cytiva

TECHNOLOGY LICENSING OPPORTUNITIES

GENOMICS RELATED LICENSING OPPORTUNITIES: SEQUENCING & DETECTION

Sequencing and Analyte Detection Platform

Cytiva has developed a broadly applicable platform technology for sequencing and analyte detection applications enabled by a new class of labelled-nucleotide poly-phosphate analogues. These analogues are superior substrates for polymerases leading to higher-throughput sequencing-related workflows.

The technology is highly suitable for:

- Rapidly sequencing long DNA lengths
- Single molecule applications

The portfolio comprises nine patent families and includes new compositions of matter for labelled nucleosides polyphosphates and new methods for sequencing and real-time nucleic acid detection, characterization and quantification.

These technologies can be licensed as a package or on an individual basis.

Terminal-Phosphate-Labelled Nucleotides and Methods of Use

Summary

Increasing the number of phosphate units of a terminal-phosphate-labeled nucleoside polyphosphate from three to 4 or more increases their incorporation efficiency by polymerases. The labels employed are chemiluminescent, fluorescent, electrochemical and chromophoric moieties as well as mass tags and include those that are directly detectable, detectable after enzyme activation or feed into other processes to generate a different signal. Methods of using these nucleotides by nucleic acid polymerases for detection, characterization or quantification of DNA or RNA have been identified.

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Background

Methods are known for detecting specific nucleic acids or analytes in a sample with high specificity and sensitivity. Such methods generally require first amplifying the nucleic acid sequence based on the presence of a specific target sequence or analyte. Following amplification, the amplified sequences are detected and quantified. Conventional detection systems for nucleic acids include detection of fluorescent labels, colored dyes, fluorescent enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.

One disadvantage of currently used detection methods is the need to separate labeled starting materials from a final labeled product or by-product. Such separations generally require gel electrophoresis or immobilization of a target sequence onto a membrane for detection. Moreover, there are often numerous reagents and/or incubation steps required for detection.

DNA and RNA polymerases are able to recognize and utilize nucleosides with a modification at or in place of the gamma position of the triphosphate moiety. It is also known that the ability of various polymerases to recognize and utilize gamma-modified nucleotide triphosphates (NTP's) appears to vary depending on the moiety attached to the gamma phosphate. In general, RNA polymerases are more promiscuous than DNA polymerases. Even so, the efficiency of incorporation is significantly reduced compared to normal nucleotides.

There is a need for terminal phosphate labeled nucleoside polyphosphates with readily detectable labels and which are better substrates for nucleic acid polymerases.

It would also be desirable to provide nucleoside polyphosphates that are substrates for polymerases where the label on the terminal-phosphate could be varied to allow for chemiluminescent and fluorescent detection, analysis by mass or reduction potential, as well as for improved calorimetric detection, enabling the use of routine methods and instrumentation for detection.

Technology

This Cytiva technology provides new compositions of matter in the form of labeled nucleoside polyphosphates with four or more phosphates. In addition compositions of nucleoside polyphosphates with four or more phosphates that are substrates for nucleic acid polymerases with enhanced substrate properties and methods of using these nucleoside polyphosphates for nucleic acid detection, characterization and quantification are described. The compositions include nucleoside polyphosphate, dideoxynucleoside polyphosphate, or deoxynucleoside polyphosphate analogues which have colorimetric, chemiluminescent, or fluorescent moieties, mass tags or an electrochemical tags attached to the terminal-phosphate. When a nucleic acid polymerase uses this analogue as a substrate, an enzyme-activatable label would be present on the inorganic polyphosphate by-product of phosphoryl transfer. Removal of the polyphosphate product of phosphoryl transfer via phosphate or polyphosphate transferring enzyme leads to a detectable change in the label

attached thereon. When the polymerase assay is performed in the presence of a phosphatase, it allows for a convenient method for real-time monitoring of DNA or RNA synthesis and detection of a target nucleic acid.

