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(54) **METHODE DE DETECTION DE L'ACIDE NUCLEIQUE**

(54) **METHOD OF DETECTING NUCLEIC ACID**

(57) A method of detecting a nucleic acid which comprises bringing a solid carrier suspected to carry or contain a nucleic acid into contact with a polyamine to which a label capable of generating a detectable signal or a precursor of a label is bound, to form a complex between said nucleic acid and said polyamine, if a precursor is used, converting the precursor into said label, removing polyamine which has not formed a complex before or after the conversion of said precursor and detecting said label.

ABSTRACT

A method of detecting a nucleic acid which comprises bringing a solid carrier suspected to carry or contain a nucleic acid into contact with a polyamine to which a label capable of generating a detectable signal or a precursor of a label is bound, to form a complex between said nucleic acid and said polyamine, if a precursor is used, converting the precursor into said label, removing polyamine which has not formed a complex before or after the conversion of said precursor and detecting said label.

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METHOD OF DETECTING NUCLEIC ACID

The present invention relates to a method of detecting a nucleic acid using property in which a polyamine binds electrostatically to a nucleic acid.

A conventional method of detection of nucleic acids in gels and plates involves the use of ethidium bromide. This method exploits the intercalation of ethidium bromide into a nucleic acid. Subsequently, fluorescence measurements can be used to determine the location of a nucleic acid. A disadvantage of this kind of method is that fluorescence can only be detected in the dark, and the location of nucleic acids in a gel can only be detected by referencing the original gel to a photograph thereof. The photograph must be taken under ultraviolet radiation in the dark. In addition, ethidium bromide requires careful handling because of its strong carcinogenic activity. Takagi, Yasuhiro, Gene Operation Manual, p. 2, lines 16-21, Kodansha Scientific Press, (1982). Alternatively, methods using anionic gold colloid and cationic iron colloid cacodylate are also known. Saman, E., Gene Anal. Techn., Vol. 3, p. 1-5, "A Simple and Sensitive Method for Detection of Nucleic Acids Fixed on Nylon-based Filters", (1986). However, the sensitivity of such methods is low. In addition, since methods using gold colloid involves a kind of background staining system, detection of a nucleic acid is not easy and hybridization after the staining is inhibited. Similarly, a method using iron colloid, suffers the disadvantage that staining cannot be carried out after hybridization. The development of an easy, safe, simple and reproducible method for detecting a nucleic acid has been needed for a long time. It is already known that a polyamine can bind to DNA and also has affinity for RNA (cf. Biochemical Journal, 123, 811-815 (1967), Journal of Molecular Biology, 24, 113-122 (1967), ibid., 42, 363-373 (1969) and ibid., 121, 327-337 (1987)). In order to detect target polynucleotides, it is known to use probes which bond

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covalently to polynucleotides, complementary to proteins modified with polyamine (Japanese Patent Publication (in Japanese) A: TOKUKOHYOSHO 60-501488 (1985), to which corresponding, Japanese Patent Publication B: TOKUKOHEI 2-59720 (1990) and its divisional application, Japanese Patent Publication A: TOKUKAIHEI 1-124400 (1989)). However, a method of detecting a nucleic acid which comprises forming a complex of a labeled polyamine and a nucleic acid is not known.

SUMMARY OF THE INVENTION

In one aspect the invention provides

(1) a method of detecting a nucleic acid which comprises bringing a solid carrier suspected to carry or contain a nucleic acid into contact with a polyamine to which a label capable of generating a detectable signal or a precursor of such a label is bound, to form an electrostatically bonded complex between said nucleic acid and said polyamine, if a precursor of a label is used, converting the precursor to said label, removing polyamine which has not formed any electrostatically bonded complex before or after the conversion of said precursor and then detecting said label.

In a further aspect, the invention provides

(2) a method of differentiating a target nucleic acid from any nucleic acid other than said target nucleic acid which comprises combining the following steps (i) and (ii) in any order:

(i) bringing a solid carrier suspected to carry or contain a target nucleic acid, into contact with a probe to which a label capable of generating a detectable signal or a precursor of such a label is bound, the probe being capable of hybridizing with said target nucleic acid under hybridization conditions allowing a hybrid to form, if a precursor is used, converting the precursor into said label, removing probe which has not formed any hybrid before or after the conversion of said precursor and then detecting said label, and

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(ii) bringing a solid carrier suspected to carry or contain a target nucleic acid, into contact with a polyamine to which a label capable of generating a detectable signal or a precursor of such a label is bound, to form an electrostatically bonded complex between said nucleic acid and said polyamine, if a precursor is used, converting the precursor into said label, removing polyamine which has not formed any electrostatically bonded complex before or after the conversion of said precursor and then detecting said label.

In yet another aspect the invention provides

(3) A kit for detecting a nucleic acid comprising

(i) a polyamine enzyme conjugate

(ii) a chromogen which generates a signal when acted on enzymatically.

The terms used herein are illustrated as follows:

The term "a nucleic acid" means a polymer in which nucleotides consisting of a purine or pyrimidine base, a pentose and phosphoric acid as a basic unit are polymerized by ester linkages of the phosphoric acids. As a base, there are included adenine, cytosine, quanine, thymine and uracil, and modified bases thereof. Nucleic acids are distinguished by a sugar moiety, into DNA and RNA, by the number of strands, into single and double stranded nucleic acids and also by steric structure.

As a "solid carrier", there may be exemplified a thin plate, a sheet, a strip, a slab, film, membrane, etc. There are typically mentioned agarose gel, nylon membrane, filter paper, nitrocellulose membrane, etc.

The term "a label" means a substance which generates a measurable signal and includes an enzyme (in conjunction with a substrate), a spin compound, a radioactive nuclear atom, a fluorescent substance, a chemiluminescent substance, an absorption substance, etc. An enzyme is preferred. The enzyme mentioned is made of peroxidase, ß-galactosidase, glucose oxidase, alkaline phosphatase, glucose-6-phosphate

