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(54) **Title**: SEQUENCES AND THEIR USE FOR DETECTION AND CHARACTERIZATION OF STEC BACTERIA

(57) **Abstract**: This invention relates to a rapid method for detection and characterization of STEC bacteria 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. This method is preferably employed to detect STEC bacteria in a food or water sample, such as a beef enrichment. The present invention further relates to isolated polynucleotides, replication compositions, kits, and reagent tablets for carrying out the method of the present invention.

TITLE

SEQUENCES AND THEIR USE FOR DETECTION AND CHARACTERIZATION OF STEC BACTERIA

FIELD OF INVENTION

This invention relates to methods for detection and characterization of Shiga toxin producing *Escherichia coli* (STEC) bacteria based on the presence of nucleic acid sequences, preferably PCR-based methods for detection, and to oligonucleotide molecules and reagents and kits useful therefor.

BACKGROUND OF INVENTION

Escherichia coli (*E. coli*) is a gram-negative, rod-shaped bacterium. Although most strains of *E. coli* are benign and are found as normal intestinal flora of humans and other animals, some strains are pathogenic and can lead to disease. Different strains of pathogenic *E. coli* differ in their epidemiology, clinical course and potential for causing outbreaks of disease.

Shiga toxin producing *Escherichia coli* (STEC) is a type of enterohemorrhagic bacteria that can cause mild to severe intestinal disease, kidney problems, and even central nervous system effects. The pathogenicity of these bacterial strains is due, in large part, to their production of Shiga-like toxins Stx1 and Stx2. Additionally, production of the intimin adherence protein encoded by the eae gene has been linked to pathogenicity of bacteria, as has possession of certain surface antigens, including the O26, O111, O121, O45, O103, and O145 antigens.

One well-known serotype of STEC bacteria is *E. coli* serotype O157:H7, which has been associated with several food and water borne outbreaks. This bacterial serotype is regulated as an adulterant in ground beef by the U.S. Department of Agriculture (USDA) with a zero tolerance standard. Because of its tight regulation, numerous tests for *E. coli* O157:H7 exist in the market. However, numerous other STEC bacteria strains also can cause disease, making detection of other STEC bacterial serotypes, or STEC bacteria in general, important for improved food safety.

It is desirable, therefore, to have a test for the accurate detection and characterization of STEC bacteria in a sample.

SUMMARY OF INVENTION

One aspect of the present invention is a method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising:

(a) providing a reaction mixture comprising suitable primer pairs for amplification of at least a portion of one or more of the eae gene, the Stx1A gene, and the Stx2A gene;

(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).

In certain examples, the invention relates to a method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising

(a) providing a reaction mixture comprising a first primer, a second primer, and a probe for amplification and detection of at least a portion of SEQ ID NO: 688 (eae); wherein each of said first primer, second primer, and probe comprises a 5' end and a 3' end; wherein said first primer comprises at least 15 contiguous nucleotides of SEQ ID NO: 145 or a sequence complementary thereto; and wherein said probe comprises at least 15 contiguous nucleotides of SEQ ID NO: 160 or a sequence complementary thereto;

(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).

In certain embodiments, the second primer comprises a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 191 or a sequence

complementary thereto. In further embodiments, the first primer comprises a sequence selected from the group consisting of SEQ ID NOs: 146-159, the second primer comprises a sequence selected from the group consisting of SEQ ID NOs: 192-205, and the probe comprises a sequence selected from the group consisting of SEQ ID NOs: 161-175. In additional embodiments, the 3' end of the probe is directly or indirectly attached to the 5' end of said the primer forming a primer-probe complex. In still further embodiments, the primer-probe complex can be detectably labeled. In additional embodiments, the reaction mixture further comprises a quencher oligonucleotide comprising at least 15 contiguous nucleotides of SEQ ID NO: 176. In some embodiments, the sample comprises a food sample or a water sample.

In other examples, the invention relates to a method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising

(a) providing a reaction mixture comprising a first primer, a second primer, and a probe for amplification and detection of at least a portion of SEQ ID NO: 686 (Stx1A); wherein each of said first primer, second primer, and probe comprises a 5' end and a 3' end; and wherein said first primer and probe are selected from the group consisting of:

(I) a first primer comprising at least 15 contiguous nucleotides of SEQ ID NO: 1 or a sequence complementary thereto and a probe comprising at least 15 contiguous nucleotides of SEQ ID NO: 16 or a sequence complementary thereto;

(II) a first primer comprising at least 15 contiguous nucleotides of SEQ ID NO: 1 or a sequence complementary thereto and a probe comprising at least 15 contiguous nucleotides of SEQ ID NO: 49 or a sequence complementary thereto; and

(III) a first primer comprising SEQ ID NO: 55 or a sequence complementary thereto and probe comprising SEQ ID NO: 56 or a sequence complementary thereto;

(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).

In certain embodiments, the second primer comprises a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 58 or comprising SEQ ID NO: 73. In other embodiments, the first primer comprises a sequence selected from the group consisting of SEQ ID NOs: 2-15, 48; the second primer comprises a sequence selected from the group consisting of SEQ ID NOs: 59-72; and the probe comprises a sequence selected from the group consisting of SEQ ID NOs: 17-30, 49-52. In further embodiments, the 3' end of the probe is directly or indirectly attached to the 5' end of the first primer forming a primer-probe complex. In still further embodiments, the primer-probe complex can be detectably labeled. In additional embodiments, the reaction mixture further comprises a quencher oligonucleotide comprising SEQ ID NO: 53, 54, or 57, or comprising at least 15 contiguous nucleotides of SEQ ID NO: 31. In some embodiments, the sample comprises a food sample or a water sample.