Terminal-Phosphate-Labelled Nucleotides and Methods of Use

Summary

Improved methods of detecting a target using a labeled substrate or substrate analog. The improvement involves reacting the substrate or substrate analog in an enzyme-catalyzed reaction which produces a labeled moiety with independently detectable signal only when such substrate or substrate analog reacts. The invention principally relates to methods of detecting a polynucleotide in a sample, based on the use of terminal-phosphate-labeled nucleotides including three or more phosphates as substrates for nucleic acid polymerases. The labels employed are dyes, which undergo a chemical change and become a fluorescent or color producing reagent only upon the action of the polymerase.

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Background

Methods are known for detecting specific nucleic acids or analytes in a sample with high specificity and sensitivity. Such methods generally require first amplifying the nucleic acid sequence based on the presence of a specific target sequence or analyte. Following amplification, the amplified sequences are detected and quantified. Conventional detection systems for nucleic acids include detection of fluorescent labels, colored dyes, fluorescent enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.

One disadvantage of currently used detection methods is the need to separate labeled starting materials from a final labeled product or by-product. Such separations generally require gel electrophoresis or immobilization of a target sequence onto a membrane for detection. Moreover, there are often numerous reagents and/or incubation steps required for detection.

A previously reported colorimetric assay for monitoring RNA synthesis from RNA polymerases in presence of a gamma-phosphate modified nucleotide describes RNA polymerase reactions performed in the presence of a gamma-modified, alkaline phosphatase resistant nucleotide triphosphate which was modified at its gamma-phosphate with a dinitrophenyl group. When RNA polymerase reactions were performed in the presence of this gamma-modified NTP as the sole nucleotide triphosphate and a homopolymeric template, it was found that RNA polymerase could recognize and utilize the modified NTP. Additionally, when the polymerase reactions were performed in the presence of an alkaline phosphatase, which digested the p-nitrophenyl pyrophosphate aldo-product of phosphoryl transfer to the chromogenic p-nitrophenylate, an increase in absorbence was reported. A disadvantage of this detection method is that the real-time colorimetric assay, performed in the presence of an alkaline phosphatase, only works with a homopolymeric template.

It would, therefore, be of benefit to provide a method for detecting RNA in the presence of a heteropolymeric template, where the method would not be restricted to using a single terminal-phosphate modified nucleotide as the sole nucleotide that is substantially non-reactive to alkaline phosphatase. This would enable a single-tube assay for real-time monitoring of RNA synthesis using hetero-polymeric templates.

Generally, the fluorescent dyes in the assays are quenched by a molecule placed in close proximity to them in the labeled entity, and the detectable signal is produce when the structure is altered and the quencher is either removed, moved away from the dye, or otherwise rendered inactive. At that point a detectable signal is produced. However, since quenching is not absolute, the dynamic range of such assays is limited.

It would also be beneficial to provide similar assays for RNA wherein the identity of the label on the terminal-phosphate is varied to allow for better recognition and utilization by RNA polymerase. Additionally, it would be desirable for the label on the terminal-phosphate to be varied to allow for chemiluminescent and fluorescent detection, or reduction

potential, as well as for improved calorimetric detection, wherein only simple and routine instrumentation would be required for detection, and increased dynamic range.

Technology

This Cytiva technology describes methods of detecting a nucleic acid in a sample, based on the use of terminal-phosphate-labeled nucleotides as substrates for nucleic acid polymerases. The methods provided by this invention utilize a nucleoside polyphosphate, dideoxynucleoside polyphosphate, or deoxynucleoside polyphosphate analogue which has a colorimetric dye, chemiluminescent, or fluorescent moiety, a mass tag or an electrochemical tag attached to the terminal-phosphate. When a nucleic acid polymerase uses this analogue as a substrate, an enzyme-activatable label would be present on the inorganic polyphosphate by-product of phosphoryl transfer. Cleavage of the polyphosphate product of phosphoryl transfer via phosphatase leads to a detectable change in the attached label attached. When the polymerase assay is performed in the presence of a phosphatase, there is provided a convenient method for real-time monitoring of DNA or RNA synthesis and detection of a target nucleic acid.