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dehydrogenase, etc. As preferred methods for determination of enzyme activity, there may be mentioned absorption method, fluorescence method and chemiluminescence methods. For example, in the absorption method, peroxidase is used in conjunction with a chromogen, such as 2,2'-azinodi(3-ethylbenzthiazolin)-6'-sulfonate (ABTS) and the color developed is measured by an absorption method. Glucose oxidase produces hydrogen peroxide in the presence of glucose as a substrate and therefore, it can be determined by conjugating with peroxidase, followed by the above-mentioned method for peroxidase. ß-Galactosidase can be determined using o-nitrophenyl-ß-D-galactoside as a substrate. Amongst fluorescence methods, mention is made of the determination of peroxidase using p-hydroxyphenylpropionic acid as a substrate. ß-Galactosidase can be determined using fluorescein-ß-galactosidase or 4-methylumbelliferyl-ß-D-galactoside as a substrate and phosphatase can be measured using umbelliferyl phosphate or bromochloroindolyl phosphate/nitroblue-tetrazolium as a substrate. In the chemiluminescence method, for example, peroxidase can be measured using luminol and hydrogen peroxide as substrates. A label (e.g. an enzyme) may be bound to a polyamine by a cross-linking agent such as benzoquinone (quinhydrone), bis[2-(succinimidocarbonyl-oxy)ethyl]sulfon (BSOCOES), bis(sulfosuccinimidyl)-suberate (BS3), 1,2-difluoro-2,4-dinitrobenzene (DFDNB), 4,4'-diisothiocyano-2,2'-disulfostilbene disodium salt (CDIDS), dimethyl adipin-imidate dihydrochloride, N-(γ-maleimidobutyryloxy)succinimide (GMBS), N-(4-azidophenylthio)-phthalimide (APTP), N-succinimidyl-6-(4'-azidophenyl)-1,3'-dithiopropionate (SADP), pyridine disulfide, thiophthalimide, etc.

The term "a precursor of a label" means, for example, a label blocked with a blocking substance. The precursor can be converted into the detectable label.

The term "signal" means visible light, fluorescence, radioactive radiation, or other electromagnetic waves and the

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like. A measured value of signal is to be related to an amount of a substance which emits the signal.

The term "detecting a label" means detecting a signal by physical or technical means.

The term "polyamine" herein means a compound which has an aliphatic backbone having 2 or more primary amino groups. This includes naturally occurring amines and synthetic amines. It is preferred that primary amino groups are at both ends of a chain of 3 to 50 carbon atoms, preferably, about 6 to about 15 or 20 carbon atoms. The chain may be interrupted by one or more imino groups. As a typical naturally occurring polyamine, there may be included 1,3-diaminopropane, putrescine, cadaverine, norspermidine, spermidine, homospermidine, aminopropylcadaverine, telmine, spermine, thermospermine, canavalmine, aminopentylnorspermidine, N,N'-bis(aminopropyl)cadaverine, caldopentamine, homocaldopentamine, caldohexamine, etc. A typical synthetic polyamine includes diethylenetriamine, triethylenetetramine, tetraethylenepentamine, pentaethylenehexamine, hexamethylene-diamine, polyethyleneimine (an average molecular weight is about 10,000 - 200,000, preferably, about 20,000 - 100,000, for example, Polymin™ G 35 by BASF AG, with a molecular weight of about 50,000 - 60,000.

The word "complex" (a verb) means to bind electrostatically and/or physically. The word "complex" (a noun) means an electrostatically and/or physically bonded product. In the context of this description, it is not meant to include covalent bonding. The "complexes" formed by "complexing" are generally reversible, and can be easily dissociated.

The term "hybridize" or "hybridization" means that, when two single-stranded polynucleotides are complementary or almost complementary (for example, more than 70%, preferably, more than 80 or 85%, especially, more than 90 or 95%), they align through Watson-Crick base pairing to form a double-stranded polynucleotide.

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"Under hybridization conditions" means the conditions under which polynucleotides capable of hybridizing can produce a hybrid. In general, the conditions include a temperature which is below about 70°C, preferably below about 60°C, generally below about 40 to 55°C and a period which is long enough for hybridization.

"Target" means a molecule which is to be detected or measured.

"Probe" is a DNA which is highly complementary to the target DNA and a part thereof and, may be shorter than the target DNA and has about 5 - about 50 bases, preferably about 10 - about 40 bases, for example, about 20 - about 30 bases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the result of detection of λDNA in Example 1.

Figure 2 shows the result of detection of Escherichia coli rRNA in Example 1.

Figure 3 represents a photograph of electrophoresis of λHind III DNA in Example 1.

Figure 4 represents a photograph of electrophoresis showing the result (the color development pattern according to the invention) of cleaving a DNA fragment inserted into pBR322 with a restriction enzyme.

Figure 5 represents a photograph of electrophoresis showing the result (the X-ray profile) of cleaving a DNA fragment inserted into pBR322 with a restriction enzyme.

Figure 6 represents a photograph of electrophoresis of λHind III DNA in Example 1.

Figure 7 like Figure 6 in Example 1 represents the photograph of electrophoresis of λHind III DNA in Example 1.

Figure 8 shows the difference in color in Figure 7.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A typical method of detecting a nucleic acid according to the invention may be summarized as follows:

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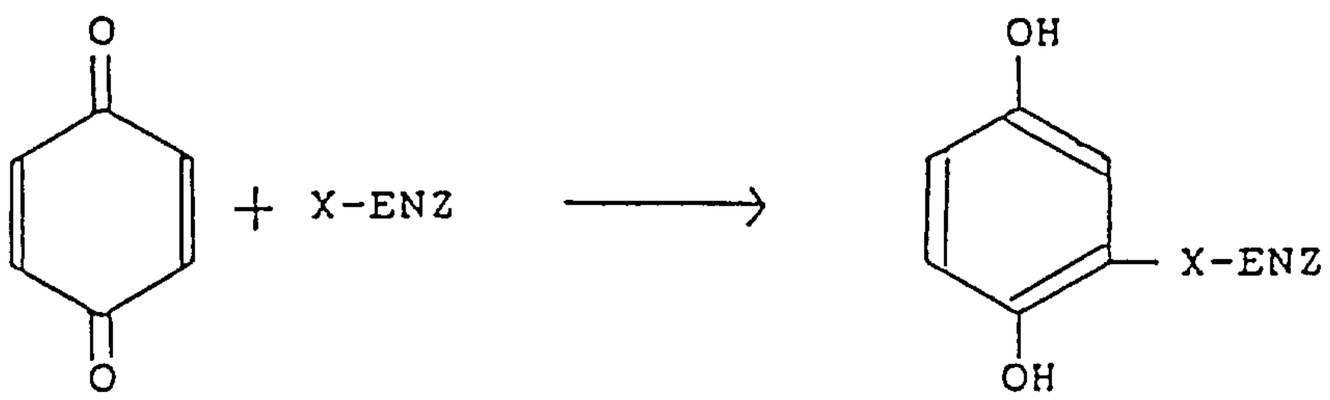
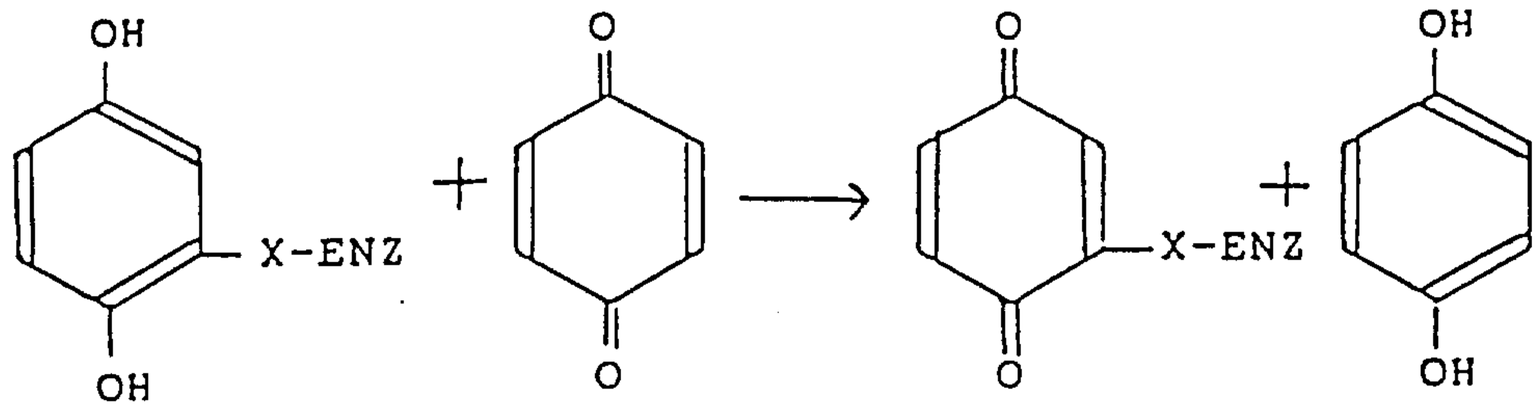
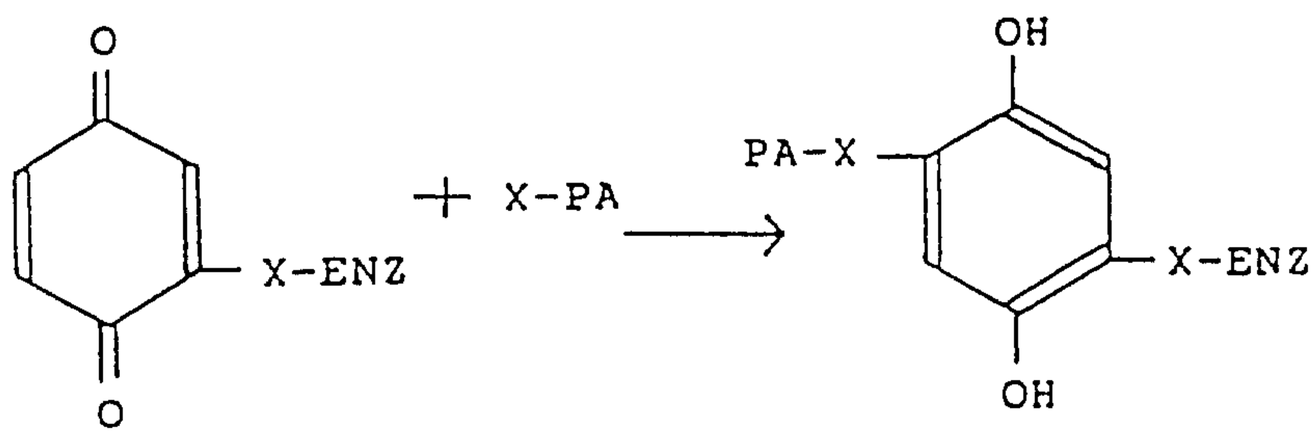
In an example of a complex consisting of a polyamine and an enzyme using benzoquinone, the reaction is considered to proceed according to the following scheme.







in which ENZ represents the radical resulting from removal of an amino group from an enzyme; PA represents the radical resulting from removal of an amino group from a polyamine; X represents a primary amino group or a secondary amino group.

The reaction is, for example, carried out as follows:

To benzoquinone (about 1 part) is added an enzyme (about 150 parts) which has been previously purified, (for example, by such a mean as dialysis). The reaction is preferably carried out at a somewhat elevated temperature to give a reaction mixture of wine-red color, which is subjected to such a method as gel permeation chromatography and fractionated into a purple, a yellow and a wine-red fraction. The wine-red fraction is reacted with a polyamine in the presence of a weak base while heating slightly to give an enzyme labeled-polyamine after suitable purification.

Detection of a nucleic acid is for example performed as follows:

(a) A nucleic acid specimen is dot-spotted on a membrane or is electrotransferred to a membrane after being subjected to gel electrophoresis and then the membrane is fixed by baking. After the membrane is treated with bovine serum albumin in a buffer, a labeled polyamine or a label-precursor labeled polyamine is applied. After washing, the label is detected, if a label-precursor is used, the label-precursor is converted into the label, and the label is then detected.

(b) DNA fragments are subjected to agarose gel electrophoresis and then Southern blotting. After prehybridization with radio-labeled probes while heating, the DNA fragments are hybridized. After washing, the position of DNA is detected using an X-ray film. Then the DNA is reacted with a labeled or a precursor-labeled polyamine and then a product thereof is developed with a suitable detection system.

(c) DNA fragments are fixed on a membrane by dot blotting or by Southern blotting after subjecting to agarose gel electrophoresis, and after prehybridization, are hybridized with Biotin DNA probes. After washing and blocking, the

resulting product is reacted with an enzyme-labeled streptavidin and after washing and preincubation, in addition, another enzyme-labeled polyamine is reacted therewith. After washing, the resultant hybrid is subjected to color developments for each enzyme to give two different color patterns corresponding to hybridized DNA and non-hybridized DNA. For example, alkaline phosphatase/BCIP-NBT and peroxidase/DAB systems are developed into purple and brown, respectively. In such a method, the order of treatments with a probe and a polyamine may be reversed.

Such membrane as treated above develop color by itself, and not as an X-ray film and therefore, a subject nucleic acid can be detected directly on the membrane. In addition, in PCR method, amplification products having similar molecular weights, but having different sequences, can be distinguished from each other by hybridization.

The method of detecting a nucleic acid according to the invention has the following advantages:

The method requires no dangerous reagents and has a high sensitivity (i.e. in the order of picogram in DNA and 10 picogram in RNA). The use of the method is very easy, and if the reagents to be used in the method are incorporated into a kit, it becomes easier.

Examples

The present invention is illustrated by the following specific embodiments.

