect of immunotherapy on a cancer patient. For example, IL-6 protein in blood may be used as a biomarker. The larger level of the IL-6 protein in the blood of a patient than the standard value of the long-lived group can serve as an index for predicting the patient not to respond to the immunotherapy. The standard value of the level of IL-6 protein in blood may be set to, for example, 4.8 pg/ml for the short-lived group and 3.3 pg/ml for the long-lived group with reference to Figure 8.

[0032] In one aspect, the present invention also provides a probe, primers or an antibody that is available for measurement of the expression level of each gene in the gene set of the present invention or its gene expression product. The probe and the primers for each gene can be synthesized by a conventional method on the basis of sequence information about the gene. The probe and the primers have, for example, a sequence partially complementary to the sequence of each gene and can specifically hybridize to the gene.

As used herein, the term "specifically hybridize" refers to, for example, "hybridizing under stringent conditions". The "stringent conditions" can be determined appropriately by those skilled in the art with reference to, for example, Molecular Cloning: A Laboratory Manual, 3rd edition (2001). Examples thereof include 0.2 x SSC, 0.1% SDS, and 65°C.

The primers can be designed to be capable of being used in PCR for amplifying each gene or a portion thereof. The sequence information about each gene can be obtained according to GenBank Accession numbers described in the tables of the present specification. Also, a method for preparing the antibody is well known in the art ("Antibodies: A Laboratory Manual", Lane, H. D. et al. eds., Cold Spring Harbor Laboratory Press, New York, 1989).

For example, the respective probes and primers of DEFA1, DEFA4, CEACAM8 and MPO may be the oligonucleotides of SEQ ID NOs: 1 to 8 described in Examples.

The antibody may be a polyclonal antibody or may be a monoclonal antibody. Alternatively, the antibody may be an antibody fragment such as Fab, $F(ab')_2$ and Fv.

[0033] In one aspect, the present invention also provides a kit for predicting effect of immunotherapy on a cancer patient, comprising the above-described probe, the above-described primers and/or the above-described antibody.

The kit of the present invention is a kit that is used for, for example, DNA microarray, DNA chip, PCR (including real-time PCR), Northern blot, fluorescent antibody, enzyme antibody and Western blot methods. Examples of the kit for the DNA microarray method include those comprising a microarray comprising the above-described probe immobilized on an appropriate substrate.

The kit may also comprise, for example, an anti-IL-6 polyclonal antibody or an anti-IL-6 monoclonal antibody for determining the level of IL-6 protein in blood.

The kit may also comprise other necessary reagents according to the measurement method.

[0034] In one aspect, the present invention also provides a method for selecting a gene set for predicting effect of immunotherapy on a cancer patient. The method for selecting a gene set comprises, for example, step 1: a step of determining an expression level of a gene expressed in a sample derived from the cancer patient group where immunotherapy is effective for the patient (long-lived group) and where immunotherapy is not effective for the patient (short-lived group); step 2: a step of selecting a gene capable of serving as a marker for predicting effect of immunotherapy, on the basis of the difference in gene expression level between the group where immunotherapy is effective for the patient (long-lived group) and where immunotherapy is not effective for the patient (short-lived group) and statistically significant difference thereof; and step 3: a step of determining the best combination for predicting effect of immunotherapy by variable selection from the selected genes.

The difference in gene expression level may be evaluated, for example, on the basis of a value of $\log_2$ "expression level in a short-lived group / expression level in a long-lived group" after determining the expression level in the short-lived group / the expression level in the long-lived group.

The statistically significant difference may be determined by t-test and/or Wilcoxon test or may be determined by the Limma method (see Smyth. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Statistical applications in genetics and molecular biology (2004) vol. 3, pp. Article).

For example, in the above-described step 2, a gene that satisfies the conditions of $\log_2$ "expression level in the short-lived group / expression level in the long-lived group" < -1.0 or > 1.0 and P-value (limma) < 0.01 may be selected. The variable selection can be carried out appropriately by those skilled in the art. For example, the stepwise discriminant analysis (SDA) described in Sungwoo Kwon et al: DNA Microarray Data Analysis for Cancer Classification Based on Stepwise Discriminant Analysis and Bayesian Decision Theory. Genome Informatics 12: 252-254 (2001) may be carried out. Examples of SDA include the implementation of the following (i) to (iii):

(i) when Wilk's lambda is evidently decreased by adding one gene to a gene set, adding the gene to the gene set;
(ii) when Wilk's lambda is slightly increased by removing one gene from a gene set, removing the gene from the gene set; and
(iii) repeating the steps (i) and (ii) until the Wilk's lambda no longer changes from a statistical standpoint.

[0035] Hereinafter, the present invention will be further described with reference to Examples. However, the present invention is not intended to be limited to them.

Example

Example 1

1: DNA microarray study of gene expression profile before peptide vaccine therapy

[0036] The patient-derived samples used were peripheral blood that was obtained from each prostate cancer patient who gave informed consent according to a protocol approved by the ethics committee of Kurume University when the patient was diagnosed as having recurrent prostate cancer in the past clinical trial. 40 prostate cancer patients were examined for their gene expression profiles before peptide vaccine therapy using DNA microarrays (HumanWG-6 v3.0 Expression BeadChip manufactured by Illumina, Inc.). The prostate cancer patients were 20 individuals in a good prognosis group (survival time of 900 days or longer after peptide vaccine therapy) and 20 individuals in a poor prognosis group (survival time of 300 days or shorter after peptide vaccine therapy).

(I) RNA extraction and purification from peripheral blood of patient

[0037]

1. To the peripheral blood sample of each patient, TRIzol LS (manufactured by Invitrogen Corp.) was added at a ratio of 1:3 and mixed therewith.
2. 200 μL of chloroform with respect to 750 μL of the TRIzol LS solution was added and mixed therewith, followed by centrifugation.
3. The supernatant was transferred to a new tube, to which ethanol was added in an amount of 0.55 times the volume of the supernatant.
4. The sample of 3 was placed on the column of SV Total RNA Isolation System (manufactured by Promega Corp.) and applied to a filter.
5. The filter was washed with 500 μL of Wash Buffer.
6. Total RNA was eluted with 80 μL of Nuclease Free Water.
7. The concentration of the RNA was measured using a spectrophotometer, and the quality of the RNA was checked by electrophoresis using Experion System (manufactured by Bio-Rad Laboratories, Inc.).

(II) Synthesis of cRNA for microarray using Illumina TotalPrep RNA Amplification Kit (manufactured by Life Technologies Corp. (Ambion))

(1) Synthesis of single-stranded cDNA by reverse transcription

[0038]

1. Nuclease Free Water was added to 500 μg of each total RNA to adjust the volume to 11 μL.
2. 9 μL of Reverse Transcription Master Mix was added to the solution of 1, and the mixture was incubated at 42°C for 2 hours.

(2) Synthesis of double-stranded cDNA

[0039]

1. 80 μL of Second Strand Master Mix was added to each tube of (1)2.

2. Each tube was incubated at 16°C for 2 hours.

(3) cDNA purification

[0040]

1. 250 μL of cDNA Binding Buffer was added to each tube.
2. The solution of 1 was placed on cDNA Filter Cartridge and applied to a filter by centrifugation.
3. The filter was washed with 500 μL of Wash Buffer.
4. cDNA was eluted with 19 μL of Nuclease Free Water preheated to 50 to 55°C.

(4) cRNA synthesis through *in vitro* transcription reaction

[0041]

1. 7.5 μL of IVT Master Mix was added to the cDNA sample obtained in (3)4.
2. The tube of 1 was incubated at 37°C for 14 hours.
3. 75 μL of Nuclease Free Water was added to the tube of 2.

(5) cRNA purification

[0042]

1. 350 μL of cRNA Binding Buffer was added to each tube.
2. 250 μL of 100% ethanol was added to each tube and mixed.
3. The sample of 2 was placed on cRNA Filter Cartridge and applied to a filter by centrifugation.
4. The filter was washed with 650 μL of Wash Buffer.
5. cRNA was eluted with 100 μL of Nuclease Free Water preheated to 50 to 55°C.
6. The concentration of the cRNA was measured on the basis of OD, and the cRNA was then used as a hybridization sample.

(III) Microarray hybridization

[0043]

(1) Preparation of cRNA for hybridization

1. Nuclease Free Water was added to 500 μg of each total RNA to adjust the volume to 10 μL.
2. 20 μL of GEX-HYB was added to the solution of 1, and the mixture was incubated at 65°C for 5 minutes.

(2) Hybridization

1. The prepared cRNA sample was applied to HumanWG-6 v3.0 Expression BeadChip loaded in a specific chamber.
2. The lid of the specific chamber was closed, followed by incubation at 55°C for 18 hours.

(IV) Microarray washing and staining

[0044]

(1) Washing of array

1. The cover of the microarray was removed in Wash E1BC solution.
2. The array was immediately loaded in a slide rack and washed for 10 minutes in 1 x High-Temp Wash buffer preheated to 55°C.
3. The array was washed for 5 minutes in Wash E1BC solution.
4. The array was washed for 5 minutes in ethanol.
5. The array was washed for 5 minutes in Wash E1BC solution.
6. 4 ml of Block E1 buffer was prepared in a staining-specific tray, and each array was loaded therein one by one and blocked at room temperature for 10 minutes.
7. 2 μL of streptavidin-Cy3 with respect to 2 ml of Block E1 buffer was added to the staining-specific tray, and each array was loaded therein one by one, followed by staining at room temperature for 10 minutes.
8. The array was washed for 5 minutes in Wash E1BC solution and then dried by centrifugation.

(V) Scanning and quantification

[0045]

1. The array was loaded in a specific scanner manufactured by Illumina, Inc. and scanned in a standard mode.

2. After the completion of scanning, each spot on the microarray was quantified using specific software BeadStudio.

[0046] The obtained microarray data was normalized using VST (variance stabilizing transformation) and RSN (robust spline normalization). A gene expression level with presence probability < 0.05 with respect to a negative control (gene expression level measured using a probe for a gene that was absent on the microarray) was determined to be significant. Genes with presence probability < 0.05 in 70% or more of the 40 patients were used in the following experiments:

2: Selection of gene for use in prediction

[0047] The prostate cancer patients of Example 1 were classified on the basis of a survival time after peptide vaccine therapy into a good prognosis group (long-lived group) with a survival time of 480 days or longer and a poor prognosis group (short-lived group) with a survival time shorter than 480 days. Genes that could significantly differentiate between two groups were selected. Analysis was conducted using t-test and Wilcoxon test.
Specifically, 16968 genes were subjected to t-test and Wilcoxon test in the short-lived group (S) and the long-lived group (L) (Figures 1 and 2). As a result, 54 genes with a level of significance "p <= 0.001" or "fold change of 2 or more" demonstrated by the t-test and the Wilcoxon test were extracted (Table 1). The expression levels (fluorescence reader-measured values) of these 54 genes are shown in the columns "Mean of short-lived group (S)" and "Mean of long-lived group (L)" of Table 1.

[Table 1]

OBS	Probe_ID	Symbol	Accession	Mean of short-lived group (S)	Mean of long-lived group (L)	S-L	Pr > |t|	P-value, Kruskal-Waills Test
2	150706	UGP2	NM_006759.3	7.9670	8.3141	-0.3471	0.0008	0.00088
4	510208	LOC643310	XM_926856.1	9.2936	9.6892	-0.3958	0.0005	0.00021
7	770400	LOC653600	XM_928349.1	9.8511	8.6911	1.1600	0.0416	0.08342
8	840064	LOC645489	XM_928514.1	7.2404	7.4395	-0.1990	0.0005	0.00097
11	840601	MEGF10	NM_032446.1	6.6521	6.6044	0.0477	0.0005	0.00097
12	870477	LOC728358	NM_001042500.1	11.6369	10.2926	1.3443	0.0219	0.03726
13	990315	TCP1	NM_030752.2	8.0893	8.3461	-0.2568	0.0008	0.00065
16	1400240	LDHB	NM_002300.4	10.8411	11.4466	-0.6055	<.0001	0.00008
17	1500047	RIN1	NM_004292.2	6.8923	8.7602	0.1322	0.0004	0.00088
18	1500735	CTSG	NM_001911.2	8.7810	7.5263	1.2547	0.0048	0.00869
19	1570392	IL21R	NM_181078.1	6.6201	6.5630	0.0571	0.0002	0.00088
23	1780709	DDX17	NM_030881.2	6.6347	9.0780	-0.4433	0.0005	0.00072
24	1780719	PTGES3	NM_006601.4	8.3710	8.8997	-0.5287	0.0002	0.00036
25	2030332	PTPN18	NM_014369.2	6.6984	6.6146	0.0838	<.0001	0.00003
28	2340091	LOC646135	XM_933437.1	6.8649	6.7536	0.1113	0.0006	0.00097
29	2360672	TNFRSF19	NM 148957.2	6.6360	6.5650	0.0710	0.0006	0.00080
30	2370764	G3BP2	NM_012297.3	6.6111	6.5557	0.0553	0.0002	0.00021
31	2480600	LOC728358	MM_001042500.1	11.7328	10.2900	1.4428	0.0148	0.02655
33	2680440		BF338865	6.6258	6.5641	0.0617	0.0005	0.00048
35	2900255	ZBT645	NM_032792.2	6.8759	6.7543	0.1246	0.0003	0.00097
37	2970747	DEFA3	NM_005217.2	11.8854	10.4815	1.4039	0.0171	0.02476
38	3060288	NAIP	NM_004536.2	6.6667	6.5931	0.0736	<.0001	0.00017
39	3130296	AMY2A	NM_000699.2	7.2523	7.5351	-0.2828	0.0007	0.00065
40	3130370	ZNF83	NM_018300.2	7.4687	7.7744	-0.3057	0.0002	0.00023

(continued)

OBS	Probe_ID	Symbol	Accession	Mean of short-lived group (S)	Mean of long-lived group (L)	S-L	Pr > |t|	P-value, Kruskal-Waills Test
41	3130477	C7orf28A	XM_001133729.1	7.7594	8.1429	-0.3834	0.0003	0.00080
43	3360228	RPS20	NM_001023.2	14.5907	14.7537	-0.1630	0.0007	0.00097
44	3390368	POP2	NM_020786.1	6.6320	6.5632	0.0688	<.0001	0.00006
45	3420136	C6orf222	NM_001010903.3	6.5946	6.5441	0.0505	0.0004	0.00097
46	3440189	ZBTB20	NM_015642.3	7.5534	7.8405	-0.2871	0.0002	0.00044
48	3610521	PCDHGB6	NM_018928.2	6.8835	6.7067	0.1768	<.0001	0.00023
51	3990608	MAN2A1	NM_002372.2	8.5259	8.8533	-0.3274	0.0001	0-00021
52	4010632	LOC642946	XM_927142.1	6.6135	6.5408	0.0727	0.0002	0.00019
53	4120056	IQSEC2	NM_015075.1	5.6354	6.5576	0.0777	0.0002	0.00054
55	4150408	P2RY2	NM_002564.2	6.8606	6.5785	0.1821	<.0001	0.00002
58	4220731	P4HA1	NM_000917.2	7.8835	8.1913	-0.3078	0.0003	0.00039
59	4250154	LOC648749	XM_937834.2	6.5599	6.6215	-0.0617	0.0003	0.00044
60	4260767	GP1BA	NM_000173.4	7.0478	6.9170	0.1309	0.0004	0.00064
62	4540239	DEFA1	NM_004084.2	12.6997	11.3300	1.3597	0.0171	0.02655
65	4810072	TUSC2	NM_007275.1	7.3719	7.2093	0.1626	0.0002	0.00032
66	4830255	DPP4	NM_001935.3	6.8716	7.1790	-0.3074	<.0001	0.00019
68	5080692	HLA-A29.1	NM_001080840.1	11.3133	9.8801	1.4332	0.0823	0.11667
69	6290358	CPT1A	NM_001031847.1	6.9679	6.8024	0.1656	0.0003	0.00059
70	5550711	SYNE1	NM_182961.2	6.6852	6.6097	0.0755	0.0004	0.00072
71	5860075	CAMP	NM_004345.3	9.9646	8.9316	1.0328	0.0129	0.02000
72	6860465	USPSY	NM_004654.3	6.6293	6.7238	-0.0945	0.0001	0.00054
73	6900129	CROP	NM_006107.2	7.9041	8.3479	-0.4438	0.0006	0.00066
74	5960072		BY797688	6.6802	6.7772	-0.0970	0.0003	0.00002
75	6110630	HIST1H2BK	NM_080593.1	10.7582	10.3790	0.3791	<.0001	0.00023

(continued)

OBS	Probe_ID	Symbol	Accession	Mean of short-lived group (S)	Mean of long-lived group (L)	S-L	Pr > |t|	P-value, Kruskal-Waills Test
78	6420446	CMPK1	NM_016308.1	8.2399	8.6386	-0.3986	0.0007	0.00072
79	6550154	DEFA4	NM_001925.1	8.6054	7.5272	1.2782	0.0029	0.00380
83	6590484	NAP1L1	NM_139207.1	7.5255	7.8829	-0.3574	0.0006	0.00039
88	6940433	STAT5B	NM_012448.3	8.5812	8.8213	-0.2402	0.0007	0.00088
88	7150170	LOC728358	NM_001042500.1	11.6943	10.3009	1.3933	0.0209	0.04248
93	7650497	ELA2	NM_001972.2	8.9421	7.6222	1.3199	0.0022	0.00414

[0048] The selected 54 gene was further subjected to variable selection using t-test and Wilcoxon test (Figures 3 and 4) to select 13 genes (Table 2).

[Table 2]

Probe_ID	Symbol	Accession	Pr > |t|	P-value, Kruskal-WallisTest
770400	LOC653600	XM_928349.1	0.0416	0.08342
2030332	PTPN18	NM 014369.2	<.0001	0.00003
2360672	TNF RSF 19	NM 148957.2	0.0006	0.00080
2370754	G3BP2	NM 012297.3	0.0002	0.00021
3130370	ZNF83	NM_018300.2	0.0002	0.00023
3420136	C6orf222	NM_001010903.3	0.0004	0.00097
3440189	ZBTB20	NM_015642.3	0.0002	0.00044
4220731	P4HA1	NM 000917.2	0.0003	0.00039
4260767	GP1BA	NM 000173.4	0.0004	0.00054
5080692	HLA-A29.1	NM 001080840.1	0.0823	0.11667
5550711	SYNE1	NM 182961.2	0.0004	0.00072
5960072		BY797688	0.0003	0.00002
6590484	NAP1L1	NM_139207.1	0.0006	0.00039

3: Discrimination rate based on selected gene

3-1. Calculation of discrimination rate

[0049] The discrimination rate of the short-lived group or the long-lived group based on the selected genes was calculated. Specifically, the data set was divided into training data and test data, and subjected to cross-validation for performing model construction and tests. For the cross-validation method, leave-one-out cross-validation was used, where training for the data set except for the data of one individual is performed and a discriminant model is evaluated using this one individual that has not been used in the training data; and the above-described task is repeated for all individuals.

[0050] Results of analysis using 4 genes: probe ID Nos. 770400, 3130370, 4220731 and 5550711 shown in Table 3 are shown as an example. The analysis was conducted using statistical analysis software SAS (SAS Institute Japan Ltd.) and statistical analysis software JMP (SAS Institute Japan Ltd.).

[Table 3]

Linear discriminant function for grp		
Variable	**0**	**1**
Constant	-7124	-7038
770400	10.47056	9.49131
3130370	148.16314	154.23258
4220731	62.46423	69.11172
5550711	1877	1850

[0051] Table 4 shows results of conducting leave-one-out cross validation with respect to the training data of 40 patients. Specifically, this table shows which group each case is predicted to belong to using the discriminant functions of Table 3. In the table, (Actual) represents a group to which each case actually belonged, and (Prediction) represents a group predicted using the discriminant functions. As is evident from the table, S2_pre was correctly predicted [0 (short-lived group) → 0 (short-lived group)], whereas S10_pre was incorrectly predicted [0 (short-lived group) → 1 (long-lived group)]. In the table, the symbol * represents that the case was predicted to belong to a group which is different from the actual one. A total of 4 individuals corresponded thereto.

[Table 4]

Results of data-discrimination by means of decision rule: WORK MOTHER Results of cross-validation (linear discriminant function)					
Posterior probability for grp					
Case Number	**Group (Actual): grp**	**Group (Prediction): grp**		**0**	**1**
S2_pre	**0**	**0**		0.9616	0.0384
S3_pre	**0**	**0**		0.9982	0.0018
S4 pre	**0**	**0**		0.8032	0.1968
S6_pre	**0**	**0**		0.9994	0.0006
S7_pre	**0**	**0**		0.9778	0.0222
S8_pre	**0**	**0**		0.7907	0.2093
L3 pre	**1**	**1**		0.2898	0.7001
L4_pre	**1**	**1**		0.1652	0.8348
L1_pre	**1**	**1**		0.3087	0.8913
S10_pre	**0**	**1**	*	0.1158	0.8842
S12_pre	**0**	**0**		0.9168	0.0832
S13_pre	**0**	**0**		0.9988	0.0011
S14_pre	**0**	**0**		0.9609	0.0391
S15_pre	**0**	**0**		0.9393	0.0607
S16_pre	**0**	**0**		0.9869	0.0131
S17_pre	**0**	**0**		0.9904	0.0096
S16_pre	**0**	**0**		0.9996	0.0004
S20_pre	**0**	**0**		0.9999	0.0001
S21_pre	**0**	1	*	0.4247	0.5753
S22_pre	**0**	0		0.9901	0.0099
S23_pre	**0**	**0**		0.9261	0.0739
S25_pre	**0**	**0**		0.6318	0.3682
S26_pre	**0**	**1**	*	0.021	0.979
L5_pre	**1**	**1**		0.0716	0.9284
L6_pre	**1**	**1**		0.0336	0.9664
L7_pre	**1**	**1**		0.0144	0.9856
L8_pre	**1**	**1**		0.0152	0.9848
L9_pre	**1**	**0**	*	0.9708	0.0292
L10_pre	**1**	**1**		0.0159	0.9841
L11_pre	**1**	**1**		0.3711	0.6288
L12 pre	**1**	**1**		0.0251	0.9749
L13_pre	**1**	**1**		0.0019	0.9981
L14_pre	**1**	**1**		0.006	0.995

(continued)

Results of data-discrimination by means of decision rule: WORK MOTHER Results of cross-validation (linear discriminant function)					
Posterior probability for grp					
Case Number	**Group (Actual): grp**	**Group (Prediction): grp**		**0**	**1**
L15_pre	**1**	**1**		0.0007	0.9993
L16_pre	**1**	**1**		0.178	0.822
L17_pre	**1**	**1**		0.0109	0.9891
L18-pre	**1**	**1**		0.0308	0.8692
L20_pre	**1**	**1**		0.1248	0.8752
L21_pre	**1**	**1**		0.0008	0.8992
L19_pre	**1**	**1**		0.001	0.999

[0052] Table 5 summarizes the results of Table 4 in a 2 x 2 cross table.
As is evident therefrom, 17 cases of the short-lived group were correctly discriminated with [0 → 0] (17/20 = 0.85: 85%), whereas 19 cases of the long-lived group were correctly discriminated with [1 → 1] (19/20 = 0.95: 95%).
These are discrimination rates.

