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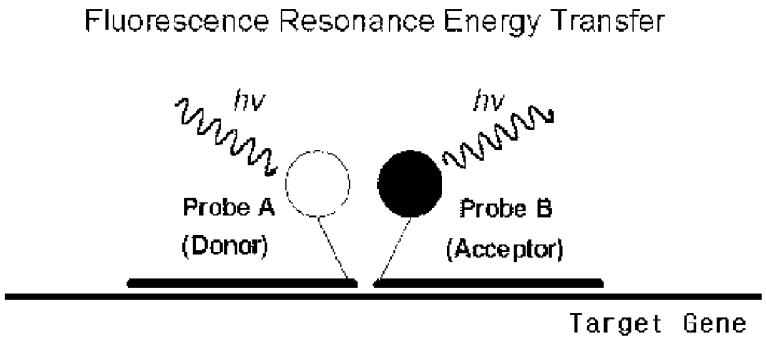
(54) **Title:** FRET GENE PROBE SELECTION METHOD USING PCR SCREENING



(57) **Abstract:** Disclosed herein is a method for screening a probe set for detecting a target gene using a FRET system, the method comprising the steps of: (A) determining one common primer to a complementary strand from the base sequence of a target gene and designing a plurality of different primers to a target strand; (B) mixing the primers with DNA containing the target gene and subjecting the mixtures to PCR so as to first screen primers to the target strand, which produce products; (C) mixing the first screened primers with one common primer to the com¬ plementary strand and DNA containing no target gene, subjecting the mixtures to PCR so as to second screen primers to the target strand, which produce no product; and (D) selecting any two primers from the group consisting of the second screened primers to the target strand and labeling the selected primers with an energy donor and energy acceptor for FRET test, re¬ spectively. The use of the method not only allows the reduction of enormous expense required for a gene labeling process but also can increase the accuracy and selectivity of a probe set to a target gene so as to perform a target gene detection process in a more rapid and easy manner.

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Description

FRET GENE PROBE SELECTION METHOD USING PCR SCREENING

Technical Field

[1] The present invention relates to a method for screening a gene detection probe set using polymerase chain reaction (PCR).

[2]

Background Art

[3] A rapid detection system for a certain gene, based on DNA amplification, is necessary for the diagnosis of various diseases or the detection of genetically modified food. To lower detection limit and obtain detection sensitivity, the existing systems for the detection of target DNA require the amplification of target DNA by PCR and/or the culture of microorganisms having target DNA. Since PCR is based on the sequence-specific hybridization of two primers to template DNA, the amplification by PCR theoretically allows the sequence-specific detection of target DNA.

[4] However, the sequence-specific PCR has a fundamental problem in that it fails to amplify because the amplication of byproduct or PCR is not an enzyme reaction conforming to a strict stoichiometry. Another problem is that it is difficult to use in the quantification of a target gene (Biosensors & Bioelectronics 14 (1999) 401-408).

[5] Diseases, such as Alzheimer's disease and cancer, occur by a certain point mutation. Because such a mutation causes a certain lesion as the number of mutated cells reaches a critical limit, the total number of cells containing a certain point mutation in tissue becomes important. Thus, it is required to exactly measure the ratio of normal and mutated cells in a given tissue. For this purpose, a quantitative analytical method for measuring the ratio of mutated cells by gene probes using fluorescence resonance energy transfer (FRET) was developed (Nucleic Acid Res. 2004 32(7) e63). Gene probes for the FRET system can be used as a tool capable of not only quantifying a certain gene but also detecting a certain gene in an easy and precise manner.

[6] The FRET (fluorescence resonance energy transfer) system uses a process of transferring fluorescent energy while inducing resonance interaction between two chromophores, called an "energy donor" and an "nergy acceptor", and is based on the Forster's theory explaining that energy transfer efficiency is in inverse proportion to the 6^{th} power of the distance between the energy donor and acceptor (Forster theory; J.R. Lakowicz. *Principles of Fluorescence Spectroscopy, Plenum Press, New York and London, 1983).*

[7] When the fluorescence resonance energy transfer system is used to label two

chromophores corresponding to the energy donor and energy acceptor, respectively, the distance between the two molecules can be measured with a resolution of a few within a range of 1-8 nm. According to the Forster's theory, the energy transfer efficiency between the energy donor and the energy acceptor can be expressed by the following equation 1:

[8] **Equation 1**

[9]

$$E_{trans} = (R_0/d)^6/[(R_0/d)^6 + 1] \qquad (1)$$

$$R_0 = 9.79 \times 10^3 \ (\kappa^2 \Phi J n^{-4})^{1/6}$$

[10] κ : dipole-dipole orientation factor

[11] Φ : fluorescence quantum yield of energy donor

[12] J : spectral overlap integral

[13] n : refractive index of medium

[14]

[15] wherein R_0 is a distance where a possibility for the donor energy to be transferred to the acceptor is 50%, and it has a value in a range of 3-4 nm, depending on a combination of the energy donor and the energy acceptor. A change in distance in a range of about $½R_0$-$2R_0$ can be experimentally measured.