Example 1

Production of peroxidase- or alkaline phosphatase-labeled polyethyleneimine

Alkaline phosphatase (CIP, Boehringer-Mannheim GmbH, Grade 1), 0.81 ml/4.05 mg/7500 unit or horseradish peroxidase (Boehringer-Mannheim GmbH) was dialyzed against 0.1 M phosphate buffer at 40°C over night and according to a method as described in Immuno Chemistry, Vol. 14, 767-774 (1977), was reacted with p-benzoquinone (120 µl/30 mg/ethanol) at 37°C for 60 minutes in the dark. After gel permeation chromatography

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on Sephadex™ G-25, wine-red fractions (2.7 ml) were taken up and 300 μl of 1M-$NaHCO_3$ (pH 9.0), 30 μl of polyethyleneimine (Epomin) were added thereto and thoroughly mixed. The resultant mixture was reacted at 37°C in the dark during one night, and then dialyzed against 5 mM phosphate buffer (pH 6.8) to give an enzyme-polyethyleneimine-benzoquinone conjugate, which was stable at 4°C for more than 1 year.

Nucleic acid blotting on membrane

Denatured λHind III DNA or Escherichia coli K-12 rRNA was dot blotted on a nylon membrane (Biodyne™ A, Pall Co. Ltd.) (Maniatis, T., Fritsch, E.F. and Sambrook, J., Molecular Cloning, A Laboratory Manual, p. 382-389, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA (1982). A clone of Staphylococcus aureus genome DNA, which was randomly cloned into a vector pBR322, was amplified according to a method known to those skilled in the art, extracted and then cleaved with Hind III. Specimens were fractionated by 1% agarose gel electrophoresis and the isolates were electro-blotted on a membrane (40V, for 4 hours). λHind III DNA was also blotted as above. The DNA or RNA specimen was baked (80°C, 2 hours) to fix on the membrane.

Detection of nucleic acid blotted on membrane

A membrane containing fixed DNA or RNA was soaked in 5mM phosphate buffer-1% bovine serum albumin (BSA, Sigma, Fraction V), incubated at room temperature for 60 minutes and alkaline phosphatase (or peroxidase)-labeled polyethyleneimine was added in a ratio of 100 μl/20 ml thereto and incubated at 37°C for 120 minutes. The membrane was washed 3 times with 5mM phosphate buffer-1% BSA-1% Tween™ 20 for 10 minutes and rinsed with 0.1 M Tris HCl (pH 9.5)-10 mM-$MgCl_2$.

The membrane was developed with 50 ml of a staining solution consisting of 0.1M Tris HCl (pH 9.5)-10 mM-$MgCl_2$ containing each 300 μl of bromochloroindolyl phosphate (BCIP: 75 mg/ml) and nitroblue tetrazolium (NBT: 50 mg/ml).

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Southern Blotting Hybridization

A Nick translation reaction was performed using Staphylococcus aureus genome kbp fragment DNA according to a conventional technique with ^{32}P-dCTP or Biotin-11-dUTP (BRL) to prepare ^{32}P-DNA or Biotin-DNA. The membrane was treated for hybridization with hybridization buffer (5 x SSC, 0.1% BSA, 0.1% Tween™ 20) containing denatured probes at 42 - 55°C during one night. Then the membrane was washed with 0.16 x SSC-0.1% Tween™ 20 at 55°C for 30 minutes (the endpoint of washing is referred to as "the point A").

When ^{32}P-DNA probe was used, at the point A, the moist membrane was wrapped with polyethylene film and the signal detected by exposure to an X-ray film. Then, the membrane was treated for blocking with 3% BSA - 0.1% Tween™ 20 - phosphate buffer saline (PBS) at 42°C for more than 3 hours, followed by adding 0.1% BSA - 0.1% Tween™ - PBS containing 100 μl of peroxidase-labeled polyethyleneimine per sheet, and reacting the membrane for 2 - 3 hours. The membrane was washed 3 times for 20 minutes with 0.1% Tween™ 20 - PBS and developed with a diaminobenzidine (DAB) - H_2O_2 system.

When a Biotin - DNA probe was used, after the point A, the membrane was treated with PBS - 0.5% Tween™ 20 at room temperature for 60 minutes. After treatment with PBS - 0.05% Tween™ 20 containing alkaline phosphatase-labeled streptavidine at room temperature for 20 minutes, the membrane was washed 2 times with PBS - 0.5% Tween™ 20 for 10 minutes. After preincubation with PBS - 0.1% BSA - 0.1% Tween™ 20 at room temperature for 20 minutes, and addition of peroxidase-labeled polyethyleneimine thereto, the membrane was reacted at 42°C for 3 hours. After the membrane was washed 3 times with PBS - 0.1% Tween™ 20 for 10 minutes, it was rinsed with 0.1 M Tris HCl (pH 9.5)-10 mM-$MgCl_2$ and then developed with BCIP - NBT system at room temperature for 20 minutes. The membrane was stored after washing with PBS - 0.1% Tween™ 20.

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Results

(i) λDNA or E. coli rRNA was dot-spotted with detected by alkaline phosphatase-labeled polyethyleneimine. The results are shown in Figures 1 and 2. The amounts of λDNA spots are (1) 4.7 μg, (2) 470 ng, (3) 47 ng, (4) 4.7 ng, (5) 470 pg, (6) 47 pg, (7) 4.7 pg, (8) 470 fg from the top in Fig. 1. The amounts of γRNA spots are (1) 5 μg, (2) 500 ng, (3) 50 ng, (4) 5 ng, (5) 500 pg, (6) 50 pg from the top in Fig. 2.

(ii) After serial dilutions, λHind III DNA was fractionated by agarose gel electrophoresis and electroblotted from the gel to a membrane, each specimen was detected by alkalinephosphatase-labeled polyethyleneimine. The results are shown in Fig. 3. The results show that the profile obtained by an ethidium bromide method after agarose gel electrophoresis is identical to the pattern obtained by electroblotting of the specimen of the serial dilution (0.06 μg), and thus confirm the results obtained with the method of the invention.

(iii) DNA fragments inserted in pBR322, containing a size marker of λHind III DNA, were split with a restriction enzyme, fractionated by agarose gel electrophoresis, transferred onto a membrane by electroblotting, and hybridized with ^{32}P-DNA probe. The membrane was exposed to an X-ray film to obtain signals (Fig. 5). In order to identify a kind of hybridized DNA, the membrane was treated with peroxidase-labeled polyethyleneimine and positions of all blotted DNA were visualized (Fig. 4).

