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(54) Title: SEQUENCES AND THEIR USE FOR DETECTION OF SALMONELLA

(57) Abstract: This invention relates to a rapid method for detection of Salmonella in a sample based on the presence of nucleic acid sequences, in particular, to a PCR-based method for detection, and to oligonucleotide molecules and reagents and kits useful therefore. In certain embodiments, the method is employed to detect Salmonella in a food or water sample. The present invention further relates to isolated polynucleotides, replication compositions, and kits for carrying out the method of the present invention.

TITLE

SEQUENCES AND THEIR USE FOR DETECTION OF SALMONELLA

FIELD OF INVENTION

This invention relates to a rapid method for detection of *Salmonella* in a sample based on the presence of nucleic acid sequences, in particular, to a PCR-based method for detection, and to oligonucleotide molecules and reagents and kits useful therefore. In certain embodiments, the method is employed to detect *Salmonella* in a food or water sample. The present invention further relates to isolated polynucleotides, replication compositions, and kits for carrying out the method of the present invention.

BACKGROUND OF INVENTION

Salmonella is a genus of rod-shaped, gram-negative bacteria that is known to cause numerous illnesses, including food poisoning and typhoid fever. *Salmonella* infections can be transferred from animals to humans and can be acquired through the ingestion of food contaminated with *Salmonella*. In infections involving entiridis-type *Salmonella*, which is responsible for food poisoning, the organism typically enters the digestive tract through ingestion. In healthy adults, *Salmonella* generally must be ingested in large numbers to cause any disease. However, in young children, ingestion of a relatively small number of bacteria has been shown to cause disease due to the increased susceptibility of this population. With regard to the course of *Salmonella* infection, the bacteria typically incubate for up to one day before symptoms of infection appear. After that incubation period, intestinal inflammation occurs, resulting in diarrhea that is often bloody. Symptoms are generally mild with no sepsis, though sepsis can occur in immunocompromised individuals. Additionally, *Salmonella* meningitis can occur in children.

Because of its mode of transmission and the seriousness of some infections, detection of *Salmonella* in samples, such as food or beverage samples, is critical to

the safety of the population. As such, it is desirable to have a test for the rapid and accurate detection of *Salmonella* in a sample.

SUMMARY OF INVENTION

One aspect of this invention is a method for detecting the presence of *Salmonella* in a sample, the sample comprising nucleic acids, the method comprising (a) providing a reaction mixture comprising at least one primer and probe, wherein the primer is at least 11 nucleotides in length and the probe is at least 14 nucleotides in length, and wherein (i) the primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and the probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and the probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto; and (b) performing PCR amplification of the nucleic acids of the sample using the reaction mixture of step (a); and (c) detecting the amplification of step (b).

In certain embodiments, the reaction mixture comprises both the primer and probe of (i) and the primer and probe of (ii). In certain other embodiments the primer and probe each possess a 3' terminus and a 5' terminus, and the 3' terminus of said probe is directly or indirectly attached to the 5' terminus of the primer, thereby forming a primer-probe complex. In still further embodiments, the primer-probe complex further comprises a 5' Stem Sequence and a 3' Stem Sequence, wherein the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe, wherein the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer. In additional embodiments, the primer-probe complex comprises a detectable label. In

further embodiments, the reaction mixture further comprises a quencher oligonucleotide capable of selectively hybridizing under stringent conditions to the probe.

In certain examples, the primer capable of selectively hybridizing to SEQ ID NO: 1 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 40-54, and the probe capable of selectively hybridizing to SEQ ID NO: 2 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6-39. In other examples, the primer capable of selectively hybridizing to SEQ ID NO: 3 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111, and wherein said probe capable of selectively hybridizing to SEQ ID NO: 4 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94. In further examples, the 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 112-119, and wherein said 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 120-130.

In another aspect, the invention relates to an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 6-111. In other embodiments, the invention relates to an isolated polynucleotide, wherein the polynucleotide comprises a primer-probe complex, wherein the primer-probe complex comprises a nucleic acid primer portion and a nucleic acid probe portion, wherein the primer portion is at least 11 nucleotides in length and the probe portion is at least 14 nucleotides in length, wherein the 3' terminus of the probe portion is directly or indirectly attached to the 5' terminus of the primer portion, and wherein (i) the primer portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and the probe portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer portion comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and the probe portion comprises a nucleic acid

sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto. In certain examples, the primer portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 40-54, and/or the probe portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 6-39. In other examples, the primer portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111, and/or the probe portion comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94.

In additional embodiments, the primer-probe complex further comprises a 5' Stem Sequence and a 3' Stem Sequence, wherein the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe portion, wherein the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe portion, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer portion. In certain examples, the 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 112-119, and/or the 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 120-130.

In other aspects, the invention relates to a reagent tablet or kit for detection of *Salmonella* in a sample.

The present invention provides a method for detecting the presence of *Salmonella* in a sample, said sample comprising nucleic acids, said method comprising: (a) providing a reaction mixture comprising: (i) one primer comprising a nucleic acid sequence as set forth in SEQ ID NO:47 capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:1 and one probe comprising a nucleic acid sequence as set forth in SEQ ID NO:29 capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:2; and (ii) at least one additional primer and probe, wherein said additional primer is at least 11 nucleotides in length and said additional probe is at least 14 nucleotides in length, and wherein said additional primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and said additional probe comprises a nucleic

acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto; wherein said primer and probe of (i) and (ii) each possess a 3' terminus and a 5' terminus, and wherein the 3' terminus of a 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe, wherein the 5' terminus of a 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer, thereby forming a primer-probe complex; and (b) performing PCR amplification of said nucleic acids of said sample using the reaction mixture of step (a); and (c) detecting the amplification of step (b).

SUMMARY OF THE SEQUENCES

SEQ ID NOs: 1-5 are the nucleotide sequences of portions of the *Salmonella* genome that are useful for detecting the presence of *Salmonella* in a sample. In certain examples, a primer directed toward SEQ ID NO: 1 is used in conjunction with a probe directed toward SEQ ID NO: 2. In other examples, a primer directed toward SEQ ID NO: 3 is used in conjunction with a probe directed toward SEQ ID NO: 4 or 5. In certain other examples, the primer and probe are attached so as to form a primer-probe complex, wherein the 3' terminus of the probe portion is directly or indirectly attached to the 5' terminus of the primer portion. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 1 include

SEQ ID NOs: 40-54. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 2 include SEQ ID NOs: 6-39. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 3 include SEQ ID NOs: 95-111. Primers and/or probes capable of selectively hybridizing under stringent conditions to SEQ ID NO: 4 include SEQ ID NOs: 55-94.

SEQ ID NOs: 6-39 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 2.

SEQ ID NOs: 40-54 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 1.

SEQ ID NOs: 55-94 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 4 or .

SEQ ID NOs: 95-111 are nucleotide sequences capable of use as primers or probes for selectively hybridizing under stringent conditions, and ultimately detecting, the sequence of SEQ ID NO: 3.

SEQ ID NOs: 112-119 are nucleotide sequences capable of use as a 5' Stem Sequence, for example, in conjunction with a suitable probe sequence, such as those described by SEQ ID NOs: 6-39 and 55-94. In certain examples, the 5' Stem Sequence is directly or indirectly attached to a probe sequence, such that the 3' terminus of the 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe sequence.

SEQ ID NOs: 120-130 are nucleotide sequences capable of use as a 3' Stem Sequence, for example, in conjunction with a suitable probe sequence, such as those described by SEQ ID NOs: 6-39 and 55-94. In certain examples, the 3' Stem Sequence is directly or indirectly attached to a probe sequence and a primer sequence, such that the 5' terminus of the 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe sequence and the 3' terminus of the 3' Stem Sequence is directly or indirectly attached to the 5' terminus of the primer sequence.

SEQ ID NO: 131 is the nucleotide sequence that comprises a synthetic SV40 ("sSV40") sequence which can be effectively employed, for example, as a target for a positive control amplification reaction. In certain embodiments, this sequence can be used as a "spiked" control and can be amplified and detected using SEQ ID NOs: 132-136.

SEQ ID NOs: 132-136 are nucleotide sequences useful for amplifying and detecting the Positive Control sequence of SEQ ID NO: 131. In certain embodiments, SEQ ID NO: 132 is a 5' stem sequence, SEQ ID NO: 133 is a probe sequence, SEQ ID NO: 134 is a 3' stem sequence, SEQ ID NO 135 is a forward primer sequence, and SEQ ID NO: 136 is a reverse primer sequence. In other embodiments, SEQ ID NOs: 132-135 are combined so as to form a primer-probe complex capable of forming a stem-loop structure. In other embodiments that primer-probe complex is used as a forward primer/probe in conjunction with SEQ ID NO: 136 as reverse primer to amplify and detect SEQ ID NO: 131.

DETAILED DESCRIPTION

Applicants specifically incorporate the entire contents of all cited references in this disclosure. Further, when an amount, concentration, or other value or parameter is given as either a range, preferred range, or a list of upper preferable values and lower preferable values, this is to be understood as specifically disclosing all ranges formed from any pair of any upper range limit or preferred value and any lower range limit or preferred value, regardless of whether ranges are separately disclosed. Where a range of numerical values is recited herein, unless otherwise stated, the range is intended to include the endpoints thereof, and all integers and fractions within the range. It is not intended that the scope of the invention be limited to the specific values recited when defining a range.

Definitions

In this disclosure, a number of terms and abbreviations are used. The following definitions are provided.

As used herein, the term “about” or “approximately” means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

The term “comprising” is intended to include embodiments encompassed by the terms “consisting essentially of” and “consisting of.” Similarly, the term “consisting essentially of” is intended to include embodiments encompassed by the term “consisting of.”

“Polymerase chain reaction” is abbreviated PCR.

The term "isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The terms “polynucleotide,” “polynucleotide sequence,” “nucleic acid sequence,” “nucleic acid fragment,” and “oligonucleotide” are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural, or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more strands of cDNA, genomic DNA, synthetic DNA, or mixtures thereof.