[16] This principle of the fluorescence resonance energy transfer can be used in methods of analyzing the base sequence of a certain gene. As mentioned above, in order for the fluorescence resonance energy transfer to arise, the two chromophores corresponding to the energy donor and the energy acceptor, respectively, must be located in a very short distance, i.e., in a range of 3-4 nm. Accordingly, this principle can be effectively used in a process of screening and quantifying a gene with a certain base sequence in a given solution.

[17] Generally, the energy donor and energy acceptor which freely float in a solution do not emit the FRET fluorescence because the average distance therebetween is long. However, when an oligomer (a kind of primer) labeled with the energy donor and acceptor is hybridized to a target gene (DNA or RNA) containing a certain base sequence, the FRET fluorescence will be emitted because two probes become closer through their binding to the gene, as shown in FIG. 1. When the fluorescence resonance energy transfer occurs as described above, the fluorescent spectrum of the gene will be changed, resulting in an actual change in the color of the solution. Accordingly, the method of inducing FRET fluorescence by two probes and observing a change in fluorescent spectrum is also called "dual probe color change-FRET"(hereinafter, referred to as "DPCC-FRET". In the DPCC-FRET method, two independent probes corresponding to the donor and the acceptor are used and consist of

the same base number (n), and thus, only when base sequences of 2n for the two probes complementarily match with target gene, the FRET phenomenon will appear. In the DPCC-FRET method, to observe the FRET fluorescence using designed oligomers each consisting of a gene probe having, for example, 12 bases, total 24 base sequences of the probes must match with the target gene, and thus, it is possible to detect single nucleotide polymorphism (SNP) on the human genome.

[18] In addition, the method for detecting a gene using DPCC-FRET has the following advantages.

[19] First, since it is possible to make the gene probe length (the number of bases in sequences) short, the method has excellent ability to identify the mismatch in base sequence between a target gene and gene probes. If the length of probes becomes longer, the ability to identify the mismatch in base sequences therebetween will be weaken, because the hybridization will easily occur as shown in FIG. 2 even when not all the base sequences match between the probe and target gene.

[20] Second, the two gene probes should all be hybridized to the target gene to detect the FRET fluorescence. Thus, even when short gene probes containing 12-18 base sequences are used, the target gene can be detected with the same scarcity or selectivity in base sequence as gene probes containing two times longer gene sequences.

[21] Third, since gene probes do not emit the FRET fluorescence in a reaction solution but emit the fluorescence only in a state hybridized to the target gene, their use in array-type DNA chips does not require a process of washing the fluorescent gene probes. Accordingly, errors in quantitative analysis, which can occur in the process of washing the gene probes, can be eliminated, making more accurate analysis possible.

[22] However, in the DPCC-FRET method, those obtained by labeling an oligomer of DNA/RNA or new substance peptide nucleic acid (PNA) with two fluorescent substances corresponding to the energy donor and acceptor, respectively, are mainly used as a set of gene probes, and the fluorescence-labeled DNA/RNA or PNA oligomer is purchased with many costs. Particularly, because the PNA oilgomer requires many production costs even in an unlabeled state, exorbitant cost is required to purchase the fluorescence-labeled PNA oligomer.

[23] Meanwhile, a case can occur where primers constructed based on only the base sequence of the target gene do not effectively bind to the target gene so that the gene is not well amplified by polymerase chain reaction. Since the primers used in this case do not require many costs to produce or purchase, the loss of cost caused by trial and error is not significant even when polymerase chain reaction is not well made so that primers with the corresponding base sequences cannot be used. For this reason, to obtain efficient primers, repeated test procedures of examining reaction efficiency by repeating polymerase chain reaction and agarose gel electrophoresis are generally

carried out.

[24] Also, even if labeled probes are used in the DPCC-FRET method, a case where the gene probes are not well hybridized to a target gene can occur, but this case can be detected only when the FRET fluorescence is observed directly with fluorescence-labeled probes constructed expensively. If the hybridization between a target gene and gene probes does not effectively occur, the cost required to detect the ineffective hybridization will be too large, and it will become larger when a more variety of gene probes are to be used.

[25] Accordingly, there is a need for the development of a method for screening gene probes for detecting a target gene using the FRET system, in which the hybridization between a target gene and gene probes efficiently occurs.

[26]

Disclosure of Invention

Technical Problem

[27] It is an object of the present invention to provide a method for screening a gene detection probe set for the FRET system using polymerase chain reaction.

[28] Another object of the present invention is to provide a method for screening a probe set for detecting a foreign gene introduced into animals/plants, using the FRET system.

[29] Still another object of the present invention is to provide a method for screening a probe set for detecting an herbicide-resistant gene EPSPS introduced into plants, using the FRET system.

[30] Yet another object of the present invention is to provide a method for detecting a gene using said gene detection probe set by the FRET system.