(iv) The DNA fragments which were treated as described in step (iii) were hybridized with a Biotin DNA probe system to simultaneously detect both hybridized DNA and non-hybridized DNA. By suitable choice of Biotin DNA probe and each enzyme in alkalinephosphatase-labeled streptavidin and peroxidase-labeled polyethyleneimine, hybridized DNA and a non-hybridized DNA were differentiated by a double staining system. The results are shown in Fig. 7 and the difference in color is shown in Fig. 8. In the figure, the shaded portion represents brown color (the peroxidase-labeled polyethyleneimine system)

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and the black portion represents purple color. The patterns were identical to the results (Fig. 6) obtained by ethidium bromide staining after the same treatment as described above.

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CLAIMS

1. A method of detecting a nucleic acid which comprises bringing a solid carrier suspected to carry or contain a nucleic acid into contact with a polyamine to which a label capable of generating a detectable signal or a precursor of a label is bound, to form an electrostatically bonded complex between said nucleic acid and said polyamine, converting a precursor into said label if a precursor is used, removing polyamine which has not formed an electrostatically bonded complex before or after the conversion of said precursor, and then detecting said label.

2. The method of detecting a nucleic acid according to claim 1, wherein said label capable of generating a detectable signal or a precursor of a label is an enzyme.

3. The method of detecting a nucleic acid according to claim 2, wherein said enzyme is alkaline phosphatase or peroxidase.

4. The method of detecting a nucleic acid according to claim 2 or 3, wherein said enzyme is bound to said polyamine through a cross-linking agent.

5. The method of detecting a nucleic acid according to claim 4, wherein the cross-linking agent is benzoquinone.

6. The method of detecting a nucleic acid according to any one of claims 1 to 5, wherein said polyamine is a naturally occurring polyamine or a synthetic polyamine, having an aliphatic chain of 3 to 50 carbon atoms and primary amino groups at both ends of said chain, and the chain may be interrupted by one or more imino groups.

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7. The method of detecting a nucleic acid according to any one of claims 1 to 6, wherein said polyamine is a polyamine having an aliphatic chain of 6 to 15 carbon atoms.

8. The method of detecting a nucleic acid according to any one of claims 1 to 7, wherein said polyamine is a synthetic polyamine.

9. The method of detecting a nucleic acid according to any one of claims 1 to 5, wherein said polyamine is polyethyleneimine.

10. A method of distinguishing a target nucleic acid from any nucleic acid other than said target nucleic acid comprising the following steps (i) and (ii) in any order,

(i) bringing a solid carrier suspected to carry or contain a target nucleic acid, into contact with a DNA probe to which a label capable of generating a detectable signal or a precursor of a label is bound, the DNA probe being capable of hybridizing with said target nucleic acid under hybridization conditions, to form a hybrid, converting a precursor into said label if a precursor is used, removing probe which has not formed any hybrid before or after the conversion of said precursor and detecting said label, and

(ii) bringing a solid carrier suspected to carry or contain a target nucleic acid into contact with a polyamine to which a label capable of generating a detectable signal or a precursor of a label is bound, to form an electrostatically bonded complex between said nucleic acid and said polyamine, converting the precursor into said label if a precursor is used, removing polyamine which has not formed any electrostatically bonded complex, before or after the conversion of said precursor, and detecting said label.

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11. The method of distinguishing a nucleic acid according to claim 10, wherein step (i) is carried out by Southern blot hybridization.

12. The method of distinguishing a nucleic acid according to claim 10, wherein said label capable of generating a detectable signal or the precursor of a label is an enzyme.

13. The method of distinguishing a nucleic acid according to claim 12, wherein the enzyme is alkaline phosphatase or peroxidase.

14. The method of distinguishing a nucleic acid according to claim 12 or 13, wherein said enzyme is bound to said polyamine through a cross-linking agent.

15. The method of distinguishing a nucleic acid according to claim 14, wherein said cross-linking agent is benzoquinone.

16. The method of distinguishing a nucleic acid according to any one of claims 10 to 15, wherein said polyamine is a naturally occurring polyamine or a synthetic polyamine, having an aliphatic chain of 3 to 50 carbon atoms and primary amino groups at both ends of said chain, and the chain may be interrupted by one or more imino groups.

17. The method of distinguishing a nucleic acid according to any one of claims 10 to 16, wherein said polyamine is a polyamine having an aliphatic chain of 6 to 15 carbon atoms.

18. The method of distinguishing a nucleic acid according to any one of claims 10 to 17, wherein said polyamine is a synthetic polyamine.

19. The method of distinguishing a nucleic acid according to any one of claims 10 to 15, wherein said polyamine is polyethyleneimine.

20. A kit for detecting a nucleic acid comprising

(i) a polyamine-enzyme conjugate and

(ii) a chromogen which generates a signal when acted on enzymatically.

21. The kit for detecting a nucleic acid according to claim 20, wherein said enzyme is alkaline phosphatase or peroxidase.

22. The kit for detecting a nucleic acid according to claim 20, wherein said enzyme is bound to said polyamine through a cross-linking agent.

23. The kit for detecting a nucleic acid according to claim 22, wherein said cross-linking agent is benzoquinone.

24. The kit for detecting a nucleic acid according to claim 20 or 22, wherein said polyamine is a naturally occurring polyamine or a synthetic polyamine, having an aliphatic chain of 3 to 50 carbon atoms and primary amino groups at both ends, and said chain may be interrupted by one or more imino groups.

25. The kit for detecting a nucleic acid according to claim 24, wherein said polyamine is a polyamine having an aliphatic chain of 6 to 15 carbon atoms.