[Table 5]

Results of data-discrimination by means of decision rule: WORK MOTHER Abstracts of cross-validation (linear discriminant function)			
Number of observation of grp-discrimination and discrimination rate			
Group (Actual): grp	**0**	**1**	**Total**
0	17 85	3 15	20 100
1	1 5	18 95	20 100
Total	18 45	22 55	40 100
Priors	0.5	0.5	

[0053] Table 6 shows results of conducting discriminant analysis using the discriminant functions of Table 3 with respect to the test data of 11 patients. Like Table 4, Table 6 shows the results of predicting which group each case belongs to. Only one case (4818441059_E) with the symbol * was incorrectly discriminated with [1 (long-lived group) → 0 (short-lived group)].

[Table 6]

Results of discrimination of test data: WORK.TEST3 Results of discrimination (linear discriminant function)					
Posterior probability for grp					
Case Number	**Group (Actual): grp**	**Group (Prediction): grp**		**0**	**1**
48184411050_A	**1**	**1**		0	1
4818441050_B	**1**	**1**		0.0209	0.9791
48184411050_C	**0**	**0**		0.8852	0.1048
488441050_D	**1**	**1**		0.34	0.66

(continued)

Results of discrimination of test data: WORK.TEST3 Results of discrimination (linear discriminant function)					
				Posterior probability for grp	
Case Number	**Group (Actual): grp**	**Group (Prediction): grp**		**0**	**1**
4818441050_E	**0**	**0**		0.5882	0.4118
4818441050_F	**0**	**0**		0.9995	0.0005
4818441052_A	**0**	**0**		0.797	0.203
4818441059_C	**0**	**0**		0.6135	0.3865
4818441059_D	**0**	**0**		0.9108	0.0692
4818441059_E	**1**	**0**	*	0.9728	0.0272
4818441059_F	**1**	**1**		0.2318	0.7684

[0054] Table 7 summarizes the results of Table 6 in a 2 x 2 cross table.
As is evident therefrom,
6 cases of the short-lived group were correctly discriminated with [0 (short-lived group) → 0 (short-lived group)] (6/6 = 1.00: 100%), whereas 4 cases of the long-lived group were correctly discriminated with [1 (long-lived group) → 1 (long-lived group)] (4/5 = 0.80: 80%).

[Table 7]

Results of discrimination of test data: WORK.TEST3 Abstracts of discrimination (linear discriminant function)			
Number of observation of grp-discrimination and discrimination rate			
Group (Actual):grp	**0**	**1**	**Total**
0	6 100	0 0	6 100
1	1 20	4 80	5 100
Total	7 63.64	4 36.35	11 100
Priors	05	05	

[0055] These results demonstrated that the group to which each case belongs could be discriminated (predicted) with 80% or more accuracy for both the training data and the test data by discriminant analysis using the 4 genes, i.e., probe ID NOs. 770400, 3130370, 4220731 and 5550711.

3-2. Discrimination rate based on selected gene

[0056] Subsequently, the discrimination rate was calculated using combinations of the 13 genes selected in the above-described item 2.

(1) Discrimination rate based on 1 gene

[0057] A study was made on whether or not long life or short life could be discriminated (discrimination rate: 80% or more) on the basis of one out of the 13 genes using training data (40 individuals) and test data (11 individuals).
As shown in Table 8, one gene 5960072 (BY797688) out of the 13 genes permitted prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life. Prediction based on this gene resulted in a short life discrimination rate (ccvP0) of 85% using the training data (40 individuals) and a short life discrimination rate (ctcP0) of 67% using the test data (11 individuals). On the other hand, the prediction

resulted in a long life discrimination rate (ccvP1) of 85% using the training data and a long life discrimination rate (ctcP1) of 80% using the test data.

[Table 8]

OBS	rep	index	pbnum	pb1	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	13	12	1	5960072	85	66.67	85	80	0	1
2	13	4	1	2370754	65	83.33	90	20	0	0
3	13	2	1	2030332	75	50	75	60	0	0
4	13	3	1	2360672	60	33. 33	75	60	0	0
5	13	9	1	4260767	70	100	75	20	0	0
6	13	13	1	6590484	85	0	70	80	0	0
7	13	7	1	3440189	70	66.67	70	60	0	0
8	13	8	1	4220731	80	66.67	70	40	0	0
9	13	5	1	3130370	75	83.33	65	60	0	0
10	13	6	1	3420136	65	83. 33	65	40	0	0
11	13	11	1	5550711	80	66.67	65	20	0	0
12	13	1	1	7.70400	45	16.67	60	100	0	0
13	13	10	1	5080692	85	33. 33	45	60	0	0

Pbnum: The number of probe (gene)
Pb1: Probe ID NO.
ccvP0: 0 (short life) → 0 (short life) discrimination rate obtained using training data (40 individuals)
ctcP0: 0 (short life) → 0 (short life) discrimination rate obtained using test data (11 individuals)
ccvP1: 0 (long life) → 0 (long life) discrimination rate obtained using training data
ctcP1: 0 (long life) → 0 (long life) discrimination rate obtained using test data
flg0: The short life discrimination rate was 80% or more for both training data and test data
flg1: The long life discrimination rate was 80% or more for both training data and test data

(2) Discrimination rate based on 2 genes

[0058] The number of combinations of 2 genes from the 13 genes is 78, and some of them are shown in Table 9. For example, the sets of 2 gene probes that permitted prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life were the following 6 sets (Table 9: 1 to 6) (indicated by probe 1 / probe 2 [short life discrimination rate (ccvP0) obtained using training data (40 individuals) / short life discrimination rate (ctcP0) obtained using test data (11 individuals); long life discrimination rate (ccvP1) obtained using training data / long life discrimination rate (ctcP1) obtained using test data]: 2360672/3130370 (85/66.67; 85/80), 770400/2360672 (70/33.33; 80/80), 770400/5960072 (90/66.67; 80/80), 2360672/5080692 (65/50; 80/80), 2360672/5960072 (95/66.67; 80/80) and 2360672/6590484 (75/33.33; 80/80). On the other hand, the sets of 2 gene probes that permitted prediction with a discrimination rate of 80% or more as to the discrimination of short life were the following 15 sets (Table 9: 7 to 21) (probe 1 / probe 2 [short life discrimination rate (ccvP0) obtained using training data (40 individuals) / short life discrimination rate (ctcP0) obtained using test data (11 individuals); long life discrimination rate (ccvP1) obtained using training data / long life discrimination rate (ctcP1) obtained using test data]: 2370754/5960072 (80/83.33; 95/40), 3440189/5550711 (85/83.33; 90/60), 3420136/5960072 (90/83.33; 90/40), 2370754/4260767 (80/100; 90/0), 2030332/5550711 (90/83.33; 85/60), 3420136/3440189 (85/83.33; 85/60), 2370754/3130370 (80/83.33; 85/40), 2360672/4220731 (80/83.33; 80/60), 3440189/4220731 (8583.33/; 80/60), 4220731/4260767 (85/100; 80/20), 4220731/5550711 (85/83.33; 75/60), 3420136/4220731 (95/83.33; 75/40), 2370754/4220731 (95/83.33; 75/20), 2370754/5550711 (85/83.33; 7520/) and 4260767/6590484 (80/100; 75/20).

[Table 9]

OBS	rep	index	pbnum	pb1	pb2	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	78	25	2	2360672	3130370	85	66.67	85	80	0	1
2	78	2	2	770400	2360672	70	33.33	80	80	0	1
3	78	11	2	770400	5960072	90	66.67	80	80	0	1
4	78	30	2	2360672	5080692	65	50	80	80	0	1
5	78	32	2	2360672	5960072	95	66.67	80	80	0	1
6	78	33	2	2360672	6590484	75	33.33	80	80	0	1
7	78	41	2	2370754	5960072	80	83.33	95	40	1	0
8	78	61	2	3440189	5550711	85	83.33	90	60	1	0
9	78	56	2	3420136	5960072	90	83.33	90	40	1	0
10	78	38	2	2370754	4260767	80	100	90	0	1	0
11	78	21	2	2030332	5550711	90	83.33	85	60	1	0
12	78	51	2	3420136	3440189	85	83.33	85	60	1	0
13	78	34	2	2370754	3130370	80	83.33	85	40	1	0
14	78	28	2	2360672	4220731	80	83.33	80	60	1	0
15	78	58	2	3440189	4220731	85	83.33	80	60	1	0
16	78	64	2	4220731	4260767	85	100	80	20	1	0
17	78	66	2	4220731	5550711	85	83.33	75	60	1	0
18	78	52	2	3420136	4220731	95	83.33	75	40	1	0
19	78	37	2	2370754	4220731	95	83.33	75	20	1	0
20	78	40	2	2370754	5550711	85	83.33	75	20	1	0
21	78	72	2	4260767	6590484	80	100	75	20	1	0
22	78	14	2	2030332	2370754	80	66.67	95	60	0	0
23	78	16	2	2030332	3420136	90	66.67	95	60	0	0
24	78	1	2	770400	2030332	85	50	90	60	0	0
25	78	19	2	2030332	4260767	75	83.33	90	40	0	0
26	78	7	2	770400	4220731	70	66.67	85	60	0	0
27	78	15	2	2030332	3130370	80	66.67	85	60	0	0
28	78	17	2	2030332	3440189	90	50	85	60	0	0
29	78	22	2	2030332	5960072	95	50	85	60	0	0
30	78	23	2	2030332	6590484	85	50	85	60	0	0
31	78	31	2	2360672	5550711	75	83.33	85	60	0	0
32	78	78	2	5960072	6590484	80	50	85	60	0	0
33	78	3	2	770400	2370754	55	66.67	85	40	0	0
34	78	13	2	2030332	2360672	80	50	85	-40	0	0
35	78	18	2	2030332	4220731	80	66.67	85	40	0	0
36	78	24	2	2360672	2370754	70	66.67	85	40	0	0
37	78	67	2	4220731	5960072	75	83.33	85	40	0	0
38	78	76	2	5550711	5960072	80	66.67	85	40	0	0

(continued)

OBS	rep	index	pbnum	pb1	pb2	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
39	78	10	2	770400	5550711	80	66.67	80	60	0	0
40	78	36	2	2370754	3440189	75	100	80	60	0	0
41	78	48	2	3130370	5550711	65	100	80	60	0	0
42	78.	49	2	3130370	5960072	80	66.67	80	60	0	0
43	78	62	2	3440189	5960072	85	66.67	80	60	0	0
44	78	77	2	5550711	6590484	90	66.67	80	60	0	0
45	78	29	2	2360672	4260767	75	100	80	40	0	0
46	78	45	2	3130370	4220731	75	66.67	80	40	0	0
47	78	55	2	3420136	5550711	70	83.33	80	40	0	0
48	78	59	2	3440189	4260767	75	100	80	40	0	0
49	78	8	2	770400	4260767	70	100	80	20	0	0

(3) Discrimination rate based on 3 genes

[0059] The number of combinations of 3 genes from the 13 genes is 286, and some of them are shown in Table 10. For example, the set of 3 gene probes that permitted prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life was only one set: 2360672/3440189/4220731 (Table 10: 1). Prediction based on this gene set resulted in a short life discrimination rate (ccvP0) of 90% using the training data (40 individuals) and a short life discrimination rate (ctcP0) of 83.33% using the test data (11 individuals). On the other hand, the prediction resulted in a long life discrimination rate (ccvP1) of 90% using the training data and a long life discrimination rate (ctcP1) of 80% using the test data. Likewise, examples of the probe sets that permit prediction of long life with a discrimination rate of 80% or more include 16 sets (Table 10: 2 to 17). In addition, examples of the probe sets that permits prediction of short life with a discrimination rate of 90% or more include the following gene probe sets: 2030332/2370754/4260767, 2030332/2370754/3440189, 3440189/5080692/5550711, 2360672/4220731/5550711, 3440189/4220731/5550711, 2370754/3440189/5550711, 2370754/3440189/4220731, 2370754/4220731/4260767, 3420136/4220731/5550711, 3420136/4220731/4260767 and 3130370/3420136/4220731.

[Table 10]

OBS	rep	index	pbnum	pb1	pb2	pb3	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	286	146	3	2360672	3440189	4220731	90	83.33	90	80	1	1
2	286	33	3	770400	3130370	4220731	75	66.67	90	80	0	1
3	286	65	3	770400	5550711	6590484	85	33.33	90	80	0	1
4	286	30	3	770400	2370754	6590484	85	66.67	85	80	0	1
5	286	46	3	770400	3440189	4220731	75	66.67	85	80	0	1
6	286	66	3	770400	5960072	6590484	80	33.33	85	80	0	1
7	286	130	3	2360672	2370754	6590484	80	66.67	85	80	0	1
8	286	162	3	2360672	5080692	5960072	85	50	85	80	0	1
9	286	165	3	2360672	5550711	6590484	85	66.67	85	80	0	1
10	286	166	3	2360672	5960072	6590484	90	50	85	80	0	1
11	286	20	3	770400	2360672	5960072	80	66.67	80	100	0	1
12	286	21	3	770400	2360672	6590484	75	16.67	80	100	0	1
13	286	63	3	770400	5080692	6590484	70	0	80	100	0	1
14	286	40	3	770400	3420136	4220731	80	66.67	80	80	0	1
15	286	53	3	770400	4220731	5080692	75	33.33	80	80	0	1
16	286	62	3	770400	5080692	5960072	80	50	80	80	0	1
17	286	138	3	2360672	3130370	6590484	90	50	80	80	0	1
18	286	78	3	2030332	2370754	3420136	85	83.33	100	60	1	0
19	286	96	3	2030332	3420136	4260767	90	83.33	100	60	1	0
20	286	234	3	3420136	3440189	5550711	85	83.33	100	60	1	0
21	286	274	3	4220731	5550711	5960072	90	83.33	100	40	1	0
22	286	81	3	2030332	2370754	4260767	95	100	100	20	1	0
23	286	36	3	770400	3130370	5550711	85	83.33	95	60	1	0
24	286	49	3	770400	3440189	5550711	95	83.33	95	60	1	0
25	286	77	3	2030332	2370754	3130370	90	83.33	95	60	1	0
26	286	84	3	2030332	2370754	5960072	95	83.33	95	60	1	0
27	286	113	3	2030332	4260767	5550711	80	100	95	40	1	0
28	286	143	3	2360672	3420136	5550711	80	83.33	95	40	1	0
29	286	191	3	2370754	4220731	5960072	90	83.33	95	40	1	0
30	286	249	3	3420136	5550711	5960072	85	83.33	95	40	1	0
31	286	243	3	3420136	4260767	5550711	80	100	95	20	1	0
32	286	269	3	4220731	4260767	5960072	90	83.33	95	20	1	0
33	286	7	3	770400	2030332	4260767	80	83.33	90	60	1	0
34	286	67	3	2030332	2360672	2370754	90	83.33	90	60	1	0
35	286	79	3	2030332	2370754	3440189	100	100	90	60	1	0
36	286	83	3	2030332	2370754	5550711	90	83.33	90	60	1	0
37	286	95	3	2030332	3420136	4220731	90	83.33	90	60	1	0
38	286	104	3	2030332	3440189	5550711	90	83.33	90	60	1	0
39	286	122	3	2360672	2370754	3130370	85	83.33	90	60	1	0
40	286	213	3	3130370	3440189	5550711	80	83.33	90	60	1	0
41	286	228	3	3130370	5550711	5960072	85	83.33	90	60	1	0
42	286	258	3	3440189	4260767	5550711	85	100	90	60	1	0
43	286	261	3	3440189	5080692	5550711	90	100	90	60	1	0
44	286	264	3	3440189	5550711	5960072	80	83.33	90	60	1	0
45	286	265	3	3440189	5550711	6590484	80	83.33	90	60	1	0
46	286	154	3	2360672	4220731	5550711	90	100	90	40	1	0
47	286	173	3	2370754	3130370	5960072	80	83.33	90	40	1	0
48	286	219	3	3130370	4220731	5960072	85	83.33	90	40	1	0
49	286	240	3	3420136	4220731	5960072	85	100	90	40	1	0

(4) Discrimination rate based on 4 genes

[0060] The number of combinations of 4 genes from the 13 genes is 715, and some of them are shown in Table 11. For example, the sets of 4 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 18 sets [Table 11: 1 to 18].

[Table 11-1]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	715	152	4	770400	3130370	4220731	5550711	85	100	95	80	1	1
2	715	188	4	770400	3440189	4220731	5550711	95	100	95	80	1	1
3	715	70	4	770400	2360672	3130370	5550711	90	83.33	90	80	1	1
4	715	88	4	770400	2360672	4220731	5550711	80	83.33	90	80	1	1
5	715	165	4	770400	3420136	3440189	4220731	90	100	90	80	1	1
6	715	420	4	2360672	2370754	5550711	6590484	90	83.33	90	80	1	1
7	715	437	4	2360672	3130370	4220731	5550711	90	100	90	80	1	1
8	715	473	4	2360672	3440189	4220731	5550711	90	100	90	80	1	1
9	715	494	4	2360672	4220731	5550711	6590484	90	100	90	80	1	1
10	715	59	4	~~770400~~	~~2360672~~	~~2370754~~	~~4220731~~	~~80~~	~~83.33~~	~~85~~	~~80~~	~~1~~	~~1~~
11	715	74	4	770400	2360672	3420136	4220731	90	83.33	85	80	1	1
12	715	83	4	770400	2360672	3440189	5550711	90	83.33	85	80	1	1
13	715	138	4	770400	3130370	3420136	4220731	90	83.33	85	80	1	1
14	715	215	4	770400	4260767	5550711	6590484	90	83.33	85	80	1	1
15	715	401	4	2360672	2370754	3440189	4220731	100	100	85	80	1	1
16	715	423	4	2360672	3130370	3420136	4220731	90	83.33	85	80	1	1
17	715	56	4	770400	2360672	2370754	3130370	80	100	80	80	1	1
18	715	457	4	2360672	3420136	4220731	5080692	90	83.33	80	80	1	1
19	715	205	4	770400	4220731	5080692	5550711	85	66.67	95	80	0	1
20	715	209	4	770400	4220731	5550711	6590484	80	66.67	95	80	0	1
21	715	64	4	770400	2360672	2370754	6590484	75	50	90	80	0	1
22	715	99	4	770400	2360672	5550711	6590484	85	33.33	90	80	0	1
23	715	135	4	770400	2370754	5550711	6590484	80	50	90	80	0	1
24	715	163	4	770400	3130370	5550711	6590484	90	66.67	90	80	0	1
25	715	184	4	770400	3420136	5550711	6590484	90	50	90	80	0	1
26	715	199	4	770400	3440189	5550711	6590484	90	66.67	90	80	0	1
27	715	100	4	770400	2360672	5960072	6590484	85	50	85	100	0	1
28	715	58	4	770400	2360672	2370754	3440189	75	83.33	85	80	0	1
29	715	80	4	770400	2360672	3440189	4220731	85	66.67	85	80	0	1
30	715	150	4	770400	3130370	4220731	4260767	75	100	85	80	0	1
31	715	151	4	770400	3130370	4220731	5080692	70	66.67	85	80	0	1
32	715	154	4	770400	3130370	4220731	6590484	75	66.67	85	80	0	1
33	715	190	4	770400	3440189	4220731	6590484	75	66.67	85	80	0	1
34	715	218	4	770400	5080692	5550711	6590484	85	33.33	85	80	0	1
35	715	418	4	2360672	2370754	5080692	6590484	85	50	85	80	0	1
36	715	429	4	2360672	3130370	3440189	4220731	90	66.67	85	80	0	1
37	715	436	4	2360672	3130370	4220731	5080692	85	66.67	85	80	0	1
38	715	472	4	2360672	3440189	4220731	5080692	90	66.67	85	80	0	1
39	715	490	4	2360672	4220731	5080692	5550711	85	66.67	85	80	0	1
40	715	85	4	770400	2360672	3440189	6590484	85	33.33	80	100	0	1
41	715	97	4	770400	2360672	5080692	6590484	85	33.33	80	100	0	1
42	715	65	4	770400	2360672	3130370	3420136	80	66.67	80	80	0	1
43	715	67	4	770400	2360672	3130370	4220731	75	66.67	80	80	0	1
44	715	73	4	770400	2360672	3420136	3440189	80	66.67	80	80	0	1
45	715	87	4	770400	2360672	4220731	5080692	75	66.67	80	80	0	1
46	715	90	4	770400	2360672	4220731	6590484	70	50	80	80	0	1
47	715	96	4	770400	2360672	5080692	5960072	85	50	80	80	0	1
48	715	133	4	770400	2370754	5080692	6590484	80	50	80	80	0	1
49	715	170	4	770400	3420136	3440189	6590484	95	50	80	80	0	1

(5) Discrimination rate based on 5 genes

[0061] The number of combinations of 5 genes from the 13 genes is 1287, and some of them are shown in Table 12. For example, the sets of 5 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 55 sets [Table 12: 1 to 55].