[31]

Technical Solution

[32] To achieve the above object, the present invention provides a method for screening a probe set for detecting a target gene using the FRET system.

[33] More specifically, the present invention provides a method for screening a probe set for detecting a target gene using the FRET system, the method comprising the steps of: (A) determining one common primer to a complementary strand from the base sequence of a target gene and designing a plurality of different primers to a target strand; (B) mixing the primers with DNA containing the target gene, and subjecting the mixtures to PCR so as to first screen primers to the target strand, which produce products; (C) mixing the first screened primers with one common primer to the complementary strand and DNA containing no target gene, subjecting the mixtures to PCR so as to second screen primers to the target strand, which produce no product; and (D)

selecting any two primers from the group consisting of the second screened primers to the target strand and labeling the two selected primers with an energy donor and energy acceptor for FRET test, respectively.

[34] In the inventive method, the two primers selected from the second screened primers to the target strand are located so close that a change in fluorescent spectrum occurs by the FRET system. Thus, when the two selected primers bind to the target gene, the interval between the 3 -terminal end of a primer bound to the forward portion of the target gene and the 5 -terminal end of a primer bound to the reverse portion of the target gene will preferably be 1-8 nm (an interval of 3-24 bases), and more preferably 3-4 nm (an interval of 9-12 bases).

[35] Also, the present invention provides a method for detecting a target gene using the FRET system. In other words, when the target gene detection probe set selected by the above-described method is mixed with a certain DNA, the primer set (two primers, i.e., probes) will bind to the target strand if the target strand exists in the DNA. Thus, the energy donor and acceptor bound to the probes become closer to each other within a given distance while emitting fluorescence upon irradiation with laser light. Accordingly, the measurement of a change in fluorescent spectrum can determine if the target gene exists in the DNA.

[36] Although the target "gene" is described for convenience in the present invention, it is to be understood that the definition of the target gene is not limited only to genes, and any DNA or mRNA having a certain sequence can also be detected according to the present invention. Also, the target gene may be selected from various kinds of genes. For example, genes showing pathogenicity, genes inducing mutation or genes introduced for the transformation of animals and plants may be included in the definition of the target gene. More specifically, according to the present invention, it is also possible to screen a probe set for detecting herbicide-resistant gene EPSPS introduced into plants. In this case, the probe set may be selected from primers forth in SEQ ID NOS: 1, 2, 3, and 5 to 11.

[37] The inventive method for screening the gene detection probe set can be effectively used in the expensive DPCC-FRET labeling method, since only the primers selected by the screening of the primer sequences are used after treatment (labeling of the energy donor and acceptor as described below). Particularly, the method of estimating the binding efficiency of primers using polymerase chain reaction is cost-effective as compared to a case where a gene probe set labeled by the DPCC-FRET system is directly used.

[38] In another aspect, the present invention provides a target gene detection method which allows whether a target gene exists in a certain DNA to be determined in a simple and easy manner, the method comprising mixing the target gene detection

probe set selected by the above-described method with a certain DNA and subjecting the mixture to PCR. For example, the present invention provides a method for screening a target gene, which comprises mixing the target gene detection probe set selected by the above method with a certain DNA isolated from a sample and measuring a change in the fluorescent spectrum of the mixture to determine if a target gene exists in the DNA.

[39] By the screening method according to the present invention, a gene detection probe set capable of inducing a false-positive signal can be detected in advance.

[40] For reference, if a target gene containing a certain base sequence (hereinafter, referred to as "gene B" is to be detected from a mixture of a gene which is not an object to be detected (hereinafter, referred to as "gene A" and the gene B, a false signal caused by the gene A should not be generated. When polymerase chain reaction on the gene A is performed using the primer set as probes, a possibility for a false-positive signal caused by the gene A to be generated will be very high upon gene detection if the gene amplification effectively occurs. This suggests that the primer set is not suitable as probes for the detection of the gene B. Thus, if a primer set efficient for the detection of the gene B is first screened by polymerase chain reaction and is then used to perform PCR reaction for another gene (e.g., gene A) to confirm that it shows no amplification action for the another gene, whether the signal of the gene A, which is not to be generated, can occur, can be determined in advance by polymerase chain reaction.

[41] Also, in the inventive screening method, gene detection probes which are used on DNA chips can be effectively selected.

[42] For reference, a method for detecting a target gene using a gene and probes fixed onto a DNA chip still has a problem in that a false-positive signal and a false-negative signal cannot be distinguished from each other due to various factors. For this reason, whether the gene detection probes properly bind to the target gene needs to be determined in advance by a method for analyzing the false-positive and false-negative signals.

[43] Hereinafter, the present invention will be described in detail.

[44] The present invention provides a method for screening gene detection probes by observing gene products produced by polymerase chain reaction and estimating the binding efficiency of detection probes.

[45] In the present invention, to screen probes for detecting a gene containing a certain base sequence, one common primer to a complementary strand on DNA is used and a plurality of different primers to a target strand are designed and used. If all these primers are used to perform polymerase chain reaction and gene products obtained by this PCR reaction are analyzed by agarose gel electrophoresis, results as shown in FIG.

