



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
 Bert Vogelstein, et. al.) Group Art Unit: 1656
 Serial No. 09/613,826) Examiner: J Siew
 Filing Date: July 11, 2000) Docket No. 01107.00031

For: **DIGITAL AMPLIFICATION**

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AMENDMENT

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JUL 17 2001

Assistant Commissioner for Patents
Washington, D.C. 20231

TECH CENTER 1600/2900

Sir:

In response to the Office Action mailed April 12, 2001, applicants request entry of the following amendments and request reconsideration of the claims. Claims 1-64 are pending in the application. Claims 2 and 38-64 are allowable over the prior art. If any additional fee is due please change our Deposit Account No. 19-0733.

IN THE CLAIMS

Please add new claims 65-69.

65. (New) A molecular beacon probe comprising:
 an oligonucleotide comprising a stem and a loop structure and having a

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photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop comprises 16 base pairs and has a T_m of 50-51°C, and wherein the stem comprises 4 base pairs having a sequence 5'-CACG-3'.

66. (New) The molecular beacon probe of claim 65, wherein the probe detects a wild-type nucleic acid better than a mutant nucleic acid.

67. (New) The molecular beacon probe of claim 65, wherein the probe detects a mutant nucleic acid better than a wild-type nucleic acid.

68. (New) A molecular beacon probe comprising:
an oligonucleotide comprising a stem and a loop structure and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the loop comprises 19-20 base pairs and has a T_m of 54-56°C, and wherein the stem comprises 4 base pairs having a sequence 5'-CACG-3'.

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cont*

69. (New) A pair of molecular beacon probes comprising:
a first oligonucleotide comprising a first stem and a first loop structure and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the first loop comprises 16 base pairs and has a T_m of 50-51°C, and wherein the first stem comprises 4 base pairs having a sequence 5'-CACG-3'; and
a second oligonucleotide comprising a second stem and a second loop structure

and having a photoluminescent dye at one of the 5' or 3' ends and a quenching agent at the opposite 5' or 3' end, wherein the second loop comprises 19-20 base pairs and has a T_m of 54-56°C, and wherein the second stem comprises 4 base pairs having a sequence 5'-CACG-3'.

IN THE SPECIFICATION

Please replace the paragraph beginning on page 4, line 5, with the following paragraph.

FIG. 1A, 1B, 1C. Schematic of experimental design. (A) The basic two steps involved: PCR on diluted DNA samples is followed by addition of fluorescent probes which discriminate between WT and mutant alleles and subsequent fluorometry. (B) Principle of molecular beacon analysis. In the stem-loop configuration, fluorescence from a dye at the 5' end of the oligonucleotide probe is quenched by a Dabcyl group at the 3' end. Upon hybridization to a template, the dye is separated from the quencher, resulting in increased fluorescence. Modified from Marras *et al.* (C) Oligonucleotide design. Primers F1 and R1 are used to amplify the genomic region of interest. Primer INT is used to produce single stranded DNA from the original PCR products during a subsequent asymmetric PCR step (see Materials and Methods). MB-RED is a Molecular Beacon which detects any appropriate PCR product, whether it is WT or mutant at the queried codons. MB-GREEN is a Molecular Beacon which preferentially detects the WT PCR product.

Please replace the paragraph beginning on page 14, line 29 with the following paragraph.

Oligonucleotides and DNA sequencing. Primer F1:

5'-CATGTTCTAATATAGTCACATTTTCA-3' (SEQ ID NO: 1); Primer R1:

5'-TCTGAATTAGCTGTATCGTCAAGG-3' (SEQ ID NO: 2); Primer INT:

5'-TAGCTGTATCGTCAAGGCAC-3' (SEQ ID NO: 3); MB-RED:

5'-Cy3-CACGGGCCTGCTGAAAATGACTGCGTG-Dabcyl-3' (SEQ ID NO: 4);

MB-GREEN: 5'-Fluorescein-CACGGGAGCTGGTGGCGTAGCGTG-Dabcyl-3' (SEQ ID NO: 5). Molecular Beacons (33,34) were synthesized by Midland Scientific and other

oligonucleotides were synthesized by Gene Link (Thornwood, NY). All were dissolved at 50 uM in TE (10 mM Tris, pH 8.0/ 1 mM EDTA) and kept frozen and in the dark until

use. PCR products were purified using QIAquick PCR purification kits (Qiagen). In the relevant experiments described in the text, 20% of the product from single wells was used for gel electrophoresis and 40% was used for each sequencing reaction. The primer used for sequencing was 5'-CATTATTTTATTATAAGGCCTGC-3' (SEQ ID NO: 6).

Sequencing was performed using fluorescently-labeled ABI Big Dye terminators and an ABI 377 automated sequencer.

SEQUENCE LISTING

Please enter the enclosed paper copy of the Sequence Listing after the claims. A computer readable copy of the Sequence Listing is also enclosed herewith to comply with 37 § CFR 1.821(e). The content of the paper and computer readable copy of the

Sequence Listing, submitted in accordance with 37 CFR § 1.821 (c) and (e), respectively, are identical. The submitted Sequence Listing, filed in accordance with 37 CFR § 1.821 (g) herein does not include new matter.

REMARKS

The Invention

The invention is directed to a method for determining the ratio of a selected genetic sequence in a population of genetic sequences. Nucleic acid template molecules in a biological sample are diluted to form a set comprising a plurality of assay samples. The diluted nucleic acid template molecules are amplified to form a population of amplified molecules in the assay samples of the set. The amplified molecules are analyzed to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence. The first number and the second number are compared to ascertain a ratio that reflects the composition of the biological sample (claim 1).

The invention is also drawn to a method for determining the ratio of a selected genetic sequence in a population of genetic sequences. Template molecules within a set which comprises a plurality of assay samples are amplified to form a population of amplified molecules in each of the assay samples of the set. The amplified molecules in the assay samples of the set are analyzed to determine a first number of assay samples which contain the selected genetic sequence and a second number of assay samples which contain a reference genetic sequence. At least one-fiftieth of the assay samples in the set

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