[Table 12-1]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	pb5	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	1287	337	5	770400	2370754	3440189	4220731	5550711	95	100	95	80	1	1
2	1287	378	5	770400	3130370	3420136	4220731	5550711	85	83.33	95	80	1	1
3	1287	393	5	770400	3130370	3440189	4220731	5550711	90	100	95	80	1	1
4	1287	407	5	770400	3130370	4220731	4260767	5550711	85	100	95	80	1	1
5	1287	410	5	770400	3130370	4220731	5080692	5550711	85	83.33	95	80	1	1
6	1287	414	5	770400	3130370	4220731	5550711	6590484	85	100	95	80	1	1
7	1287	426	5	770400	3420136	3440189	4220731	4260767	85	100	95	80	1	1
8	1287	428	5	770400	3420136	3440189	4220731	5550711	90	83.33	95	80	1	1
9	1287	469	5	770400	3440189	4220731	5550711	6590484	90	100	95	80	1	1
10	1287	171	5	770400	2360672	2370754	3130370	5550711	90	83.33	90	80	1	1
11	1287	186	5	770400	2360672	2370754	3440189	6590484	85	83.33	90	80	1	1
12	1287	189	5	770400	2360672	2370754	4220731	5550711	85	83.33	90	80	1	1
13	1287	191	5	770400	2360672	2370754	4220731	6590484	80	83.33	90	80	1	1
14	1287	212	5	770400	2360672	3130370	3440189	5550711	90	83.33	90	80	1	1
15	1287	217	5	770400	2360672	3130370	4220731	5550711	85	100	90	80	1	1
16	1287	218	5	770400	2360672	3130370	4220731	5960072	85	83.33	90	80	1	1
17	1287	224	5	770400	2360672	3130370	5080692	5550711	85	83.33	90	80	1	1
18	1287	228	5	770400	2360672	3130370	5550711	6590484	90	83.33	90	80	1	1
19	1287	253	5	770400	2360672	3440189	4220731	5550711	90	100	90	80	1	1
20	1287	267	5	770400	2360672	4220731	4260767	5550711	80	100	90	80	1	1
21	1287	280	5	770400	2360672	4260767	5550711	6590484	85	83.33	90	80	1	1
22	1287	465	5	770400	3440189	4220731	5080692	5550711	95	100	90	80	1	1
23	1287	485	5	770400	4220731	4260767	5550711	6590484	85	83.33	90	80	1	1
24	1287	898	5	2360672	2370754	4220731	5550711	6590484	85	83.33	90	80	1	1
25	1287	933	5	2360672	3130370	3440189	4220731	5550711	85	100	90	80	1	1
26	1287	950	5	2360672	3130370	4220731	5080692	5550711	90	83.33	90	80	1	1
27	1287	1002	5	2360672	3440189	4220731	4260767	5550711	90	100	90	80	1	1
28	1287	1009	5	2360672	3440189	4220731	5550711	6590484	90	100	90	80	1	1
29	1287	168	5	770400	2360672	2370754	3130370	4220731	90	100	85	80	1	1
30	1287	175	5	770400	2360672	2370754	3420136	4220731	85	83.33	85	80	1	1
31	1287	206	5	770400	2360672	3130370	3420136	5550711	85	83.33	85	80	1	1
32	1287	251	5	770400	2360672	3440189	4220731	4260767	80	83.33	85	80	1	1
33	1287	254	5	770400	2360672	3440189	4220731	5960072	85	83.33	85	80	1	1
34	1287	255	5	770400	2360672	3440189	4220731	6590484	80	83.33	85	80	1	1
35	1287	257	5	770400	2360672	3440189	4260767	5550711	90	83.33	85	80	1	1
36	1287	314	5	770400	2370754	3420136	3440189	4220731	100	100	85	80	1	1
37	1287	427	5	770400	3420136	3440189	4220731	5080692	90	83.33	85	80	1	1
38	1287	430	5	770400	3420136	3440189	4220731	6590484	80	100	85	80	1	1
39	1287	864	5	2360672	2370754	3420136	4220731	6590484	80	100	85	80	1	1
40	1287	954	5	2360672	3130370	4220731	5550711	6590484	85	100	85	80	1	1
41	1287	181	5	770400	2360672	2370754	3440189	4220731	100	100	80	80	1	1
42	1287	203	5	770400	2360672	3130370	3420136	4220731	85	83.33	80	80	1	1
43	1287	230	5	770400	2360672	3420136	3440189	4220731	90	83.33	80	80	1	1
44	1287	237	5	770400	2360672	3420136	4220731	5080692	90	83.33	80	80	1	1
45	1287	259	5	770400	2360672	3440189	4260767	6590484	85	83.33	80	80	1	1
46	1287	278	5	770400	2360672	4260767	5080692	6590484	80	83.33	80	80	1	1
47	1287	287	5	770400	2370754	3130370	3420136	4220731	90	83.33	80	80	1	1
48	1287	336	5	770400	2370754	3440189	4220731	5080692	95	100	80	80	1	1
49	1287	370	5	770400	3130370	3420136	3440189	4220731	90	100	80	80	1	1

[Table 12-2]

50	1287	377	5	770400	3130370	3420136	4220731	5080692	85	83.33	80	80	1	1
51	1287	380	5	770400	3130370	3420136	4220731	6590484	90	83.33	80	80	1	1
52	1287	861	5	2360672	2370754	3420136	4220731	5080692	85	83.33	80	80	1	1
53	1287	910	5	2360672	3130370	3420136	3440189	4220731	95	100	80	80	1	1
54	1287	970	5	2360672	3420136	3440189	4220731	6590484	90	100	80	80	1	1
55	1287	987	5	2360672	3420136	4220731	5080692	6590484	90	83.33	80	80	1	1
56	1287	462	5	770400	3440189	4220731	4260767	5550711	95	66.67	95	80	0	1
57	1287	200	5	770400	2360672	2370754	5550711	6590484	80	50	90	80	0	1
58	1287	249	5	770400	2360672	3420136	5550711	6590484	85	66.67	90	80	0	1
59	1287	250	5	770400	2360672	3420136	5960072	6590484	85	50	90	80	0	1
60	1287	264	5	770400	2360672	3440189	5550711	6590484	90	66.67	90	80	0	1
61	1287	270	5	770400	2360672	4220731	5080692	5550711	75	66.67	90	80	0	1
62	1287	274	5	770400	2360672	4220731	5550711	6590484	80	66.67	90	80	0	1
63	1287	367	5	770400	2370754	5080692	5550711	6590484	80	50	90	80	0	1
64	1287	449	5	770400	3420136	4220731	5550711	6590484	85	66.67	90	80	0	1
65	1287	488	5	770400	4220731	5080692	5550711	6590484	85	50	90	80	0	1
66	1287	180	5	770400	2360672	2370754	3420136	6590484	85	50	85	80	0	1
67	1287	198	5	770400	2360672	2370754	5080692	6590484	75	50	85	80	0	1
68	1287	215	5	770400	2360672	3130370	4220731	4260767	75	83.33	85	80	0	1
69	1287	216	5	770400	2360672	3130370	4220731	5080692	80	66.67	85	80	0	1
70	1287	219	5	770400	2360672	3130370	4220731	6590484	75	66.67	85	80	0	1
71	1287	283	5	770400	2360672	5080692	5550711	6590484	80	33.33	85	80	0	1
72	1287	284	5	770400	2360672	5080692	5960072	6590484	85	50	85	80	0	1
73	1287	412	5	770400	3130370	4220731	5080692	6590484	65	66.67	85	80	0	1
74	1287	932	5	2360672	3130370	3440189	4220731	5080692	90	66.67	85	80	0	1
75	1287	265	5	770400	2360672	3440189	5960072	6590484	85	50	80	100	0	1
76	1287	170	5	770400	2360672	2370754	3130370	5080692	75	83.33	80	80	0	1
77	1287	173	5	770400	2360672	2370754	3130370	6590484	85	66.67	80	80	0	1
78	1287	188	5	770400	2360672	2370754	4220731	5080692	80	66.67	80	80	0	1
79	1287	205	5	770400	2360672	3130370	3420136	5080692	80	66.67	80	80	0	1
80	1287	208	5	770400	2360672	3130370	3420136	6590484	80	50	80	80	0	1
81	1287	211	5	770400	2360672	3130370	3440189	5080692	85	50	80	80	0	1
82	1287	235	5	770400	2360672	3420136	3440189	6590484	90	50	80	80	0	1
83	1287	252	5	770400	2360672	3440189	4220731	5080692	80	66.67	80	80	0	1
84	1287	272	5	770400	2360672	4220731	5080692	6590484	75	50	80	80	0	1
85	1287	391	5	770400	3130370	3440189	4220731	4260767	75	83.33	80	80	0	1
86	1287	406	5	770400	3130370	4220731	4260767	5080692	70	66.67	80	80	0	1
87	1287	409	5	770400	3130370	4220731	4260767	6590484	75	100	80	80	0	1
88	1287	464	5	770400	3440189	4220731	4260767	6590484	75	100	80	80	0	1
89	1287	483	5	770400	4220731	4260767	5080692	6590484	70	83.33	80	80	0	1
90	1287	896	5	2360672	2370754	4220731	5080692	6590484	85	66.67	80	80	0	1
91	1287	1001	5	2360672	3440189	4220731	4260767	5080692	80	66.67	80	80	0	1
92	1287	1007	5	2360672	3440189	4220731	5080692	6590484	80	66.67	80	80	0	1
93	1287	1028	5	2360672	4220731	5080692	5550711	6590484	85	66.67	80	80	0	1
94	1287	4	5	770400	2030332	2360672	2370754	4220731	95	83.33	100	60	1	0
95	1287	5	5	770400	2030332	2360672	2370754	4260767	90	100	100	60	1	0
96	1287	46	5	770400	2030332	2370754	3130370	3420136	90	83.33	100	60	1	0
97	1287	49	5	770400	2030332	2370754	3130370	4260767	90	100	100	60	1	0
98	1287	55	5	770400	2030332	2370754	3420136	4220731	95	83.33	100	60	1	0
99	1287	62	5	770400	2030332	2370754	3440189	4260767	90	100	100	60	1	0

(6) Discrimination rate based on 6 genes

[0062] The number of combinations of 6 genes from the 13 genes is 1716, and some of them are shown in Table 13. For example, the sets of 6 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 71 sets [Table 13: 1 to 71].

[Table 13-1]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	pb5	pb6	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	1716	473	6	770400	2360672	3420136	3440189	4220731	5550711	90	83.33	95	80	1	1
2	1716	510	6	770400	2360672	3440189	4220731	5080692	5550711	95	100	95	80	1	1
3	1716	549	6	770400	2370754	3130370	3420136	4220731	5550711	85	83.33	95	80	1	1
4	1716	564	6	770400	2370754	3130370	3440189	4220731	5550711	90	100	95	80	1	1
5	1716	599	6	770400	2370754	3420136	3440189	4220731	5550711	90	100	95	80	1	1
6	1716	633	6	770400	2370754	3440189	4220731	4260767	5550711	95	100	95	80	1	1
7	1716	669	6	770400	3130370	3420136	3440189	4220731	5550711	90	83.33	95	80	1	1
8	1716	683	6	770400	3130370	3420136	4220731	4260767	5550711	85	83.33	95	80	1	1
9	1716	686	6	770400	3130370	3420136	4220731	5080692	5550711	85	83.33	95	80	1	1
10	1716	690	6	770400	3130370	3420136	4220731	5550711	6590484	85	83.33	95	80	1	1
11	1716	710	6	770400	3130370	3440189	4220731	5550711	6590484	85	100	95	80	1	1
12	1716	726	6	770400	3130370	4220731	4260767	5550711	6590484	85	100	95	80	1	1
13	1716	745	6	770400	3420136	3440189	4220731	5550711	6590484	90	100	95	80	1	1
14	1716	776	6	770400	3440189	4220731	4260767	5550711	6590484	85	83.33	95	80	1	1
15	1716	346	6	770400	2360672	2370754	3130370	4220731	5550711	85	83.33	90	80	1	1
16	1716	382	6	770400	2360672	2370754	3440189	4220731	5550711	95	100	90	80	1	1
17	1716	393	6	770400	2360672	2370754	3440189	5550711	6590484	85	83.33	90	80	1	1
18	1716	399	6	770400	2360672	2370754	4220731	5080692	5550711	80	83.33	90	80	1	1
19	1716	403	6	770400	2360672	2370754	4220731	5550711	6590484	85	83.33	90	80	1	1
20	1716	423	6	770400	2360672	3130370	3420136	4220731	5550711	85	83.33	90	80	1	1
21	1716	438	6	770400	2360672	3130370	3440189	4220731	5550711	85	100	90	80	1	1
22	1716	449	6	770400	2360672	3130370	3440189	5550711	6590484	90	83.33	90	80	1	1
23	1716	452	6	770400	2360672	3130370	4220731	4260767	5550711	85	100	90	80	1	1
24	1716	459	6	770400	2360672	3130370	4220731	5550711	6590484	85	100	90	80	1	1
25	1716	465	6	770400	2360672	3130370	4260767	5550711	6590484	85	83.33	90	80	1	1
26	1716	514	6	770400	2360672	3440189	4220731	5550711	6590484	90	100	90	80	1	1
27	1716	520	6	770400	2360672	3440189	4260767	5550711	6590484	90	83.33	90	80	1	1
28	1716	530	6	770400	2360672	4220731	4260767	5550711	6590484	80	83.33	90	80	1	1
29	1716	581	6	770400	2370754	3130370	4220731	5080692	5550711	90	83.33	90	80	1	1
30	1716	659	6	770400	2370754	4220731	5080692	5550711	6590484	85	83.33	90	80	1	1
31	1716	706	6	770400	3130370	3440189	4220731	5080692	5550711	95	100	90	80	1	1
32	1716	722	6	770400	3130370	4220731	4260767	5080692	5550711	85	100	90	80	1	1
33	1716	740	6	770400	3420136	3440189	4220731	4260767	6590484	80	100	90	80	1	1
34	1716	761	6	770400	3420136	4220731	4260767	5550711	6590484	85	83.33	90	80	1	1
35	1716	779	6	770400	3440189	4220731	5080692	5550711	6590484	90	100	90	80	1	1
36	1716	1424	6	2360672	3130370	3440189	4220731	5550711	6590484	90	100	90	80	1	1
37	1716	1443	6	2360672	3130370	4220731	5080692	5550711	6590484	85	100	90	80	1	1
38	1716	1490	6	2360672	3440189	4220731	4260767	5550711	6590484	90	100	90	80	1	1
39	1716	332	6	770400	2360672	2370754	3130370	3420136	4220731	85	100	85	80	1	1
40	1716	348	6	770400	2360672	2370754	3130370	4220731	6590484	85	100	85	80	1	1
41	1716	359	6	770400	2360672	2370754	3420136	3440189	4220731	95	100	85	80	1	1
42	1716	369	6	770400	2360672	2370754	3420136	4220731	6590484	80	83.33	85	80	1	1
43	1716	434	6	770400	2360672	3130370	3420136	5550711	6590484	90	83.33	85	80	1	1
44	1716	439	6	770400	2360672	3130370	3440189	4220731	5960072	80	83.33	85	80	1	1
45	1716	455	6	770400	2360672	3130370	4220731	5080692	5550711	80	83.33	85	80	1	1
46	1716	474	6	770400	2360672	3420136	3440189	4220731	5960072	95	83.33	85	80	1	1
47	1716	528	6	770400	2360672	4220731	4260767	5080692	6590484	80	83.33	85	80	1	1
48	1716	541	6	770400	2370754	3130370	3420136	3440189	4220731	100	100	85	80	1	1
49	1716	668	6	770400	3130370	3420136	3440189	4220731	5080692	90	83.33	85	80	1	1

[Table 13-2]

50	1716	729	6	770400	3130370	4220731	5080692	5550711	6590484	85	83.33	85	80	1	1
51	1716	737	6	770400	3420136	3440189	4220731	4260767	5080692	85	83.33	85	80	1	1
52	1716	743	6	770400	3420136	3440189	4220731	5080692	6590484	85	83.33	85	80	1	1
53	1716	1373	6	2360672	2370754	4220731	5080692	5550711	6590484	85	83.33	85	80	1	1
54	1716	1402	6	2360672	3130370	3420136	4220731	5080692	6590484	90	83.33	85	80	1	1
55	1716	337	6	770400	2360672	2370754	3130370	3420136	6590484	85	83.33	80	80	1	1
56	1716	338	6	770400	2360672	2370754	3130370	3440189	4220731	95	100	80	80	1	1
57	1716	343	6	770400	2360672	2370754	3130370	3440189	6590484	85	83.33	80	80	1	1
58	1716	421	6	770400	2360672	3130370	3420136	4220731	4260767	80	100	80	80	1	1
59	1716	422	6	770400	2360672	3130370	3420136	4220731	5080692	85	83.33	80	80	1	1
60	1716	425	6	770400	2360672	3130370	3420136	4220731	6590484	80	83.33	80	80	1	1
61	1716	463	6	770400	2360672	3130370	4260767	5080692	6590484	80	83.33	80	80	1	1
62	1716	471	6	770400	2360672	3420136	3440189	4220731	4260767	90	100	80	80	1	1
63	1716	472	6	770400	2360672	3420136	3440189	4220731	5080692	90	83.33	80	80	1	1
64	1716	475	6	770400	2360672	3420136	3440189	4220731	6590484	85	100	80	80	1	1
65	1716	485	6	770400	2360672	3420136	3440189	5960072	6590484	85	83.33	80	80	1	1
66	1716	486	6	770400	2360672	3420136	4220731	4260767	5080692	85	83.33	80	80	1	1
67	1716	489	6	770400	2360672	3420136	4220731	4260767	6590484	80	100	80	80	1	1
68	1716	618	6	770400	2370754	3420136	4220731	5080692	6590484	95	83.33	80	80	1	1
69	1716	667	6	770400	3130370	3420136	3440189	4220731	4260767	85	100	80	80	1	1
70	1716	671	6	770400	3130370	3420136	3440189	4220731	6590484	85	100	80	80	1	1
71	1716	1332	6	2360672	2370754	3420136	4220731	5080692	6590484	85	83.33	80	80	1	1
72	1716	412	6	770400	2360672	2370754	5080692	5550711	6590484	75	33.33	95	80	0	1
73	1716	503	6	770400	2360672	3420136	5080692	5550711	6590484	85	33.33	95	80	0	1
74	1716	537	6	770400	2360672	4260767	5080692	5550711	6590484	85	66.67	95	80	0	1
75	1716	703	6	770400	3130370	3440189	4220731	4260767	5550711	90	66.67	95	80	0	1
76	1716	738	6	770400	3420136	3440189	4220731	4260767	5550711	90	66.67	95	80	0	1
77	1716	357	6	770400	2360672	2370754	3130370	5550711	6590484	90	66.67	90	80	0	1
78	1716	378	6	770400	2360672	2370754	3420136	5550711	6590484	85	50	90	80	0	1
79	1716	507	6	770400	2360672	3440189	4220731	4260767	5550711	90	66.67	90	80	0	1
80	1716	526	6	770400	2360672	4220731	4260767	5080692	5550711	75	83.33	90	80	0	1
81	1716	533	6	770400	2360672	4220731	5080692	5550711	6590484	75	50	90	80	0	1
82	1716	620	6	770400	2370754	3420136	4220731	5550711	6590484	85	66.67	90	80	0	1
83	1716	764	6	770400	3420136	4220731	5080692	5550711	6590484	85	66.67	90	80	0	1
84	1716	772	6	770400	3440189	4220731	4260767	5080692	5550711	95	66.67	90	80	0	1
85	1716	788	6	770400	4220731	4260767	5080692	5550711	6590484	85	66.67	90	80	0	1
86	1716	345	6	770400	2360672	2370754	3130370	4220731	5080692	90	66.67	85	80	0	1
87	1716	420	6	770400	2360672	3130370	3420136	3440189	6590484	85	50	85	80	0	1
88	1716	432	6	770400	2360672	3130370	3420136	5080692	6590484	80	50	85	80	0	1
89	1716	451	6	770400	2360672	3130370	4220731	4260767	5080692	75	66.67	85	80	0	1
90	1716	457	6	770400	2360672	3130370	4220731	5080692	6590484	80	66.67	85	80	0	1
91	1716	468	6	770400	2360672	3130370	5080692	5550711	6590484	90	50	85	80	0	1
92	1716	509	6	770400	2360672	3440189	4220731	4260767	6590484	75	83.33	85	80	0	1
93	1716	1422	6	2360672	3130370	3440189	4220731	5080692	6590484	90	66.67	85	80	0	1
94	1716	355	6	770400	2360672	2370754	3130370	5080692	6590484	90	50	80	80	0	1
95	1716	366	6	770400	2360672	2370754	3420136	4220731	5080692	85	66.67	80	80	0	1
96	1716	376	6	770400	2360672	2370754	3420136	5080692	6590484	90	33.33	80	80	0	1
97	1716	401	6	770400	2360672	2370754	4220731	5080692	6590484	80	66.67	80	80	0	1
98	1716	437	6	770400	2360672	3130370	3440189	4220731	5080692	75	66.67	80	80	0	1
99	1716	440	6	770400	2360672	3130370	3440189	4220731	6590484	80	66.67	80	80	0	1

(7) Discrimination rate based on 7 genes

[0063] The number of combinations of 7 genes from the 13 genes is 1715, and some of them are shown in Table 13. For example, the sets of 7 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life are 63 sets [Table 14: 1 to 63].