The term “amplification product” or “amplicon” refers to nucleic acid fragments produced during a primer-directed amplification reaction. Typical methods of primer-directed amplification include polymerase chain reaction (PCR), ligase chain reaction (LCR), or strand displacement amplification (SDA). If PCR methodology is selected, the replication composition may comprise the components for nucleic acid replication, for example: nucleotide triphosphates, two (or more) primers or primer-probe complexes with appropriate sequences, thermostable polymerase, buffers, solutes, and proteins. These reagents and details describing procedures for their use in amplifying nucleic acids are provided in U.S. Patent No. 4,683,202 (1987, Mullis, et al.) and U.S. Patent No. 4,683,195 (1986, Mullis, et al.). If LCR methodology is

selected, then the nucleic acid replication compositions may comprise, for example: a thermostable ligase (*e.g.*, *Thermus* aquaticus ligase), two sets of adjacent oligonucleotides (wherein one member of each set is complementary to each of the target strands), Tris-HCl buffer, KCl, EDTA, NAD, dithiothreitol, and salmon sperm DNA. See, for example, Tabor et al., *Proc. Natl. Acad. Sci. U.S.A.* 82:1074-1078 (1985).

The term "primer" refers to an oligonucleotide (synthetic or occurring naturally) that is capable of acting as a point of initiation of nucleic acid synthesis or replication along a complementary strand when placed under conditions in which synthesis of a complementary strand is catalyzed by a polymerase. A primer can further contain a detectable label, for example a 5' end label. In certain embodiments, primers of the present invention are 8-60 nucleic acids in length. In other embodiments, primers are 10-50, 14-40, or 20-30 nucleic acids in length. In certain specific embodiments, primers are at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

The term "probe" refers to an oligonucleotide (synthetic or occurring naturally) that is complementary (though not necessarily fully complementary) to a polynucleotide of interest and forms a duplexed structure by hybridization with at least one strand of the polynucleotide of interest. A probe or primer-probe complex can further contain a detectable label. In certain embodiments, probes of the present invention are 8-60 nucleic acids in length. In other embodiments, probes are 10-50, 14-40, or 20-30 nucleic acids in length. In certain specific embodiments, probes are at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

A probe can either be an independent entity or complexed with or otherwise attached to a primer, such as where a probe is connected, directly or indirectly, via its 3' terminus to a primer's 5' terminus. In some examples, the probe and primer are attached through a linker, which may be a nucleotide or non-nucleotide linker and which may be a non-amplifiable linker, such as a hexethylene glycol (HEG) or 18-carbon linker. In such a case, this would be termed a "primer-probe complex." One example of such primer-probe complexes can be found in U.S. Patent No. 6,326,145,

incorporated herein by reference in its entirety, which are frequently referred to as “Scorpion probes” or “Scorpion primers.” In a typical primer probe complex, the primer portion can be, for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length, while the probe portion can be, for example, at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols, semiconductor nanocrystals, ligands (e.g., biotin, avidin, streptavidin, or haptens), and the like. A detectable label can also include a combination of a reporter and a quencher.

The term "reporter" refers to a substance or a portion thereof that is capable of exhibiting a detectable signal, which signal can be suppressed by a quencher. The detectable signal of the reporter is, e.g., fluorescence in the detectable range. The term “quencher” refers to a substance or portion thereof that is capable of suppressing, reducing, inhibiting, etc., the detectable signal produced by the reporter.

As used herein, the terms "quenching" and "fluorescence energy transfer" refer to the process whereby, when a reporter and a quencher are in close proximity, and the reporter is excited by an energy source, a substantial portion of the energy of the excited state nonradiatively transfers to the quencher where it either dissipates nonradiatively or is emitted at a different emission wavelength than that of the reporter.

Preferably, the reporter may be selected from fluorescent organic dyes modified with a suitable linking group for attachment to the oligonucleotide, such as to the terminal 3' carbon or terminal 5' carbon. The quencher may also be selected from organic dyes, which may or may not be fluorescent, depending on the embodiment of the present invention. Generally, whether the quencher is fluorescent or simply releases the transferred energy from the reporter by non-radiative decay, the absorption band of the quencher should at least substantially overlap the fluorescent emission band of the reporter to optimize the quenching. Non-fluorescent

quenchers or dark quenchers typically function by absorbing energy from excited reporters, but do not release the energy radiatively.

Selection of appropriate reporter-quencher pairs for particular probes may be undertaken in accordance with known techniques. Fluorescent and dark quenchers and their relevant optical properties from which exemplary reporter-quencher pairs may be selected are listed and described, for example, in Berlman, Handbook of Fluorescence Spectra of Aromatic Molecules, 2nd ed., Academic Press, New York, 1971, the content of which is incorporated herein by reference. Examples of modifying reporters and quenchers for covalent attachment via common reactive groups that can be added to an oligonucleotide in the present invention may be found, for example, in Haugland, Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes of Eugene, Oreg., 1992, the content of which is incorporated herein by reference.

Preferred reporter-quencher pairs may be selected from xanthene dyes including fluoresceins and rhodamine dyes. Many suitable forms of these compounds are available commercially with substituents on the phenyl groups, which can be used as the site for bonding or as the bonding functionality for attachment to an oligonucleotide. Another preferred group of fluorescent compounds for use as reporters is the naphthylamines, having an amino group in the alpha or beta position. Included among such naphthylamino compounds are 1-dimethylaminonaphthyl-5 sulfonate, 1-anilino-8-naphthalene sulfonate and 2-p-touidinyl-6-naphthalene sulfonate. Other dyes include 3-phenyl-7-isocyanatocoumarin; acridines such as 9-isothiocyanatoacridine; N-(p-(2-benzoxazolyl)phenyl)maleimide; benzoxadiazoles; stilbenes; pyrenes and the like.

Most preferably, the reporters and quenchers are selected from fluorescein and rhodamine dyes. These dyes and appropriate linking methodologies for attachment to oligonucleotides are well known in the art.

Suitable examples of quenchers may be selected from 6-carboxy-tetramethyl-rhodamine, 4-(4-dimethylaminophenylazo) benzoic acid (DABYL), tetramethylrhodamine (TAMRA), BHQ-0™, BHQ-1™, BHQ-2™, and BHQ-3™, each of which are available from Biosearch Technologies, Inc. of Novato, Calif., QSY-7™,

QSY-9™, QSY-21™ and QSY-35™, each of which are available from Molecular Probes, Inc., and the like.

Suitable examples of reporters may be selected from dyes such as SYBR green, 5-carboxyfluorescein (5-FAM™ available from Applied Biosystems of Foster City, Calif.), 6-carboxyfluorescein (6-FAM), tetrachloro-6-carboxyfluorescein (TET), 2,7-dimethoxy-4,5-dichloro-6-carboxyfluorescein, hexachloro-6-carboxyfluorescein (HEX), 6-carboxy-2',4,7,7'-tetrachlorofluorescein (6-TET™ available from Applied Biosystems), carboxy-X-rhodamine (ROX), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (6-JOE™ available from Applied Biosystems), VIC™ dye products available from Molecular Probes, Inc., NED™ dye products available from available from Applied Biosystems, and the like.

One example of a probe that contains a reporter and a quencher is a Scorpion probe in either a unimolecular or bimolecular conformation. In a unimolecular Scorpion, the probe portion of the primer-probe complex is flanked by self-complementary regions, which allow the probe to form into a stem-loop structure when the probe is unbound from its target DNA. In certain embodiments, these are termed the 5' Stem Sequence, which has its 3' terminus attached to the 5' terminus of the probe, and the 3' Stem Sequence, which has its 5' terminus attached to the 3' terminus of the probe and its 3' terminus attached to the primer. These attachments can be either direct or indirect, such as through a linker. Further, in a unimolecular Scorpion, a reporter is typically attached at or near one of the self-complementary regions, such as at the 5' terminus of the Scorpion probe, and a quencher is attached at or near the other self-complementary region, such as immediately 5' to the non-amplifiable linker, such that the quencher is in sufficiently close proximity to the reporter to cause quenching when the probe is in its stem-loop conformation. In a bimolecular Scorpion, self-complementary flanking regions are not typically employed, but rather a separate "blocking oligonucleotide" or "quenching oligonucleotide" is employed in conjunction with the Scorpion probe. This blocking oligonucleotide is capable of hybridizing to the probe region of the Scorpion probe when the probe is unbound from its target DNA. Further, in a bimolecular Scorpion, the reporter is typically attached to the probe region of the Scorpion probe, such as at

the 5' terminus of the Scorpion probe, while the quencher is attached to the blocking oligonucleotide, such as at the 3' terminus of the blocking oligonucleotide, such that the quencher is in sufficiently close proximity to the reporter to cause quenching when the probe is unbound from its target DNA and is instead hybridized to the blocking oligonucleotide.

Another example of a probe that contains a reporter and a quencher is a probe that is to be used in a 5'-exonuclease assay, such as the TaqMan® real-time PCR technique. In this context, the oligonucleotide probe will have a sufficient number of phosphodiester linkages adjacent to its 5' end so that the 5' to 3' nuclease activity employed can efficiently degrade the bound probe to separate the reporters and quenchers. Yet another example of a probe that contains a reporter and quencher is a Molecular Beacon type probe, which contains a probe region flanked by self-complementary regions that allow the probe to form a stem-loop structure when unbound from the probe's target sequence. Such probes typically have a reporter attached at or near one terminus and a quencher attached at or near the other terminus such that the quencher is in sufficiently close proximity to the reporter to cause quenching when the probe is in its unbound, and thus stem-loop, form.

The term "replication inhibitor moiety" refers to any atom, molecule or chemical group that is attached to the 3' terminal hydroxyl group of an oligonucleotide that will block the initiation of chain extension for replication of a nucleic acid strand. Examples include, but are not limited to: 3'-deoxynucleotides (e.g., cordycepin), dideoxynucleotides, phosphate, ligands (e.g., biotin and dinitrophenol), reporter molecules (e.g., fluorescein and rhodamine), carbon chains (e.g., propanol), a mismatched nucleotide or polynucleotide, or peptide nucleic acid units. The term "non-participatory" refers to the lack of participation of a probe or primer in a reaction for the amplification of a nucleic acid molecule. Specifically a non-participatory probe or primer is one that will not serve as a substrate for, or be extended by, a DNA or RNA polymerase. A "non-participatory probe" is inherently incapable of being chain extended by a polymerase. It may or may not have a replication inhibitor moiety.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic

acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified, for example, in Sambrook, J., Fritsch, E. F. and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T*m* of 55°C, can be used, e.g., 5X SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5X SSC, 0.5% SDS. Moderate stringency hybridization conditions correspond to a higher T*m*, e.g., 40% formamide, with 5X or 6X SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although, depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T*m* for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T*m*) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T*m* have been derived (see Sambrook et al., *supra*, 9.50-9.51). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*, 11.7-11.8). In one preferred embodiment, the length for a hybridizable nucleic acid is at least about 10 nucleotides. More preferably a minimum length for a hybridizable nucleic acid is at least about 11 nucleotides, at least about 12 nucleotides, at least about 13 nucleotides, at least about 14 nucleotides, at least about 15 nucleotides, at least about 16 nucleotides, at least about 17 nucleotides, at least about 18 nucleotides, at least about 19 nucleotides, at least about 20 nucleotides, at least about 21 nucleotides, at least about 22 nucleotides, at least about 23 nucleotides, at least

about 24 nucleotides, at least about 25 nucleotides, at least about 26 nucleotides, at least about 27 nucleotides, at least about 28 nucleotides, at least about 29 nucleotides, or, most preferably, at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

In certain embodiments, primers probes are able to selectively hybridize to a target nucleic acid sequence under selective (*e.g.*, stringent) hybridization conditions. The term "selectively hybridize" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing nucleic acid sequences typically have about at least 70% sequence identity, preferably at least 80% sequence identity, and most preferably 90%, 95%, 97%, 99%, or 100% sequence identity with each other.

