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(54) **SYSTEM, ARRAY AND NON-POROUS SOLID SUPPORT COMPRISING FIXED OR IMMOBILIZED NUCLEIC ACIDS**

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(63) Continuation of application No. 07/967,646, filed on Oct. 28, 1992, now abandoned, which is a continuation of application No. 07/607,347, filed on Oct. 30, 1990, now abandoned, which is a continuation of application No. 07/385,986, filed on Jul. 20, 1989, now Pat. No. 4,994,373, which is a continuation of application No. 06/732,374, filed on May 9, 1985, now abandoned, which is a continuation-in-part of application No. 06/461,469, filed on Jan. 27, 1983, now abandoned.

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See application file for complete search history.

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(57) **ABSTRACT**

Nucleic acids are fixed or immobilized to non-porous solid supports (substrates), and include systems containing such supports and arrays with fixed or immobilized nucleic acids. These compositions are useful for nucleic acid analyses and a host of applications, including, for example, detection, mutational analysis and quantification. The non-porous solid supports can be transparent or translucent, and the surfaces can be treated with agents to fix or immobilize the nucleic acids. Such agents include, for example, amine providing compounds, epoxy compounds and acid solutions. The fixed or immobilized nucleic acids can be unlabeled, or labeled with at least one non-radioactive signaling moiety, such as the case when the nucleic acids are double-stranded.

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SYSTEM, ARRAY AND NON-POROUS SOLID SUPPORT COMPRISING FIXED OR IMMOBILIZED NUCLEIC ACIDS

CROSS-REFERENCE TO OTHER RELATED APPLICATIONS

This is a continuation application of U.S. Patent Application Ser. No. 07/967,646, filed on Oct. 28, 1992, now abandoned, which application is a continuation application of U.S. Patent Application Ser. No. 07/607,347, filed on Oct. 30, 1990, also abandoned. Ser. No. 07/607,347 is a continuation of U.S. Patent Application Ser. No. 07/385,986, filed on Jul. 20, 1989, now U.S. Pat. No. 4,994,373 issued on Feb. 19, 1991. Ser. No. 07/385,986 is a continuation of U.S. Patent Application Ser. No. 06/732,374, filed on May 9, 1985, also abandoned, which application is a continuation-in-part of U.S. Patent Application Ser. No. 06/461,469, filed on Jan. 27, 1983, also abandoned.

TECHNICAL FIELD OF INVENTION

The present invention relates generally to the detection of genetic material by polynucleotide probes. More specifically, it relates to a method for quantifiably detecting a targeted polynucleotide sequence in a sample of biological and/or nonbiological material employing a probe capable of generating a soluble signal. The method and products disclosed herein in accordance with the invention are expected to be adaptable for use in many laboratory, industrial, and medical applications wherein quantifiable and efficient detection of genetic material is desired.

BACKGROUND OF THE INVENTION

In the description, the following terms are employed:

Analyte—A substance or substances, either alone or in admixtures, whose presence is to be detected and, if desired, quantitated. The analyte may be a DNA or RNA molecule of small or high molecular weight, a molecular complex including those molecules, or a biological system containing nucleic acids, such as a virus, a cell, or group of cells. Among the common analytes are nucleic acids (DNA and RNA) or segments thereof, oligonucleotides, either single- or double-stranded, viruses, bacteria, cells in culture, and the like. Bacteria, either whole or fragments thereof, including both gram positive and gram negative bacteria, fungi, algae, and other microorganisms are also analytes, as well as animal (e.g., mammalian) and plant cells and tissues.

Probe—A labelled polynucleotide or oligonucleotide sequence which is complementary to a polynucleotide or oligonucleotide sequence of a particular analyte and which hybridizes to said analyte sequence.

Label—That moiety attached to a polynucleotide or oligonucleotide sequence which comprises a signalling moiety capable of generating a signal for detection of the hybridized probe and analyte. The label may consist only of a signalling moiety, e.g., an enzyme attached directly to the sequence. Alternatively, the label may be a combination of a covalently attached bridging moiety and signalling moiety or a combination of a non-covalently bound bridging moiety and signalling moiety which gives rise to a signal which is detectable, and in some cases quantifiable.

Bridging Moiety—That portion of a label which on covalent attachment or non-covalent binding to a polynucle-

Signalling Moiety—That portion of a label which on covalent attachment or non-covalent binding to a polynucleotide or oligonucleotide sequence or to a bridging moiety attached or bound to that sequence provides a signal for detection of the label.

Signal—That characteristic of a label or signalling moiety that permits it to be detected from sequences that do not carry the label or signalling moiety.

The analysis and detection of minute quantities of substances in biological and non-biological samples has become a routine practice in clinical, diagnostic and analytical laboratories. These detection techniques can be divided into two major classes: (1) those based on ligand-receptor interactions (e.g., immunoassay-based techniques), and (2) those based on nucleic acid hybridization (polynucleotide sequence-based techniques).

Immunoassay-based techniques are characterized by a sequence of steps comprising the non-covalent binding of an antibody and antigen complementary to it. See, for example, T. Chard, *An Introduction To Radioimmunoassay And Related Techniques* (1978).

Polynucleotide sequence-based detection techniques are characterized by a sequence of steps comprising the non-covalent binding of a labelled polynucleotide sequence or probe to a complementary sequence of the analyte under hybridization conditions in accordance with the Watson-Crick base pairing of adenine (A) and thymine (T), and guanine (G) and cytosine (C), and the detection of that hybridization. [M. Grunstein and D. S. Hogness, "Colony Hybridization: A Method For The Isolation Of Cloned DNAs That Contain A Specific Gene", *Proc. Natl. Acad. Sci. USA,* 72, pp. 3961–65 (1975)]. Such polynucleotide detection techniques can involve a fixed analyte [see, e.g., U.S. Pat. No. 4,358,535 to Falkow et al], or can involve detection of an analyte in solution [see U.K. patent application 2,019, 408 A].

The primary recognition event of polynucleotide sequence-based detection techniques is the non-covalent binding of a probe to a complementary sequence of an analyte, brought about by a precise molecular alignment and interaction of complementary nucleotides of the probe and analyte. This binding event is energetically favored by the release of non-covalent bonding free energy, e.g., hydrogen bonding, stacking free energy and the like.

In addition to the primary recognition event, it is also necessary to detect when binding takes place between the labelled polynucleotide sequence and the complementary sequence of the analyte. This detection is effected through a signalling step or event. A signalling step or event allows detection in some quantitative or qualitative manner, e.g., a human or instrument detection system, of the occurrence of the primary recognition event.

The primary recognition event and the signalling event of polynucleotide sequence based detection techniques may be coupled either directly or indirectly, proportionately or inversely proportionately. Thus, in such systems as nucleic acid hybridizations with sufficient quantities of radiolabeled probes, the amount of radio-activity is usually directly proportional to the amount of analyte present. Inversely proportional techniques include, for example, competitive immuno-assays, wherein the amount of detected signal decreases with the greater amount of analyte that is present in the sample.

Amplification techniques are also employed for enhancing detection wherein the signalling event is related to the

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