[Table 14-1]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	pb5	pb6	pb7	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	1716	521	7	770400	2360672	2370754	3420136	3440189	4220731	5550711	90	100	95	80	1	1
2	1716	558	7	770400	2360672	2370754	3440189	4220731	5080692	5550711	95	100	95	80	1	1
3	1716	574	7	770400	2360672	2370754	4220731	4260767	5080692	5550711	85	100	95	80	1	1
4	1716	663	7	770400	2360672	3420136	3440189	4220731	5080692	5550711	90	100	95	80	1	1
5	1716	667	7	770400	2360672	3420136	3440189	4220731	5550711	6590484	90	100	95	80	1	1
6	1716	701	7	770400	2360672	3440189	4220731	5080692	5550711	6590484	90	100	95	80	1	1
7	1716	717	7	770400	2370754	3130370	3420136	3440189	4220731	5550711	90	100	95	80	1	1
8	1716	738	7	770400	2370754	3130370	3420136	4220731	5550711	6590484	85	83.33	95	80	1	1
9	1716	751	7	770400	2370754	3130370	3440189	4220731	4260767	5550711	90	100	95	80	1	1
10	1716	786	7	770400	2370754	3420136	3440189	4220731	4260767	5550711	90	100	95	80	1	1
11	1716	849	7	770400	3130370	3420136	3440189	4220731	5550711	6590484	90	100	95	80	1	1
12	1716	865	7	770400	3130370	3420136	4220731	4260767	5550711	6590484	85	83.33	95	80	1	1
13	1716	868	7	770400	3130370	3420136	4220731	5080692	5550711	6590484	85	83.33	95	80	1	1
14	1716	880	7	770400	3130370	3440189	4220731	4260767	5550711	6590484	85	83.33	95	80	1	1
15	1716	471	7	770400	2360672	2370754	3130370	3420136	4220731	5550711	85	100	90	80	1	1
16	1716	486	7	770400	2360672	2370754	3130370	3440189	4220731	5550711	90	100	90	80	1	1
17	1716	500	7	770400	2360672	2370754	3130370	4220731	4260767	5550711	85	100	90	80	1	1
18	1716	507	7	770400	2360672	2370754	3130370	4220731	5550711	6590484	85	100	90	80	1	1
19	1716	555	7	770400	2360672	2370754	3440189	4220731	4260767	5550711	95	100	90	80	1	1
20	1716	562	7	770400	2360672	2370754	3440189	4220731	5550711	6590484	95	100	90	80	1	1
21	1716	578	7	770400	2360672	2370754	4220731	4260767	5550711	6590484	90	100	90	80	1	1
22	1716	581	7	770400	2360672	2370754	4220731	5080692	5550711	6590484	80	83.33	90	80	1	1
23	1716	591	7	770400	2360672	3130370	3420136	3440189	4220731	5550711	90	83.33	90	80	1	1
24	1716	605	7	770400	2360672	3130370	3420136	4220731	4260767	5550711	85	100	90	80	1	1
25	1716	612	7	770400	2360672	3130370	3420136	4220731	5550711	6590484	85	83.33	90	80	1	1

[Table 14-2]

26	1716	625	7	770400	2360672	3130370	3440189	4220731	4260767	5550711	85	83.33	90	80	1	1
27	1716	628	7	770400	2360672	3130370	3440189	4220731	5080692	5550711	95	100	90	80	1	1
28	1716	632	7	770400	2360672	3130370	3440189	4220731	5550711	6590484	85	100	90	80	1	1
29	1716	638	7	770400	2360672	3130370	3440189	4260767	5550711	6590484	90	83.33	90	80	1	1
30	1716	648	7	770400	2360672	3130370	4220731	4260767	5550711	6590484	85	100	90	80	1	1
31	1716	694	7	770400	2360672	3440189	4220731	4260767	5080692	5550711	95	83.33	90	80	1	1
32	1716	698	7	770400	2360672	3440189	4220731	4260767	5550711	6590484	90	83.33	90	80	1	1
33	1716	710	7	770400	2360672	4220731	4260767	5080692	5550711	6590484	80	83.33	90	80	1	1
34	1716	812	7	770400	2370754	3420136	4220731	5080692	5550711	6590484	85	83.33	90	80	1	1
35	1716	883	7	770400	3130370	3440189	4220731	5080692	5550711	6590484	90	100	90	80	1	1
36	1716	913	7	770400	3420136	4220731	4260767	5080692	5550711	6590484	85	83.33	90	80	1	1
37	1716	919	7	770400	3440189	4220731	4260767	5080692	5550711	6590484	95	83.33	90	80	1	1
38	1716	1552	7	2360672	3130370	3440189	4220731	4260767	5550711	6590484	90	100	90	80	1	1
39	1716	463	7	770400	2360672	2370754	3130370	3420136	3440189	4220731	90	100	85	80	1	1
40	1716	470	7	770400	2360672	2370754	3130370	3420136	4220731	5080692	85	100	85	80	1	1
41	1716	473	7	770400	2360672	2370754	3130370	3420136	4220731	6590484	85	100	85	80	1	1
42	1716	503	7	770400	2360672	2370754	3130370	4220731	5080692	5550711	85	83.33	85	80	1	1
43	1716	505	7	770400	2360672	2370754	3130370	4220731	5080692	6590484	85	100	85	80	1	1
44	1716	523	7	770400	2360672	2370754	3420136	3440189	4220731	6590484	95	100	85	80	1	1
45	1716	604	7	770400	2360672	3130370	3420136	4220731	4260767	5080692	80	83.33	85	80	1	1
46	1716	608	7	770400	2360672	3130370	3420136	4220731	5080692	5550711	80	83.33	85	80	1	1
47	1716	621	7	770400	2360672	3130370	3420136	5080692	5550711	6590484	85	83.33	85	80	1	1
48	1716	644	7	770400	2360672	3130370	4220731	4260767	5080692	5550711	85	100	85	80	1	1
49	1716	651	7	770400	2360672	3130370	4220731	5080692	5550711	6590484	80	83.33	85	80	1	1

[Table 14-3]

50	1716	659	7	770400	2360672	3420136	3440189	4220731	4260767	5080692	85	83.33	85	80	1	1
51	1716	820	7	770400	2370754	3440189	4220731	4260767	5080692	5550711	95	83.33	85	80	1	1
52	1716	847	7	770400	3130370	3420136	3440189	4220731	5080692	6590484	85	83.33	85	80	1	1
53	1716	892	7	770400	3130370	4220731	4260767	5080692	5550711	6590484	85	100	85	80	1	1
54	1716	899	7	770400	3420136	3440189	4220731	4260767	5080692	6590484	80	83.33	85	80	1	1
55	1716	520	7	770400	2360672	2370754	3420136	3440189	4220731	5080692	90	100	80	80	1	1
56	1716	560	7	770400	2360672	2370754	3440189	4220731	5080692	6590484	95	100	80	80	1	1
57	1716	589	7	770400	2360672	3130370	3420136	3440189	4220731	4260767	90	100	80	80	1	1
58	1716	590	7	770400	2360672	3130370	3420136	3440189	4220731	5080692	90	83.33	80	80	1	1
59	1716	607	7	770400	2360672	3130370	3420136	4220731	4260767	6590484	80	100	80	80	1	1
60	1716	627	7	770400	2360672	3130370	3440189	4220731	4260767	6590484	80	100	80	80	1	1
61	1716	662	7	770400	2360672	3420136	3440189	4220731	4260767	6590484	90	100	80	80	1	1
62	1716	665	7	770400	2360672	3420136	3440189	4220731	5080692	6590484	80	83.33	80	80	1	1
63	1716	681	7	770400	2360672	3420136	4220731	4260767	5080692	6590484	85	83.33	80	80	1	1
64	1716	551	7	770400	2360672	2370754	3420136	5080692	5550711	6590484	80	50	95	80	0	1
65	1716	660	7	770400	2360672	3420136	3440189	4220731	4260767	5550711	90	66.67	95	80	0	1
66	1716	842	7	770400	3130370	3420136	3440189	4220731	4260767	5550711	90	66.67	95	80	0	1
67	1716	901	7	770400	3420136	3440189	4220731	4260767	5550711	6590484	90	66.67	95	80	0	1
68	1716	686	7	770400	2360672	3420136	4220731	5080692	5550711	6590484	80	66.67	90	80	0	1
69	1716	876	7	770400	3130370	3440189	4220731	4260767	5080692	5550711	95	66.67	90	80	0	1
70	1716	897	7	770400	3420136	3440189	4220731	4260767	5080692	5550711	90	66.67	90	80	0	1
71	1716	516	7	770400	2360672	2370754	3130370	5080692	5550711	6590484	90	66.67	85	80	0	1
72	1716	646	7	770400	2360672	3130370	4220731	4260767	5080692	6590484	75	83.33	85	80	0	1
73	1716	540	7	770400	2360672	2370754	3420136	4220731	5080692	6590484	85	66.67	80	80	0	1
74	1716	624	7	770400	2360672	3130370	3440189	4220731	4260767	5080692	75	66.67	80	80	0	1
75	1716	630	7	770400	2360672	3130370	3440189	4220731	5080692	6590484	80	66.67	80	80	0	1

(8) Discrimination rate based on 8 genes

[0064] The number of combinations of 8 genes from the 13 genes is 1287, and some of them are shown in Table 15. For example, the sets of 8 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 45 sets [Table 15:

1 to 45].

[Table 15-1]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	pb5	pb6	pb7	pb8	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	1287	534	8	770400	2360672	2370754	3420136	3440189	4220731	4260767	5550711	90	100	95	80	1	1
2	1287	541	8	770400	2360672	2370754	3420136	3440189	4220731	5550711	6590484	90	100	95	80	1	1
3	1287	568	8	770400	2360672	2370754	3440189	4220731	4260767	5080692	5550711	95	83.33	95	80	1	1
4	1287	575	8	770400	2360672	2370754	3440189	4220731	5080692	5550711	6590484	95	100	95	80	1	1
5	1287	584	8	770400	2360672	2370754	4220731	4260767	5080692	5550711	6590484	85	100	95	80	1	1
6	1287	593	8	770400	2360672	3130370	3420136	3440189	4220731	5080692	5550711	90	100	95	80	1	1
7	1287	597	8	770400	2360672	3130370	3420136	3440189	4220731	5550711	6590484	90	100	95	80	1	1
8	1287	649	8	770400	2360672	3420136	3440189	4220731	4260767	5550711	6590484	90	83.33	95	80	1	1
9	1287	652	8	770400	2360672	3420136	3440189	4220731	5080692	5550711	6590484	90	100	95	80	1	1
10	1287	674	8	770400	2370754	3130370	3420136	3440189	4220731	4260767	5550711	90	100	95	80	1	1
11	1287	733	8	770400	2370754	3420136	3440189	4220731	4260767	5550711	6590484	90	100	95	80	1	1
12	1287	761	8	770400	3130370	3420136	3440189	4220731	4260767	5550711	6590484	90	83.33	95	80	1	1
13	1287	786	8	770400	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	95	80	1	1
14	1287	465	8	770400	2360672	2370754	3130370	3420136	3440189	4220731	5550711	85	100	90	80	1	1
15	1287	467	8	770400	2360672	2370754	3130370	3420136	3440189	4220731	6590484	90	100	90	80	1	1
16	1287	479	8	770400	2360672	2370754	3130370	3420136	4220731	4260767	5550711	85	100	90	80	1	1
17	1287	482	8	770400	2360672	2370754	3130370	3420136	4220731	5080692	5550711	80	100	90	80	1	1
18	1287	486	8	770400	2360672	2370754	3130370	3420136	4220731	5550711	6590484	85	100	90	80	1	1
19	1287	499	8	770400	2360672	2370754	3130370	3440189	4220731	4260767	5550711	90	100	90	80	1	1
20	1287	502	8	770400	2360672	2370754	3130370	3440189	4220731	5080692	5550711	95	100	90	80	1	1
21	1287	506	8	770400	2360672	2370754	3130370	3440189	4220731	5550711	6590484	85	100	90	80	1	1
22	1287	522	8	770400	2360672	2370754	3130370	4220731	4260767	5550711	6590484	85	100	90	80	1	1
23	1287	525	8	770400	2360672	2370754	3130370	4220731	5080692	5550711	6590484	80	83.33	90	80	1	1
24	1287	539	8	770400	2360672	2370754	3420136	3440189	4220731	5080692	6590484	90	100	90	80	1	1
25	1287	572	8	770400	2360672	2370754	3440189	4220731	4260767	5550711	6590484	95	100	90	80	1	1

[Table 15-2]

26	1287	613	8	770400	2360672	3130370	3420136	4220731	4260767	5550711	6590484	85	100	90	80	1	1
27	1287	624	8	770400	2360672	3130370	3440189	4220731	4260767	5080692	5550711	95	83.33	90	80	1	1
28	1287	628	8	770400	2360672	3130370	3440189	4220731	4260767	5550711	6590484	85	83.33	90	80	1	1
29	1287	631	8	770400	2360672	3130370	3440189	4220731	5080692	5550711	6590484	90	100	90	80	1	1
30	1287	667	8	770400	2360672	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	90	80	1	1
31	1287	779	8	770400	3130370	3440189	4220731	4260767	5080692	5550711	6590484	95	83.33	90	80	1	1
32	1287	464	8	770400	2360672	2370754	3130370	3420136	3440189	4220731	5080692	90	100	85	80	1	1
33	1287	518	8	770400	2360672	2370754	3130370	4220731	4260767	5080692	5550711	80	100	85	80	1	1
34	1287	570	8	770400	2360672	2370754	3440189	4220731	4260767	5080692	6590484	90	100	85	80	1	1
35	1287	609	8	770400	2360672	3130370	3420136	4220731	4260767	5080692	5550711	80	100	85	80	1	1
36	1287	616	8	770400	2360672	3130370	3420136	4220731	5080692	5550711	6590484	80	100	85	80	1	1
37	1287	640	8	770400	2360672	3130370	4220731	4260767	5080692	5550711	6590484	80	100	85	80	1	1
38	1287	647	8	770400	2360672	3420136	3440189	4220731	4260767	5080692	6590484	85	83.33	85	80	1	1
39	1287	708	8	770400	2370754	3130370	3440189	4220731	4260767	5080692	5550711	95	83.33	85	80	1	1
40	1287	751	8	770400	2370754	3440189	4220731	4260767	5080692	5550711	6590484	95	83.33	85	80	1	1
41	1287	484	8	770400	2360672	2370754	3130370	3420136	4220731	5080692	6590484	85	100	80	80	1	1
42	1287	589	8	770400	2360672	3130370	3420136	3440189	4220731	4260767	5080692	85	83.33	80	80	1	1
43	1287	592	8	770400	2360672	3130370	3420136	3440189	4220731	4260767	6590484	90	100	80	80	1	1
44	1287	595	8	770400	2360672	3130370	3420136	3440189	4220731	5080692	6590484	85	83.33	80	80	1	1
45	1287	759	8	770400	3130370	3420136	3440189	4220731	4260767	5080692	6590484	85	100	80	80	1	1
46	1287	645	8	770400	2360672	3420136	3440189	4220731	4260767	5080692	5550711	90	66.67	95	80	0	1
47	1287	590	8	770400	2360672	3130370	3420136	3440189	4220731	4260767	5550711	90	66.67	90	80	0	1
48	1287	757	8	770400	3130370	3420136	3440189	4220731	4260767	5080692	5550711	95	66.67	90	80	0	1
49	1287	7	8	770400	2030332	2360672	2370754	3130370	3420136	4220731	4260767	90	100	100	60	1	0

(9) Discrimination rate based on 9 genes

[0065] The number of combinations of 9 genes from the 13 genes is 715, and some of them are shown in Table 16. For example, the sets of 9 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 22 sets [Table 16: 1 to 22].

[Table 16]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	pb5	pb6	pb7	pb8	pb9	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	715	339	9	770400	2360672	2370754	3130370	3420136	3440189	4220731	5550711	6590484	90	100	95	80	1	1
2	715	387	9	770400	2360672	2370754	3420136	3440189	4220731	4260767	5080692	5550711	90	83.33	95	80	1	1
3	715	391	9	770400	2360672	2370754	3420136	3440189	4220731	4260767	5550711	6590484	90	100	95	80	1	1
4	715	419	9	770400	2360672	3130370	3420136	3440189	4220731	4260767	5550711	6590484	90	83.33	95	80	1	1
5	715	444	9	770400	2360672	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	95	80	1	1
6	715	455	9	770400	2370754	3130370	3420136	3440189	4220731	4260767	5550711	6590484	90	100	95	80	1	1
7	715	332	9	770400	2360672	2370754	3130370	3420136	3440189	4220731	4260767	5550711	85	100	90	80	1	1
8	715	355	9	770400	2360672	2370754	3130370	3420136	4220731	4260767	5550711	6590484	85	100	90	80	1	1
9	715	358	9	770400	2360672	2370754	3130370	3420136	4220731	5080692	5550711	6590484	80	100	90	80	1	1
10	715	366	9	770400	2360672	2370754	3130370	3440189	4220731	4260767	5080692	5550711	95	83.33	90	80	1	1
11	715	370	9	770400	2360672	2370754	3130370	3440189	4220731	4260767	5550711	6590484	85	100	90	80	1	1
12	715	373	9	770400	2360672	2370754	3130370	3440189	4220731	5080692	5550711	6590484	95	100	90	80	1	1
13	715	382	9	770400	2360672	2370754	3130370	4220731	4260767	5080692	5550711	6590484	80	100	90	80	1	1
14	715	409	9	770400	2360672	2370754	3440189	4220731	4260767	5080692	5550711	6590484	95	83.33	90	80	1	1
15	715	422	9	770400	2360672	3130370	3420136	3440189	4220731	5080692	5550711	6590484	90	100	90	80	1	1
16	715	437	9	770400	2360672	3130370	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	90	80	1	1
17	715	480	9	770400	2370754	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	90	80	1	1
18	715	488	9	770400	3130370	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	90	80	1	1
19	715	337	9	770400	2360672	2370754	3130370	3420136	3440189	4220731	5080692	6590484	90	100	85	80	1	1
20	715	431	9	770400	2360672	3130370	3420136	4220731	4260767	5080692	5550711	6590484	80	100	85	80	1	1
21	715	473	9	770400	2370754	3130370	3440189	4220731	4260767	5080692	5550711	6590484	95	83.33	85	80	1	1
22	715	417	9	770400	2360672	3130370	3420136	3440189	4220731	4260767	5080692	6590484	85	83.33	80	80	1	1
23	715	415	9	770400	2360672	3130370	3420136	3440189	4220731	4260767	5080692	5550711	90	66.67	95	80	0	1
24	715	7	9	770400	2030332	2360672	2370754	3130370	3420136	3440189	4260767	5550711	90	100	100	60	1	0
25	715	10	9	770400	2030332	2360672	2370754	3130370	3420136	3440189	5080692	5550711	95	100	100	60	1	0

(10) Discrimination rate based on 10 genes

[0066] The number of combinations of 10 genes from the 13 genes is 286, and some of them are shown in Table 17. For example, the sets of 10 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 7 sets [Table 17: 1 to 7].

[Table 17]

OBS	rep	index	pbnum	pb1	pb2	pb3	pb4	pb5	pb6	pb7	pb8	pb9	pb10	ccvP0	ctcP0	ccvP1	ctcP1	flg0	flg1
1	286	166	10	770400	2360672	2370754	3130370	3420136	3440189	4220731	4260767	5080692	5550711	90	83.33	95	80	1	1
2	286	170	10	770400	2360672	2370754	3130370	3420136	3440189	4220731	4260767	5550711	6590484	90	100	95	80	1	1
3	286	195	10	770400	2360672	2370754	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	95	80	1	1
4	286	162	10	770400	2360672	2370754	3130370	3420136	4220731	4260767	5080692	5550711	6590484	80	100	90	80	1	1
5	286	203	10	770400	2360672	3130370	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	90	80	1	1
6	286	212	10	770400	2370754	3130370	3420136	3440189	4220731	4260767	5080692	5550711	6590484	90	83.33	90	80	1	1
7	286	188	10	770400	2360672	2370754	3130370	3440189	4220731	4260767	5080692	5550711	6590484	95	83.33	85	80	1	1
8	286	5	10	770400	2030332	2360672	2370754	3130370	3420136	3440189	4220731	5080692	5550711	95	100	100	60	1	0
9	286	11	10	770400	2030332	2360672	2370754	3130370	3420136	3440189	4260767	5080692	5550711	85	100	100	60	1	0
10	286	14	10	770400	2030332	2360672	2370754	3130370	3420136	3440189	4260767	5550711	5960072	95	100	100	60	1	0
11	286	15	10	770400	2030332	2360672	2370754	3130370	3420136	3440189	4260767	5550711	6590484	90	83.33	100	60	1	0
12	286	17	10	770400	2030332	2360672	2370754	3130370	3420136	3440189	5080692	5550711	5960072	95	83.33	100	60	1	0
13	286	22	10	770400	2030332	2360672	2370754	3130370	3420136	4220731	4260767	5080692	5960072	95	83.33	100	60	1	0
14	286	31	10	770400	2030332	2360672	2370754	3130370	3420136	4260767	5080692	5550711	5960072	95	100	100	60	1	0
15	286	37	10	770400	2030332	2360672	2370754	3130370	3440189	4220731	4260767	5080692	5960072	95	83.33	100	60	1	0
16	286	42	10	770400	2030332	2360672	2370754	3130370	3440189	4220731	5080692	5550711	5960072	100	100	100	60	1	0
17	286	46	10	770400	2030332	2360672	2370754	3130370	3440189	4260767	5080692	5550711	5960072	95	100	100	60	1	0
18	286	49	10	770400	2030332	2360672	2370754	3130370	3440189	4260767	5550711	5960072	6590484	100	83.33	100	60	1	0
19	286	56	10	770400	2030332	2360672	2370754	3130370	4260767	5080692	5550711	5960072	6590484	100	83.33	100	60	1	0
20	286	63	10	770400	2030332	2360672	2370754	3420136	3440189	4220731	5080692	5550711	5960072	100	100	100	60	1	0
21	286	67	10	770400	2030332	2360672	2370754	3420136	3440189	4260767	5080692	5550711	5960072	95	100	100	60	1	0
22	286	71	10	770400	2030332	2360672	2370754	3420136	3440189	5080692	5550711	5960072	6590484	100	83.33	100	60	1	0
23	286	91	10	770400	2030332	2360672	3130370	3420136	3440189	4220731	5080692	5550711	5960072	100	83.33	100	60	1	0
24	286	124	10	770400	2030332	2370754	3130370	3420136	3440189	4220731	4260767	5550711	5960072	100	83.33	100	60	1	0
25	286	127	10	770400	2030332	2370754	3130370	3420136	3440189	4220731	5080692	5550711	5960072	100	100	100	60	1	0

(11) Discrimination rate based on 11 genes

[0067] The number of combinations of 11 genes from the 13 genes is 78. For example, the sets of 11 gene probes that permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life include 1 set [770400/2360672/2370754/3130370/3420136/3440189/4220731/4 260767/5080692/5550711/6590484.

[0068] A study was made on combinations of 12 or 13 genes selected from the 13 genes in the same way as above. No gene probe set was found to permit prediction with a discrimination rate of 80% or more in training data (40 individuals) and test data (11 individuals) as to the discrimination of long life or short life.

(14) Selection of gene advantageous for discrimination

[0069] As described above, when 1, 2, ..., 13 genes are used from the 13 genes, 4 genes that offered a favorable discrimination rate (highly frequent genes used in the gene sets that resulted in a discrimination rate of 80% or more) were selected (probe ID NOs : 770400, 2360672, 4220731 and 5550711). Table 18 and Figure 5 show the appearance frequency of each probe obtained from the combinations of probes that result in both flg0 and flg1 of 1 (discrimination rate of 80% or more in both training data and test data). The combinations involving these 4 genes exhibited high prognostic predictability.

[Table 18]

OBS	Probe_ID	Symbol	Accession	frequency
1	770400	LOC653600	XM_928349.1	257
2	2030332	PTPN18	NM_014369.2	0
3	2360672	TNFRSF19	NM_148957.2	203
4	2370754	G3BP2	NM_012297.3	112
5	3130370	ZNF83	NM_018300.2	157
6	3420136	C6orf222	NM_001010903.3	140
7	3440189	ZBTB20	NM_015642.3	161
8	4220731	P4HA1	NM_000917.2	257
9	4260767	GP1BA	NM_000173.4	112
10	5080692	HLA-A29.1	NM_001080840.1	115
11	5550711	SYNE1	NM_182961.2	186
12	5960072		BY797688	5
13	6590484	NAP1L1	NM_139207.1	151

Example 2

1: Selection of immunologically relevant gene

[0070] In the same way as in Example 1, the short-lived group and the long-lived group were subjected to t-test with focusing on 748 immunologically relevant genes from the 16968 genes. As a result, top 100 genes with a small P-value (large significant difference) were selected as candidates (Table 19). The expression levels (fluorescence reader-measured values) of these 100 genes are shown in the columns "0" (short-lived group) and "1" (long-lived group) of Table 19.