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by, e.g., Sambrook et al. (supra); and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience (1987).

Oligonucleotides

Methods have been developed for detecting *Salmonella* bacteria in a sample by detecting one or more target nucleic acid sequences. In certain embodiments, the methods involve isolated polynucleotides and/or reaction mixtures comprising a primer and a probe, wherein (i) the primer is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 1 or a sequence complementary thereto and the probe is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 3 or a sequence complementary thereto and the probe is capable of selectively hybridizing under stringent conditions to SEQ ID NO: 4 or a sequence complementary thereto. In some

embodiments, probes capable of selectively hybridize to SEQ ID NO: 1 include SEQ ID NOs: 40-54. In other embodiments, primers capable of selectively hybridize to SEQ ID NO: 2 include SEQ ID NOs: 6-39. In further embodiments, probes capable of selectively hybridize to SEQ ID NO: 3 include SEQ ID NOs: 95-111. In still further embodiments, primers capable of selectively hybridize to SEQ ID NO: 4 include SEQ ID NOs: 55-94.

In some embodiments, the primer and probe are directly or indirectly attached, thereby forming a primer-probe complex. In some examples, the primer-probed complex is formed by directly or indirectly attaching 3' terminus of the probe to the 5' terminus of the primer. These primer probe complexes of the instant invention can also contain a non-amplifiable linker that connects the 3' terminus of the probe region to the 5' terminus of the primer region. This non-amplifiable linker stops extension of a complementary strand from proceeding into the probe region of the primer-probe complex. Examples of such non-amplifiable linkages include hexethylene glycol (HEG) and, preferably, 18-carbon linkers.

Primer-probe complexes of the present invention can also contain a self-complementary region, including a 3' Stem Sequence and a 5' Stem Sequence, that allows the primer-probe complex to form a stem-loop structure when the probe is unbound from its target DNA, which may be useful, for example, in bringing the reporter and quencher into sufficiently close proximity to one another to cause the reporter signal to be quenched. In some embodiments, the 5' Stem Sequence is one of SEQ ID NOs: 112-119, and the 3' Stem Sequence is one of SEQ ID NOs: 120-130.

In additional embodiments, the primers, probes, or primer-probe complexes further comprise a detectable label, such as a 5' end label or a reporter-quencher pair. In some instances, a quencher oligonucleotide can be employed with a probe or primer-probe complex, which quencher oligonucleotide is capable of hybridizing to the probe or probe region of the primer-probe complex when the probe is unbound from its target DNA. If the reporter is attached to the probe or primer-probe complex and the quencher is attached to the blocking oligonucleotide, this can bring the

reporter and quencher into sufficiently close proximity to one another to allow quenching to occur.

In certain embodiments, the primer or primer-probe complex is used in conjunction with a reverse primer. In still further embodiments, two such primer-probe complexes are employed, one as a forward primer-probe complex and the other as a reverse primer-probe complex. Exemplary combinations of probe portions, primer portions, 5' and 3' Stem Sequences, linking moieties, and detectable labels are provided in **Figs. 1A-1C**.

In addition to their usefulness in PCR, these primer-probe complexes may also be useful for other nucleic acid amplification methods such as the ligase chain reaction (LCR) (Backman et al., 1989, EP 0 320 308; Carrino et al., 1995, *J. Microbiol. Methods* 23: 3-20); nucleic acid sequence-based amplification (NASBA), (Carrino et al., 1995, *supra*); and self-sustained sequence replication (3SR) and 'Q replicase amplification' (Pfeffer et al., 1995 *Veterinary Res. Comm.* 19: 375-407).

In addition, oligonucleotides of the present invention also may be used as hybridization probes. Hybridization using DNA probes has been frequently used for the detection of pathogens in food, clinical and environmental samples, and the methodologies are generally known to one skilled in the art. It is generally recognized that the degree of sensitivity and specificity of probe hybridization is lower than that achieved through the previously described amplification techniques.

Assay Methods

Detection of the selected gene targets, and subsequent detection of the presence of *Salmonella* in a sample, may be accomplished in any suitable manner. Preferred methods are primer-directed amplification methods and nucleic acid hybridization methods. These methods may be used to detect *Salmonella* in a sample that is either a complex matrix or a purified culture, e.g., from an animal, environmental, or food source suspected of contamination.

A preferred embodiment of the instant invention comprises (1) culturing a complex sample mixture in a non-selective growth media to resuscitate the target bacteria, (2) releasing total target bacterial DNA, and (3) subjecting the total DNA to

an amplification protocol with a primer and probe, or a primer-probe complex, of the invention and a reverse primer, or two primer-probe complexes of the invention (one acting as a forward primer and a second acting as a reverse primer.

Primer-Directed Amplification Assay Methods

A variety of primer-directed nucleic acid amplification methods are known in the art which can be employed in the present invention, including thermal cycling methods (e.g., PCR, RT-PCR, and LCR), as well as isothermal methods and strand displacement amplification (SDA). The preferred method is PCR. In one preferred embodiment, the primer-probe complexes set forth in **Figs. 1A-1C** may be used as primers for use in primer-directed nucleic acid amplification for the detection of the target nucleic acid(s) and, ultimately, the detection of *Salmonella*.

Sample Preparation:

The oligonucleotides and methods according to the instant invention may be used directly with any suitable clinical or environmental samples, without any need for sample preparation. In order to achieve higher sensitivity, and in situations where time is not a limiting factor, it is preferred that the samples be pre-treated and that pre-amplification enrichment is performed.

The minimum industry standard for the detection of food-borne bacterial pathogens is a method that will reliably detect the presence of one pathogen cell in 25 g of food matrix as described in Andrews et al., 1984, "Food Sample and Preparation of Sample Homogenate", Chapter 1 in *Bacteriological Analytical Manual,* 8th Edition, Revision A, Association of Official Analytical Chemists, Arlington, VA. In order to satisfy this stringent criterion, enrichment methods and media have been developed to enhance the growth of the target pathogen cell in order to facilitate its detection by biochemical, immunological or nucleic acid hybridization means. Typical enrichment procedures employ media that will enhance the growth and health of the target bacteria and also inhibit the growth of any background or non-target microorganisms present. For example, the USDA has set forth a protocol for

enrichment of samples of ground beef to be tested for pathogenic *E. coli* (U.S. Food and Drug Administration, Bacterial Analytical Manual).

Selective media have been developed for a variety of bacterial pathogens and one of skill in the art will know to select a medium appropriate for the particular organism to be enriched. A general discussion and recipes of non-selective media are described in the FDA Bacteriological Analytical Manual. (1998) published and distributed by the Association of Analytical Chemists, Suite 400, 2200 Wilson Blvd, Arlington, VA 22201-3301.

After selective growth, a sample of the complex mixtures is removed for further analysis. This sampling procedure may be accomplished by a variety of means well known to those skilled in the art. In a preferred embodiment, 5 µl of the enrichment culture is removed and added to 200 µl of lysis solution containing protease. The lysis solution is heated at 37°C for 20 min followed by protease inactivation at 95°C for 10 min as described in the BAX® System User's Guide, DuPont Qualicon, Inc., Wilmington, DE.

PCR Assay Methods:

A preferred method for detecting the presence of the present invention's target nucleic acids and subsequently *Salmonella* in a sample comprises (a) performing PCR amplification using a primer and probe, or a primer-probe complex, of the present invention, such as those described in **Figs. 1A-1C**, and a suitable reverse primer; and (b) detecting the amplification, whereby a positive detection of the amplification indicates the presence of *Salmonella* in the sample. In another embodiment, PCR amplification is performed using two different primer-probe complexes of the present invention that have primer binding regions that are sufficiently separated such that one primer-probe complex acts as a forward primer and the second primer-probe complex acts as a reverse primer. Looking at **Figs. 1A-1C**, examples of such forward-acting primer-probe complexes include S35C610-1, S35C610-2, S35C610-2a, S35C610-3a, S35C610-3a, S35C610-3a, S35C610-3b, S35C610-4b, S35C610-5b, S35FAM-3a, S35Q670-2a, S35TEX1, S35TEX1a, S35TEX2, S35TEX2, S35TEX2b, S35TEX2c, S35TEX3, S35TEX3a, S35TEX3a,

S35TEX-3a, and SB35C610, while examples of such reverse-acting primer-probe complexes include S761aC610-4d, S761bC610-4g, S761bC610-5g, S761C610-3, S761C610-3a, S761C610-3b, S761C610-3c, S761C610-4c, S761C610-4d, S761C610-4e, S761C610-4f, S761C610-4g, S761C610-5f, S761C610-5g, SB761C610, and SB761C610-g.

In another preferred embodiment, prior to performing PCR amplification, a step of preparing the sample may be carried out. The preparing step may comprise at least one of the following processes: (1) bacterial enrichment, (2) separation of bacterial cells from the sample, (3) cell lysis, and (4) total DNA extraction.

Amplification Conditions:

A skilled person will understand that any generally acceptable PCR conditions may be used for successfully detecting the nucleic acid targets and the target *Salmonella* bacteria using the oligonucleotides of the instant invention, and depending on the sample to be tested and other laboratory conditions, routine optimization for the PCR conditions may be necessary to achieve optimal sensitivity and specificity.

Detection/Examination/Analysis:

Primer-directed amplification products produced by the methods of the present invention can be analyzed using various methods. Homogenous detection refers to a preferred method for the detection of amplification products where no separation (such as by gel electrophoresis) of amplification products from template or primers is necessary. Homogeneous detection is typically accomplished by measuring the level of fluorescence of the reaction mixture during or immediately following amplification. In addition, heterogeneous detection methods, which involve separation of amplification products during or prior to detection, can be employed in the present invention.