4 can be obtained. Primers corresponding to sequences well amplified in the polymerase chain reaction are determined to have a high efficiency of binding to a target gene (first screening of primers).

[46] Also, in the present invention, the gene detection probes screened by the above-described procedures were labeled with fluorescence by the DPCC-FRET system and used to detect a target gene in DNA, and the results are shown in FIG. 6. As can be seen in FIG. 6, if the target gene DNA is contained in a sample, the fluorescent spectrum will change by the corresponding gene probes. Accordingly, in DPCC-FRET fluorescence tests, the screening method according to the present invention can be effectively used to design and develop the optimal gene detection probe set.

[47] Furthermore, according to the inventive screening method, whether primers have specific selectivity to a gene containing a certain base sequence can be determined in advance. Namely, the first screened primers to a target strand, the one common primer to a complementary strand, and (1) DNA containing a target gene or (2) DNA containing no target gene, were mixed with each other and subjected to a conventional polymerase chain reaction. As a result, as shown in FIG. 5, the resulting products were different between the case of DNA containing a target gene and the case of DNA containing no target gene. Namely, it could be seen that the first screened primers (SEQ ID NO: 4 was out of the question because it was excluded in the first selection) could act specifically only on the target gene to produce PCR products. If PCR products are produced by a non-target gene in a large amount, it can be seen that the gene detection primers well bind to the non-target gene. In this case, although the probe set has high efficiency for amplifying the target gene, the probe set is determined to be unsuitable for the detection of the target gene (second screening).

[48]

Brief Description of the Drawings

[49] FIG. 1 is a conceptual diagram showing a principle for detecting the base sequence of a certain gene using a fluorescence resonance energy transfer (FRET) labeling method.

[50] FIG. 2 is a conceptual diagram showing possible hybrids if there is a mismatch in base sequence between primers and a target gene.

[51] FIG. 3 is a conceptual diagram showing the inventive primer design process used in polymerase chain reaction for testing the binding efficiency of gene detection probes.

[52] FIG. 4 is a photograph showing electrophoresis results for PCR products obtained by the inventive method.

[53] FIG. 5 is a photograph showing electrophoresis results for the PCR products of genes containing the base sequence of a target gene and the PCR products of genes

containing no base sequence of a target gene.

[54] FIG. 6 is a graphic diagram showing results for the detection of a target gene by the use of gene detection probes obtained by the inventive screening method and labeled by the fluorescence resonance energy transfer method.

[55]

Mode for the Invention

[56] Hereinafter, the present invention will be described in more detail by examples. It is to be understood, however, that theses examples are given for illustrative purpose only and are not construed to limit the scope of the present invention. In the following examples, although tests were performed using herbicide-resistant gene EPSPS as a target gene to be detected, it will be obvious to a person skilled in the art that other genes can be used as target genes to construct a suitable probe set by the same principle.

[57] Example 1: Screening of gene probes containing certain base sequence

[58] Using herbicide-resistant gene EPSPS introduced into Roundup Ready®RR) soybean, a genetically modified organism, as a target gene, the utility of gene probes containing a certain gene sequence was examined.

[59] To secure DNA containing an EPSPS base sequence to be detected, the genomic DNA of RR soybean, as a template, was subjected to PCR using primers capable of amplifying a fragment of about 2 kbp corresponding to the EPSPS gene. In the PCR amplification, 35S3F (18-mer; AAGATGCCTCTGCCGACA (SEQ ID NO: 13)) was used as the forward primer, and NOS3R (25mer; ATG-TATAATTGCGGGACTCTAATCA (SEQ ID NO: 14)) was used as the reverse primer.

[60] The DNA having the EPSPS gene, a common primer (SEQ ID NO: 12) to a complementary strand on the DNA, and each of 30 primers to a target strand, were used to perform PCR amplification. 11 of the 30 primers to the target strand, used in the test, are shown in Table 1 below, and the shown primers have varying lengths and ranges.

[61] 3 □ of EPSPS gene solution (10ng/□), 10 □ of dNTP (25mM), 10 □ of 10 x buffer (Bioneer Premix; Cat. No. K-2016, 20 □), 16 □ of $MgCl_2$(25mM), and 5 □ of each of Taq polymerase (5U/□), primers to a complementary strand (20 μM) and primers to a target strand (100 μM), were placed in a PCR tube to which distilled water was added to make a total of 100 □ of reaction solution. PCR reaction was performed in gradient cycler PTC-0225 set to the following PCR conditions: denaturation at 94℃ for 5 min; amplification of 35 cycles each consisting of 20 sec at 94℃, 30 sec at 45℃, and 2 min at 72℃; and final elongation at 72℃ for 10 min.