[Table 19-1]

OBS	ProbeID	Symbol	Accession	_0	_1	_diT01	Method	Variances	tValue	DF	Probt	P_KW	flg1	flg2	flg3	FC	_lpval_t	_lpval_w
1	4830255	DPP4	NM_001935.3	6.8716	7.179	-0.3074	Pooled	Equal	-4.52	38	<.0001	0.00019	1	1	2	-0.3074	4.23172	3.7229
2	1110091	TIAL1	NM_001033925.1	7.6534	7.8873	-0.2339	Pooled	Equal	-3.9	38	0.0004	0.00141	1	1	2	-0.23391	3.42498	2.84975
3	6940433	STAT5B	NM 012448.3	8.5812	8.8213	-0.2402	Pooled	Equal	-3.67	38	0.0007	0.00088	1	1	2	-0.24015	3.12747	3.05682
4	2640025	HP	NM_005143.2	7.3689	6.9621	0.4068	Pooled	Equal	3.6	38	0.0009	0.00187	1	1	2	0.40682	3.03732	2.72907
5	3520601	MPO	NM_000250.1	8.129	7.1635	0.9655	Pooled	Equal	3.39	38	0.0016	0.0008	1	1	2	0.96555	2.7846	3.09913
6	6550600	MYC	NM_002467.3	7.5767	7.914	-0.3374	Pooled	Equal	-3.37	38	0.0017	0.00048	1	1	2	-0.33736	2.75912	3.31516
7	3610735	F12	NM_000505.3	6.8105	6.7151	0.0954	Pooled	Equal	3.09	38	0.0037	0.00245	1	1	2	0.09539	2.43139	2.61106
8	5900100	BCR	NM 021574.2	6.6621	6.5878	0.0743	Pooled	Equal	3.07	38	0.004	0.00204	1	1	2	0.07433	2.4012	2.68944
9	3130669	SATB1	NM_002971.2	7.0787	7.2949	-0.2162	Pooled	Equal	-3.05	38	0.0041	0.00803	1	1	2	-0.21617	2.38317	2.09542
10	1500735	CTSG	NM_001911.2	8.781	7.5263	1.2547	Pooled	Equal	3	38	0.0048	0.00869	1	1	2	1.25466	2.3209	2.06078
11	5420095	MYC	NM_002467.3	8.2483	8.6315	-0.3832	Pooled	Equal	-2.91	38	0.0061	0.00451	1	1	2	-0.38321	2.21614	2.34605
12	5670739	AZU1	NM_001700.3	7.3525	6.7459	0.6066	Pooled	Equal	2.89	38	0.0063	0.00741	1	1	2	0.60663	2.20215	2.13034
13	10358	SPN	NM_001030288.1	8.4201	8.0743	0.3457	Pooled	Equal	2.86	38	0.0069	0.00803	1	1	2	0.34574	2.16135	2.09542
14	770021	PRKRA	NM_003690.3	8.4218	8.6512	-0.2294	Pooled	Equal	-2.82	38	0.0075	0.00803	1	1	2	-0.22936	2.12341	2.09542
15	2940767	CEBPE	NM_001805.2	6.7703	6.625	0.1453	Pooled	Equal	2.78	38	0.0084	0.00224	1	1	2	0.14529	2.0756	2.65011
16	4250577	HSPD1	NM_002156.4	6.5931	6.5566	0.0365	Pooled	Equal	2.76	38	0.0088	0.02655	1	1	2	0.0365	2.05747	1.57598
17	940356	IL15RA	NM_002189.2	6.8246	6.7111	0.1135	Pooled	Equal	2.76	38	0.0088	0.0186	1	1	2	0.1135	2.05697	1.73038
18	6250615	PGLYRP1	NM_005091.1	8.0051	7.3418	0.6634	Pooled	Equal	2.76	38	0.0088	0.01018	1	1	2	0.66337	2.05532	1.99238
19	4390398	LCN2	NM 005564.3	9.4014	8.4624	0.9391	Pooled	Equal	2.76	38	0.0089	0.02476	1	1	2	0.93905	2.04895	1.60628
20	5490403	CD1E	NM_001042588.1	6.8164	6.9676	-0.1512	Pooled	Equal	-2.73	38	0.0094	0.01018	1	1	2	-0.15121	2.02472	1.99238
21	6900634	CD69	NM_001781.1	8.5701	9.0537	-0.4836	Pooled	Equal	-2.65	38	0.0117	0.011	1	1	2	-0.48356	1.93088	1.95862
22	6400176	IRF7	NM_004029.2	9.7006	9.3658	0.3348	Pooled	Equal	2.64	38	0.0118	0.02149	1	1	2	0.3348	1.92745	1.66776
23	2030767	CD48	NM_001 778.2	11.9662	12.2108	-0.2446	Pooled	Equal	-2.63	38	0.0124	0.02845	1	1	2	-0.24 463	1.90778	1.54595
24	5360719	MAPK9	NM_002752.3	6.8029	6.9261	-0.1232	Pooled	Equal	-2.62	38	0.0124	0.01188	1	1	2	-0.12321	1.90534	1.92515
25	5860075	CAMP	NM 004345.3	9.9646	8.9318	1.0328	Pooled	Equal	2.61	38	0.0129	0.02	1	1	2	1.03277	1.89047	1.69892

(continued)

OBS	ProbeID	Symbol	Accession	_0	_1	_diT01	Method	Variances	tValue	DF	Probt	P_KW	flg1	flg2	flg3	FC	_lpval_t	_lpval_w
26	3170543	TIAL1	NM 001033925.1	8.8499	9.0269	-0.1771	Pooled	Equal	-2.59	38'	0.0135	0.02476	1	1	2	-0.17707	1.86892	1.60628
27	360719	CD44	NM_000610.3	7.2686	7.5049	-0.2363	Pooled	Equal	-2.58	38	0.0139	0.01282	1	1	2	-0.23628	1.8562	1.89196
28	5870138	VWF	NM_000552.3	6.8198	6.6721	0.1477	Pooled	Equal	2.58	38	0.0139	0.05146	1	0	1	0.14772	1.85561	1.28851
29	6370435	ETS1	NM_005238.2	10.2	10.6878	-0.4678	Pooled	Equal	-2.57	38	0.0141	0.0058	1	1	2	-0.46775	1.85086	2.23688
30	3170017	MAP2K3	NM 002756.3	8.5386	8.2263	0.3122	Pooled	Equal	2.54	38	0.0152	0.00451	1	1	2	0.31225	1.81911	2.34605
31	1400079	CRHR1	NM_004382.3	6.9215	6.8466	0.0749	Pooled	Equal	2.54	38	0.0153	0.01282	1	1	2	0.07494	1.81484	1.89196
32	1240450	CD27	NM_001242.4	8.27	8.6406	-0.3706	Pooled	Equal	-2.5	38	0.017	0.01383	1	1	2	-0.37061	1.76963	1.85907
33	6960072	HSPD1	NM_002156.4	8.7781	9.056	-0.2779	Pooled	Equal	-2.49	38	0.0172	0.02655	1	1	2	-0.27785	1.76391	1.57598
34	5260315	ZBTB7B	NM_015872.1	6.901	6.7793	0.1217	Pooled	Equal	2.47	38	0.0183	0.011	1	1	2	0.12174	1.73678	1.95862
35	70605	HSPD1	NM_002158.4	9.9989	10.2759	-0.2769	Pooled	Equal	-2.45	38	0.019	0.01491	1	1	2	-0.27691	1.72222	1.82646
36	5420441	TNFSF9	NM_003811.2	6.7756	6.6769	0.0987	Pooled	Equal	2.44	38	0.0195	0.0186	1	1	2	0.09871	1.70969	1.73038
37	270156	UBE2N	NM 003348.3	9.546	9.757	-0.2109	Pooled	Equal	-2.43	38	0.02	0.02	1	1	2	-0.21093	1.69802	1.69892
38	3610709	PAG1	NM_018440.3	8.3033	8.4991	-0.1958	Pooled	Equal	-2.42	38	0.0205	0.02476	1	1	2	-0.19579	1.68762	1.60628
39	1770386	MALT1	NM_006785.2	8.068	8.2886	-0.2205	Pooled	Equal	-24	38	0.0214	0.00941	1	1	2	-0.22 051	1.67057	2.02644
40	4780075	CEACAM8	NM_001816.2	7.982	7.2856	0.6964	Pooled	Equal	2.38	38	0.0222	0.02149	1	1	2	0.69644	1.65321	1.66776
41	1690626	CMKLR1	NM_004072.1	6.7231	6.6274	0.0956	Pooled	Equal	2.38	38	0.0223	0.011	1	1	2	0.09561	1.65075	1.95862

[Table 19-21]

42	780465	SLC11A1	NM_000578.3	9.8466	9.4391	0.4075	Pooled	Equal	2.36	38	0.0235	0.0186	1	1	2	0.4075	1.629	1.73038
43	1430709	SPACA3	NM_173847.3	6.6516	6.5805	0.071	Pooled	Equal	2.29	38	0.0277	0.00533	1	1	2	0.07103	1.55822	2.27297
44	870193	SP140	NM_001005176.1	7.4131	7.2877	0.1255	Pooled	Equal	2.28	38	0.0286	0.06586	1	0	1	0.12546	1.54419	1.18141
45	5290040	TNFRSF4	NM_003327.2	6.5992	6.5555	0.0437	Pooled	Equal	2.24	38	0.0313	0.04831	1	1	2	0.04373	1.50467	1.31599
46	1050482	CD46	NM_172358.1	6.8321	6.9637	-0.1316	Pooled	Equal	-2.23	38	0.032	0.02655	1	1	2	-0.13161	1.49515	1.57598
47	2140707	SLPI	NM_003064.2	7.3095	6.923	0.3864	Poole d	Equal	2.21	38	0.0332	0.04248	1	1	2	0.38644	1.47913	1.37178
48	4560193	CD44	NM_001001392.1	8.9576	9.1696	-0.212	Pooled	Equal	-2.2	38	0.0342	0.03046	1	1	2	-0.21201	1.46649	1.51622
49	4200746	BPI	NM_001725.1	7.8696	7.3048	0.5649	Pooled	Equal	2.18	38	0.0352	0.03487	1	1	2	0.56488	1.45399	1.45759
50	4590026	IMPDH2	NM_000884.2	9.3239	9.5538	-0.2299	Pooled	Equal	-2.17	38	0.0366	0.02845	1	1	2	-0.22987	1.43659	1.54595
51	5420091	LTB	NM_002341.1	9.8693	10.162	-0.2927	Pooled	Equal	-2.14	38	0.0384	0.03046	1	1	2	-0.29269	1.41519	1.51622
52	5910019	C1QB	NM_000491.3	7.4045	7.1647	0.2398	Pooled	Equal	2.11	38	0.0417	0.04532	1	1	2	0.23979	1.37949	1.34375
53	6840435	LILRB1	NM_001081637.1	12.6694	12.9059	-0.2366	Pooled	Equal	-2.09	38	0.0429	0.04532	1	1	2	-0.23657	1.3676	1.34375
54	6290343	CRH	NM_000756.1	6.6596	6.623	0.0366	Pooled	Equal	2.07	38	0.0451	0.02476	1	1	2	0.03659	1.34582	1.60628
55	7200392	C1QBP	NM_001212.3	9.8569	10.0245	-0.1675	Pooled	Equal	-2.06	38	0.046	0.06993	1	0	1	-0.16752	1.33722	1.15532
56	4120379	GFI1	NM_005263.2	7.8526	7.6175	0.235	Pooled	Equal	2.06	38	0.0463	0.05479	1	0	1	0.23505	1.33486	1.26132
57	3990703	IL10	NM_000572.2	10.7567	11.1219	-0.3652	Pooled	Equal	-2.05	38	0.047	0.05146	1	0	1	-0.36524	1.32783	1.28851
58	1230767	IFITM2	NM_006435.2	12.8638	12.476	0.3878	Pooled	Equal	2.05	38	0.0473	0.04831	1	1	2	0.38784	1.3249	1.31599
59	6400386	MAP4K2	NM_004579.2	9.8522	9.6326	0.2196	Pooled	Equal	2.05	38	0.0474	0.05479	1	0	1	0.21964	1.3242	1.26132
60	10142	CD164	NM_006016.3	6.8434	6.9864	-0.143	Pooled	Equal	-2.03	38	0.0489	0.02307	1	1	2	-0.14303	1.31036	1.63688
61	3180494	BCL2	NM_000633.2	8.2881	8.6083	-0.3202	Pooled	Equal	-2.03	38	0.0493	0.0398	1	1	2	-0.32016	1.30743	1.4001
62	290592	CASP8	NM_033356.3	6.7671	6.6696	0.0975	Pooled	Equal	2.03	38	0.0499	0.02845	1	1	2	0.09746	1.30167	1.54595
63	7200386	GFI1B	NM_004188.3	6.6923	6.6295	0.0628	Pooled	Equal	2.01	38	0.0519	0.09893	0	0	0	0.06283	1.28501	1.00466
64	5090368	HSPA4	NM_198431.1	9.2162	9.405	-0.1888	Pooled	Equal	-1.99	38	0.0543	0.03726	0	1	1	-0.18878	1.26483	1.42871
65	6330445	CASP3	NM_004346.3	6.6553	6.6183	0.037	Pooled	Equal	1.98	38	0.0548	0.12982	0	0	0	0.03704	1.2616	0.88665
66	4810333	IL12RB1	NM_153701.1	7.4262	7.2541	0.1721	Pooled	Equal	1.98	38	0.055	0.08342	0	0	0	0.17215	1.25985	1.07876
67	3520167	CD63	NM_001040034.1	8.9275	8.6779	0.2495	Pooled	Equal	1.98	38	0.0553	0.06586	0	0	0	0.24953	1.25701	1.18141

(continued)

68	2490537	TNFRSF1B	NM_001066.2	9.3305	9.0642	0.2663	Pooled	Equal	1.98	38	0.0554	0.05479	0	0	0	0.26631	1.25678	1.26132
69	5570730	TICAM1	NM_182919.1	8.2608	7.9802	0.2806	Pooled	Equal	1.98	38	0.0554	0.15167	0	0	0	0.28059	1.25666	0.8191
70	2900451	MR1	NM_001531.1	8.1442	7.9975	0.1467	Pooled	Equal	1.91	38	0.0557	0.12311	0	0	0	0.14673	1.25425	0.90971
71	3290441	BMPR1A	NM_004329.2	6.8959	6.9693	-0. 0734	Pooled	Equal	-1.96	38	0.0575	0.06566	0	0	0	-0.07343	1.24053	1.18141
72	6650242	IFITM3	NM 021034.2	11.7018	11.2156	0.4862	Pooled	Equal	1.94	38	0.0594	0.04248	0	1	1	0.48619	1.22644	1.37178
73	5310053	LTB	NM_002341.1	12.2713	12.556	-0.2846	Pooled '	Equal	-1.94	38	0.0603	0.09352	0	0	0	-0.28465	1.21984	1.02909
74	1010246	IFI6	NM_022872.2	9.0927	8.5078	0.5849	Pooled	Equal	1.94	38	0.0603	0.06586	0	0	0	0.58489	1.21951	1.18141
75	3140242	KIR2DL3	NM_014511.3	9.2033	8.6994	0.504	Pooled	Equal	1.91	38	0.0635	0.08835	0	0	0	0.50399	1.19711	1.05378
76	2970201	ABHD2	NM_152924.3	6.9306	6.8658	0.0648	Pooled	Equal	1.9	38	0.0655	0.06586	0	0	0	0.06478	1.18408	1.18141
77	4180088	ABL1	NM_005157.3	7.3424	7.1924	0.15	Pooled	Equal	1.89	38	0.066	0.07421	0	0	0	0.14999	1.18021	1.12953
78	3800725	SPHK2	NM 020126.3	7.9083	7.7437	0.1646	Pooled	Equal	1.89	38	0.0666	0.10459	0	n	0	0.1646	1.17643	0.98052
79	5310754	VNN1	NM 004666.1	7.0161	6.888	0.128	Pooled	Equal	1.88	38	0.0679	0.0398	0	1	1	0.12802	1.16809	1.4001
88	430142	HSPA4	NM_002154.3	7.086	7.2329	-0.147	Pooled	Equal	-1.85	38	0.0717	0.05829	0	0	0	-0.14695	1.14455	1.2344
81	1030270	FPR1	NM_002029.3	11.6868	11.3479	0.3389	Pooled	Equal	1.85	38	0.0725	0.09893	0	0	0	0.33894	1.1395	1.00466

[Table 19-3]

82	540671	ULRB2	NM_001080978.1	9.4993	9.272	0.2273	Pooled	Equal	1.83	38	0.0744	0.02149	0	1	1	0.22727	1.12837	1.66776
83	610601	LYST	NM_000081.2	7.7998	7.9303	-0.1905	Pooled	Equal	-1.82	38	0.0763	0.0398	0	1	1	-0.1905	1.11768	1.4001
84	4810474	IL18	NM_001562.2	13.2815	13.4723	-0.1908	Pooled	Equal	-1.81	38	0.0774	0.08835	0	0	0	-0.1 908	1.11102	1.05378
85	2810156	IGF2R	NM_000876.2	9.8756	9.6428	0.2328	Pooled	Equal	1.81	38	0.0788	0.1105	0	0	0	0.23285	1.10327	0.95664
86	1190519	MS4A1	NM_021950.3	6.7216	6.8175	-0.096	Pooled	Equal	-1.8	38	0.0791	0.23397	0	0	0	-0.09595	1.10207	0.63085
87	5390246	CCR7	NM_001838.2	9.9724	10.3942	-0.42128	Pooled	Equal	-1.8	38	0.0792	0.09352	0	0	0	-0.42181	1.10921	1.02909
88	4250136	LTB4R	NM_181657.1	7.8286	7.6741	0.1546	Pooled	Equal	1.79	38	0.0813	0.06993	0	0	0	0.15 458	1.08977	1.15533
89	610113	TNFSF14	NM_003807.2	7.4507	7.2152	0.2355	Pooled	Equal	1.78	38	0.0834	0.07421	0	0	0	0.23552	1.079	1.12953
90	3400392	FPR2	NM_001462.3	7.9307	7.6552	0.2754	Pooled	Equal	1.78	38	0.0836	0.1441	0	0	0	0.27 545	1.07776	0.84135
91	2060377	TLR3	NM_003265.2	6.6058	6.5672	0.0385	Pooled	Equal	1.77	38	0.084	0.05146	0	0	0	0.03854	1.07572	1.28851
92	2070037	IC OS	NM 012092.2	8.6581	8.9752	-0.317	Pooled	Equal	-1.77	38	0.0847	0.09352	0	0	0	-0.31702	1.07188	1.02909
93	830324	FLT3LG	NM_001459.2	7.9726	8.1475	-0.175	Pooled	Equal	-1.77	38	0.0852	0.12982	0	0	0	-0.17496	1.06948	0.88665
94	3610440	MAF	NM_005360.3	9.0803	8.81 46	0.2657	Pooled	Equal	1.77	38	0.0853	0.09352	0	0	0	0.26566	1.0688	1.02909
95	6520215	ANXA1	NM_000700.1	12.4165	12.6172	-0.2006	Pooled	Equal	-1.76	38	0.0869	0.08342	0	0	0	-0.20065	1.06109	1.07876
96	5220189	PIK3AP1	NM_152309.2	7.5028	1.6559	-0.1531	Pooled	Equal	-1.75	38	0.0876	0.12311	0	0	0	-0.1531	1.05764	0.90971
97	620717	CCL5	NM_002985.2	13.3773	13.0768	0.3005	Pooled	Equal	1.75	38	0.0889	0.02845	0	1	1	0.30045	1.05105	1.54595
98	6110343	CCL23	NM_145898.1	7.3429	7.2314	0.1115	Pooled	Equal	1.74	38	0.0891	0.15954	0	0	0	0.11147	1.04993	0.79712
99	5490750	RELA	NM_021975.2	7.1608	7.006	0.1547	Pooled	Equal	1.74	38	0.0893	0.09352	0	0	0	0.15475	1.04908	1.02909
100	160601	ST6GA L1	NM_173216.1	6.7798	6.8588	-0.079	Pooled	Equal	-1.74	38	0.0903	0.15167	0	0	0	-0.07899	1.04414	0.8191

[0071] As a result of performing variable selection using the 100 immunologically relevant genes, 29 genes were selected (Table 20). The gene set of these 29 genes permitted prediction with a discrimination rate of 100% in training data (40 individuals) as to the discrimination of long life or short life. The discrimination of test data (11 individuals) based on this gene set resulted in a short life discrimination rate (ctcP0) of 83.33% and a long life discrimination rate (ctcP1) of 80% (Table 21).

[Table 20]

OBS	probeID	Symbol	Accession
1	610113	TNFSF14	NM_003807.2
2	610170	EREG	NM_001432.2
3	870156	CD1A	NM_001763.2
4	940356	IL15RA	NM_002189.2
5	1110091	TIAL1	NM_001033925.1
6	1300274	ANXA11	NM_001157.2
7	1450008	IL16	NM_172217.1
8	2260731	ERAP2	NM_022350.2
9	2640025	HP	NM_005143.2
10	2750324	PRKCZ	NM_002744.4
11	2760500	CD38	NM_001775.2
12	3420026	FAS	NM_152877.1
13	3520601	MPO	NM_000250.1
14	4210612	AP3D1	NM_003938.5
15	4220152	SIRPG	NM_018556.3
16	4290736	MAP2K2	NM_030662.2
17	4390241	BCL2L11	NM_207002.2
18	4830255	DPP4	NM_001935.3
19	5080608	LAT	NM_014387.3
20	5420095	MYC	NM_002467.3
21	5810685	THBS1	NM_003246.2
22	5900136	CLEC4C	NM_130441.2
23	6220639	HSF1	NM_005526.2
24	6450390	IL2RG	NM_000206.1
25	6520725	TNFRSF14	NM_003820.2
26	6580408	CTSW	NM_001335.3
27	6900424	TYK2	NM_003331.3
28	7210543	PLD2	NM_002663.2
29	7610390	NOD1	NM_006092.1

[Table 21]

pbnum	ccvP0	ccvP1	ctcP0	ctcP1	flg_ccv	flg_ctc
29	100	100	83.33	80	1	1

[0072] From these 29 immunologically relevant genes, top 11 genes that were considered particularly relevant and exhibited the high correlation between survival time and gene expression were selected, and addition of a TNF-related gene (2360672) (a total of 12 genes) resulted in selecting 12 genes (Table 22).

[Table 22]

OBS	probeID	Symbol	Accession
1	610113	TNFSF14	NM_003807.2
2	940356	IL15RA	NM_002189.2
3	1110091	TIAL1	NM_001033925.1
4	2360672	TNFRSF19	NM_148957.2
5	2640025	HP	NM_005143.2
6	3420026	FAS	NM_152877.1
7	3520601	MPO	NM_000250.1
8	4830255	DPP4	NM_001935.3
9	5420095	MYC	NM_002467.3
10	5960136	CLEC4C	NM_130441.2
11	6450390	IL2RG	NM_000206.1
12	6520725	TNFRSF14	NM_003820.2

2: Discrimination rate based on selected gene

(1) Discrimination rate based on combinations of 1 to 12 genes

[0073] Of the 12 genes, one gene probe 4830255 (Table 23) permitted prediction with a high rate (ccvP0 + ccvP1 + ctcP0 + ctcP1 > 290) by itself. This gene probe permitted prediction with 90% probability in the training data (40 individuals) and 50% probability in the test data (11 individuals) as to the discrimination of short life and with 70% probability in the training data and 80% probability in the test data as to the discrimination of long life.