Homogenous detection may be employed to carry out “real-time” primer-directed nucleic acid amplification and detection, using primer-probe complexes of the instant invention (e.g., “real-time” PCR and “real-time” RT-PCR). A particularly

preferred "real-time" detection method is the Scorpion probe assay as set forth in U.S. Patent No. 6,326,145, which is hereby incorporated by reference in its entirety. In the Scorpion probe assay, PCR amplification is performed using a Scorpion probe (either unimolecular or bimolecular) as a primer-probe complex, the Scorpion probe possessing an appropriate reporter-quencher pair to allow the detectable signal of the reporter to be quenched prior to elongation of the primer. Post-elongation, the quenching effect is eliminated and the amount of signal present is quantitated. As the amount of amplification product increases, an equivalent increase in detectable signal will be observed, thus allowing the amount of amplification product present to be determined as a function of the amount of detectable signal measured. When more than one Scorpion probe is employed in a Scorpion probe assay of present invention, each probe can have the same detectable label attached or a different detectable label attached, thus allowing each probe to be detected independently of the other probes.

Another preferred "real-time" detection method is the 5'-exonuclease detection method, as set forth in U.S. Patent Nos. 5,804,375, 5,538,848, 5,487,972, and 5,210,015, each of which is hereby incorporated by reference in its entirety. In the 5'-exonuclease detection assay, a modified probe is employed during PCR that binds intermediate to or between the two members of an amplification primer pair. The modified probe possesses a reporter and a quencher and is designed to generate a detectable signal to indicate that it has hybridized with the target nucleic acid sequence during PCR. As long as both the reporter and the quencher are on the probe, the quencher stops the reporter from emitting a detectable signal. However, as the polymerase extends the primer during amplification, the intrinsic 5' to 3' nuclease activity of the polymerase degrades the probe, separating the reporter from the quencher, and enabling the detectable signal to be emitted. Generally, the amount of detectable signal generated during the amplification cycle is proportional to the amount of product generated in each cycle.

It is well known that the efficiency of quenching is a strong function of the proximity of the reporter and the quencher, i.e., as the two molecules get closer, the quenching efficiency increases. As quenching is strongly dependent on the physical

proximity of the reporter and quencher, the reporter and the quencher are preferably attached to the probe within a few nucleotides of one another, usually within 30 nucleotides of one another, more preferably with a separation of from about 6 to 16 nucleotides. Typically, this separation is achieved by attaching one member of a reporter-quencher pair to the 5' end of the probe and the other member to a nucleotide about 6 to 16 nucleotides away.

Again, when more than one TaqMan® probe is employed in a 5'-exonuclease detection assay of present invention, such as one directed to two or more of SEQ ID NOs: 686-696, each probe can have a different detectable label (e.g., reporter-quencher pair) attached, thus allowing each probe to be detected independently of the other probes.

In addition to homogenous detection methods, a variety of other heterogeneous detection methods are known in the art that can be employed in the present invention, including standard non-denaturing gel electrophoresis (e.g., acrylamide or agarose), denaturing gradient gel electrophoresis, and temperature gradient gel electrophoresis. Standard non-denaturing gel electrophoresis is a simple and quick method of PCR detection, but may not be suitable for all applications.

Instrumentation:

When homogenous detection is employed, the level of fluorescence is preferably measured using a laser fluorometer such as, for example, an ABI Prism Model 7500 Fast Sequence Detector. However, similar detection systems for measuring the level of fluorescence in a sample are included in the invention.

Reagents and Kits:

Any suitable nucleic acid replication composition ("replication composition") in any format can be used. A typical replication composition for PCR amplification may comprise, for example, dATP, dCTP, dGTP, and dTTP; target specific primers, probes, or primer-probe complexes; and a suitable polymerase.

If the replication composition is in liquid form, suitable buffers known in the art may be used (Sambrook, J. et al., supra).

Alternatively, if the replication composition is contained in a tablet form, then typical tabletization reagents may be included such as stabilizers and binding agents. Preferred tabletization technology is set forth in U.S. Patent Nos. 4,762,857 and 4,678,812, each of which is hereby incorporated by reference in its entirety.

In certain embodiments, the replication composition of the instant invention comprises at least one primer and probe and a thermostable DNA polymerase, wherein the primer is at least 10 nucleotides in length and the probe is at least 10 nucleotides in length, and wherein (i) the primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 or a sequence complementary thereto, and the probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 or a sequence complementary thereto; or (ii) the primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and the probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto. In certain specific embodiments, the primer is at least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In further embodiments, the probe is at least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In some examples, the primer and probe are directly or indirectly attached, thereby forming a primer-probe complex. In other examples , the primer-probe complex involves direct or indirect attachment of the 3' terminus of the probe to the 5' terminus of the primer. In further examples, the probe portion of the primer-probe complex is flanked by a 5' Stem Sequence, such as set forth in SEQ ID NOs: 112-119, and a 3' Stem Sequence, such as set forth in SEQ ID NOs: 120-130.

In some embodiments, the primer portion capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 1 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 40-54. In other embodiments, the primer portion capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 2 comprises a nucleic acid sequence

selected from the group consisting of SEQ ID NOs: 5-39. In further embodiments, the primer portion capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 3 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 95-111. In still further embodiments, the primer portion capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 55-94.

In other specific embodiments, the replication composition of the instant invention comprises (a) at least one primer-probe complex selected from **Figs. 1A-1C**, and (b) thermostable DNA polymerase. Another preferred replication composition comprises (a) at least two primer-probe complexes selected from **Figs. 1A-1C**, each directed toward a different target DNA region such that one complex acts as a forward primer and the other acts as a reverse primer; and (b) thermostable DNA polymerase. In certain examples, the forward-acting primer-probe complex is selected from the group consisting of S35C610-1, S35C610-2, S35C610-2a, S35C610-3a, S35C610-3a, S35C610-3a, S35C610-3b, S35C610-4b, S35C610-5b, S35FAM-3a, S35Q670-2a, S35TEX1, S35TEX1a, S35TEX2, S35TEX2, S35TEX2b, S35TEX2c, S35TEX3, S35TEX3a, S35TEX3a, S35TEX-3a, and SB35C610, while the reverse-acting primer-probe complex is selected from the group consisting of S761aC610-4d, S761bC610-4g, S761bC610-5g, S761C610-3, S761C610-3a, S761C610-3b, S761C610-3c, S761C610-4c, S761C610-4d, S761C610-4e, S761C610-4f, S761C610-4g, S761C610-5f, S761C610-5g, SB761C610, and SB761C610-g. In certain examples, the replication composition further comprises a suitable quencher oligonucleotide capable of binding to, and quenching the signal of, the probe portion of the primer-probe complex.

A preferred kit of the instant invention comprises any one of the above replication compositions. A preferred tablet of the instant invention comprises any one of the above replication compositions. More preferably, a kit of the instant invention comprises the foregoing preferred tablet.

In some instances, an internal positive control can be included in the reaction. The internal positive control can include control template nucleic acids (e.g. DNA or

RNA), control primers, and a control nucleic acid probe. The advantages of an internal positive control contained within a PCR reaction have been previously described (U.S. Patent No. 6,312,930 and PCT Application No. WO 97/11197, each of which is hereby incorporated by reference in its entirety), and include: (i) the control may be amplified using a single primer; (ii) the amount of the control amplification product is independent of any target DNA or RNA contained in the sample; (iii) the control DNA can be tableted with other amplification reagents for ease of use and high degree of reproducibility in both manual and automated test procedures; (iv) the control can be used with homogeneous detection, i.e., without separation of product DNA from reactants; and (v) the internal control has a melting profile that is distinct from other potential amplification products in the reaction and/or a detectable label on the control nucleic acid that is distinct from the detectable label on the nucleic acid probe directed to the target.

Control DNA will be of appropriate size and base composition to permit amplification in a primer-directed amplification reaction. The control template DNA sequence may be obtained from any suitable source, but must be reproducibly amplified under the same conditions that permit the amplification of the target amplification product.

Preferred control sequences include, for example, control primers and probes directed toward SV40 DNA.

The control reaction is useful to validate the amplification reaction. Amplification of the control DNA occurs within the same reaction tube as the sample that is being tested, and therefore indicates a successful amplification reaction when samples are target negative, i.e. no target amplification product is produced. In order to achieve significant validation of the amplification reaction, a suitable number of copies of the control DNA template must be included in each amplification reaction.

In some instances, it may be useful to include an additional negative control replication composition. The negative control replication composition will contain the same reagents as the replication composition but without the polymerase. The primary function of such a control is to monitor spurious background fluorescence in a homogeneous format when the method employs a fluorescent means of detection.

Replication compositions may be modified depending on whether they are designed to be used to amplify target DNA or the control DNA. Replication compositions that will amplify the target DNA (test replication compositions) may include (i) a polymerase (generally thermostable), (ii) a primer pair capable of hybridizing to the target DNA and (iii) necessary buffers for the amplification reaction to proceed. Replication compositions that will amplify the control DNA (positive control, or positive replication composition) may include (i) a polymerase (generally thermostable) (ii) the control DNA; (iii) at least one primer capable of hybridizing to the control DNA; and (iv) necessary buffers for the amplification reaction to proceed. In addition, the replication composition for either target DNA or control DNA amplification can contain a nucleic acid probe, preferably possessing a detectable label.

EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only.

EXAMPLE 1

Determination of inclusivity/exclusivity of the individual targets via Scorpion® assay

Samples of organisms were analyzed to establish inclusivity and exclusivity of numerous Scorpion® probes of the present invention. For inclusivity, independent, bona fide *Salmonella* isolates were used. For exclusivity, closely related non-target organisms were used to ensure that the assay would discriminate the target organisms from other non-target organisms.

DNA lysate preparation

Material tested was overnight growth pure cultures of the target and non-target organisms grown at 37 °C in BHI media. Pure cultures grown overnight to cell densities of approximately $1X10^9$ cfu/ml. For exclusivity, 1:10 dilutions of overnight cultures were tested. For inclusivity, overnight cultures were diluted approximately 1:10,000 into TSB. 5 µl of the material to be tested was added to 200 µl of BAX®

lysis reagent (DuPont Qualicon, Wilmington, DE). The mixture was incubated at 37 °C for 20 minutes, then further incubated at 95 °C for 10 minutes, and finally cooled to 5 °C.