[62] Each of all the 30 primers for target strands was used to perform the polymerase

chain reaction, and gene products obtained by this reaction were analyzed by agarose gel electrophoresis. Electrophoresis results for SEQ ID NOS: 1 to 7 among these primers are shown in FIG. 4. In FIG. 4, "M"designates a gene selection marker, and (+) designates a positive control group. For reference, although reaction yield does not depend on only binding efficiency since various steps are involved in the progression of polymerase chain reaction, it can be understood that when only the base sequences of primers are changed under the same condition, the binding efficiency will be shown to be high if the amplification of genes is well made.

[63] As could be seen in FIG. 4, polymerase chain reaction for a primer corresponding to SEQ ID NO: 4 was not well made. Accordingly, this primer can be estimated to be low in the efficiency of binding to a target gene and can be determined to be unsuitable as a sequence for detecting a target gene using the FRET method. Namely, SEQ ID NO: 4 was excluded in the first screening.

[64] Table 1

SEQ ID No.	Sequence
1	TCCATGAACTCCGGG
2	TCGTGGCGATCATCG
3	ACGTCTCGCCCTCAT
4	AAGCTCATGGCGATG
5	GCAATCCACGCCATT
6	CGCTTTCCTTGACGC
7	AGTTCTTCCAGACCG
8	CATCGTCCACCGTGA
9	GGGTTTTCCGACACG
10	TCTCGCCCTCATCGCAAT
11	ATCCACGCCATTGAG
12	CATGGGCGCCAGGATCCGTAAG

[65] Example 2: Increase of selectivity to gene containing certain base sequence

[66] To examine the extent to which the inventive screening method screens a gene containing a certain base sequence, (1) a chromosome containing the base sequence of the target gene and (2) a chromosome containing no target gene were subjected to polymerase chain reaction in the same conditions as in Example 1 using primers to be used as gene probes. By this process, it could be determined if primers to be used as gene probes have the property of binding only to the target gene. The target gene used was genomic DNA extracted from GM soybean, and the gene having no connection with the target gene was genomic DNA extracted from nonGM soybean.

[67] As a result, as shown in FIG. 5, the results of polymerase chain reaction were positively distinguished between the chromosome containing the target gene and the chromosome containing no target gene. This suggests that the first screened primers

specifically bind only to the target gene and can be used as detection probe sets (namely, in this Example, all the first screened primers passed the second screening). In this Example, all the first screened primers accidentally passed the second selection. However, it is to be understood that there can occasionally be primers which pass the first screening but do not pass the second screening.

[68] If products from polymerase chain reaction for the chromosome containing no target gene are produced at large amounts like the case of the chromosome containing the target gene, it means that the gene probe set used in that case also well bind to the non-target gene. This case can cause a false-positive reaction in a detection process, and thus, it can be determined in advance that it is preferable that the base sequence of the corresponding gene should not be used for the construction of gene probes (accordingly, these primers are excluded in the second screening).

[69] Example 3: Application of gene probes screened by inventive screening method

[70] For the actual application of the inventive method for screening gene probes, a gene probe set so selected that it has high amplification efficiency and does not show a false-positive reaction was labeled by the DPCC-FRET and used to detect target gene DNA.

[71] Because DNA probes and PNA probes act in the same manner if their sequences are common, the PNA probes were used in this Example. The DPCC-FRET probes were constructed by a process where a probe set designed only with the base sequence of a target gene was first screened by polymerase chain reaction and then labeled with fluorescent substances. Namely, genomic DNA extracted from GM soybean was used as a target protein, and four sets of target gene detection PNA probes selected from the primers for target gene, which have been prescreened by the methods described in Examples 1 and 2, were used (Table 2). The used probes lacked some of bases at the 5 and/or 3-terminal ends of the relevant sequences shown in FIG. 2. This means that these probes have the omission of about 3 in base number due to a problem in PNA construction technology and can be used as probes even in this state, and this possibility was verified in advance.

[72] The selected sets of PNA probes were labeled with fluorescein (Fl) and rhodamine (Rh) which serve as an energy donor and an energy acceptor, respectively. Fl binds to the 3-terminal end of the probes, and Rh binds to the 5-terminal end of the probes.

[73] Table 2

Probe Set	Probe Type	Sequence	SEQ ID No.
A	energy donor	ATGAACTCCGGG	1
	energy acceptor	TCGTGGCGATCA	2
B	energy donor	CGCTTTCCTTGA	6
	energy acceptor	GAGTTCTTCCAG	7
C	energy donor	ATCGTCCACCGT	8
	energy acceptor	GGGTTTTCCGAC	9
D	energy donor	TCTCGCCCTCAT	10
	energy acceptor	ATCCACGCCATT	11

[74] More specifically, the PNA probes were constructed using 5-carboxyfluorescein, succinimidyl ester for the introduction of Fl (energy donor) and 5-carboxytetramethylrhodamine, succinimidyl ester for the introduction of Rh (energy acceptor). 12 ml of each of the constructed probe sets was used alone or in a mixture with 12 ml of DNA containing a target gene, and allowed to react in the following conditions for 24 hours. Then, fluorescence emission intensity of the reaction solution was measured using a FRET signal detector (see Journal of Photoscience 11(2), 47-53, (2004)) manufactured by the present inventors (FIG. 6).