[Table 23]

pb1	ccvP0	ccvP1	ctcP0	ctcP1	sumP	flg_ccv	fig_ctc
4830255	90	70	50	80	290	0	0

Of the 12 genes, examples of 2 to 11 gene probes that permitted prediction with a high rate (ccvP0 + ccvP1 + ctcP0 + ctcP1 > 290) are shown in the following Tables:

[Table 24]

Combination of 2 genes								
pb1	**pb2**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sumP**	**flg_ccv**	**flg_ctc**
2360672	5420095	75	85	50	100	310	0	0
4830255	64503090	90	70	67	80	307	0	0
940356	4830255	85	70	67	80	302	0	0
6010113	4830255	90	70	50	80	290	0	0

[0074]

[Table 25]

Combination of 3 genes									
pb1	**pb2**	**pb3**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sumP**	**flg_ccv**	**flg_ctc**
940356	2360672	4830255	95	80	67	80	322	1	0
610113	1110091	2360672	85	80	50	100	315	1	0
610113	2360672	4830255	85	80	67	80	312	1	0
2360672	7640025	4830255	85	80	67	80	312	1	n
2360672	3420026	5420095	75	85	50	100	310	0	0
1110091	2380672	4830255	85	90	33	100	308	1	0
610113	4830255	6450390	90	70	67	80	307	n	0
2360672	3420026	4830255	90	85	50	80	305	1	0
2360672	4830255	6520725	90	85	50	80	305	1	0
610113	2360672	2640025	65	75	83	80	303	0	1
610113	940356	4830255	85	70	67	80	302	0	0
3420026	4830255	6450390	90	65	67	80	302	0	n
4830255	6450390	6520725	85	70	67	80	302	0	0
2360672	5420095	5960136	70	80	50	100	300	0	0
11110091	2360672	2640025	85	80	33	100	298	1	0
1110091	2380672	5420095	75	90	33	100	298	0	0
2380672	5420095	6460390	70	75	50	100	295	0	0
1110091	2360672	6459390	80	80	33	100	293	1	0
610113	3520601	5420095	70	90	33	100	293	0	0
2360672	3520601	5420095	75	85	33	100	293	0	0
3520601	5420095	5960136	70	90	33	100	293	0	0
610113	940356	2360672	70	75	67	80	292	0	0
2360672	2640025	6520725	65	80	67	80	292	0	0
3420026	4830255	5950136	90	70	50	80	290	0	0

[0075]

[Table 26]

Combination of 4 genes										
ph1	**pb2**	**pb3**	**pb4**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**cumP**	**flg_ccv**	**flg_ctc**
940356	2360872	4830255	6520725	85	70	83	80	328	0	1
610113	11100191	2360672	3420026	85	80	50	100	315	1	0
610113	11100191	2360672	5960136	85	80	50	100	315	1	0
610113	11100191	2360672	6520725	85	80	50	100	315	1	0
610113	2360672	2640025	4830256	85	80	67	80	312	1	0
2300672	2640025	4830255	6520725	85	80	67	80	312	1	0
610113	940356	2360672	4830255	80	75	67	80	31.2	0	0
940356	2360657.2	4830255	5960136	98	70	67	80	312	0	0
610113	1110091	2360672	6450390	80	80	50	100	310	1	0
610113	1110091	2360672	5420095	75	85	50	100	310	0	0
1110091	2360672	4830255	5960136	85	90	33	100	308	1	0
2360672	2640025	4830255	5960136	80	80	67	80	307	1	0
610113	2360672	4830255	6460390	85	75	67	80	307	0	0
610113	2360672	4830255	6520725	85	75	67	80	307	0	0
2360672	2640025	3420026	4830255	85	75	67	80	307	0	0
2360672	2640025	4830255	6450390	85	75	67	80	307	0	0
2360672	3420026	4830255	6450390	90	85	50	80	305	1	0
2360672	53420026	4830255	6520725	90	85	50	80	305	1	0
2360672	483025	6450390	6520725	90	85	50	80	305	1	0
2360672	3420026	5420095	5960136	70	85	50	100	305	0	0
2360672	3520601	5420095	5960136	70	85	50	100	305	0	0
610113	1110091	2360672	4830255	85	85	33	100	303	1	0
940356	1110091	2360672	3520601	90	80	33	100	303	1	0
1110091	3420026	3520601	4830255	90	80	33	100	303	1	0

(continued)

Combination of 4 genes										
ph1	**pb2**	**pb3**	**pb4**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**cumP**	**flg_ccv**	**flg_ctc**
940356	238067.2	2640025	3520601	85	85	50	80	300	1	0
940356	2360672	2640025	5420085	85	85	50	80	300	1	0
2360672	483025.5	5960138	6520725	90	80	50	80	300	1	0
2360672	3420026	5420095	5520725	70	80	50	100	300	0	0
610113	940356	1110091	2360672	85	80	33	100	298	1	0
1110091	2360672	2640025	6520725	85	80	33	100	298	1	0
1110091	2360672	3520601	5420095	90	80	33	100	298	1	0
1110091	236087.2	5420095	6450390	80	80	33	100	298	1	0
610113	2360672	2640025	5960136	80	75	83	80	298	0	0
610113	2360672	2640025	6450390	65	70	83	100	298	0	1
1110091	2360672	3420026	5420095	75	90	33	100	298	0	0
1110091	2360672	5420095	5960136	75	90	33	100	298	0	0
1110091	2360672	5420095	6520725	75	90	33	100	298	0	0
610113	2360672	2840025	3420025	75	75	67	80	297	0	0
610113	2640025	2640025	4830255	85	85	67	80	297	0	0
610113	3420026	4830255	6450390	85	65	67	80	297	0	0
610113	2360672	3420026	4830255	85	80	50	80	295	1	0
2360672	2640025	3520601	4830255	85	80	50	80	295	1	0
610113	940356	2360672	3420026	90	75	50	80	295	0	0
610113	940356	3520601	54830255	65	80	50	100	295	0	0
940358	2236067	2640025	4830255	90	75	50	80	295	0	0

[0076]

[Table 27]

Combination of 5 genes											
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**flg_ccv**	**flg_ctc**
610113	940356	2360672	4830255	483025	95	70	83	80	32.8	0	1
2360372	2640025	4830255	6450390	6520725	90	80	67	80	317	1	0
610113	1110091	2360672	3420026	5960136	85	80	50	100	315	1	0
610113	1110091	2360672	3420026	6520725	85	80	50	100	315	1	0
610113	1110091	2360672	5960136	6520725	85	80	50	100	315	1	0
610113	1110091	2360672	6450390	6520725	85	80	50	100	315	1	0
61113	2360672	2640025	4830255	8520725	85	80	67	80	312	1	0
2360672	2640025	4830255	4830255	6520725	85	80	67	80	312	1	0
2360672	2640025	3520601	5960136	6450390	85	80	67	80	312	1	0
610113	2360672	2640025	3420026	3520601	65	80	67	100	312	0	0
610113	1110091	1110091	2640025	5960136	80	80	50	100	310	1	0
610113	1110091	2360672	3420026	6450390	80	80	50	100	310	1	0
610113	1110091	2360672	3420026	6450390	75	5	50	100	310	0	0
610113	1110091	2360672	5420095	5960136	75	85	50	100	310	0	0
610113	1110091	2360672	5420095	6450390	75	85	50	100	310	0	0
1110091	2360672	4830255	6450390	6520725	85	90	33	100	308	1	0
610113	2360672	2640025	4830255	59601	86	80	67	80	307	1	0
610113	2360672	2640025	3420026	4830255	85	75	67	80	307	0	0
610113	2360672	2640025	4830255	6450390	85	75	80	80	307	0	0
2360672	2640025	3420026	4830255	6450390	85	75	67	80	307	0	0
2360672	2640025	3420026	4830255	6520725	85	75	67	80	307	0	0
2360672	2640025	4830255	5960136	6450390	85	75	67	80	307	0	0
940356	2360672	2640025	2640025	6450390	90	85	50	80	305	0	0
940356	2360672	2670025	4830255	8520725	95	80	50	80	305	1	0

(continued)

Combination of 5 genes											
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**flg_ccv**	**flg_ctc**
1110091	2360672	2640025	4830255	5960136	90	85	85	80	80	1	0
1110091	2360672	2640025	4830255	6450390	90	95	50	90	305	1	0
2360672	3420026	4630255	6450390	6520725	90	85	50	80	305	1	0
610113	1110091	2360672	5420095	6520725	75	80	50	100	305	0	0

[0077]

[Table 28]

Combination of 6 genes												
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**ccvP0**	**ccvP1**	**ctcP1**	**sunP**	**flg_ccv**	**flg_ctc**	**fig_ctc**
2360872	3420026	5420095	3420026	5420095	6450390	75	85	67	100	327	0	0
610113	1110081	2360672	5420085	5960136	6450390	70	85	67	100	322	0	0
610113	1110091	2360672	5420095	6450390	6520725	80	75	67	100	322	0	0
610113	1110091	2360672	4830255	6450390	6520725	85	85	50	100	320	1	0
610013	2360672	2640025	4630255	6450390	6520725	90	80	67	80	317	1	0
1110091	2360672	2640025	3420026	4830255	6450390	90	80	67	60	317	1	0
610113	1110081	2360672	3420026	5960136	6520725	95	80	50	100	315	1	0
610113	1110091	2360672	6450390	645390	6520725	85	80	50	100	315	1	0
610113	1110091	2350672	5960136	6450390	6520725	85	80	50	100	315	1	0
1110091	2380672	3420026	4830255	6450390	6520725	80	85	50	100	100	1	0
1110091	2360672	3520601	4830255	6450390	6520725	80	85	50	100	315	1	0
610113	2360672	2640025	3520601	4830255	6450390	85	80	67	80	312	1	0
2360672	2640025	3420026	3520601	4830255	6450390	85	80	67	80	312	1	0
2360572	2640025	3420026	3520601	4830255	8520725	85	80	67	80	312	1	0
610113	1110081	2360672	610113	6450380	6520725	90	75	67	80	312	0	0
610113	1110081	2360672	2640025	3420026	5960136	80	80	50	100	310	1	0
610113	111008	2380672	2640025	5960136	6520725	0	80	50	100	310	1	0
610113	1110091	2360672	3420026	5420095	5960136	75	85	50	100	310	0	0
610113	1110091	3420026	3520601	4830255	6520725	90	85	33	100	308	1	0
940356	1110091	2360672	2640025	3520601	6520725	90	85	33	100	308	1	0
1110091	2360672	4830255	5960136	6450390	6520725	85	90	33	100	308	1	0
610113	2360672	2640025	4830255	5950136	6520725	80	80	67	60	307	1	0
940356	1110091	3420026	3520601	4830255	5520725	100	100	17	100	307	1	0
2360672	2640025	3520601	4830255	8450390	6450390	80	80	67	80	307	1	0

(continued)

Combination of 6 genes												
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**ccvP0**	**ccvP1**	**ctcP1**	**sunP**	**flg_ccv**	**flg_ctc**	**fig_ctc**
610113	2360672	2640025	3420026	4830255	6450390	85	75	67	80	307	0	0
610113	2360672	2640025	4830255	5960136	6450390	95	75	67	80	307	0	0
610113	2360672	2640025	4830255	596016	6450390	85	75	67	80	307	0	0
940355	2360672	2640025	5960136	5450390	6520725	85	75	67	80	307	0	0
2360672	2640025	3420026	4830255	5960136	6450390	85	75	67	80	307	0	0
2360572	2640025	3420026	4830255	5960136	6520725	85	75	67	80	307	0	0
2360672	2640025	3420026	4830255	6450390	6520725	85	75	67	80	307	0	0
2360672	2640025	3520601	4830255	6450390	6450390	85	75	67	80	307	0	0
610113	940358	2360672	2640025	3520801	4830255	90	75	85	80	305	1	0
610113	940358	2360672	2640025	4830255	6520725	95	80	50	80	305	1	0
610113	1110091	2360672	2640025	4830255	5960136	90	80	85	80	305	1	0
610113	1110091	2640025	2640025	4830255	6450390	90	85	50	80	305	1	0
1110091	2380572	313520601	4830255	4630255	5450390	90	85	50	80	305	1	0

[0078]

[Table 29]

Combination of 7 genes													
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pv7**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**flg_ccv**	**flg_ctc**
610113	1110091	3420026	3520601	4330255	6450390	6520725	90	85	50	100	325	1	0
610113	1110091	2360672	4830255	5960136	6450390	6520725	85	85	50	100	320	1	0
610113	2360672	2640025	3420026	4930255	4030255	6450390	90	80	67	60	317	1	0
1110091	2350672	2640025	3420026	4330255	5900136	6450390	90	80	67	80	317	1	0
610113	1110091	2360672	3420026	5420095	5960136	6450390	70	80	67	100	317	0	0
610113	1110091	2360672	3420026	5420095	6450390	6520725	75	75	67	100	317	0	0
610113	1110091	2360572	5420095	5960136	6450390	6520725	75	75	67	100	317	0	0
610113	1110091	2350672	3420026	4830255	6450390	6520725	80	85	50	100	315	1	0
1110091	2360672	2640025	3520601	4830255	6450390	6520725	80	85	50	100	315	1	0
610113	2360672	2640025	3420026	3520601	4830255	6450390	85	80	67	80	312	1	0
610113	2360672	2640025	3420026	3520601	4830255	5520725	85	80	67	80	312	1	0
940356	2360672	2640025	4830255	5420095	5960136	6520725	85	80	67	80	312	1	0
610113	2360672	2640025	4830255	5960136	6450390	6520725	90	75	67	80	312	0	0
2360672	2640025	3420026	4830255	5960136	6450390	6520725	90	75	67	80	312	0	0
610113	1110091	2360672	2640025	3420026	5950136	6520725	80	80	50	100	310	1	0
610113	1110091	2380672	3420028	5960138	8450390	5520725	80	80	50	100	310	1	0
610113	1110091	2360672	3520601	4830255	6450390	5520725	80	80	50	100	310	1	0
610113	2360672	2640025	3520601	4830255	6450390	8520725	80	80	67	80	307	1	0
2360672	2640025	3420026	3520601	4330255	6450390	6520725	80	80	67	80	307	1	0
610113	2380672	2640025	3420026	4830255	5960136	6520725	85	75	67	80	307	0	0
610113	2360672	2640025	3420026	4830255	6450390	6520725	85	75	67	80	307	0	0
610113	2360672	2640025	3520601	4830255	5980136	6450390	95	75	67	80	307	0	0
2360672	2640025	3420026	3520601	4330255	5960136	6450390	85	75	67	80	307	0	0
810113	1110091	2380672	2870025	4830255	5960136	5450390	90	85	50	80	305	1	0

(continued)

Combination of 7 genes													
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pv7**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**flg_ccv**	**flg_ctc**
940356	1110091	2360672	2640025	3420026	4830255	5960136	95	80	50	80	305	1	0
610113	1110091	2350672	2640025	3420026	3520601	5960136	75	80	50	100	305	0	0
610113	1110091	2360672	3420026	5420095	5960136	6520725	75	80	80	100	305		0
610113	940358	1110091	2360672	3420026	3520501	6520725	90	80	33	100	303	1	0
610113	1110091	2360672	2640025	4830255	5960136	6520725	85	85	33	100	303	1	0
1110091	2360672	3420026	3520601	4830255	6450390	6520725	85	85	33	100	303	1	0
610113	940356	1110091	3520601	4330255	5960136	8520725	95	90	17	100	302	1	0

[0079]

[Table 30]

Combination of 8 genes													
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pb7**	**pb8**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**flg_ccv**
610113	1110091	2360672	2640025	3430026	4630255	5960136	6450390	90	80	66.67	80	317	1
610113	1110091	3420026	3520801	4830255	5960136	6450390	6520725	85	80	50	100	315	1
610113	2360672	2640025	3420026	4830255	5960136	6450390	6520125	90	75	66.67	80	312	0
610113	1110091	2360672	3420036	4830255	5960136	6450390	6520725	80	80	50	100	310	1
610113	1110091	2640025	3420026	3520601	4830255	6450350	6520725	80	80	50	100	310	1
1110091	2360672	2640025	3520601	4831255	5960136	6450390	6520725	80	80	50	100	310	1
940356	1110061	2360672	2840025	3520601	5420095	5960136	6520725	80	85	33.33	100	308	1
610113	2360672	2640025	3420026	3520601	4630255	6450390	6520725	80	80	68.67	80	307	1
610113	940356	2360672	2640025	4830255	5420095	5960136	6520725	85	75	66.67	80	307	0
610113	2360872	2640025	3420026	3520601	4830255	5960136	6450390	85	75	68.67	80	307	0
610113	1110091	2360672	2640025	3420026	3520601	5980136	6520725	75	80	50	100	305	0
610113	1110091	2380872	2640025	3520601	4830255	6450390	6520725	80	75	50	100	305	0
610113	940356	1110091	2360672	2640025	3420026	4830256	5960136	95	80	50	80	305	1
610113	940356	1110091	2360672	264025	4830255	5960136	6450390	85	80	50	80	305	1
940356	1110091	2360672	2640025	3420025	4830255	5980136	6450390	85	80	50	80	305	1
610113	940356	1110091	2380672	2640025	3520601	5980136	650725	85	85	33.33	100	303	1
940356	1110091	2360872	2640025	3520601	5420095	6450390	6520725	85	85	33.33	100	303	1
610113	940356	1110091	2360672	2640025	3420026	3520601	6520725	90	80	33.33	100	303	1
940356	1110091	2360672	2640025	34220026	3520601	5420095	6520725	90	80	33.33	100	303	1
940356	1110091	2360672	3420026	4830255	5960136	6450380	6520725	90	80	33.33	100	303	1
610113	940356	1110091	2640025	3520601	4830255	5980136	6520725	95	90	16.67	100	302	1
610113	940356	1110091	2360872	3420026	3520601	4830256	6520725	100	85	16.67	100	302	1
610113	2360672	2640025	3520601	4830255	5960136	645039	6520725	80	75	66.67	80	302	0
2360672	2640025	3420026	3520601	4830255	5960136	6450390	6520725	80	75	66.67	80	302	0

(continued)

Combination of 8 genes													
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pb7**	**pb8**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**flg_ccv**
610113	2360672	2640025	3420026	3520601	5420085	596013	6520725	70	80	50	100	300	0
610113	1110091	2360672	3420026	5420095	596013	6450390	6520725	75	75	50	100	300	0
1110091	2360672	2640025	3520601	420095	5960136	6450390	6520725	75	75	50	100	300	0
610113	940356	236072	2840025	5420085	5960136	6450380	6520725	85	85	50	80	300	1
610113	940356	2360672	2640025	3520601	4830255	5960136	6520725	90	80	50	80	300	1
610113	1110091	2360872	2640025	3420026	3520601	4830255	6450390	90	80	50	80	300	1

[0080]

[Table 31]

Combination of 9 genes															
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pb7**	**pb8**	**pb9**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sumP**	**flg_ccv**	**flg_ctc**
610113	940356	1110091	2360672	2640025	3520601	5420095	5960136	6520725	90	85	33	100	308	1	0
940356	1110091	2360672	2640025	3420026	3520601	5420095	5960136	6520725	90	85	33	100	308	1	0
610113	940356	1110091	2360672	2640025	3420025	4830255	5960136	6450390	95	80	50	80	305	1	0
610113	1110091	2360672	2640025	3520601	4830255	5960136	6450390	6520725	80	75	50	100	305	0	0
610113	1110091	2640025	3420025	3520601	4830255	5960136	6450390	6520725	80	75	50	100	305	0	0
610113	940356	1110091	2360672	2640025	3520601	5420095	6450390	6520725	85	85	33	100	303	1	0
610113	940356	1110091	2360672	3420026	48302255	5420095	6450390	8520725	95	85	33	100	303	1	0
610113	2350672	2640025	3420026	3520601	4830255	5960136	6450390	6520725	80	75	67	80	302	0	0
610013	940356	2360672	2640025	3520601	4830255	5960136	6450390	6520725	90	80	50	80	300	1	0
610013	940356	1110091	2360672	2640025	3420026	3520601	5420095	6520725	95	80	33	100	298	1	0
610013	940356	1110091	2360672	2640025	3420025	3520601	5960136	6520725	95	80	33	100	298	1	0
610013	1110091	2360672	2640025	3420025	3520601	4830255	6450390	6520725	85	80	33	100	298	1	0
940356	1110091	2360672	2640025	3420026	3520601	5420095	6450390	6520725	85	80	33	100	298	1	0
940356	1110091	2360672	2640025	3420026	3520601	5960136	6450390	6520725	80	95	33	100	298	1	0 0
610113	940356	1110091	2360672	3420026	4830255	5960136	6450390	6520725	90	75	33	100	298	0	0
610113	940356	1110091	2360672	2640025	3420026	3520601	4830255	6520725	90	85	17	100	297	1	0
610113	940356	1110091	2360672	3420026	3520601	5420095	5960136	6520725	90	90	17	100	297	1	0
610113	940356	1110091	2360672	3520601	4830256	5420095	5960136	6520725	95	85	17	100	297	1	0
940356	1110091	2360672	3420026	3520601	4830255	5420095	5960136	6520725	95	85	17	100	297	1	0
610113	1110091	2360672	2640025	3420026	3520601	4830255	5960636	6450390	85	80	50	80	295	1	0
940356	2360672	2640025	3420026	4830255	5420095	5980136	6450390	6520725	85	80	50	80	295	1	0
610113	940356	1110091	2360672	2640025	4830255	5420095	5960136	6520725	90	75	50	80	295	1	0
610113	940356	2360672	2640025	3420026	4830255	5960136	6450390	6520725	90	75	50	80	295	0	0

(continued)

