

[0016] In some embodiments, the present invention provides a probe comprising an isolated nucleic acid molecule of the present invention, a reporter molecule, and a quencher molecule. In preferred embodiments, the reporter molecule produces a signal upon the separation of the reporter molecule and the quencher molecule. In preferred embodiments, the quencher molecule is capable of quenching the signal of the reporter molecule. In some embodiments, the reporter molecule is a fluorophore such as FAM, ROX, Texas Red, TET, TAMRA, JOE, HEX, CAL Red, and VIC, preferably the fluorophore is FAM, ROX, or Texas Red. In some embodiments, the probe is capable of being cleaved by a protein thereby separating the reporter molecule from the quencher molecule. In preferred embodiments, the protein is Taq polymerase.

[0017] In some embodiments, the present invention provides an assay which comprises using a probe of the present invention. In preferred embodiments, the assay is a nucleic acid hybridization assay such as a TaqMan® based assay. In some embodiments, the assay further comprises conducting PCR amplification. The assay may further comprise detecting the presence or measuring the amount of the probe and detecting the presence or measuring the amount of a target nucleic acid molecule. In preferred embodiments of the present invention, the absence of the target nucleic acid molecule and the absence of the probe indicate a true negative result for the target nucleic acid molecule and the absence of the target nucleic acid molecule and the presence of the probe indicate a false negative result for the target nucleic acid molecule.

[0018] In some embodiments, the present invention provides a kit for a probe-based nucleic acid assay comprising an isolated nucleic acid molecule of the present invention packaged with instructions for use. In preferred embodiments, the isolated nucleic acid molecule contains a label such as a reporter molecule and a quencher molecule. In some embodiments, the probe-based nucleic acid assay is for the detection of an organism such as one belonging to *Bacillus*, *Mycobacterium*, *Francisella*, *Bruceella*, *Clostridium*, *Yersinia*, *Variola*, *Orthopox*, or *Burkholderia*. The kit of the present invention may, further include reagents or components for detecting the presence of a nucleic acid molecule belonging to the organism.

[0019] In some embodiments, the present invention also provides a method of making an internal positive control nucleic acid molecule for a probe-based nucleic acid molecule assay which comprises creating a first DNA fragment and a second DNA fragment from a template DNA and first set of primers and a second set of primers; creating a third DNA fragment and a fourth DNA fragment from the first DNA fragment and the second DNA fragment with a third set of primers and a second set of primers; hybridizing the third DNA fragment and the fourth DNA fragment to obtain a first hybridized DNA; using a fifth primer set to create a fifth DNA fragment from the first hybridized DNA; using a sixth primer set and a seventh primer set to create a sixth DNA fragment and a seventh DNA fragment from the fifth DNA fragment; creating an eighth DNA fragment and a ninth DNA fragment from the sixth DNA fragment and the seventh DNA fragment using an eighth primer set and a ninth primer set; hybridizing the eighth DNA fragment and the ninth DNA fragment to obtain a second hybridized DNA; creating a tenth DNA fragment and an eleventh DNA

fragment from the second hybridized DNA using a tenth set of primers and an eleventh set of primers; creating a twelfth DNA fragment and a thirteenth DNA fragment from the tenth DNA fragment and the eleventh DNA fragment using a twelfth set of primers and a thirteenth set of primers; hybridizing the twelfth DNA fragment and the thirteenth DNA fragment to obtain the internal positive control nucleic acid molecule. In some preferred embodiments, the method of making an internal positive control nucleic acid molecule for a probe-based nucleic acid molecule assay, wherein the internal positive control nucleic acid molecule contains a sequence that has a sequence identity of at least about 70% over the 548 bp region of SEQ ID NO: 49. In preferred embodiments, the sequence identity is at least about 80%, preferably at least about 90%, more preferably at least about 95%.

[0020] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed. The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute part of this specification, illustrate several embodiments of the invention, and together with the description serve to explain the principles of the invention.

#### DESCRIPTION OF THE DRAWINGS

[0021] This invention is further understood by reference to the drawings wherein:

[0022] **FIG. 1** is a schematic showing an example of a TaqMan® based assay.

[0023] **FIG. 2** schematically shows the site-directed mutagenesis process used to generate the IPC nucleic acid molecule of the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION

[0024] The present invention provides an internal positive control (IPC) for use in nucleic acid hybridization assays, preferably probe-based nucleic acid assays such as TaqMan® based assays. An example of a TaqMan® based assay is schematically shown in **FIG. 1**. In particular, the present invention provides an oligonucleotide (IPC oligonucleotide) having a reporter molecule and a quencher molecule. The IPC oligonucleotide specifically anneals between the forward and reverse primers of a target sequence. The IPC oligonucleotide is cleaved by the 5' nuclease activity of Taq polymerase during PCR amplification and the reporter molecule is then separated from the quencher molecule to generate a sequence specific signal. With each amplification cycle, additional reporter molecules are separated from the quencher molecules. The intensity of a signal, such as fluorescence, may be monitored before, during, or after PCR amplification or a combination thereof.

[0025] The IPC nucleic acid molecule of the present invention may be used to distinguish a true negative result from a false negative result. As used herein, a "true negative" result correctly indicates that a sample lacks a target nucleic acid sequence. A "false negative" result incorrectly indicates the absence of a target nucleic acid sequence which may result from PCR inhibitors present in the sample or technical error.