[75] (1) The following test tubes are prepared: a test tube containing only fluorescence detector reagents (donor concentration: 1 μM, and acceptor concentration: 3 μM); and two test tubes each containing a mixture of a target gene (concentration: 1,250 ng/mL) and fluorescence detection reagents (donor concentration: 1 μM, and acceptor concentration: 3 μM) mixed at a volume ratio of 1:1:1 or 12:12:12 (DNA: donor: acceptor).

[76] (2) The target gene contained in the solution cannot easily bind to the fluorescence detection reagents as it is, because it has a double-stranded structure. For this reason, to help the target gene binding to the fluorescence detection reagents, the mixture solution is heated at 95℃ for 3 minutes to make the double-stranded structure of the target gene loose.

[77] (3) The mixture solution is cooled to a temperature of 45℃ at a rate of -6℃/min.

[78] (4) The mixture solution is incubated at 45℃ for 24 hours such that the gene detection reagents can well bind to the target locations of the target gene having loose strands.

[79] (5) After 24 hours of the incubation, the solution is collected and measured for fluorescence spectra while exciting with a laser light of 442 nm.

[80] (6) For the measurement of fluorescence spectra, a CCD light detector mounted on the output stage of a spectrograph with a focus distance of 150 mm, which is equipped with a grating of 150 grooves/mm, is used to observe spectra in a range of 280-850 nm

at the same time. For the recording of the fluorescence spectra, the sample solution is irradiated with laser light for 30 seconds while emitted fluorescence is measured two times, and then, the average of the measured values is calculated and recorded.

[81] In this test, if a primer set (two primers, i.e., probes) to a target strand binds to the target strand, the energy donor and energy acceptor bound to the probes will become closer to each other within a given distance so that they will emit fluorescence upon irradiation with laser light.

[82] As a result, as could be seen in FIG. 6, the case of containing target gene DNA showed a change in fluorescence spectrum by the corresponding gene probe set. Although FIG. 6 shows a test result for probe set B, the same result was also shown for other probe sets (data not shown).

[83]

Industrial Applicability

[84] As described above, according to the inventive method, the detection of a target gene can be performed by DPCC-FRET in an easy and cost-effective manner. Namely, it was demonstrated that the inventive method for screening gene probes using polymerase chain reaction could be effectively used to design and develop the optimal gene probes.

[85] Accordingly, the inventive screening method not only allows effective detection of a target gene but also can increase the accuracy and selectivity of probes to a target gene and can greatly reduce the cost for the development of the probes.

Claims

[1] 1. A method for screening a probe set for detecting a target gene using a FRET system, the method comprising the steps of:

(A) determining one common primer to a complementary strand from the base sequence of a target gene and designing a plurality of different primers to a target strand;

(B) mixing the primers with DNA containing the target gene and subjecting the mixtures to PCR so as to first screen primers to the target strand, which produce products;

(C) mixing the first screened primers with one common primer to the complementary strand and DNA containing no target gene and subjecting the mixtures to PCR so as to second screen primers to the target strand, which produce no product; and

(D) selecting any two primers from the group consisting of the second screened primers to the target strand and labeling the selected primers with an energy donor and energy acceptor for FRET test, respectively.

[2] The method of Claim 1, wherein the two selected primers to the target strand, when bound to the target gene, have an interval of 1-8 nm (an interval of 3-24 bases) between the 3-terminal end of a primer bound to the forward portion of the target gene and the 5-terminal end of a primer bound to the reverse portion of the target protein.

[3] The method of Claim 1 or 2, wherein the target gene is a foreign gene introduced into genetically modified animals/plants.

[4] The method of Claim 3, wherein the target gene is EPSPS, an herbicide-resistant gene.

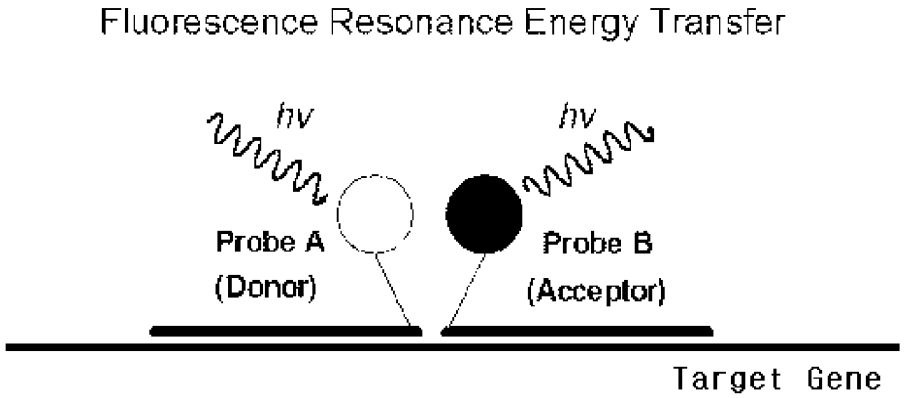
[5] The method of Claim 4, wherein the two selected primers to the target strand are selected from the group consisting of SEQ ID NOS: 1, 2, 3, and 5 to 11.