PCR conditions

30 µl of the DNA lysate was used to hydrate lyophylized PCR reaction components to obtain DNA lysate/PCR reaction component mixtures. The PCR reaction components were in the form of customized reagent containing GoTaq DNA Polymerase (Promega, Madison, WI), deoxynucleotides (Roche Diagnostics, Indianapolis, IN), BSA, and surfactamps (Sigma-Aldrich, St. Louis, MO). In addition, the primers and Scorpion® probes listed in Table 1 were included in the amounts provided. As this Table demonstrates, each of these Scorpion® probes was designed as a uni-molecular Scorpion®, such that its structure includes (in 5' to 3' order): a 5' fluorescent end label, 5' Stem Sequence, a probe sequence, a 3' Stem Sequence, an internal quencher, an 18-carbon non-amplifiable linker, and a primer sequence.

TABLE 1. Primers and Probes Used in Inclusivity/Exclusivity Study

Nucleotide Name	Target	Amt. Per Rxn.	5' End Label	5' Stem SEQ ID NO:	Probe SEQ ID NO:	3' Stem SEQ ID NO:	Internal Label / Linker	Primer SEQ ID NO:
Forward Scorpion S35C610-3a	Salmonella	50-150 nM	Cal Fluor Red 610	114	29	124	BHQ2 / 18-Carbon Linker	47
Forward Primer 35	Salmonella	250-350 nM						47
Reverse Scorpion S761C610-4d	Salmonella	50-150 nM	Cal Fluor Red 610	116	90	127	BHQ2 / 18-Carbon Linker	111
Reverse Scorpion S761C610-5g	Salmonella	50-150 nM	Cal Fluor Red 610	117	93	128	BHQ2 / 18-Carbon Linker	102
Reverse Primer 761	Salmonella	250-350 nM						111
Forward Scorpion S4219EC560-3b	Positive Control (sSV40)	10-50 nM	Cal Fluor Orange 560	132	133	134	BHQ1 / 18-Carbon Linker	135

Forward Primer 4219E	Positive Control (sSV40)	30-60 nM						135
Reverse Primer 4313E	Positive Control (sSV40)	70-125 nM						136

Amplification and testing were performed on the BAX® Q7 instrument (DuPont Qualicon, Wilmington, DE). The thermal cycling conditions were as follows: 2 minutes at 95 °C, followed by 46 cycles of 95 °C for 10 seconds and 70 °C for 50 seconds, with the fluorescent signal captured during the 70 °C step at each cycle.

Results

As can be seen in Tables 2-3, using Scorpion® probes, the method of the present invention was able to detect the various targets appropriately, including distinguishing between target and non-target organisms.

TABLE 2. Inclusivity Results

DuPont Qualicon ID #	Presumptive ID	ID Species	BAX® System Result
584	Salmonella typhi	Salmonella typhi	Positive
585	Salmonella typhi	Salmonella typhi	Positive
586	Salmonella typhimurium	Salmonella typhimurium	Positive
706	Salmonella enteritidis	Salmonella enteritidis	Positive
707	Salmonella newport	Salmonella newport	Positive
725	Salmonella arizonae	Salmonella arizonae	Positive
726	Salmonella arizonae	Salmonella arizonae	Positive
737	Salmonella enteritidis	Salmonella enteritidis	Positive
738	Salmonella virchow	Salmonella virchow	Positive
739	Salmonella stanley	Salmonella stanley	Positive
741	Salmonella gallinarum	Salmonella gallinarum	Positive
917	Salmonella choleraesuis	Salmonella choleraesuis	Positive
918	Salmonella paratyphi A	Salmonella paratyphi	Positive
919	Salmonella paratyphi A	Salmonella paratyphi	Positive
964	Salmonella bredeney	Salmonella bredeney	Positive
966	Salmonella napoli	Salmonella napoli	Positive
1084	Salmonella typhimurium	Salmonella typhimurium	Positive
1084	Salmonella typhimurium	Salmonella typhimurium	Positive
1085	Salmonella binza	Salmonella binza	Positive
1248	Salmonella panama	Salmonella panama	Positive
1251	Salmonella kedougou	Salmonella kedougou	Positive

1255	Salmonella montevideo	Salmonella montevideo	Positive
1261	Salmonella newport	Salmonella newport	Positive
1329	Salmonella braenderup	Salmonella braenderup	Positive
1331	Salmonella berta	Salmonella berta	Positive
1332	Salmonella anatum	Salmonella anatum	Positive
1333	Salmonella stanley	Salmonella stanley	Positive
1334	Salmonella anatum	Salmonella anatum	Positive
1335	Salmonella agona	Salmonella agona	Positive
1336	Salmonella thompson	Salmonella thompson	Positive
1337	Salmonella braenderup	Salmonella braenderup	Positive
1338	Salmonella brandenburg	Salmonella brandenburg	Positive
1339	Salmonella thompson	Salmonella thompson	Positive
1343	Salmonella blockley	Salmonella haardt	Positive
1352	Salmonella agona	Salmonella agona	Positive
1356	Salmonella bredeney	Salmonella bredeney	Positive
1372	Salmonella saintpaul	Salmonella saintpaul	Positive
1424	Salmonella manchester	Salmonella manchester	Positive
1429	unknown	Salmonella anfo	Positive
1467	Salmonella typhimurium	Salmonella typhimurium	Positive
1469	Salmonella ealing	Salmonella ealing	Positive
1476	Salmonella napoli	Salmonella napoli	Positive
1480	Salmonella indiana	Salmonella indiana	Positive
1482	Salmonella pullorum	Salmonella pullorum	Positive
1491	Salmonella weltevreden	Salmonella weltevreden	Positive
1492	Salmonella montevideo	Salmonella montevideo	Positive
1507	Salmonella pullorum	Salmonella pullorum	Positive
1509	Salmonella bovismorbificans	Salmonella bovismorbificans	Positive
1510	Salmonella bareilly	Salmonella bareilly	Positive
1521	Salmonella amersfoort	Salmonella abaetetuba	Positive
1523	Salmonella berkeley	Salmonella berkeley	Positive
1525	Salmonella betioky	Salmonella betioky	Positive
1526	Salmonella austin	Salmonella austin	Positive
1527	Salmonella atlanta	Salmonella atlanta	Positive
1530	Salmonella amager	Salmonella altendorf	Positive
1531	Salmonella altendorf	Salmonella altendorf	Positive
1535	Salmonella brookfield	Salmonella brookfield	Positive
1543	Salmonella adelaide	Salmonella adelaide	Positive
1547	Salmonella aberdeen	Salmonella species	Positive
1548	Salmonella abony	Salmonella abony	Positive
1551	Salmonella aequatoria	Salmonella species	Positive
1552	Salmonella alabama	Salmonella alabama	Positive
1553	Salmonella ball	Salmonella ball	Positive
1554	Salmonella banalia	Salmonella species	Positive
1555	Salmonella brancaster	Salmonella brancaster	Positive
1556	Salmonella alachua	Salmonella alachua	Positive
1557	Salmonella chicago	Salmonella chicago	Positive
1558	Salmonella canastel	Salmonella species	Positive

1560	Salmonella westpark	Salmonella species	Positive
1566	Salmonella	Salmonella	Positive
1568	Salmonella	Salmonella arizonae	Positive
1573	Salmonella	Salmonella arizonae	Positive
1576	Salmonella	Salmonella arizonae	Positive
1585	Salmonella	Salmonella arizonae	Positive
1590	Salmonella	Salmonella 3b	Positive
1592	Salmonella	Salmonella 3b	Positive
1597	Salmonella	Salmonella 3b	Positive
1598	Salmonella	Salmonella 3b	Positive
1603	Salmonella	Salmonella 3b	Positive
1608	Salmonella seminole	Salmonella seminole	Positive
1609	Salmonella wassennaar	Salmonella wassennaar	Positive
1610	Salmonella seminole	Salmonella seminole	Positive
1611	Salmonella	Salmonella kralendyk	Positive
1613	Salmonella tuindorp	Salmonella kralendyk	Positive
1615	Salmonella chameleon	Salmonella kralendyk	Positive
1616	Salmonella houten	Salmonella houten	Positive
1620	Salmonella carmel	Salmonella carmel	Positive
1621	Salmonella carrau	Salmonella carrau	Positive
1623	Salmonella champaign	Salmonella champaign	Positive
1624	Salmonella chandans	Salmonella chandans	Positive
1625	Salmonella chester	Salmonella species	Positive
1628	Salmonella colorado	Salmonella species	Positive
1632	Salmonella cubana	Salmonella cubana	Positive
1635	Salmonella daytona	Salmonella daytona	Positive
1638	Salmonella derby	Salmonella derby	Positive
1641	Salmonella durban	Salmonella species	Positive
1644	Salmonella ealing	Salmonella ealing	Positive
1650	Salmonella livingstone	Salmonella livingstone	Positive
1652	Salmonella london	Salmonella london	Positive
1653	Salmonella manhattan	Salmonella yovokome	Positive
1655	Salmonella reading	Salmonella reading	Positive
1657	Salmonella sandiego	Salmonella reading	Positive
1658	Salmonella schwarzengrund	Salmonella schwarzengrund	Positive
1659	Salmonella shangani	Salmonella shangani	Positive
1660	Salmonella sundsvall	Salmonella sundsvall	Positive
1661	Salmonella tennessee	Salmonella tennessee	Positive
1665	Salmonella colombo	Salmonella colombo	Positive
1668	Salmonella california	Salmonella california	Positive
1675	Salmonella daressalaam	Salmonella enterica	Positive
1680	Salmonella dugbe	Salmonella dugbe	Positive
1684	Salmonella emmastad	Salmonella emmastad	Positive
1686	Salmonella fayed	Salmonella fayed	Positive
1687	Salmonella ferlac	Salmonella ferlac	Positive
1689	Salmonella hartford	Salmonella species	Positive
1693	Salmonella javiana	Salmonella species	Positive