Summary of methods the invention provides:

- Improved methods of detecting a target using a labeled substrate or substrate analog that includes reacting the substrate or substrate analog in an enzyme-catalyzed reaction which produces a labeled moiety with an independently detectable signal only when such substrate or substrate analog reacts.

- Method of detecting the presence of a nucleic acid sequence including the steps of: a) conducting a nucleic acid polymerase reaction, wherein the reaction includes the reaction of a terminal-phosphate-labeled nucleotide, which reaction results in the production of labeled polyphosphate; b) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species; and c) detecting the presence of the detectable species

Terminal-Phosphate-Labelled Nucleotides with New Linkers

Summary

This invention describes methods of using terminal-phosphate-labeled nucleotides in the presence of a manganese salt to enhance their substrate properties towards various enzymes. In particular methods of detecting a nucleic acid in a sample, based on the use of terminal-phosphate-labeled nucleotides as substrates for nucleic acid polymerases, in the presence of a manganese salt. Including manganese complexes of terminal-phosphate-labeled nucleotides as well as terminal-phosphate-labeled nucleotides with new linkers with enhanced substrate properties.

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Background

Methods are known for detecting specific nucleic acids or analytes in a sample with high specificity and sensitivity. These methods generally require first amplifying the nucleic acid sequence based on the presence of a specific target sequence or analyte. Following amplification, the amplified sequences are detected and quantified. Conventional detection systems for nucleic acids include detection of fluorescent labels, fluorescent enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.

One disadvantage of these methods is that the labeled product not only requires some type of separation from the labeled starting materials but also since the label is attached to the product, it is different than the natural product to be identified. It would be beneficial to use methods and substrates that form unmodified product and at the same time generate a signal characteristic of the reaction taking place.

Gamma phosphate labeled nucleotides do provide such an opportunity. For example, incorporation of gamma phosphate labeled nucleotides into DNA or RNA by nucleic acid polymerases results in the production of unmodified DNA or RNA and at the same time the labeled pyrophosphate generated is used to detect, characterize and/or quantify the target. These nucleotides, however, are very poor substrates for various nucleic acid polymerases and it would, therefore be helpful if the rate of incorporation of these terminal-phosphate labeled nucleotides could be improved. Some rate enhancement can be achieved by increasing the length of polyphosphate chain between the label and the nucleotide. This rate enhancement although useful for some applications, for practical reasons is still insufficient for many applications where several hundred nucleotides have to be added in a short time, e.g. PCR.

It would, therefore, be of benefit to further enhance the rate of utilization of terminal phosphate labeled nucleotides. This and other concerns are addressed by this GE technology.

Technology

The Cytiva invention relates generally to the use of terminal-phosphate- labeled nucleotides with three or more phosphates as substrates for various enzymes including nucleic acid polymerases in the presence of manganese salt as cofactor for enhanced activity. The labels employed are chemiluminescent, fluorescent, electrochemical and chromogenic moieties as well as mass tags and include those that are directly detectable, as well as those that are detectable after enzyme activation or feed into other processes to generate a different signal. These terminal phosphate labeled nucleotides could be used in homogenous assays including identification of specific genotypes or genetic sequences. Also described new terminal phosphate labeled nucleoside polyphosphates with linkers that connect the label to the terminal phosphate of the nucleotides to enhance their substrate properties, i.e. to increase their rate of utilization by different enzymes.

Summary of methods the invention provides:

- several methods of use, and compositions to enhance the usefulness of terminal-phosphate labeled nucleotides (also referred to as terminal-phosphate labeled nucleoside polyphosphates)
- methods of using manganese salts in conjunction with terminal phosphate labeled nucleotides in enzymatic reactions to enhance their use by enzymes over and beyond what is observed in the absence of manganese even if other metal salts are present
- new composition of matter in the form of terminal-phosphate labeled nucleotides with linkers that enhance their substrate properties
- a method of increasing the rate of an enzyme catalyzed nucleoside monophosphate transfer from a terminal-phosphate-labeled nucleoside polyphosphate to detect the activity of said enzyme or said terminal- phosphate-labeled nucleoside polyphosphate
- a method of detecting the presence of a nucleic acid sequence including the steps of: a) conducting a nucleic acid polymerase reaction in the presence of a manganese salt to increase the rate of utilization of terminal-phosphate-labeled nucleoside polyphosphates, said polymerase reaction including reacting a terminal-phosphate-labeled nucleotide, and producing labeled polyphosphate; b) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species; and c) detecting the presence of the detectable species.