[6] A method for detecting a target gene using a FRET system, the method comprising: mixing the target gene detection probe set screened by the method of Claim 1 with a certain DNA; and measuring a change in the fluorescence spectrum of the mixture so as to determine if the target gene exists in the DNA.

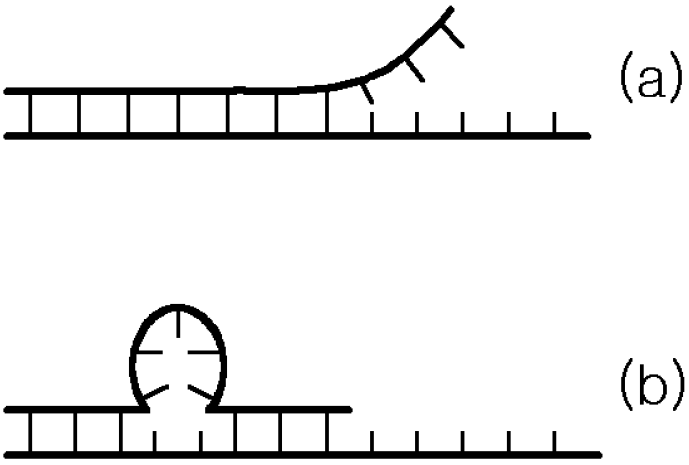
[7] The method of Claim 5, wherein the target gene is a foreign gene introduced into genetically modified animals/plants.

[8] The method of Claim 6, wherein the target gene is EPSPS, an herbicide-resistant gene.

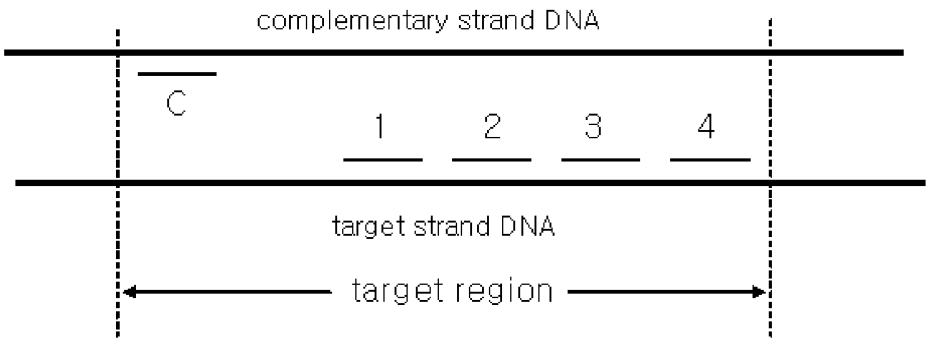
[Fig. 1]



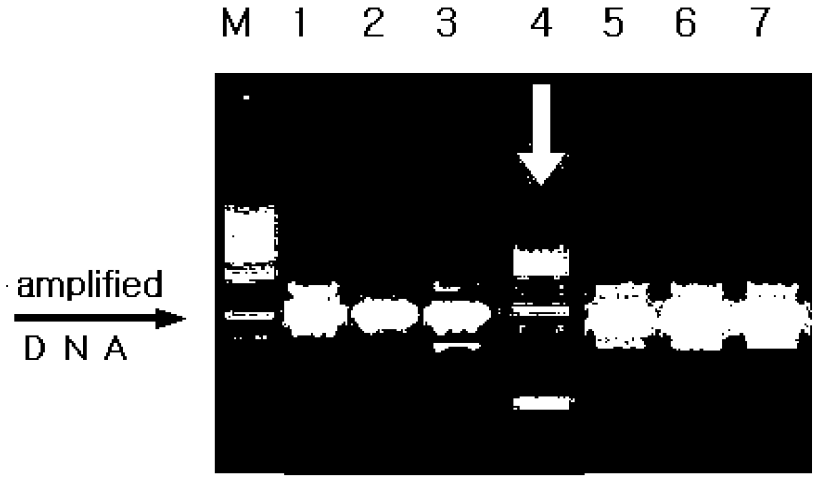
[Fig. 2]