1695	Salmonella johannesburg	Salmonella johannesburg	Positive
1698	Salmonella madelia	Salmonella madelia	Positive
1700	Salmonella meleagridis	Salmonella meleagridis	Positive
1701	Salmonella miami	Salmonella miami	Positive
1703	Salmonella mississippi	Salmonella mississippi	Positive
1704	Salmonella muenchen	Salmonella muenchen	Positive
1707	Salmonella newbrunswick	Salmonella newbrunswick	Positive
1710	Salmonella oranienburg	Salmonella oranienburg	Positive
1711	Salmonella pomona	Salmonella species	Positive
1712	Salmonella pretoria	Salmonella pretoria	Positive
1714	Salmonella wassennaar	Salmonella wassennaar	Positive
1773	Salmonella enterica	Salmonella brookfield	Positive
1775	Salmonella enterica	Salmonella typhimurium	Positive
1776	Salmonella enterica	Salmonella kralendyk	Positive
1777	Salmonella enterica	Salmonella enterica	Positive
2166	Salmonella abaetetuba	Salmonella abaetetuba	Positive
2172	Salmonella bareilly	Salmonella bareilly	Positive
2178	Salmonella california	Salmonella california	Positive
2180	Salmonella champaign	Salmonella champaign	Positive
2186	Salmonella drypool	Salmonella drypool	Positive
2189	Salmonella give	Salmonella give	Positive
2196	Salmonella kiambu	Salmonella kiambu	Positive
2199	Salmonella lexington	Salmonella lexington	Positive
2201	Salmonella madelia	Salmonella madelia	Positive
2204	Salmonella minnesota	Salmonella minnesota	Positive
2205	Salmonella mississippi	Salmonella mississippi	Positive
2215	Salmonella poona	Salmonella poona	Positive
2218	Salmonella sandiego	Salmonella sandiego	Positive
2229	Salmonella theilalle	Salmonella oranienburg	Positive
2238	Salmonella urbana	Salmonella urbana	Positive
2239	Salmonella uzaramo	Salmonella cerro	Positive
2245	Salmonella havana	Salmonella havana	Positive
2263	Salmonella lille	Salmonella lille	Positive
2274	Salmonella anatum	Salmonella anatum	Positive
2283	Salmonella newbrunswick	Salmonella newbrunswick	Positive
2289	Salmonella rubislaw	Salmonella rubislaw	Positive
2290	Salmonella hartford	Salmonella hartford	Positive
2309	Salmonella maregrosso	Salmonella species	Positive
2312	Salmonella kottbus	Salmonella kottbus	Positive
2313	Salmonella wandsworth	Salmonella wandsworth	Positive
2341	Salmonella barry	Salmonella mbandaka	Positive
2343	Salmonella bockenheim	Salmonella kralendyk	Positive
2346	Salmonella vietnam	Salmonella vietnam	Positive
2349	Salmonella drypool	Salmonella drypool	Positive
2350	Salmonella gallinarum	Salmonella gallinarum	Positive
2352	Salmonella saphra	Salmonella saphra	Positive
2353	Salmonella kristianstad	Salmonella kristianstad	Positive
2373	Salmonella species	Salmonella species	Positive

2376	Salmonella species	Salmonella sculcoates	Positive
2380	Salmonella species	Salmonella sya	Positive
2628	Salmonella kentucky	Salmonella kentucky	Positive
2629	Salmonella cerro	Salmonella cerro	Positive
2637	Salmonella schwarzengrund	Salmonella schwarzengrund	Positive
2639	Salmonella thomasville	Salmonella thomasville	Positive
2641	Salmonella schwarzengrund	Salmonella schwarzengrund	Positive
2673	Salmonella manhattan	Salmonella manhattan	Positive
2736	Salmonella oranienburg	Salmonella oranienburg	Positive
2748	Salmonella muenster	Salmonella muenster	Positive
2755	Salmonella mbandaka	Salmonella mbandaka	Positive
2757	Salmonella mbandaka	Salmonella mbandaka	Positive
2761	Salmonella mbandaka	Salmonella mbandaka	Positive
2766	Salmonella mbandaka	Salmonella mbandaka	Positive
2770	Salmonella mbandaka	Salmonella mbandaka	Positive
2774	Salmonella mbandaka	Salmonella mbandaka	Positive
2779	Salmonella mbandaka	Salmonella mbandaka	Positive
2786	Salmonella binza	Salmonella binza	Positive
2795	Salmonella berta	Salmonella berta	Positive
2813	Salmonella cerro	Salmonella cerro	Positive
2820	Salmonella braenderup	Salmonella braenderup	Positive
2867	Salmonella sya	Salmonella sya	Positive
2868	Salmonella lille	Salmonella lille	Positive
2869	Salmonella durham	Salmonella durham	Positive
2870	Salmonella corvallis	Salmonella bellevue	Positive
2935	Salmonella sandiego	Salmonella sandiego	Positive
2966	Salmonella albany	Salmonella albany	Positive
2980	Salmonella arkansas	Salmonella arkansas	Positive
2981	Salmonella arkansas	Salmonella arkansas	Positive
2992	Salmonella lille	Salmonella lille	Positive
3015	Salmonella dublin	Salmonella dublin	Positive
3017	Salmonella dublin	Salmonella dublin	Positive
3019	Salmonella dublin	Salmonella dublin	Positive
3038	Salmonella krefeld	Salmonella krefeld	Positive
3043	Salmonella johannesburg	Salmonella johannesburg	Positive
3153	Salmonella chandans	Salmonella chandans	Positive
3156	Salmonella muenchen	Salmonella muenchen	Positive
3157	Salmonella corvallis	Salmonella bellevue	Positive
3184	Salmonella sculcoates	Salmonella sculcoates	Positive
3185	Salmonella bellevue	Salmonella bellevue	Positive
3186	Salmonella sya	Salmonella sya	Positive
3187	Salmonella durham	Salmonella durham	Positive
3194	Salmonella stanleyville	Salmonella stanleyville	Positive
3217	Salmonella cotham	Salmonella cotham	Positive
3218	Salmonella agama	Salmonella agama	Positive
3432	Salmonella amager	Salmonella amager	Positive

3510	Salmonella oslo	unknown	Positive
3536	Salmonella tennessee	Salmonella tennessee	Positive
3699	Salmonella hvittingfoss	Salmonella species	Positive
3852	Salmonella indiana	Salmonella indiana	Positive
3863	Salmonella othmarschen	Salmonella oranienburg	Positive
3878	Salmonella mbandaka	Salmonella mbandaka	Positive
3882	Salmonella broughton	Salmonella minnesota	Positive
3898	Salmonella neumuenster	Salmonella thompson	Positive
3915	Salmonella haardt	Salmonella haardt	Positive
3917	Salmonella hadar	Salmonella hadar	Positive
3918	Salmonella hadar	Salmonella hadar	Positive
3924	Salmonella thomasville	Salmonella thomasville	Positive
3984	Salmonella choleraesuis	Salmonella java	Positive
3988	Salmonella choleraesuis	Salmonella choleraesuis	Positive
4011	Salmonella mbandaka	Salmonella mbandaka	Positive
4022	Salmonella enteritidis	Salmonella enteritidis	Positive
4035	Salmonella waycross	Salmonella waycross	Positive
4036	Salmonella livingstone	Salmonella livingstone	Positive
4043	Salmonella worthington	Salmonella worthington	Positive
4084	Salmonella africana	Salmonella thompson	Positive
4102	Salmonella species	Salmonella saintpaul	Positive
4558	Salmonella redlands	Salmonella redlands	Positive
5533	Salmonella infantis	Salmonella infantis	Positive
5908	Salmonella ferlac	Salmonella ferlac	Positive
6177	Salmonella species	Salmonella arkansas	Positive
6250	Salmonella santiago	Salmonella santiago	Positive
6586	Salmonella santiago	Salmonella santiago	Positive
6667	Salmonella heidelberg	Salmonella heidelberg	Positive
6696	Salmonella species	Salmonella enteritidis	Positive
6729	Salmonella manila	Salmonella manila	Positive
6735	Salmonella species	Salmonella albany	Positive
6966	Salmonella cotham	Salmonella cotham	Positive
7005	Salmonella dublin	Salmonella species	Positive
7061	Salmonella kubacha	Salmonella kubacha	Positive
7062	Salmonella kubacha	Salmonella kubacha	Positive
7072	Salmonella amsterdam	Salmonella amsterdam	Positive
7111	Salmonella infantis	Salmonella infantis	Positive
8034	Salmonella species	Salmonella saintpaul	Positive
12241	Salmonella species	Salmonella species	Positive
12904	Salmonella tranorora	Salmonella enterica	Positive
12907	Salmonella heidelberg	Salmonella heidelberg	Positive
12908	Salmonella heidelberg	Salmonella heidelberg	Positive
12909	Salmonella heidelberg	Salmonella heidelberg	Positive
12910	Salmonella heidelberg	Salmonella heidelberg	Positive
12911	Salmonella heidelberg	Salmonella heidelberg	Positive
12912	Salmonella kentucky	Salmonella kentucky	Positive
12913	Salmonella heidelberg	Salmonella heidelberg	Positive
12914	Salmonella kentucky	Salmonella kentucky	Positive

12915	Salmonella kentucky	Salmonella kentucky	Positive
12916	Salmonella kentucky	Salmonella kentucky	Positive
12917	Salmonella kentucky	Salmonella kentucky	Positive
12918	Salmonella kentucky	Salmonella kentucky	Positive
12919	Salmonella heidelberg	Salmonella heidelberg	Positive
12920	Salmonella heidelberg	Salmonella heidelberg	Positive
12921	Salmonella kentucky	Salmonella kentucky	Positive
12922	Salmonella heidelberg	Salmonella heidelberg	Positive
12925	Salmonella kentucky	Salmonella kentucky	Positive
12926	Salmonella kentucky	Salmonella kentucky	Positive
12927	Salmonella kentucky	Salmonella kentucky	Positive
12928	Salmonella heidelberg	Salmonella heidelberg	Positive
12929	Salmonella heidelberg	Salmonella heidelberg	Positive
12931	Salmonella heidelberg	Salmonella heidelberg	Positive
12932	Salmonella heidelberg	Salmonella heidelberg	Positive
12933	Salmonella heidelberg	Salmonella heidelberg	Positive
12936	Salmonella heidelberg	Salmonella heidelberg	Positive
12937	Salmonella heidelberg	Salmonella heidelberg	Positive
12941	Salmonella kentucky	Salmonella kentucky	Positive
12943	Salmonella kentucky	Salmonella kentucky	Positive
12945	Salmonella heidelberg	Salmonella heidelberg	Positive
12946	Salmonella kentucky	Salmonella kentucky	Positive
12947	Salmonella heidelberg	Salmonella heidelberg	Positive
12948	Salmonella kentucky	Salmonella kentucky	Positive
12949	Salmonella kentucky	Salmonella kentucky	Positive
12950	Salmonella kentucky	Salmonella kentucky	Positive
12951	Salmonella kentucky	Salmonella kentucky	Positive
12952	Salmonella heidelberg	Salmonella heidelberg	Positive
12953	Salmonella heidelberg	Salmonella heidelberg	Positive
12954	Salmonella heidelberg	Salmonella heidelberg	Positive
12955	Salmonella kentucky	Salmonella kentucky	Positive
12956	Salmonella kentucky	Salmonella kentucky	Positive
12957	Salmonella kentucky	Salmonella kentucky	Positive
12959	Salmonella heidelberg	Salmonella heidelberg	Positive
12960	Salmonella senftenberg	Salmonella senftenberg	Positive
12961	Salmonella senftenberg	Salmonella senftenberg	Positive
12962	Salmonella senftenberg	Salmonella senftenberg	Positive
12963	Salmonella senftenberg	Salmonella senftenberg	Positive
12964	Salmonella senftenberg	Salmonella senftenberg	Positive
12965	Salmonella senftenberg	Salmonella senftenberg	Positive
12966	Salmonella senftenberg	Salmonella senftenberg	Positive
12967	Salmonella haardt	Salmonella haardt	Positive
12968	Salmonella haardt	Salmonella haardt	Positive
12969	Salmonella haardt	Salmonella haardt	Positive
12970	Salmonella senftenberg	Salmonella senftenberg	Positive
12971	Salmonella senftenberg	Salmonella senftenberg	Positive
12972	Salmonella senftenberg	Salmonella senftenberg	Positive
12975	Salmonella senftenberg	Salmonella senftenberg	Positive

