

**INTERNAL POSITIVE CONTROL FOR  
PROBE-BASED NUCLEIC ACID MOLECULE  
ASSAYS AND METHODS OF MAKING AND USING  
THEREOF**

**CROSS REFERENCE TO RELATED  
APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/361,455 filed Mar. 4, 2002, which names Laurie J. Hartman and David A. Norwood, Jr. as co-inventors and is herein incorporated by reference in its entirety.

**ACKNOWLEDGMENT OF GOVERNMENT  
SUPPORT**

[0002] Employees of the United States Army made this invention. The government has rights in the invention.

**BACKGROUND OF THE INVENTION**

[0003] 1. Field of the Invention

[0004] The present invention generally relates to probes comprising a reporter molecule and a quencher molecule for use in nucleic acid assays. In particular, the present invention relates to a universal internal positive control that may be used in polymerase chain reaction (PCR) based assays.

[0005] 2. Description of the Related Art

[0006] Reporter molecule and quencher molecule pairs have been incorporated onto oligonucleotide probes in order to monitor, detect, or measure biological events associated with the reporter molecule and quencher molecule being separated or brought within a minimum quenching distance of each other. For example, probes have been developed where the intensity of the reporter molecule fluorescence increases due to the separation of the reporter molecule from the quencher molecule. Probes have also been developed which lose their fluorescence because the quencher molecule is brought into proximity with the reporter molecule. Reporter molecule and quencher molecule pair probes have been used to monitor hybridization assays and nucleic acid amplification reactions, such as polymerase chain reactions (PCR), by monitoring either the appearance or disappearance of the signal generated by the reporter molecule. See WO 90/03446; European Patent Application No. 0 601 889 A2; Mergney, et al., (1994) *Nucleic Acids Research* 22(6):920-928; and Arnheim and Erlich, (1992) *Ann. Rev. Biochem.* 61:131-156.

[0007] Various real time PCR amplification product assays are known in the art. See e.g. Holland et al. (1991) *PNAS* 88:7276-7280; and U.S. Pat. No. 5,210,015. One assay uses a probe having a fluorescence reporter molecule and quencher molecule pair that is cleaved apart during amplification thereby resulting in a detectable fluorescent molecule in a concentration that is proportional to the amount of double-stranded DNA. These assays are known as TaqMan® based assays. TaqMan® based assays use an oligonucleotide probe having a reporter molecule and quencher molecule pair that specifically anneals to a region of a target polynucleotide "downstream", i.e. in the direction of extension of primer binding sites. The reporter molecule and quencher molecule are positioned on the probe sufficiently close to each other such that whenever the reporter molecule

is excited, the energy of the excited state nonradiatively transfers to the quencher molecule where it either dissipates nonradiatively or is emitted at a different emission frequency than that of the reporter molecule.

[0008] During strand extension by a DNA polymerase, the probe anneals to the template where it is digested by the 5'→3' exonuclease activity of the polymerase. As a result of the probe being digested, the reporter molecule is effectively separated from the quencher molecule such that the quencher molecule is no longer close enough to the reporter molecule to quench the fluorescence of the reporter molecule. Thus, as more and more probes are digested during amplification, the number of reporter molecules in solution increases, thereby resulting in an increasing number of unquenched reporter molecules which produce a stronger and stronger fluorescent signal.

[0009] TaqMan® based assays require internal positive control reagents to help distinguish between samples that are identified as negative because the sample lacks the target sequence and samples that are identified as negative because the presence of a PCR inhibitor. A TaqMan® Exogenous Internal Positive Control kit is commercially available from Applied Biosystems (Foster City, Calif.) to distinguish true target negatives from PCR inhibition. The TaqMan® Exogenous Internal Positive Control kit distinguishes two types of negative results. A negative call for the target sequence and a positive call for the internal positive control (IPC) indicates that no target sequence is present and a negative call for the target sequence and a negative call for the IPC suggests PCR inhibition.

[0010] Unfortunately, the TaqMan® Exogenous Internal Positive Control kits allow little flexibility as the kits are made with only one fluorescent dye, VIC™ (Applied Biosystems, Foster City, Calif.), which cannot be used on all TaqMan® chemistry based instruments and the primers and probe in the kit are mixed together by the manufacturer and therefore cannot be completely optimized for use with any PCR amplification product assay.

[0011] Thus, a need exists for an internal positive control that may be used with a variety of PCR amplification product assays.

**SUMMARY OF THE INVENTION**

[0012] The present invention generally relates to a nucleic acid molecule that may be used as an internal positive control in probe-based nucleic acid assays.

[0013] In some embodiments, the present invention relates to an isolated nucleic acid molecule comprising the sequence set forth in SEQ ID NO: 49. In some embodiments, the nucleic acid molecule consists essentially of the sequence set forth in SEQ ID NO: 49. In some embodiments, the nucleic acid molecule consists of the sequence set forth in SEQ ID NO: 49.

[0014] In some embodiments, the present invention provides an isolated nucleic acid molecule 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%.

[0015] In some embodiments, the present invention provides a probe comprising an isolated nucleic acid molecule of the present invention and a label.