26. The kit for detecting a nucleic acid according to claim 25, wherein said polyamine is a synthetic polyamine.

27. The kit for detecting a nucleic acid according to claim 26, wherein said polyamine is polyethyleneimine.

Fig. 1

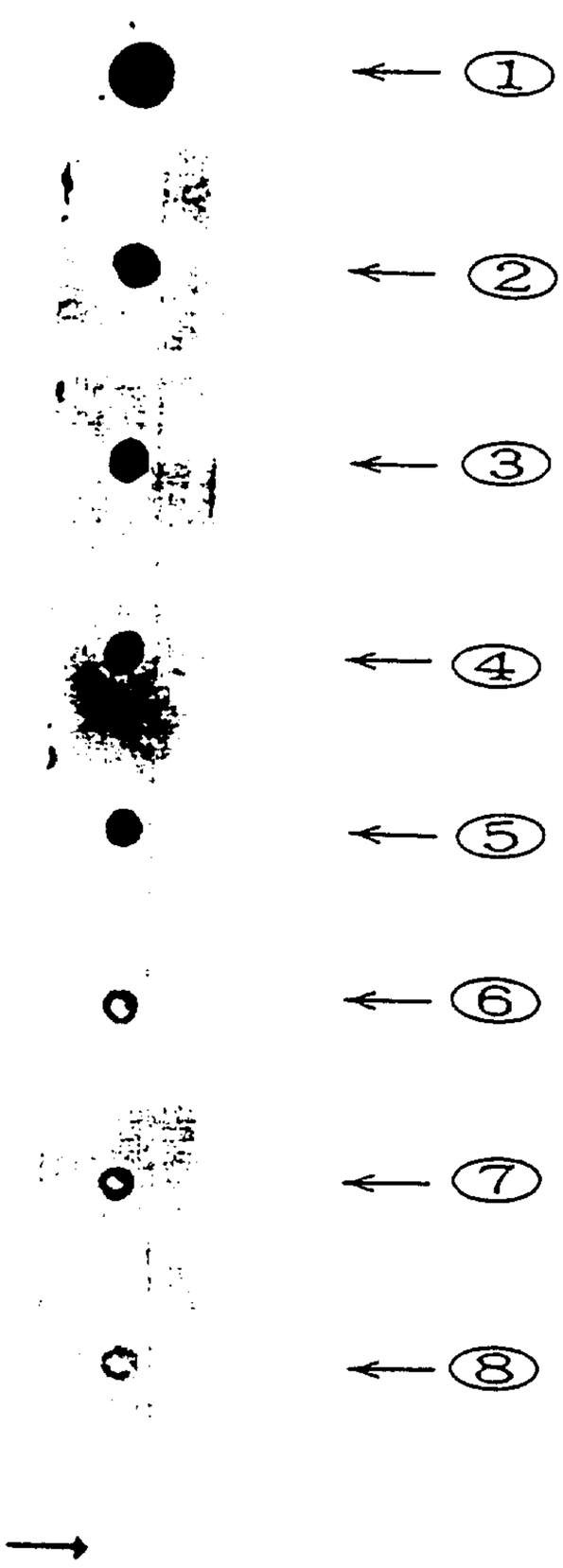
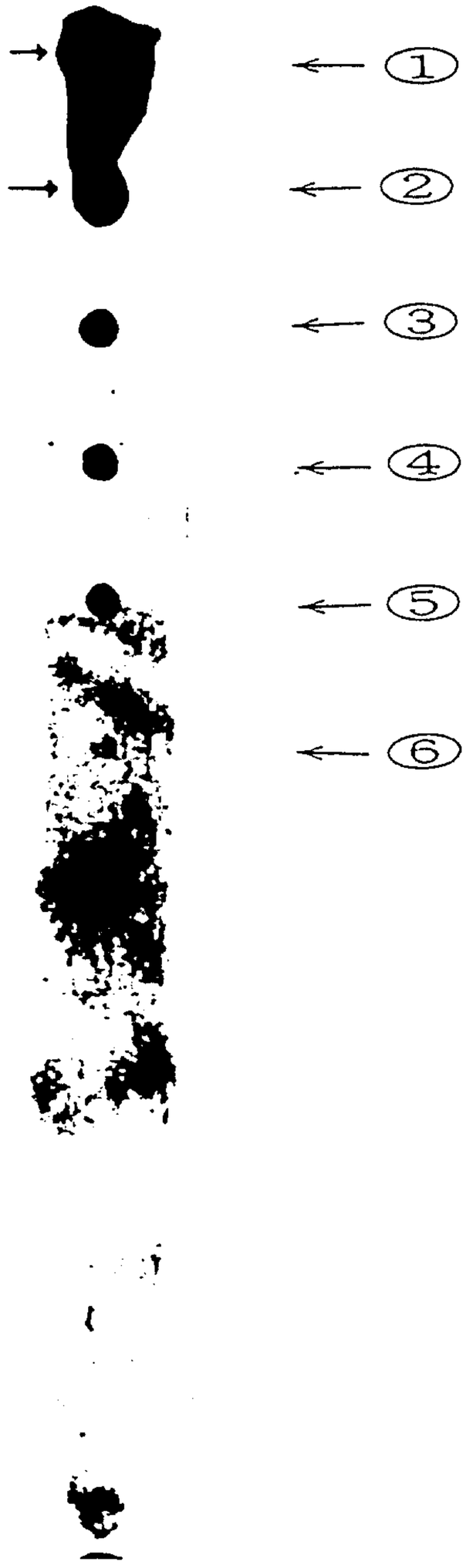