Nucleic Acid Amplification with Terminal-Phosphate Labelled Nucleotides

Summary

Methods of using terminal-phosphate labeled nucleotides (also referred to as terminal-phosphate labeled nucleoside polyphosphates) in nucleic acid amplification. Methods for the detection and quantification of a target sequence by selective amplification and for the real-time detection and quantification of a target sequence during amplification.

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Background

Methods are known for detecting specific nucleic acids or analytes in a sample with high specificity and sensitivity. These methods generally require first amplifying the nucleic acid sequence based on the presence of a specific target sequence or analyte. Following amplification, the amplified sequences are detected and quantified. Conventional detection systems for nucleic acids include detection of fluorescent labels, fluorescent enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.

One disadvantage of these methods is that the labeled product not only requires some type of separation from the labeled starting materials but also since the label is attached to the product, it is different than the natural product to be identified. It would be beneficial to use methods and substrates that form unmodified product and at the same time generate a signal characteristic of the reaction taking place. An additional benefit would be if the signal generated doesn't require separation from the starting materials.

Terminal-phosphate labeled nucleotides provide the above benefits. For example, incorporation of gamma- or delta-labeled nucleotides into DNA or RNA by nucleic acid polymerases results in the production of unmodified DNA or RNA and at the same time the labeled pyrophosphate generated can be used to detect, characterize and/or quantify the target. If these could be used in amplification reactions not only would they provide useful tools for detection and quantification of target sequence, but the amplified product, which is exact copies of the target sequence without modifications can be used in further studies.

Methods of amplification using terminal-phosphate labeled nucleoside polyphosphates where the amplification can be performed in reasonable time (similar to unmodified dNTP's) and the amount of label generated is proportional to the product formed would be attractive. Additional benefit would be found in a real time assay, where the amount of label generated can be independently detected without interference of signal from the terminal-phosphate labeled nucleotide and where where the label is completely dark until the amplification proceeds.

Technology

This Cytiva invention relates to the use of terminal-phosphate-labeled nucleotides having three or more phosphates as substrates for nucleic acid polymerases and their use in DNA amplification. The labels employed are chemiluminescent, fluorescent, electrochemical and chromogenic moieties as well as mass tags and include those that are directly detectable, detectable after enzyme activation or feed into other processes to generate a different signal. The signal generated from the attached dyes may also be used to quantify the amount of amplification. Also used are stabilizers that enhance the stability of terminal-phosphate labeled nucleoside polyphosphates in aqueous solutions and are useful for reducing non-enzymatic hydrolysis of these nucleotides, hence decrease background.

The technology incorporates a method of detecting the presence of a nucleic acid sequence including the steps of:
a) conducting a nucleic acid amplification which includes the reaction of a terminal-phosphate-labeled nucleotide, which reaction results in the production of labeled polyphosphate;
b) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species; and
c) detecting the presence of the detectable species.

Terminal phosphate blocked nucleoside polyphosphates: stabilized nucleotides for sequencing and assays

Summary

Terminal phosphate blocked nucleoside polyphosphates that are stable at high temperature and their use in nucleic acid amplification and analysis. Including modified terminal phosphate blocked nucleoside polyphosphates for improved incorporation and direct loading of nucleic acid sequencing reactions onto separating media.

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Background

DNA amplification by a number of amplification methods is performed at high temperatures. For example, in PCR, repeated cycles of denaturation at 95° C., annealing around 60° C. and extension around 70° C. causes significant breakdown of the dNTP's. This may significantly affect the yield of product in later cycles. Other amplification methods such as RCA and NASBA, although isothermal, also are conducted at higher temperatures. In case of NASBA, which is performed at 41° C., the stability of nucleotides may not be very critical. However RCA may be conducted at higher temperature depending upon the polymerase used and the complexity of sequence to be amplified. Stability of nucleotides can be an issue under these conditions. Therefore nucleotides that can survive this repeated cycling of temperature or prolonged heating at a constant yet high temperature and hence continue to give high product yields even in later cycles of amplification and possibly cut down the number of cycles/time required to achieve desirable amplification are desirable.