Combination of 9 genes															
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pb7**	**pb8**	**pb9**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sumP**	**flg_ccv**	**flg_ctc**
610113	1110091	2360672	2640025	3520601	5420095	5960136	6450390	6520725	75	70	50	100	295	0	0
1110091	2360672	2640025	3420026	3520601	5420095	5960136	6450390	6520725	75	70	50	100	295	0	0
610113	940356	1110091	2360672	2640025	3520601	5960136	6450390	6520725	80	80	33	100	293	1	0
610113	940356	1110091	2360672	3420026	3520601	5960136	6450390	6520725	80	80	33	100	293	1	0
1110091	2360672	2640025	3420026	3520601	4830255	5860136	6450390	6520725	80	80	33	100	293	1	0
610113	940356	1110081	2380872	2640025	3420026	3520601	6450390	6520725	85	75	33	100	293	0	0
610113	1110091	2360672	2640025	3520601	4830256	5420095	6450390	6520725	75	85	33	100	293	0	0
610113	1110091	2360672	3520601	4830255	5420096	5960136	6450390	6520725	75	85	33	100	293	0	0
1110091	2360672	2640025	3520601	4830255	5420096	5960136	6450390	6520725	75	85	33	100	293	0	0
610113	940356	1110091	2360672	2640025	3520601	4830255	6450390	6520725	90	85	17	100	292	1	0
610113	940356	1110091	2360672	3420025	3520601	4830255	5960136	6520725	90	85	17	100	292	1	0
610113	940356	1110091	2360672	3420026	3520601	4830255	6450390	6520725	90	85	17	100	292	1	0
610113	940356	1110091	2640025	3420026	3520601	5420095	5950136	6520725	90	85	17	100	292	1	0
610113	940356	1110091	3520601	4830255	5420095	5960136	6450390	6520725	85	80	17	100	292	1	0
940356	1110091	2360672	2640025	3420026	3520601	4830255	6450390	6520725	90	85	17	100	292	1	0
940356	1110091	2360672	2640025	3520601	4830255	5420095	5960136	6520725	90	85	17	100	292	1	0
940356	1110091	2360672	3520601	4830255	5420095	5960136	6450390	6520725	90	85	17	100	292	1	0
940356	1110091	3420026	3520601	4830255	5420095	5960136	6450390	6520725	85	90	17	100	292	1	0
610113	1110091	2360672	2640025	3420026	4830255	5420095	5960136	6450380	80	80	50	80	290	1	0
610113	940356	2350672	2640025	4830255	5420095	5960136	6450390	6520725	85	75	50	80	290	0	0
610113	1110091	2360672	2640025	3420026	4830255	5960136	6450390	6520725	85	75	50	80	290	0	0
940356	1110091	2360672	2640025	4630256	5420885	5960136	6450390	6520725	85	75	50	80	290	0	0

[0081]

[Table 32]

Combination of 10 genes																
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pb7**	**pb8**	**pb9**	**pb10**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sunP**	**fig-ccv**	**tg_ctc**
610113	940356	1110091	2360672	2640025	3420025	3520601	5420095	5960136	6520725	90	85	33	100	308	1	0
610113	940356	1110091	2360672	2640025	4830255	5420095	5960136	6450390	6520725	85	85	33	100	303	1	0
610113	940356	1110091	2360672	2640025	3420025	3520601	5420095	6450390	6520725	85	80	33	100	298	1	0
610113	940356	1110091	2360672	3420025	3520601	4830255	5420095	5960136	6520725	95	85	17	100	297	1	0
610113	940356	2360672	2640025	3420025	4830255	5420095	5960136	6450390	6520725	85	80	50	80	295	1	0
610113	1110091	2360672	2640025	3420025	3520601	5420095	5960136	6450390	6520725	75	70	50	100	295	0	0
610113	940356	1110091	2360672	2640025	3420025	3520601	5960136	6450390	6520725	80	80	33	100	293	1	0
610113	940356	1110091	2640025	3420025	3520601	4830255	5960136	6450390	6520725	80	80	33	100	293	1	0
610113	1110091	2360672	2640025	3520601	4830255	5420095	5960136	6450390	6520725	75	85	33	100	293	1	0
610113	940356	1110091	2360672	2640025	3420025	3520601	4830255	6450390	6520725	90	85	17	100	292	1	0
610113	940336	1110091	2360672	2640025	3520601	4830255	5420095	5960136	6520725	90	85	17	100	292	1	0
610113	940356	1110091	2360672	3520601	4830255	5420095	5960136	6450390	6520725	90	85	17	100	292	1	0
940356	1110091	2360672	2640025	3420025	3520601	4930255	5420095	5960136	6520725	90	85	17	100	292	1	0
940356	1110091	2360672	2640025	3520601	4630255	5420095	5960136	6450390	6520725	90	85	17	100	292	1	0
940356	1110091	2360672	3420026	3520601	4830255	5420095	5960136	6450390	6520725	90	85	17	100	292	1	0
610113	940356	1110091	2360672	2640025	4830255	5420095	5960136	6450390	6520725	85	75	50	80	290	0	0

[0082]

[Table 33]

Combination of 11 genes																	
pb1	**pb2**	**pb3**	**pb4**	**pb5**	**pb6**	**pb7**	**pb8**	**pb9**	**pb10**	**pb11**	**ccvP0**	**ccvP1**	**ctcP0**	**ctcP1**	**sumP**	**flg_ccv**	**flg_ctc**
610113	940356	1110091	2380672	3420028	3520601	4830255	5420085	5960136	6450390	6520725	90	85	17	100	292	1	0

[0083] In the above-described tables, pb1 to pb11, ccvP0, ccvP1, ctcP0, ctcP1, sumP, flg_ccv, flg_ctc, etc. are defined as follows:

pb1: Probe 1
pb2: Probe 2
pb3: Probe 3
pb4: Probe 4
pb5: Probe 5
pb6: Probe 6
pb7: Probe 7
pb8: Probe 8
pb9: Probe 9
pb10: Probe 10
pb11: Probe 11
pb12: Probe 12
ccvP0: Rate of correct determination "short life → short life" obtained using training data
ccvP1: Rate of correct determination "long life → long life" obtained using training data
ctcP0: Rate of correct determination "short life → short life" obtained using test data
ctcP1: Rate of correct determination "long life → long life" obtained using test data
sumP: ccvP0 + ccvP1 + ctcP0 + ctcP1
flg_ccv: Both ccvP0 and ccvP1 were 80% or more flg_ctc: Both ctvP0 and ctvP1 were 80% or more

Example 3

[0084] Progressive recurrent prostate cancer patients were classified into a long-lived group (16 cases) that survived for 900 days or longer after personalized peptide vaccination and a short-lived group (14 cases) that died within 300 days after the vaccination. The peptide vaccines were appropriately selected by a physician in consideration of host immunity present before the vaccination. Four peptides (3 mg each of the peptides) at the maximum were subcutaneously administered, together with Freund's incomplete adjuvants, to each patient once a week for 6 weeks. Blood was obtained from the patient before the vaccination and after the vaccination. Peripheral blood mononuclear cells (PBMCs) were prepared by density gradient centrifugation using Ficoll-Paque (GE Healthcare Life Sciences, Uppsala, Sweden). Genes that differed in expression in the peripheral blood mononuclear cells (including granulocytes, lymphocytes, etc.) of the patients between two groups (long-lived group and short-lived group) were analyzed in the same way as in Example 1 using DNA microarrays (Human WG-6 v3.0 Expression BeadChip manufactured by Illumina, Inc.). Microarray data was extracted using BeadStudio v3.0 software (Illumina, Inc.). Fold-change (FC) ranking and P-values obtained using Linear Models for Microarray Data (LIMMA) Bioconductor package were employed for evaluating the difference in gene expression between the long-lived group and the short-lived group. FC was calculated according to $\log_2 FC = \log_2 (S_S/S_L)$ wherein S_L represents the assay range of the target gene in the long-lived group-derived sample; and S_S represents the assay range of the target gene in the short-lived group-derived sample.
A volcano plot was prepared with the difference in expression level ($\log_2 FC$) as the abscissa and statistical significance (negative log P-value) as the ordinate (Figure 6). In Figure 6, for example, the area in the circle shows genes that largely differed in expression between the long-lived group and the short-lived group after the vaccination.
42 probes corresponding to 36 genes that satisfied the conditions of fold-change ranking ($\log_2 FC < -1.0$ or > 1.0) and P-value ($P < 0.01$) were identified. Of them, 1 gene (LTB) exhibited a decreased expression level in the short-lived group, whereas all the remaining 35 genes exhibited an increased expression level therein (Table 34).
[0085]

[Table 34]

Gene symbol	Gene name	[1]Fold change	[2]P-Value	[3]Expression	[4]Pre and Post
LTB	lymphotoxin beta	-1.03	2.01.E-05		
OLR1	oxidized low density lipoprotein receptor 1	1.04	3.76.E-03		
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1	1.07	3.09.E-05	*	

(continued)

Gene symbol	Gene name	[1]Fold change	[2]P-Value	[3]Expression	[4]Pre and Post
ARG1	arginase, liver	1.10	4.66.E-06	*	
MYL4	myosin, light chain 4, alkali; atrial, embryonic	1.14	7.10.E-03		
ALAS2	aminolevulinate, delta-, synthase 2	1.20	9.35E-03	**	
SLPI	secretory leukocyte peptidase inhibitor	1.22	1.58E-05	*	
SELENBP1	selenium binding protein 1	1.22	7.56.E-03		
SNCA	synuclein, alpha	1.25	7.55.E-03		
AZU1	azurocidin 1	1.25	1.89.E-06	*	#
HMGXB4	HMG box domain containing 4	1.27	1.07.E-03		
RNASE3	ribonuclease, RNase A family, 3	1.28	9.83.E-04	*	#
HBQ1	hemoglobin theta 1	1.31	1.42.E-03	**	
MMP9	matrix metallopeptidase 9	1.32	3.56.E-06	*	
GYPE	glycophorin E	1.36	5.00.E-06	**	
SNCA	synuclein, alpha	1.39	4.85.E-03		
EPB42	erythrocyte membrane protein band 4.2	1.45	3.00.E-03	**	
HP	haptoglobin	1.50	2.58.E-05	**	
IFIT1L	interferon-induced protein with tetratricopeptide repeats 1-like	1.51	2.89.E-03		
CD24	CD24 molecule	1.55	8.89.E-05	*	
BPI	bactericidal/permeability-increasing protein	1.64	1.29.E-05	*	
CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6	1.72	1.34.E-06	*	#
PGLYRP1	peptidoglycan recognition protein 1	1.80	4.59.E-05	*	#
MPO	myeloperoxidase	1.94	1.03.E-06	*	#
OLFM4	olfactomedin 4	2.01	1.34.E-04		
HBM	hemoglobin, mu	2.05	1.67.E-03	**	
ALAS2	aminolevulinate, delta-, synthase 2	2.11	4.62.E-03	**	
CEACAM8	carcinoembryonic antigen-related cell adhesion molecule 8	2.13	2.97.E-06	*	#
ERAF	erythroid associated factor	2.29	2.33.E-03	**	
CA1	carbonic anhydrase I	2.31	3.00.E-04	*	

(continued)

Gene symbol	Gene name	[1]Fold change	[2]P-Value	[3]Expression	[4]Pre and Post
HBD	hemoglobin, delta	2.37	1.83.E-03	**	
LCN2	lipocalin 2	2.40	1.94.E-05	*	#
CTSG	cathepsin G	2.40	7.60.E-07	*	#
DEFA1	defensin alphal 1	2.40	1.20.E-05	*	#
CAMP	cathelicidin antimicrobial peptide	2.41	2.81.E-05	*	#
ELA2	elastase 2, neutrophil	2.44	9.74.E-07	*	#
DEFA4	defensin, alpha 4, corticostatin	2.53	3.09.E-07	*	#
DEFA3	defensin, alpha 3, neutrophil-specific	2.65	7.04.E-06	*	#
DEFA1	defensin, alpha 1	2.65	6.70.E-06	*	#
DEFA1	defensin, alpha 1	2.67	4.96.E-06	*	#
DEFA1	defensin, alpha 1	2.68	6.24.E-06	*	#
DEFA1	defensin, alpha 1	2.87	2.15.E-06	*	#
$\log_2$(FC): [2]P-value (limma): [3]Preferential expression; *Granulocyte, **Erythroid: [4] #Identified in both pre- and post-vaccine PBMCs					

[0086] It is noteworthy that, of the 35 genes whose expression level was increased in the short-lived group, 20 were genes known to be expressed in granulocytes and in relation to their functions. For example, defensins (DEFA1, DEFA3, and DEFA4), ELA2, CTSG, CAMP, and MPO are known to be localized in granules within granulocytic cells. In addition, molecules, such as MMP9 and arginase, which play an important role in tumor growth or immunosuppression, were observed.

[0087] Different gene expressions were confirmed by renal-time PCR for 4 genes, i.e., DEFA1, DEFA4, CEACAM8 and MPO, among the genes whose expression level was larger in the short-lived group than in the long-lived group (Figure 7). The real-time PCR was performed using Thermal Cycler Dice Real Time System (Takara Bio Inc.) and also using SYBR Premix Ex Taq II kit (Takara Bio Inc.). The sequences of primers used for defensin alpha, myeloperoxidase (MPO), carcinoembryonic antigen-related cell adhesion molecule 8 (CEACAM8) and GAPDH are as described in the following (a) to (d):

(a) DEFA1

PCR primer 1: 5’-CGGACATCCCAGAAGTGGTTG-3’ (SEQ ID NO: 1)
PCR primer 2: 5’-CCCTGGTAGATGCAGGTTCCATA-3’ (SEQ ID NO: 2)

(b) DEFA4

PCR primer 1: 5’-CACTCCAGGCAAGAGGTGATGA-3’ (SEQ ID NO: 3)
PCR primer 2: 5’-GAGGCAGTTCCCAACACGAAGT-3’ (SEQ ID NO: 4)

(c) CEACAM8

PCR primer 1: 5’-TGGCACATTCCAGCAATACACA-3’ (SEQ ID NO: 5)
PCR primer 2: 5’-ATCATGATGCTGACAGTGGCTCTA-3’ (SEQ ID NO: 6)

(d) MPO

PCR primer 1: 5’-CTGCATCATCGGTACCCAGTTC-3’ (SEQ ID NO: 7)

PCR primer 2: 5'-GATGCCTGTGTTGTCGCAGA-3' (SEQ ID NO: 8)

(e) GAPDH

PCR primer 1: 5'-GCACCGTCAAGGCTGAGAAC-3' (SEQ ID NO: 9)
PCR primer 2: 5'-TGGTGAAGACGCCAGTGGA-3' (SEQ ID NO: 10)

Example 4

[0088] Genes that differed in expression between a long-lived group (20 cases) and a short-lived group (20 cases) were searched for using the peripheral mononuclear cells of patients before vaccination. Both gene expression levels (FC) and limma P-values were lower than those after vaccination (see Example 3). In fact, when genes were selected on the basis of the same values ($\log_2 FC < -1.0$ or > 1.0 and $P < 0.01$) as those after vaccination, only 5 probes derived from 5 genes were identified as different expressed genes. By contrast, 23 probes derived from 19 genes were selected on the basis of values ($\log_2 FC < -0.6$ or > 0.6 and $P < 0.05$) less strict than the same values. Of these genes, 4 genes (PRKAR1A, LRRN3, PCDH17 and TTN) were decreased in the short-lived group, while 15 genes (LAIR2, RNASE3, CEACAM6, AZU1, HIST1H4C, PGLYRP1, CEACAM8, LCN2, MPO, CAMP, DEFA1, DEFA3, CTSG, DEFA4 and ELA2) were increased therein (Table 35).

[0089]

[Table 35]

Gene symbol	Gene name	[1]Fold change	[2]P-Value	[3]Expression	[4]Pre and Post
PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha	-0.82	4.89.E-02		
LRRN3	leucine rich repeat neuronal 3	-061	8.40.E-03		
PCDH17	protocadherin 17	-0.60	2.16.E-03		
TTN	titin	-0.60	7.55.E-03		
LAIR2	leukocyte-associated immunoglobulin-like receptor 2	0.60	3.23.E-02		
RNASE3	ribonuclease, RNase A family, 3	0.63	2.01.E-02	*	#
CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6	0.65	9.92.E-03	*	#
AZU1	azurocidin 1	0.66	6.37.E-03	*	#
HISHT1H4C	histone cluster 1, H4c	0.71	2.47.E-02		
PGLYRP1	peptidoglycan recognition protein 1	0.72	7.49.E-03	*	#
CEACAMB	carcinoembryonic antigen-related cell adhesion molecule 8	0.78	1.52.E-02	*	#
LCN2	lipocalin 2	1.00	5.26.E-03	*	#
MPO	myeloperoxidase	1.04	1.10.E-03	*	#
CAMP	cathelicidin antimicrobial peptide	1.09	6.78.E-03	*	#
DEFA1	defensin, alpha 1	1.17	3.15.E-02	*	#
DEFA1	defensin, alpha 1	1.20	1.76.E-02	*	#

(continued)

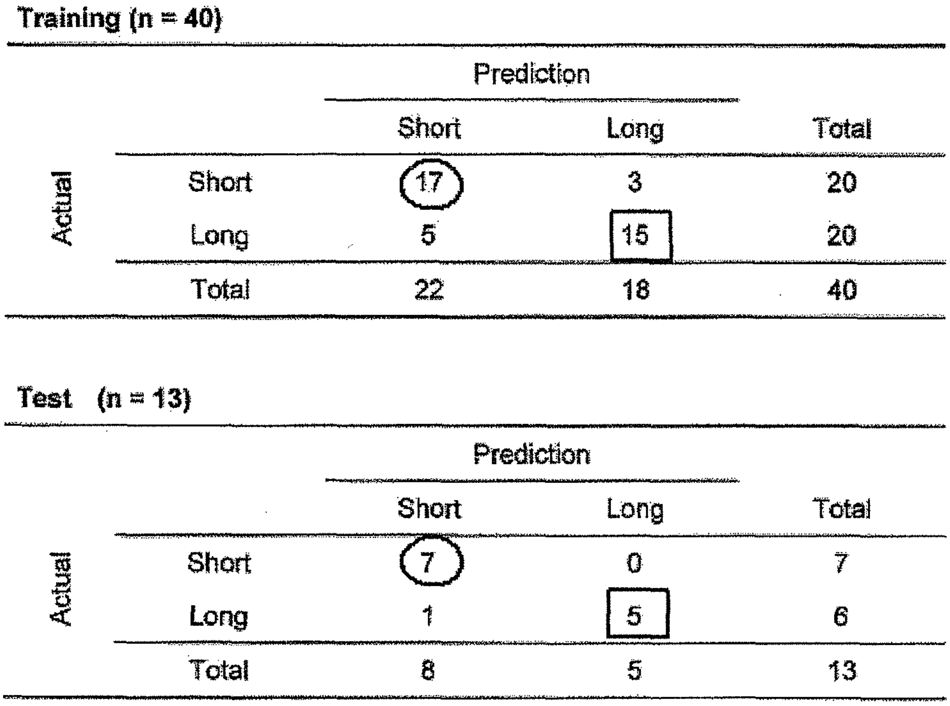
Gene symbol	Gene name	^{1}Fold change	^{2}P-Value	^{3}Expression	^{4}Pre and Post
DEFA1	defensin, alpha 1	1.26	1.76.E-02	*	#
DEFA3	defensin, alpha 3, neutrophil-specific	1.27	1.65.E-02	*	#
DEFA1	defensin, apha 1	1.27	1.97.E-02	*	#
DEFA1	defensin, alpha 1	1.30	1.54.E-02	*	#
CTSS	cathepsin G	1.32	2.77.E-03	*	#
DEFA4	defensin, alpha 4, corticostatin	1.33	2.06.E-03	*	#
ELA2	elastase 2, neutrophil	1.36	1.64.E-03	*	#
$^1\log_2$(FC): ^{2}P-value (limma): ^{3}Preferential expression; *Granulocyte: ^{4}#Identified in both pre- and post-vaccine PBMCs					

It is noteworthy that, of these 15 genes increased in the short-lived group, 13 were granulocyte-specific genes generally identified before and after vaccination.

A most important application of gene expression information based on microarrays is prediction of therapeutic effect. Thus, a study was made on whether gene expression profiles examined using cDNA microarrays in the peripheral mononuclear cells of patients before vaccination were useful in prognostic prediction after the peptide vaccination. A set of four genes (LRRN3, PCDH17, HIST1H4C and PGLYRP1) was selected by variable selection (the stepwise discriminant analysis method) from 23 probes (Table 35) that differed in expression in peripheral mononuclear cells in the 40 cases (long-lived group (20 cases) and short-lived group (20 cases)) before vaccination. This set was used to study prognostic prediction. As a result, the prognosis (long life or short life) after the vaccination could be predicted with respect to 32 patients (80%) out of the 40 patients. Sensitivity (%), specificity (%), positive predictive value, negative predictive value and accuracy (%) were 85% (17/20), 75% (15/20), 77% (17/22), 83% (15/18), and 80% (32/40), respectively (the upper table (Training) of Table 36). For validation, the determination was performed with new independent patients (13 individuals) as subjects using the 4 genes. As a result, the prognosis (long life or short life) after the vaccination could be predicted with respect to 12 patients (93%) out of the 13 patients (the lower table (Test) of Table 36). Sensitivity (%), specificity (%), positive predictive value, negative predictive value, and accuracy (%) were 100% (7/7), 83% (5/6), 88% (7/8), 100% (5/5), and 92% (12/13), respectively. In Table 36, the circled number represents the number of patients who were predicted to belong to the short-lived group and actually had short life, i.e., the number of cases in which the determination of poor prognosis before vaccination was correct, and the boxed number represents the number of patients who were predicted to belong to the long-lived group and actually had short life, i.e., the number of cases in which the determination of good prognosis before vaccination was correct.

[0090]

[Table 36]



Training (n = 40)

		Prediction		
		Short	Long	Total
Actual	Short	17	3	20
	Long	5	15	20
	Total	22	18	40

Test (n = 13)

		Prediction		
		Short	Long	Total
Actual	Short	7	0	7
	Long	1	5	6
	Total	8	5	13

[0091] The levels of cytokines, chemokines and growth factors in the plasmas of patients before vaccination were detected using bead-based multiplex assay (xMAP; Luminex Corporation, Austin, TX). The levels of cytokines, chemokines and growth factors including IL-1Rα, IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-α, IFN-γ, TNF-α, G-CSF, GM-CSF, IP-10, RANTES, Eotaxin, MIP-1α, MIP-1β, MCP-1, MIG, VEGF, EGF, HGF and FGF basic were measured using a kit (Invitrogen Corporation: Human 30-Plex).
As a result, IL-6 was present at a high content in the plasmas of the short-lived group, compared with the long-lived group (Figure 8).

Industrial Applicability

[0092] The present invention provides a prediction of patients (poor prognosis group) for whom immunotherapy is not expected to be effective and provides information useful in the selection of treatment methods for cancer patients.

[0093] The present application claims priority based on Japanese Patent Application No. 2010-147797. The contents thereof are incorporated herein by reference in its entirety.

[Sequencing Listing]

Claims

1. A method for predicting effect of immunotherapy on a cancer patient, comprising a step of measuring an expression level of each gene in a gene set consisting of at least one gene selected from the group of genes shown in Table 1 or 19 in a sample obtained from the cancer patient before the immunotherapy.