Fig. 2



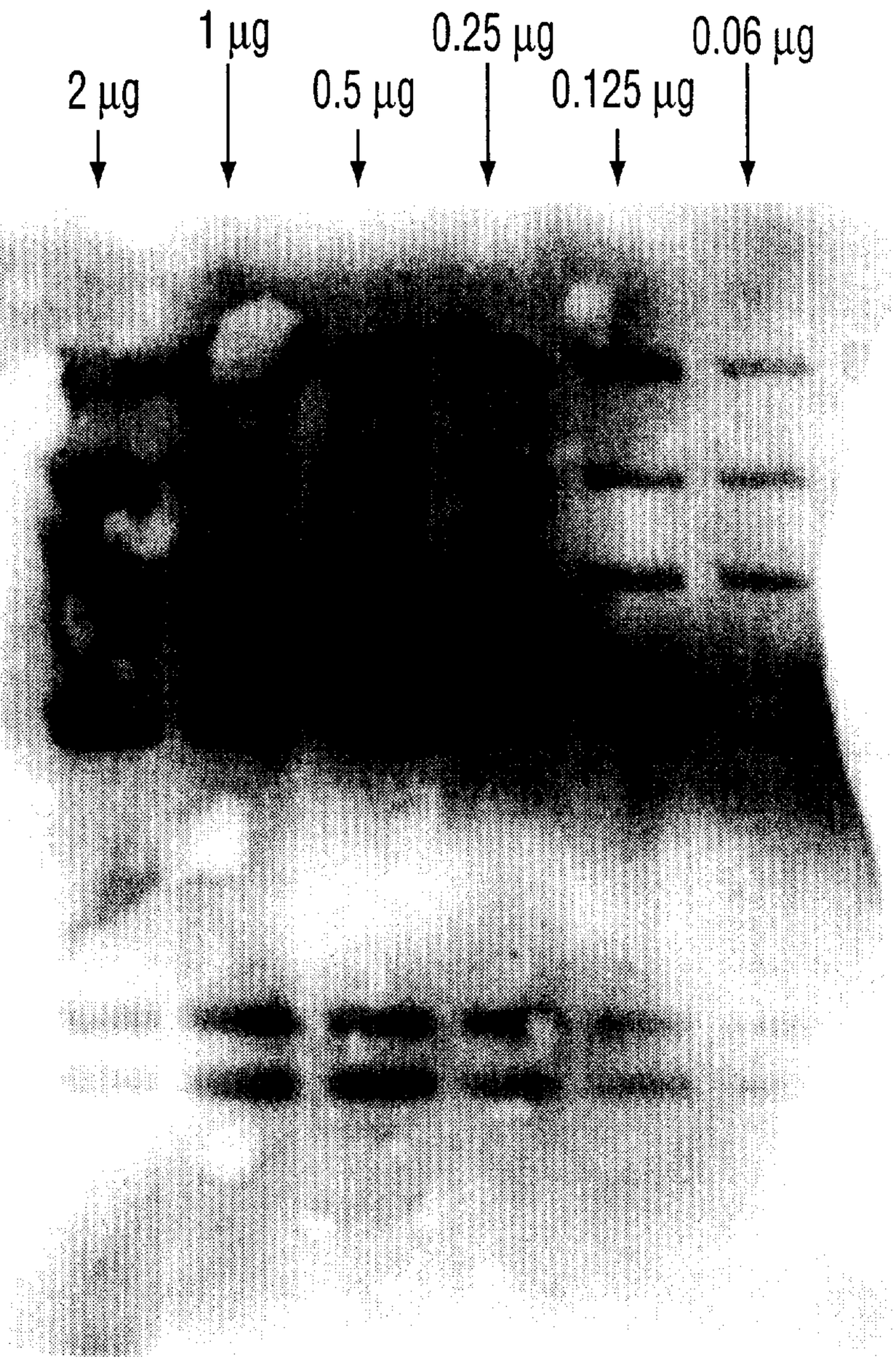


FIG. 3

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Fig. 4

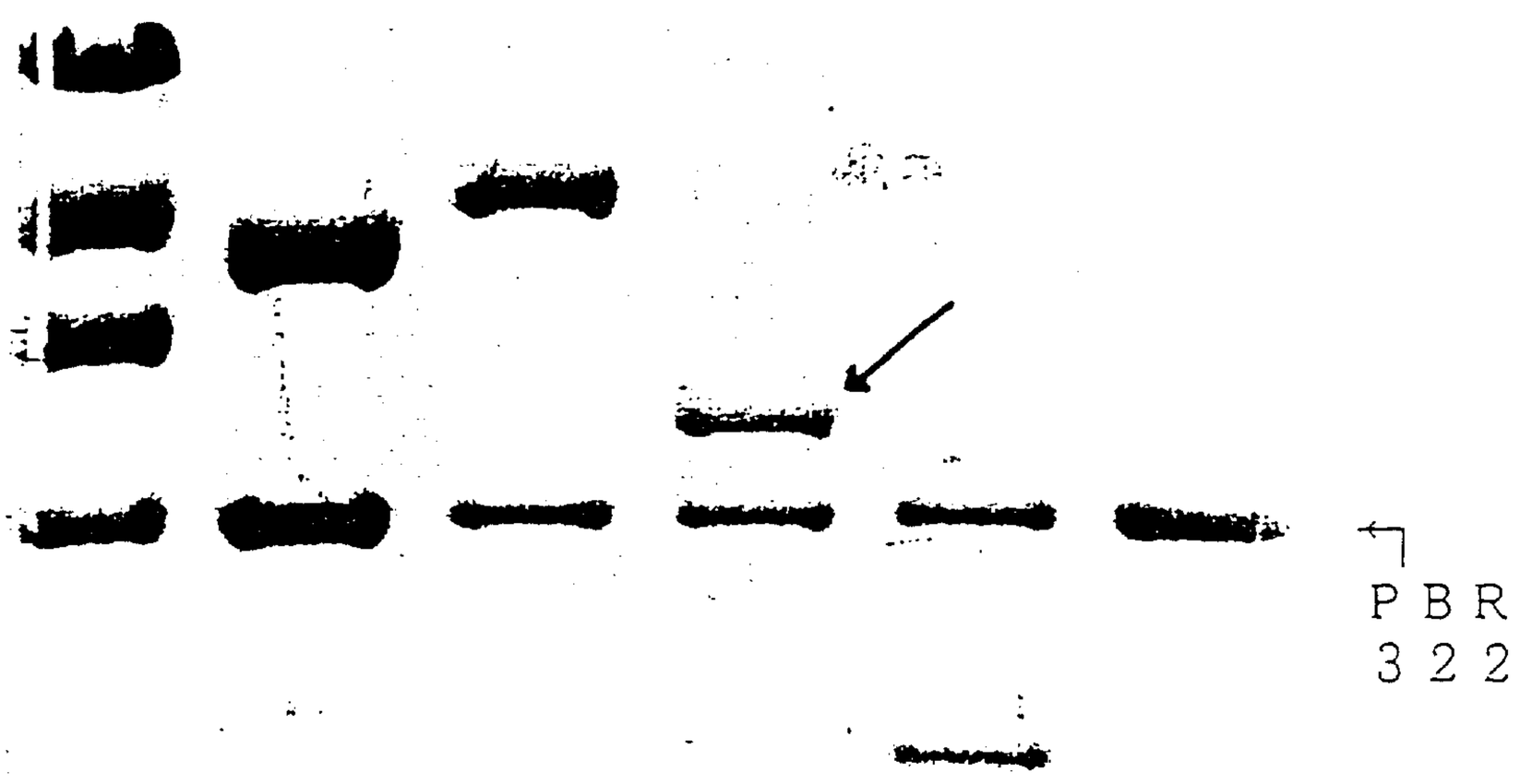
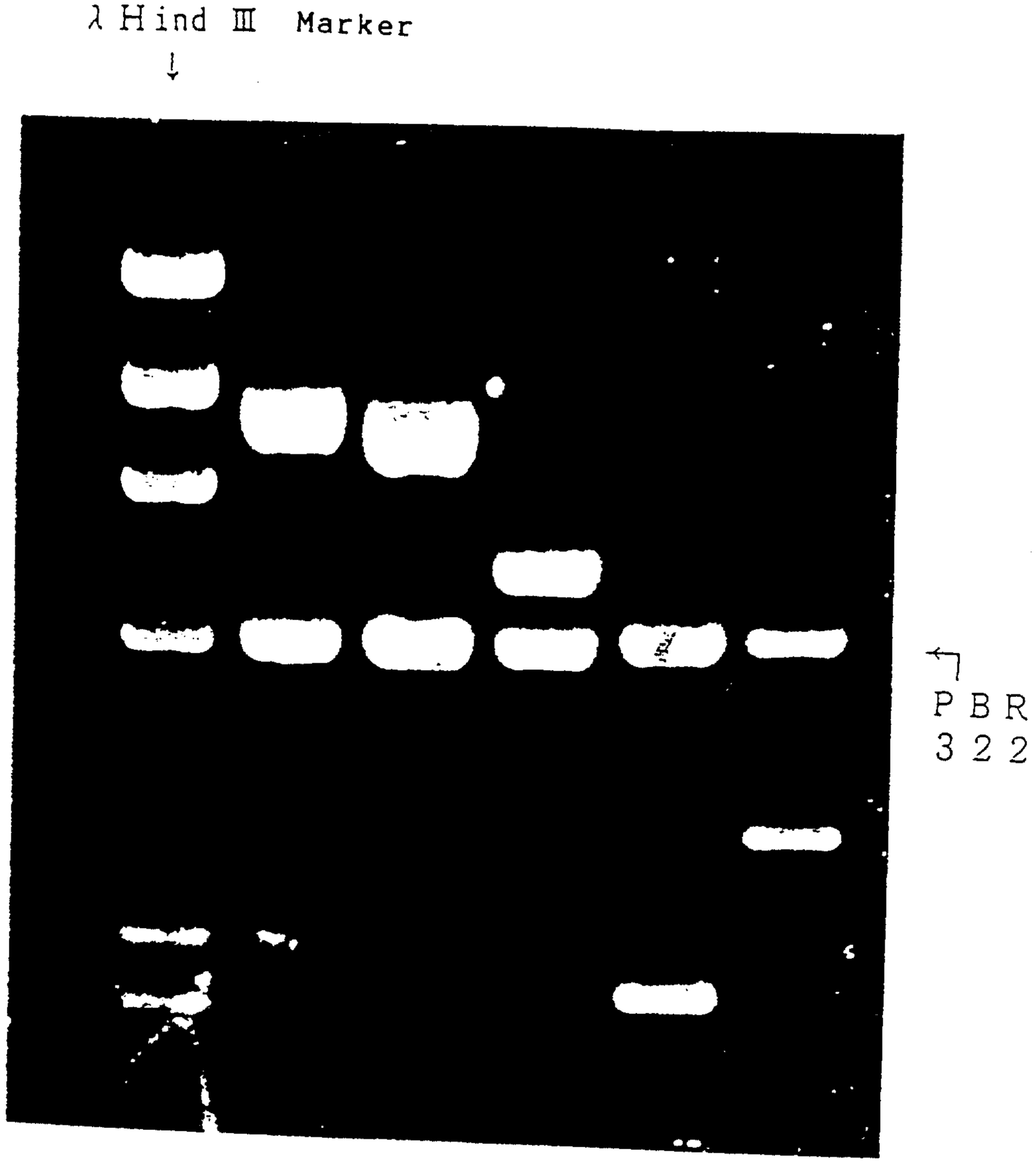