A process is needed for improving the clarity of sequencing data. Ideally, such a process would reduce sample preparation time and result in improved sequencing throughput.

Charge modified nucleoside-triphosphates that are either highly negatively charged so that they move well ahead of the sequence product fragments or highly positively charged so that they move in the opposite direction of the sequencing fragment when separated on a sequencing gel, are available. These nucleotides have a string of negatively or positively charged moieties attached to the base but once incorporated, due to the presence of string of charges on the base, they significantly affect the mobility of sequencing fragments. It is desirable to have modified nucleoside triphosphates that are either highly negatively charged or net positively charged, but after incorporation have same charge as the natural nucleotides. Therefore, mobility of the sequencing products is not affected. Even when mobility is not an issue, it is desirable to have more stable nucleoside triphosphate so that any possible complications from breakdown products are prevented.

Technology

This Cytiva invention relates to terminal phosphate blocked nucleoside polyphosphates that are stable at high temperature and their use in nucleic acid amplification and analysis. It also describes charge modified terminal phosphate blocked nucleoside polyphosphates for improved incorporation and direct loading of nucleic acid sequencing reactions onto separating media. Aspects of the technology include:

- the use of terminal phosphate blocked nucleoside polyphosphates in high temperature amplification methods such as PCR to enhance yield of amplified product
- describes labeled nucleoside polyphosphates with a blocking group on the terminal phosphate and their use in sequencing and other genotyping methods requiring high temperature or temperature cycling
- a nucleic acid amplification and/or detection kit

Terminal phosphate blocked nucleoside polyphosphates: stabilized nucleotides for sequencing and assays

Summary

A nucleic acid sequencing method and, in particular, a method that allows error correction during the sequencing of individual molecules.

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Background

Nucleic acid sequencing is routinely performed by the method of chain termination and gel separation. The method relies on the generation of a mixed population of nucleic acid fragments representing terminations at each base in the sequence. The sequence is then determined by electrophoretic separation of these fragments.

Recent efforts to increase the throughput of sequencing have resulted in the development of alternative methods that eliminate the electrophoretic separation step.

Generally, non-separation based approaches rely on the presence of large numbers of template molecules for each target sequence to generate a consensus sequence from a given target. Thus, for example, base extension reactions may be applied to multiple templates by interrogating discrete spots of nucleic acid, each comprising a multiplicity of molecules, immobilized in a spatially addressable array.

However, reactions of terminator incorporation/cleavage, or base excision are prone to errors, inefficiencies and restricted to a limited number of sequencing cycles due to deterioration of signal.

Recent advances in methods of single molecule detection make it possible to apply sequencing strategies to single molecules. However, sequencing, when applied to clonal populations of molecules, is a random process that results in some molecules undergoing reactions while others remain unmodified. Thus, in conventional sequencing methods, errors such as mis-incorporations are not normally of serious significance as the large numbers of molecules present ensure that consensus signal is obtained. When these reactions are applied to single molecules the outcomes are effectively quantized.

Technology

This technology provides a single molecule sequencing method that enables errors to be detected and allows analysis and error prevention, or correction, by monitoring the fate of individual molecules through sequencing reactions. The method steps include:

a) attaching a molecule to a solid phase

b) incubating the molecule with a first composition comprising a first reporter moiety

c) detecting incorporation of said first reporter moiety

d) performing a reaction to eliminate said first reporter moiety

e) incubating the molecule with a second composition comprising a second reporter moiety

f) detecting incorporation of said second reporter moiety characterized in that the first and second reporter moieties can be distinguished from each other.