12978	Salmonella senftenberg	Salmonella senftenberg	Positive
12980	Salmonella senftenberg	Salmonella senftenberg	Positive
12981	Salmonella kentucky	Salmonella kentucky	Positive
12982	Salmonella senftenberg	Salmonella senftenberg	Positive
12983	Salmonella senftenberg	Salmonella senftenberg	Positive
12984	Salmonella senftenberg	Salmonella senftenberg	Positive
12985	Salmonella haardt	Salmonella haardt	Positive
12986	Salmonella senftenberg	Salmonella senftenberg	Positive
12987	Salmonella senftenberg	Salmonella senftenberg	Positive
12988	Salmonella senftenberg	Salmonella senftenberg	Positive
12989	Salmonella kentucky	Salmonella kentucky	Positive
12990	Salmonella kentucky	Salmonella kentucky	Positive
12993	Salmonella kentucky	Salmonella kentucky	Positive
12995	Salmonella heidelberg	Salmonella heidelberg	Positive
12996	Salmonella heidelberg	Salmonella heidelberg	Positive
12997	Salmonella kentucky	Salmonella kentucky	Positive
12998	Salmonella heidelberg	Salmonella heidelberg	Positive
12999	Salmonella heidelberg	Salmonella heidelberg	Positive
13000	Salmonella heidelberg	Salmonella heidelberg	Positive
13001	Salmonella heidelberg	Salmonella heidelberg	Positive
13002	Salmonella kentucky	Salmonella kentucky	Positive
13003	Salmonella kentucky	Salmonella kentucky	Positive
13004	Salmonella heidelberg	Salmonella heidelberg	Positive
13005	Salmonella typhimurium	Salmonella typhimurium	Positive
13006	Salmonella kentucky	Salmonella kentucky	Positive
13007	Salmonella kentucky	Salmonella kentucky	Positive
13008	Salmonella kentucky	Salmonella kentucky	Positive
13009	Salmonella kentucky	Salmonella kentucky	Positive
13010	Salmonella kentucky	Salmonella kentucky	Positive
13011	Salmonella typhimurium	Salmonella typhimurium	Positive
13012	Salmonella kentucky	Salmonella kentucky	Positive
13013	Salmonella kentucky	Salmonella kentucky	Positive
13014	Salmonella heidelberg	Salmonella heidelberg	Positive
13015	Salmonella kentucky	Salmonella kentucky	Positive
13016	Salmonella kentucky	Salmonella kentucky	Positive
13017	Salmonella heidelberg	Salmonella heidelberg	Positive
13018	Salmonella heidelberg	Salmonella heidelberg	Positive
13019	Salmonella heidelberg	Salmonella heidelberg	Positive
13020	Salmonella heidelberg	Salmonella heidelberg	Positive
13021	Salmonella heidelberg	Salmonella heidelberg	Positive
13022	Salmonella heidelberg	Salmonella heidelberg	Positive
13035	Salmonella enterica	Salmonella enterica	Positive
13036	Salmonella enterica	Salmonella enterica	Positive
13037	Salmonella species	Salmonella species	Positive
13056	Salmonella senftenberg	Salmonella senftenberg	Positive
13057	Salmonella senftenberg	Salmonella senftenberg	Positive
13058	Salmonella senftenberg	Salmonella senftenberg	Positive
13059	Salmonella senftenberg	Salmonella senftenberg	Positive

13060	Salmonella senftenberg	Salmonella senftenberg	Positive
13061	Salmonella tennessee	Salmonella tennessee	Positive
13062	Salmonella tennessee	Salmonella tennessee	Positive
13063	Salmonella tennessee	Salmonella tennessee	Positive
13064	Salmonella tennessee	Salmonella tennessee	Positive
13065	Salmonella tennessee	Salmonella tennessee	Positive
13066	Salmonella tennessee	Salmonella tennessee	Positive
13067	Salmonella havana	Salmonella havana	Positive
13068	Salmonella lexington	Salmonella lexington	Positive
13069	Salmonella mbandaka	Salmonella mbandaka	Positive
13070	Salmonella species	Salmonella species	Positive
13075	Salmonella species	Salmonella cubana	Positive
13079	Salmonella newport	Salmonella newport	Positive
13080	Salmonella saintpaul	Salmonella saintpaul	Positive
13081	Salmonella virchow	Salmonella virchow	Positive
S-1	Salmonella	Newport	Positive
S-4	Salmonella	Typhimurium	Positive
S-45	Salmonella	V48:i:-	Positive
S-46	Salmonella	V 40:z35:-	Positive
S-47	Salmonella	V 44:z39:-	Positive
S-48	Salmonella	V 60:z41:-	Positive
S-49	Salmonella	V 66:z41>	Positive
S-5	Salmonella	Typhi	Positive
S-50	Salmonella	V48:z35:-	Positive
S-51	Salmonella	VI 6,14,25:zl0:l,(2),7	Positive
S-52	Salmonella	VI II:b:l,7	Positive
S-53	Salmonella	VI 6,7:z41:l,7	Positive
S-54	Salmonella	VI II:a:l,5	Positive
S-55	Salmonella	VI 6,14,25:a:e,n,x	Positive
S-56	Salmonella	Typhimurium / DTI 04b	Positive
S-57	Salmonella	Typhimurium	Positive
S-58	Salmonella	Typhimurium	Positive
S-59	Salmonella	Typhimurium	Positive
S-60	Salmonella	Typhimurium	Positive
S-61	Salmonella	Typhimurium	Positive
S-62	Salmonella	Typhimurium	Positive
S-63	Salmonella	Typhimurium	Positive
S-64	Salmonella	Typhimurium	Positive
S-65	Salmonella	Typhimurium	Positive
S-66	Salmonella	Typhimurium	Positive
S-67	Salmonella	Typhimurium	Positive
S-68	Salmonella	Typhimurium	Positive
S-69	Salmonella	Typhimurium	Positive
S-70	Salmonella	Typhimurium / DTI 04	Positive
S-71	Salmonella	Typhimurium / DTI 04	Positive
S-72	Salmonella	Typhimurium / DTI 04	Positive
S-8	Salmonella	Virchow	Positive
S-82	Salmonella	Saphra	Positive

S-83	Salmonella	Rubislaw	Positive
S-84	Salmonella	Michigan	Positive
S-85	Salmonella	Urbana	Positive
S-86	Salmonella	Vietnam	Positive
S-87	Salmonella	Tornow	Positive
S-92	Salmonella	Muenchen	Positive
S-93	Salmonella	Senftenberg	Positive
S-94	Salmonella	Muenster	Positive
S-95	Salmonella	Montevideo	Positive

TABLE 3. Exclusivity Results

DuPont Qualicon ID#	ID Species	BAX® System Result
DD2901	Bacillus cereus	Negative
DD2558	Citrobacter freundii	Negative
DD383	Citrobacter freundii	Negative
DD1725	E.coli O125:H19	Negative
DD2614	Edwardsiella tarda	Negative
DD11348	Enterobacter sakazakii	Negative
DD3981	Enterococcus faecalis	Negative
DD846	Escherichia blattae	Negative
DD641	Escherichia coli	Negative
DD640	Escherichia coli O157:H7	Negative
DD847	Escherichia ferguson	Negative
DD6719	Escherichia hermanii	Negative
DD849	Escherichia intermedia	Negative
DD850	Escherichia vulnaris	Negative
DD6121	Gram negative rod	Negative
DD2389	Hafnia alvei	Negative
DD5588	Hafnia alvei	Negative
DD6523	Klebsiella oxytoca	Negative
DD658	Klebsiella oxytoca	Negative
DD657	Klebsiella ozaenae	Negative
DD373	Klebsiella pneumoniae	Negative
DD7344	Lactobacillus acidophilus	Negative
DD687	Lactobacillus carnis	Negative
DD922	Listeria innocua	Negative
DD1152	Listeria monocytogenes	Negative
DD13142	Morganella morganii	Negative
DD3064	Morganella morganii	Negative
DD374	Proteus mirabilis	Negative
DD13147	Providencia rettgeri	Negative
DD13148	Pseudomonas aeruginosa	Negative
DD3982	Pseudomonas	Negative

	aeruginosa	
DD569	Pseudomonas fluorescens	Negative
DD661	Pseudomonas fluorescens	Negative
DD577	Pseudomonas stutzeri	Negative
DD2166	Salmonella abaetetuba	Negative
DD3017	Salmonella dublin	Negative
DD3019	Salmonella dublin	Negative
DD1777	Salmonella enterica	Negative
DD2416	Serratia liquefaciens	Negative
DD2417	Serratia liquefaciens	Negative
DD1081	Shigella boydii	Negative
DD6832	Shigella sonnei	Negative
DD4160	Staphylococcus aureus	Negative
DD610	Staphylococcus aureus	Negative
DD3998	Streptococcus equi	Negative
DD7083	Unknown	Negative
TD3136	Vibrio cholera	Negative
DD13249	Vibrio parahemolyticus	Negative
TD3122	Vibrio vulnificus	Negative

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The claims defining the invention are as follows:

1. A method for detecting the presence of *Salmonella* in a sample, said sample comprising nucleic acids, said method comprising:
   (a) providing a reaction mixture comprising:
      (i) one primer comprising a nucleic acid sequence as set forth in SEQ ID NO:47 capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:1 and one probe comprising a nucleic acid sequence as set forth in SEQ ID NO:29 capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:2; and
      (ii) at least one additional primer and probe, wherein said additional primer is at least 11 nucleotides in length and said additional probe is at least 14 nucleotides in length, and wherein said additional primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO: 3 or a sequence complementary thereto, and said additional probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO: 4 or a sequence complementary thereto;

   wherein said primer and probe of (i) and (ii) each possess a 3' terminus and a 5' terminus, and wherein the 3' terminus of a 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe, wherein the 5' terminus of a 3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer, thereby forming a primer-probe complex; and
   (b) performing PCR amplification of said nucleic acids of said sample using the reaction mixture of step (a); and
   (c) detecting the amplification of step (b).