Fig. 5

Fig. 6



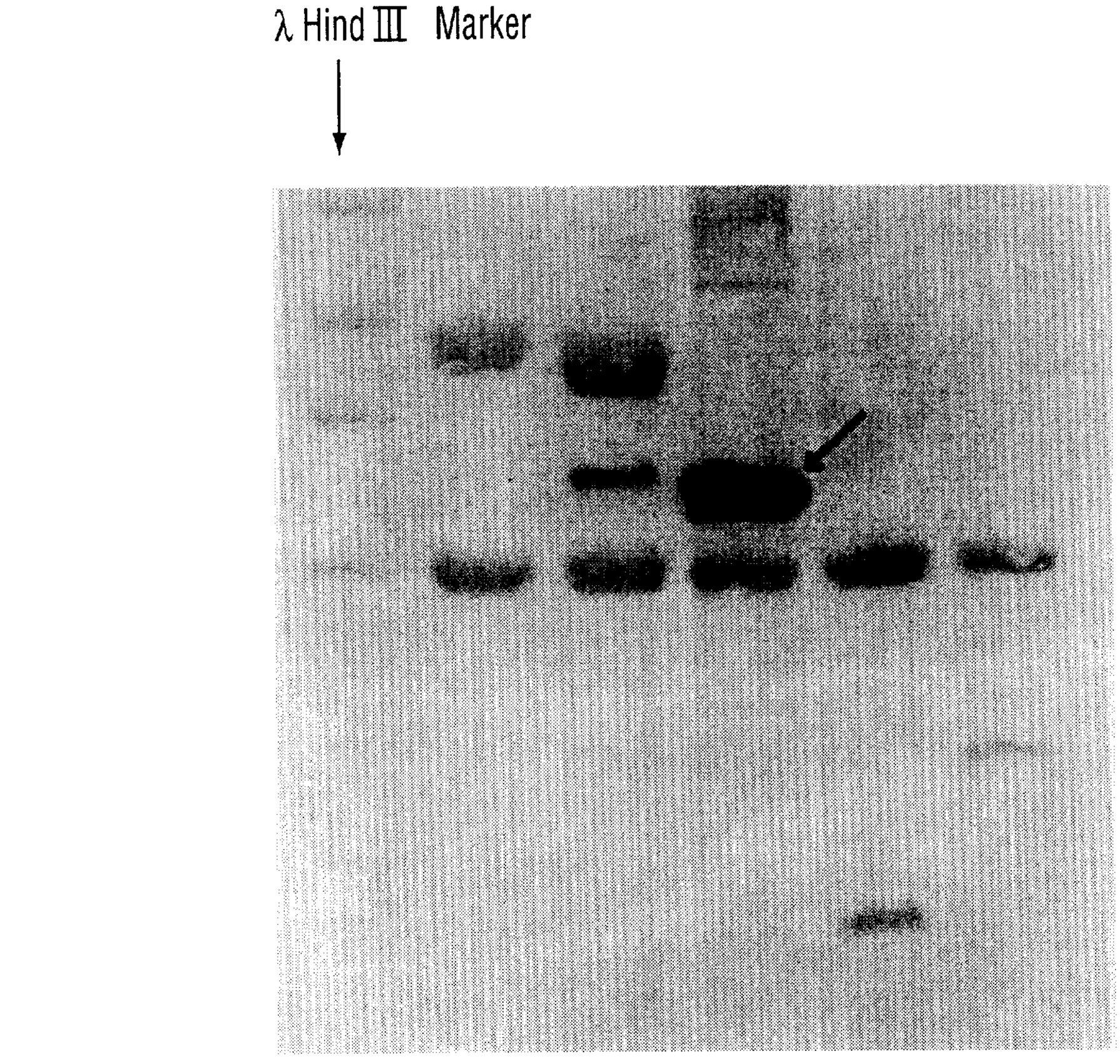


FIG. 7

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Fig. 8