Allele Specific Primer Extension

Summary

Methods of detecting and characterizing a polynucleotide in a sample, based on the use of a normal or non-hydrolyzable primer and terminal-phosphate-labeled nucleotides as substrates for DNA polymerase. Including, a method of detecting a polymorphism of a specific nucleotide base in a target polynucleotide. The labels employed are enzyme-activatable and include chemiluminescent, fluorescent, electrochemical and chromophoric moieties as well as mass-tags.

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Background

Methods are known for detecting specific nucleic acids or analytes in a sample with high specificity and sensitivity. A method of analysis that is based on the complimentarily between nucleotide sequences allows for the direct analysis of genetic characters. This provides a very useful means for identifying genetic disorders or a carcinomatous change of normal cells.

However, detection and characterization of a trace amount of a target nucleotide in a sample is difficult. Therefore, methods for direct detection of the gene generally require first amplifying a nucleic acid sequence based on the presence of a specific target sequence or analyte. Following amplification, the amplified sequences are detected and quantified. Conventional detection systems for nucleic acids include detection of fluorescent labels, fluorescent enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.

As a method of amplifying a nucleic acid sequence, the PCR (polymerase chain reaction) process is known. Presently, the PCR is the most conventional means for in vitro amplification of nucleic acid. However, the PCR has certain disadvantages, including the requirement for strict temperature control, inadequate quantification due to logarithmic amplification, and the danger of erroneous results brought about by simultaneous amplification of trace amounts of contaminated DNA.

In addition to amplification methods which involve detection and quantification of the sequences, there are signal amplification methods which detect amplified decomposition products, i.e., a product or by-product of a reaction is amplified as the signal from a target nucleic acid.

Methods of detecting and characterizing a nucleic acid, which include utilization of terminal-phosphate-labeled nucleotides as substrates for DNA polymerase in a signal amplification protocol would be highly advantageous. They would further be of benefit if the methods employed enzyme-activatable labels at the terminal phosphate of the nucleotide for production of an amplified detectable species from a target nucleic acid which would eliminate the need to separate labeled starting materials from labeled products or by-products. Real-time monitoring of a heteropolymeric target nucleic acid using routine lab instrumentation would also be desirable.

Technology

A method of characterizing a nucleic acid sample that includes the steps of: (a) conducting a DNA polymerase reaction that includes the reaction of a template, an allele specific primer, at least one terminal phosphate-labeled nucleotide, DNA polymerase, and optionally an enzyme having 3′→5′ exonuclease activity when the primer is non-hydrolyzable, which reaction results in the production of labeled polyphosphate; (b) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species; (c) detecting the detectable species; and (d) characterizing the nucleic acid sample based on such detection.

Also described is a homogenous method of detecting and characterizing a polynucleotide sequence in which terminal phosphate modified dNTP's are used in conjunction with a polymerase, a phosphatase and allele specific primers to generate an amplified signal which can be detected without separation of reaction components.

Solid Phase Sequencing

Summary

Methods of sequencing a polynucleotide in a sample, based on the use of terminal-phosphate-labeled nucleotides containing three or more phosphates as substrates for nucleic acid polymerases. The labels employed are enzyme-activatable and include chemiluminescent, fluorescent, electrochemical and chromogenic moieties as well as mass tags.

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Background

Existing methods are known for detecting specific nucleic acids or analytes in a sample with high specificity and sensitivity. These methods generally require first amplifying nucleic acid sequence based on the presence of a specific target sequence or analyte. Following amplification, the amplified sequences are detected and quantified. Conventional detection systems for nucleic acids include detection of fluorescent labels, fluorescent enzyme-linked detection systems, antibody-mediated label detection, and detection of radioactive labels.

One disadvantage of detection methods widely in use is the need to separate labeled starting materials from a final labeled product or by-product. Such separations generally require gel electrophoresis or immobilization of a target sequence onto a membrane for detection. Moreover, there are often numerous reagents and/or incubation steps required for detection.