2. The method of claim 1, wherein said primer-probe complex comprises a detectable label.

3. The method of claim 1, wherein said additional primer capable of selectively hybridizing to SEQ ID NO:3 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:95 to 111, and wherein said additional probe capable of selectively hybridizing to SEQ ID NO: 4 comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:55 to 94.

4. The method of any one of claims 1 to 3, wherein said 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:112 to 119, and wherein said 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:120 to 130.

5. The method of claim 1, wherein said reaction mixture further comprises a quencher oligonucleotide capable of selectively hybridizing under stringent conditions to the probe.

6. A kit for detection of *Salmonella* in a sample, comprising:
   (i) one primer comprising a nucleic acid sequence as set forth in SEQ ID NO:47 capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:1 and one probe comprising a nucleic acid sequence as set forth in SEQ ID NO:29 capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:2; and
   (ii) at least one additional primer and probe, wherein said additional primer is at least 11 nucleotides in length and said additional probe is at least 14 nucleotides in length, and wherein said additional primer comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the nucleic acid sequence of SEQ ID NO:3 or a sequence complementary thereto, and said additional probe comprises a nucleic acid sequence capable of selectively hybridizing under stringent conditions to the sequence of SEQ ID NO:4 or a sequence complementary thereto;

wherein said primer and probe of (i) and (ii) each possess a 3' terminus and a 5' terminus, and wherein the 3' terminus of a 5' Stem Sequence is directly or indirectly attached to the 5' terminus of the probe, wherein the 5' terminus of a

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3' Stem Sequence is directly or indirectly attached to the 3' terminus of the probe, and wherein the 3' terminus of the 3' Stem Sequence is directly or indirectly attached of the 5' terminus of the primer, thereby forming a primer-probe complex.

7. The kit of claim 6, wherein said additional primer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:95 to 111 and said additional probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:55 to 94.

8. The kit of claim 6 or claim 7, wherein said 5' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:112 to 119, and wherein said 3' Stem Sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:120 to 130.

9. The method of claim 3 or claim 4 or the kit of claim 7 or claim 8, wherein said additional primer comprises a nucleic acid sequence as set forth in SEQ ID NO:102.

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Primer-Probe Complex	5' Modification	5' Stem SEQ ID	5' Stem Sequence	Probe (Loop) SEQ ID	Probe (Loop) Sequence	3' Stem SEQ ID	3' Stem Sequence	Internal Modification	Primer SEQ ID	Primer Sequence
S35C610-1	Cal Fluor Red 610	112	AGGGACCC	13	TGCCGACACCAGCGCCCG	120	GGGTCCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35C610-2	Cal Fluor Red 610	113	AGGACCC	13	TGCCGACACCAGCGCCCG	122	GGGTCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35C610-2a	Cal Fluor Red 610	113	AGGACCC	29	CGGGCGCTGGTGTCGGCA	122	GGGTCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35C610-3a	Cal Fluor Red 610	114	AGGGCCCC	29	CGGGCGCTGGTGTCGGCA	124	GGGGCCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35C610-3b	Cal Fluor Red 610	114	AGGGCCCC	39	CAGGGGCTGGTGTCGGCA	124	GGGGCCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35C610-4b	Cal Fluor Red 610	118	AAGGGCCC	39	CAGGGGCTGGTGTCGGCA	129	GGGCCCTT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35C610-5b	Cal Fluor Red 610	119	AAGGTGCCC	39	CAGGGGCTGGTGTCGGCA	130	GGGCACCTT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35FAM-3a	Fluorescein (6-FAM)	114	AGGGCCCC	29	CGGGCGCTGGTGTCGGCA	124	GGGGCCCT	[BHQ1][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35Q670-2a	Quasar 670	113	AGGACCC	29	CGGGCGCTGGTGTCGGCA	122	GGGTCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX1	Texas Red	112	AGGGACCC	13	TGCCGACACCAGCGCCCG	121	GGGTCCC	[DabdT][HEG]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX1a	Texas Red	112	AGGGACCC	21	CCGACACCAGCGCCCG	121	GGGTCCC	[DabdT][HEG]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX2	Texas Red	113	AGGACCC	13	TGCCGACACCAGCGCCCG	123	GGGTCC	[DabdT][HEG]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX2b	Texas Red	113	AGGACCC	37	CGGGCGCTGGTGTCG	123	GGGTCC	[Dab-dT][Sp-C18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX2c	Texas Red	113	AGGACCC	38	CGGGCGCTGGT	123	GGGTCC	[Dab-dT][Sp-C18]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX3	Texas Red	114	AGGGCCCC	13	TGCCGACACCAGCGCCCG	125	GGGGCCC	[DabdT][HEG]	47	TAGCCGGGACGCTT AATGCGGTTAAC
S35TEX3a	Texas Red	114	AGGGCCCC	29	CGGGCGCTGGTGTCGGCA	125	GGGGCCC	[DabdT][HEG]	47	TAGCCGGGACGCTT AATGCGGTTAAC

FIG. 1A

Primer-Probe Complex	5' Mod-ification	5' Stem SEQ ID	5' Stem Sequence	Probe (Loop) SEQ ID	Probe (Loop) Sequence	3' Stem SEQ ID	3' Stem Sequence	Internal Modification	Primer SEQ ID	Primer Sequence
Primer-Probe Complex	5' Mod-ification	5' Stem SEQ ID	5' Stem Sequence	Probe (Loop) SEQ ID	Probe (Loop) Sequence	3' Stem SEQ ID	3' Stem Sequence	Internal Modification	Primer SEQ ID	Primer Sequence
S35TEX3a	Cy5	114	AGGGCCCC	29	CGGGCGCTGGTGTCGGCA	124	GGGGCCCT	[BHQ2][HEG]	47	TAGCCGGGACGCTTAATGCGGTTAAC
S35TEX-3a	Texas Red	114	AGGGCCCC	29	CGGGCGCTGGTGTCGGCA	124	GGGGCCCT	[BHQ2][SP-18]	47	TAGCCGGGACGCTTAATGCGGTTAAC
S761aC610-4d	Cal Fluor Red 610	116	ACGGCCGC	90	CGCGTCAATGACCCTGAGCGACGTGAAA	127	GCGGCCGT	[BHQ2][SP-18]	111	CTTTACCGCTTCCAGGGTGGCCTGAA
S761bC610-4g	Cal Fluor Red 610	116	ACGGCCGC	93	CGCGCCAATGACCATAAGCGACGTGAAA	127	GCGGCCGT	[BHQ2][SP-18]	110	TTTTACCGCGTCCAGTGTGGCCTGAA
S761bC610-5g	Cal Fluor Red 610	117	ACGGACCGC	93	CGCGCCAATGACCATAAGCGACGTGAAA	128	GCGGTCCGT	[BHQ2][SP-18]	110	TTTTACCGCGTCCAGTGTGGCCTGAA
S761C610-3	Cal Fluor Red 610	114	AGGGCCCC	62	CCTAAGCGACGGGAAAAAAT	124	GGGGCCCT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-3a	Cal Fluor Red 610	114	AGGGCCCC	80	CGCGCCAATGATCCTAAGCGACGGGAAA	124	GGGGCCCT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-3b	Cal Fluor Red 610	114	AGGGCCCC	88	GCCGTAAGACGCGCCAATGATCCTAAGCGACGGGAAAAA	124	GGGGCCCT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-3c	Cal Fluor Red 610	114	AGGGCCCC	89	GCCAATGATCCTAAGCGACGGGAAA	124	GGGGCCCT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-4c	Cal Fluor Red 610	115	AGGCGCCG	89	GCCAATGATCCTAAGCGACGGGAAA	126	CGGCGCCT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-4d	Cal Fluor Red 610	116	ACGGCCGC	90	CGCGTCAATGACCCTGAGCGACGTGAAA	127	GCGGCCGT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-4e	Cal Fluor Red 610	116	ACGGCCGC	91	CCGTTCGACGCGTCAATGACCCTGAGCGACGTGAAAAA	127	GCGGCCGT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA

FIG. 1B

Primer-Probe Complex	5' Mod-ification	5' Stem SEQ ID	5' Stem Sequence	Probe (Loop) SEQ ID	Probe (Loop) Sequence	3' Stem SEQ ID	3' Stem Sequence	Internal Modification	Primer SEQ ID	Primer Sequence

Primer-Probe Complex	5' Modification	5' Stem SEQ ID	5' Stem Sequence	Probe (Loop) SEQ ID	Probe (Loop) Sequence	3' Stem SEQ ID	3' Stem Sequence	Internal Modification	Primer SEQ ID	Primer Sequence
S761C610-4f	Cal Fluor Red 610	116	ACGGCCGC	92	GTCAATGACCCTGAGCGACGTGAAA	127	GCGGCCGT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-4g	Cal Fluor Red 610	116	ACGGCCGC	93	CGCGCCAATGACCATAAGCGACGTGAAA	127	GCGGCCGT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-5f	Cal Fluor Red 610	117	ACGGACCGC	92	GTCAATGACCCTGAGCGACGTGAAA	128	GCGGTCCGT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S761C610-5g	Cal Fluor Red 610	117	ACGGACCGC	93	CGCGCCAATGACCATAAGCGACGTGAAA	128	GCGGTCCGT	[BHQ2][SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
SB35C610	Cal Fluor Red 610			29	CGGGCGCTGGTGTCGGCA			[SP-18]	47	TAGCCGGGACGCTTAATGCGGTTAAC
SB761C610	Cal Fluor Red 610			90	CGCGTCAATGACCCTGAGCGACGTGAAA			[SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
SB761C610-g	Cal Fluor Red 610			93	CGCGCCAATGACCATAAGCGACGTGAAA			[SP-18]	102	CTTTACCGCTTCCAGTGTGGCCTGAA
S4219EC5603b	Cal Fluor Orange 560	132	AGGCGCC	133	CAGACGACCGCCGCCGCT	134	GGCGCCT	[BHQ1][SP-18]	135	GAGCATAGTTATTAATAGCAGACACTCTATGCCTGTGTG

FIG. 1C

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DeMarco, Daniel R.

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