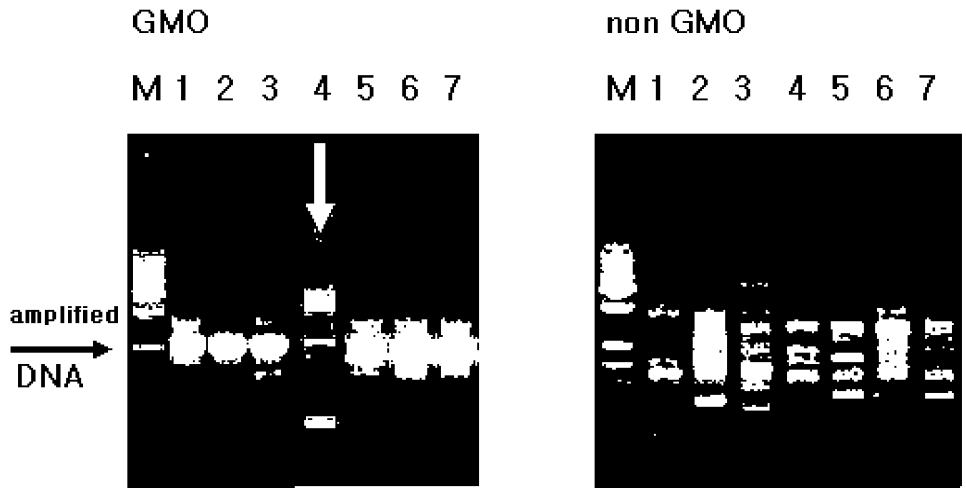
[Fig. 3]



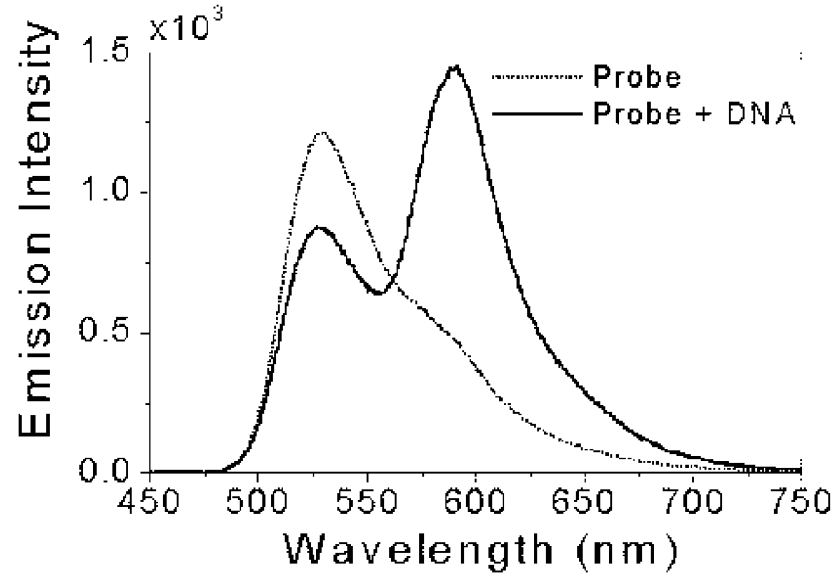
[Fig. 4]



[Fig. 5]



[Fig. 6]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/003038

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of :

   a. type of material
      - [x] a sequence listing
      - [x] table(s) related to the sequence listing

   b. format of material
      - [x] on paper
      - [x] in electronic form

   c. time of filing/furnishing
      - [x] contained in the international application as filed
      - [x] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/003038

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12Q 1/68

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)
IPC7 C12N 15/11, C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
NCBI PubMed, e-KIPASS, NDSL, "FRET, GMO, EPSPS, PCR, primer, design, etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KR 2001-0100216 A (Bioneer Corporation, KR) 14 Nov. 2001 - see the whole document	1 - 8
Y	WO 03/068964 (Hirohito Yamakawa, et al., JP) 21 Aug. 2003 - see the whole document	1 - 8
Y	Abdullag Karadag, et al., 'A novel technique based on a PNA hybridization probe and FRET principle for quantification of mutant genotype in fibrous dysplasia/McCune-Albright syndrome', In: Nucleic Acids Research, 19 April 2004, Vol.32(7) e63 - see the whole document	1 - 8
A	KR 2004-0012260 A (Bioneer Corporation, KR) 11 Feb. 2004 - see the whole document	1 - 8
A	Marc Vaitilingom, et al., 'Real-time quantitative PCR detection of genetically modified maximezer Maize and roundup ready soybean in some representative foods', In: J. Agric. Fddo Chem., 1999, Vol.47(12), p.5261-5266 - see the whole document	1 - 8
A	Irina Nazarenko, et al., 'Multiplex quantitative PCR using self-quenched primers labeled with a single fluorophore', In: Nucleic Acids Research, 2002, Vol.30(9) e37	1 - 8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 DECEMBER 2005 (21.12.2005)	**21 DECEMBER 2005 (21.12.2005)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer SHIN, Kyeong A Telephone No. 82-42-481-5589

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2005/003038

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2001-0100216 A	14 Nov. 2001	None	
WO 03/068964 A1	21 Aug. 2003	AU 2002332325 A1	04 Sep. 2003
		CA 2476557 A1	21 Aug. 2003
		CN 1620504 A	25 May 2005
		EP 1473364 A1	03 Nov. 2004
		KR 20040083427 A	01 Oct. 2004
KR 2004-0012260 A	11 Feb. 2004	None	