Technology

This Cytiva invention describes methods of sequencing a nucleic acid in a sample, based on the use of terminal-phosphate-labeled nucleotides as substrates for nucleic acid polymerases. The methods provided utilize a nucleoside polyphosphate, dideoxynucleoside polyphosphate, or deoxynucleoside polyphosphate analogue which has a calorimetric dye, chemiluminescent, or fluorescent moiety, a mass tag or an electrochemical tag attached to the terminal-phosphate. When a nucleic acid polymerase uses this analogue as a substrate, an enzyme-activatable label is present on the inorganic polyphosphate by-product of phosphoryl transfer. Cleavage of the polyphosphate product of phosphoryl transfer via phosphatase leads to a detectable change in the attached label attached. In some instances the labeled polyphosphate may be detected directly via the label and provide information on the nucleic acid. When the polymerase assay is performed in the presence of a phosphatase, a convenient method for real-time monitoring of DNA or RNA synthesis and characterization of a target nucleic acid can be used.

The method of detecting the presence of a nucleic acid sequence consists of:

a) conducting a nucleic acid polymerase reaction, wherein the reaction includes the reaction of a terminal-phosphate-labeled nucleotide, which reaction results in the production of labeled polyphosphate;

b) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species; and

c) detecting the presence of the detectable species.

The method of sequencing a target region of a nucleic acid template, comprises of the above steps plus the following:

d) continuing polymerization reaction by adding a different terminal-phosphate-labeled nucleoside polyphosphate selected from the remaining natural bases or base analogs to the reaction mixture and repeating steps b and c; and

e) identifying the target region sequence from the identity and order of addition of terminal-phosphate labeled nucleoside polyphosphates resulting in production of the detectable species.

Analyte Detection

Summary

Methods of detecting one or more analytes using terminal phosphate labeled nucleotides, including three or more phosphates as substrates for nucleic acid polymerases. The labels employed are enzyme-activatable and include chemiluminescent, fluorescent, electrochemical and chromophoric moieties as well as mass tags.

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Background

Methods are known for detecting analytes in a sample with high specificity and sensitivity. These methods include antigen-antibody assays as well DNA hybridization based assays. Detection of analytes using immunodetection is well known and these methods include direct labeling of antibodies using radioisotopes, fluorescent or chemiluminescent tags, or ELISA assays. Similar methods have been incorporated into DNA hybridization based assays, which are generally more sensitive and in most diagnostic assays can be used at an earlier stage of disease progression. Enhanced sensitivity is achieved by first amplifying nucleic acid sequence based on the presence of a specific target sequence. Following amplification, the amplified sequences are detected and quantified.

The traditional methods of amplifying the signal use enzymes linked to antibodies or polynucleotides and are limited by the amount of multiplexing one can achieve. There are only a few enzymes, such as alkaline phosphatase or horse radish peroxidase, that have been linked to antibodies or DNA probes. Other methods of signal amplification are based on nucleic acid metabolizing enzymes.

It would be beneficial to have methods of detecting and characterizing an analyte, where the methods would include utilization of terminal-phosphate-labeled nucleotides as substrates for DNA polymerase in a cycling assay by an exonuclease. Ideally the methods would employ enzyme-activatable labels at the terminal phosphate of the nucleotide for production of an amplified detectable species from a target nucleic acid which would eliminate the need to separate labeled starting materials from labeled products or by-products. It would be highly desirable if such methods would allow for real-time monitoring of a heteropolymeric target nucleic acid using routine lab instrumentation and if they could be easily multiplexed to analyze four or more analytes per reaction compartment simultaneously or in sequential manner.

Technology

A method of characterizing an analyte sample that includes the steps of: (a) anchoring the analyte to a nucleic acid template of known sequence; (b) conducting a DNA polymerase reaction that includes the reaction of a template, a non-hydrolyzable primer, at least one terminal phosphate-labeled nucleotide, DNA polymerase, and an enzyme having 3’→5’ exonuclease activity which reaction results in the production of labeled polyphosphate; (c) permitting the labeled polyphosphate to react with a phosphatase to produce a detectable species characteristic of the sample; (d) detecting the detectable species. The method may include the step of characterizing the nucleic acid sample based on the detection. The technology also describes methods of analyzing multiple analytes in a sample, and kits for characterizing analyte samples.