2. The method according to claim 1, wherein the gene set comprises LOC653600, TNFRSF19, P4HA1 and SYNE1.

3. A method for predicting effect of immunotherapy on a cancer patient, comprising a step of measuring an expression level of each gene in a gene set consisting of at least one gene selected from the group of genes shown in Table 34 in a sample obtained from the cancer patient after the immunotherapy.

4. The method according to claim 3, wherein the gene set comprises DEFA1, DEFA4, CEACAM8 and MPO.

5. A method for predicting effect of immunotherapy on a cancer patient, comprising a step of measuring an expression level of each gene in a gene set consisting of at least one gene selected from the group of genes shown in Table 35 in a sample obtained from the cancer patient before the immunotherapy.

6. The method according to claim 5, wherein the gene set comprises LRRN3, PCDH17, HIST1H4C and PGLYRP1.

7. The method according to any of claims 1 to 6, further comprising a step of determining a prognosis of the patient by discriminant analysis using the expression level.

8. The method according to any of claims 1 to 7, for predicting a poor prognosis group.

9. The method according to any of claims 1 to 8, wherein the immunotherapy is peptide vaccine therapy.

10. The method according to any of claims 1 to 9, wherein the cancer is prostate cancer.

11. The method according to any of claims 1 to 10, wherein the sample obtained from the cancer patient is blood.

12. A gene set for predicting effect of immunotherapy on a cancer patient, consisting of at least one gene selected from the group of genes shown in Table 1, 19, 34 or 35.

13. The gene set according to claim 12, wherein the gene set consists of at least one gene selected from the group of genes shown in Table 2 or 22.

14. The gene set according to claim 12 or 13, wherein the gene set comprises LOC653600, TNFRSF19, P4HA1 and SYNE1.

15. The gene set according to any of claims 12 to 14, wherein the gene set comprises DEFA1, DEFA4, CEACAM8 and MPO.

16. The gene set according to any of claims 12 to 15, wherein the gene set comprises LRRN3, PCDH17, HIST1H4C and PGLYRP1.

17. A biomarker for predicting effect of immunotherapy on a cancer patient, consisting of a gene set according to any of claims 12 to 16.

18. A probe designed to specifically hybridize to at least one gene selected from the group of genes shown in Table 1, 19, 34 or 35.

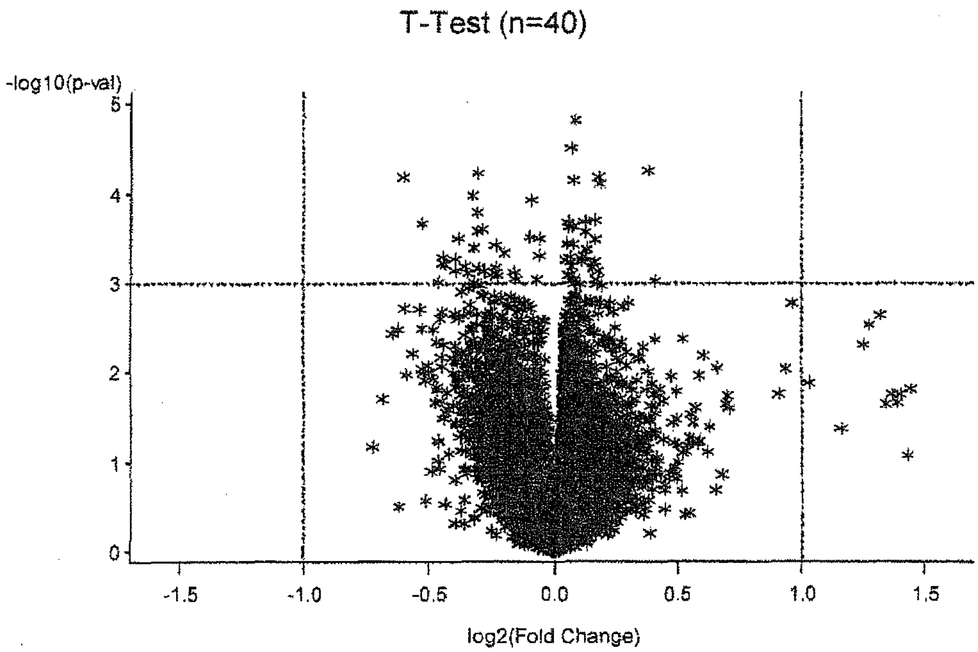
19. A kit comprising a probe according to claim 18 and/or primers specifically hybridizing to at least one gene selected from the group of genes shown in Table 1, 19, 34 or 35.

20. The kit according to claim 19, wherein the probe and the primers are any combination of the following (1) to (4) :

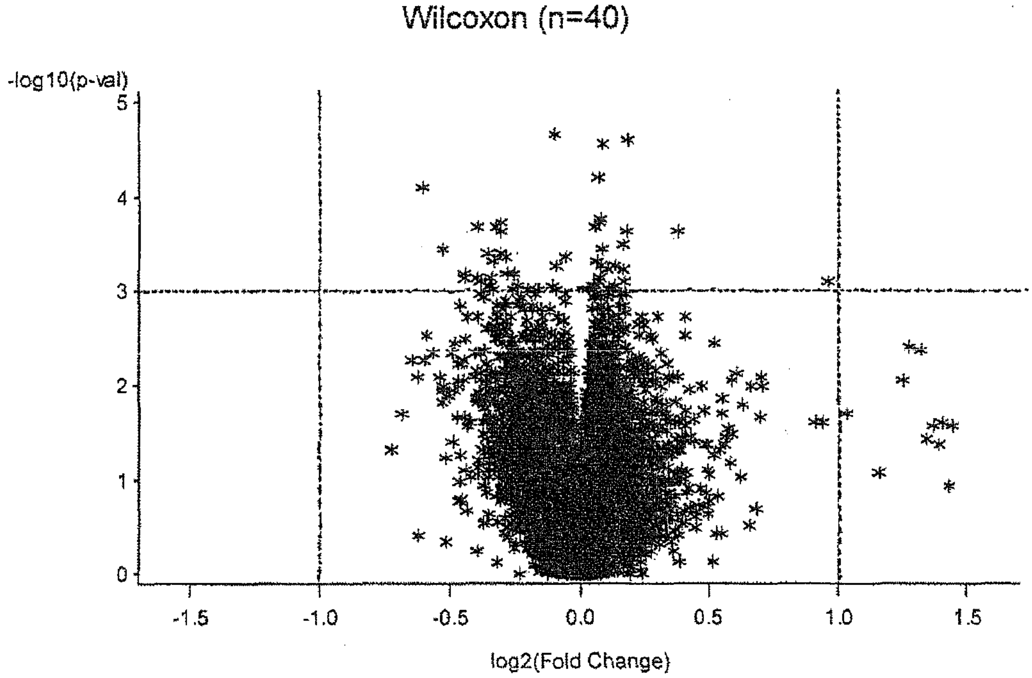
(1) a primer consisting of the sequence of SEQ ID NO: 1, a primer consisting of the sequence of SEQ ID NO: 2, and a probe consisting of the sequence of SEQ ID NO: 3;
(2) a primer consisting of the sequence of SEQ ID NO: 4, a primer consisting of the sequence of SEQ ID NO: 5, and a probe consisting of the sequence of SEQ ID NO: 6;
(3) a primer consisting of the sequence of SEQ ID NO: 7, a primer consisting of the sequence of SEQ ID NO: 8, and a probe consisting of the sequence of SEQ ID NO: 9; and
(4) a primer consisting of the sequence of SEQ ID NO: 10, a primer consisting of the sequence of SEQ ID NO: 11, and a probe consisting of the sequence of SEQ ID NO: 12.

21. A method for predicting effect of immunotherapy on a cancer patient, comprising a step of measuring an expression level of IL-6 protein in blood obtained from the cancer patient before the immunotherapy.

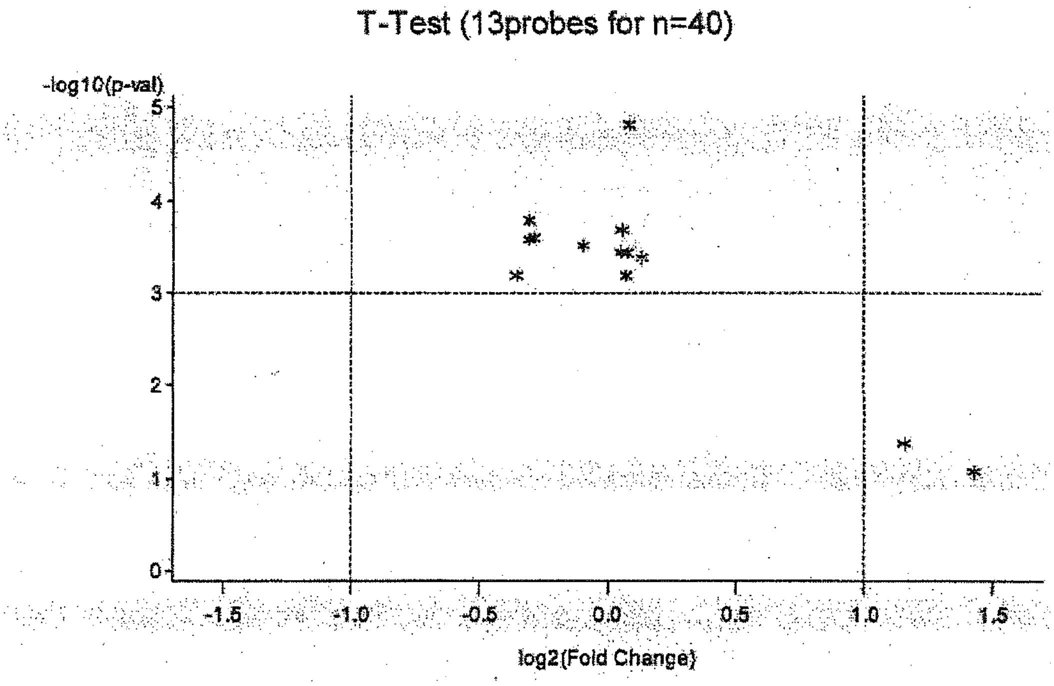
[Figure 1]



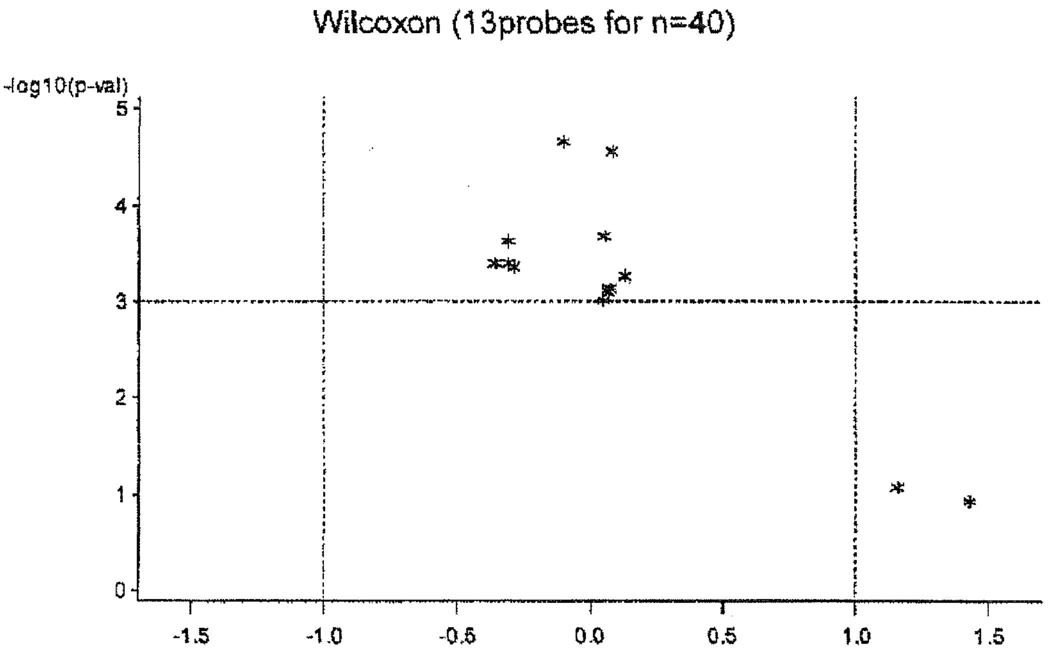
[Figure 2]



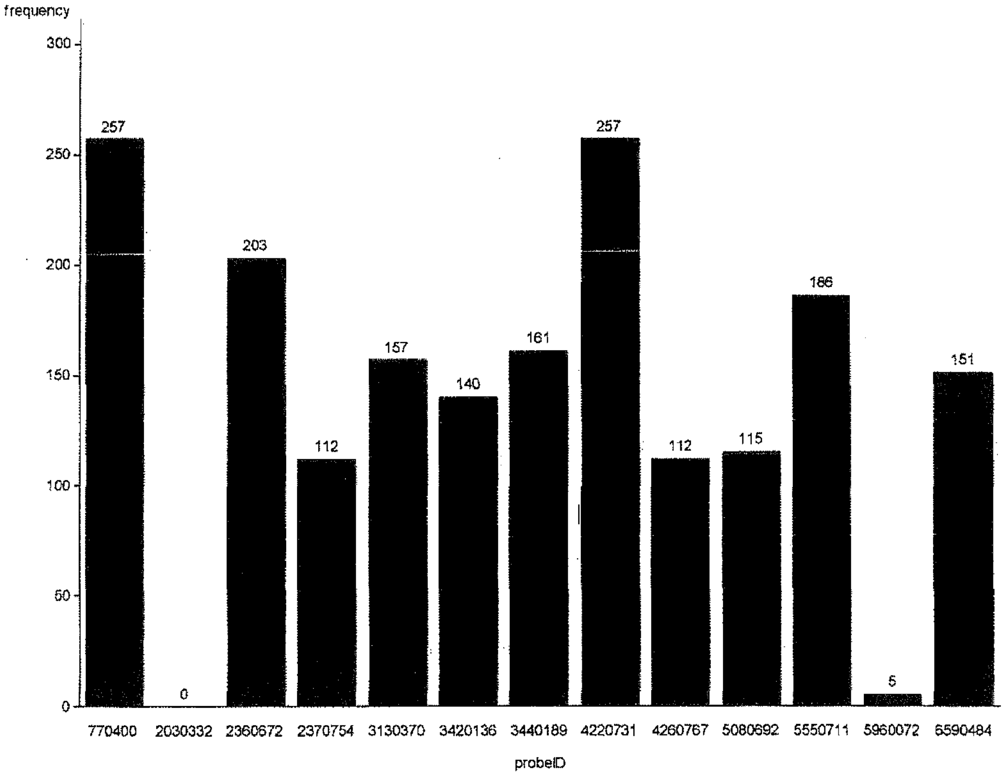
[Figure 3]



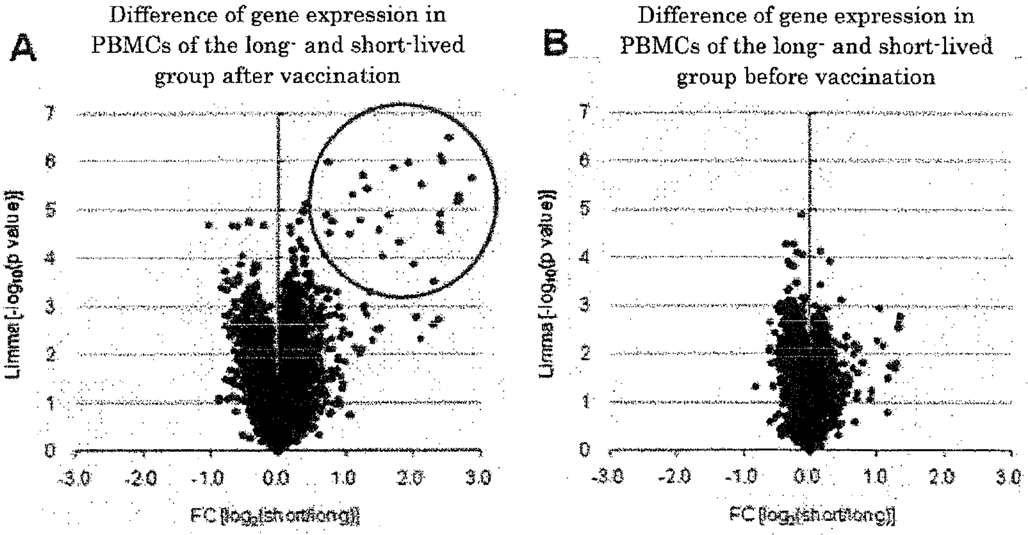
[Figure 4]



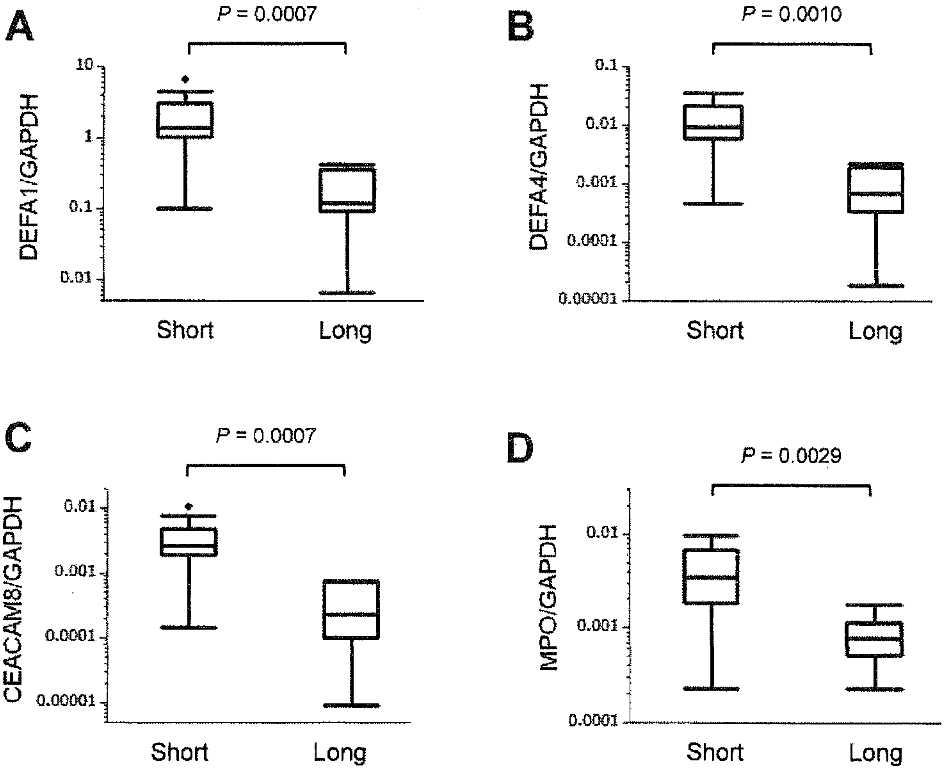
[Figure 5]



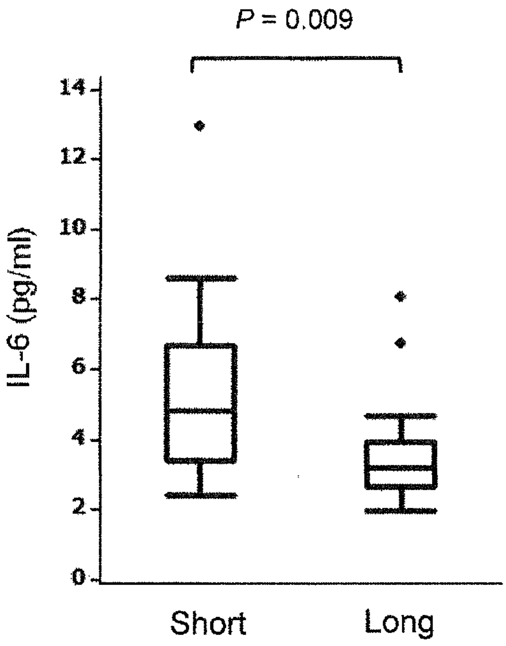
[Figure 6]



[Figure 7]



[Figure 8]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/058094

A. CLASSIFICATION OF SUBJECT MATTER
C12Q1/68(2006.01)i, *C12N15/09*(2006.01)i, *G01N33/53*(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Jitsuyo Shinan Koho	1922-1996	Jitsuyo Shinan Toroku Koho	1996-2011
Kokai Jitsuyo Shinan Koho	1971-2011	Toroku Jitsuyo Shinan Koho	1994-2011

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPlus/BIOSIS/MEDLINE/WPIDS(STN), JSTPlus/JMEDPlus/JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	SHI W. et al., Illumina WG-6 BeadChip strips should be normalized separately, BMC Bioinformatics, 2009, Vol.10, No.372, entire text	12-16,18 1-21
Y	Masanori NOGUCHI, "7. Tailor-Made-gata Peptide Vaccine Ryoho ni yoru Sainen Zenritsusen Gan no Chiryo Seiseki", Hinyokigeka, 2008, vol.21, no.8, pages 1043 to 1047, particularly, II, III	1-21
Y	JP 2009-509525 A (Digital Genomics Inc.), 12 March 2009 (12.03.2009), particularly, claims; examples & WO 2007/037611 A1 & KR 10-0617467 B1 & CN 101326290 A	1-21

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search 17 May, 2011 (17.05.11)	Date of mailing of the international search report 31 May, 2011 (31.05.11)
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/058094

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 2009-532035 A (BRISTOL-MYERS SQUIBB CO.), 10 September 2009 (10.09.2009), particularly, claims; examples & EP 1994412 A & WO 2007/117439 A2 & CA 2647565 A	1-21
Y	JP 2007-37409 A (Taiho Pharmaceutical Co., Ltd.), 15 February 2007 (15.02.2007), particularly, claims; examples (Family: none)	1-21
E,X	WO 2011/040532 A1 (Kurume University), 07 April 2011 (07.04.2011), entire text (Family: none)	1-21

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- Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0032]**
- **SMYTH.** Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical applications in genetics and molecular biology,* 2004, vol. 3 **[0034]**
- **SUNGWOO KWON et al.** DNA Microarray Data Analysis for Cancer Classification Based on Stepwise Discriminant Analysis and Bayesian Decision Theory. *Genome Informatics,* 2001, vol. 12, 252-254 **[0034]**

专利名称(译)	用于预测免疫疗法对癌症患者的治疗效果的方法，以及用于该方法的基因组和试剂盒		
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IPC分类号	C12Q1/68 C12N15/09 G01N33/53		
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代理机构(译)	法思博事务所		
优先权	2010147797 2010-06-29 JP		
其他公开文献	EP2589665A1		
外部链接	Espacenet		

摘要(译)

提供了一种基因组，其可用于预测免疫疗法对癌症患者的治疗效果。还提供了用于检查免疫疗法是否有效的方法，所述方法包括定量构成上述基因组的每种基因的表达量。该检查方法可用于确定癌症患者的治疗策略。