In still further examples, the present invention relates to a method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising

(a) providing a reaction mixture comprising a first primer, a second primer, and a probe for amplification and detection of at least a portion of SEQ ID NO: 687 (Stx2A); wherein each of said first primer, second primer, and probe comprises a 5' end and a 3' end; and wherein said first primer and probe are selected from the group consisting of:

(I) a first primer comprising at least 15 contiguous nucleotides of SEQ ID NO: 74 or a sequence complementary thereto and a probe comprising at least 15 contiguous nucleotides of SEQ ID NO: 89 or a sequence complementary thereto;

(II) a first primer comprising SEQ ID NO: 120 or a sequence complementary thereto and a probe comprising SEQ ID NO: 121 or 122 or a sequence complementary thereto; and

(III) a first primer comprising SEQ ID NO: 125 or a sequence complementary thereto and probe comprising SEQ ID NO: 126 or a sequence complementary thereto;

(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).

In certain embodiments, the second primer comprises a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 128 or comprising SEQ ID NO: 143 or 144. In other embodiments, the first primer comprises a sequence selected from the group consisting of SEQ ID NOs: 75-88; the second primer comprises a sequence selected from the group consisting of SEQ ID NOs: 129-144; and the probe comprises a sequence selected from the group consisting of SEQ ID NOs: 90-104. In additional embodiments, the 3' end of the probe is directly or indirectly attached to the 5' end of said first primer forming a primer-probe complex. In still further embodiments, the primer-probe complex can be detectably labeled. In additional embodiments, the reaction mixture further comprises a quencher oligonucleotide comprising SEQ ID NO: 123, 124, or 127, or comprising at least 15 contiguous nucleotides of SEQ ID NO: 105. In some embodiments, the sample comprises a food sample or a water sample.

In a further aspect, the invention relates to an isolated polynucleotide comprising a primer-probe complex, wherein said primer probe complex comprises:

(A) a primer region comprising a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 1 and a probe region comprising at least 15 contiguous nucleotides of SEQ ID NO: 16;

(B) a primer region comprising SEQ ID NO: 48 and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 49-52;

(C) a primer region comprising SEQ ID NO: 55, and a probe region comprising SEQ ID NO: 56;

(D) a primer region comprising at least 15 contiguous nucleotides of SEQ ID NO: 74 and a probe region comprising at least 15 contiguous nucleotides of SEQ ID NO: 89;

(E) a primer region comprising SEQ ID NO: 120 and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 121-122;

(F) a primer region comprising SEQ ID NO: 125 and a probe region comprising SEQ ID NO: 126; and

(G) a primer region comprising at least 15 contiguous nucleotides of SEQ ID NO: 145 and a probe region comprising at least 15 contiguous nucleotides of SEQ ID NO: 160;

wherein said probe region and said primer region each have a 5' and 3' terminus, wherein said 3' terminus of said probe region is attached to said 5' terminus of said primer region via a linker moiety, and wherein said primer-probe complex further comprises a detectable label.

In a further aspect, the invention relates to kits and reagent tablets for detection of STEC bacteria in a sample, comprising an isolated polynucleotide of the present invention.

A further aspect of the present invention is a method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising:

(a) providing a reaction mixture comprising suitable primer pairs for amplification of at least a portion of:

(i) the eae gene; and

(ii) one or more of the Stx1A and Stx2A genes; 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).

In certain examples, amplification of a portion of the eae gene comprises amplification of at least a portion of SEQ ID NO: 688. In other examples, amplification of a portion one or more of the Stx1A and Stx2A genes comprises amplification of at least a portion of one or both of SEQ ID NOs: 686 and 687. In certain embodiments, the reaction mixture comprises suitable primer pairs for amplification of at least a portion of all three of SEQ ID NOs: 686-688.

In additional embodiments, the reaction mixture further comprises suitable primer pairs for amplification of at least a portion of one or more of the genes encoding the O26, O111, O121, O45, O103, O145, and O157 surface antigens. In certain examples, such amplification comprises amplification of one or more nucleic acid sequences selected from the group consisting of SEQ ID NOs: 689-696.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 686 comprises (A) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-15 and 48, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 58-72; or (B) a first primer comprising SEQ ID NO: 55 and a second primer comprising SEQ ID NO: 73. In additional examples, the reaction mixture further comprises a probe, wherein: (I) where said first primer is selected from SEQ ID NOs: 1-15, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 31-47; (II) where said first primer comprises SEQ ID NO: 48, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 49-52; and (III) where said first primer comprises SEQ ID NO: 55, said probe comprises SEQ ID NO: 56. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction

mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 31-47, 53-54, and 57.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 687 comprises: (A) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 74-88, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 128-142; (B) a first primer comprising SEQ ID NO: 120 and a second primer comprising SEQ ID NO: 143; or (C) a first primer comprising SEQ ID NO: 125 and a second primer comprising SEQ ID NO: 144. In additional examples, the reaction mixture further comprises a probe, wherein: (I) where said first primer is selected from SEQ ID NOs: 74-88, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 89-104; (II) where said first primer comprises SEQ ID NO: 120, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 121-122; and (III) where said first primer comprises SEQ ID NO: 125, said probe comprises SEQ ID NO: 126. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 105-119, 123-124, and 127.

In certain examples, a said suitable primer pair for amplification of SEQ ID NO: 688 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 145-159, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 191-205. In additional examples, the reaction mixture further comprises a probe, wherein the probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 160-175. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction

mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 176-190.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 689 comprises: (A) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 206-220, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 258-272; (B) a first primer comprising SEQ ID NO: 251, and a second primer comprising SEQ ID NO: 273; or (C) a first primer comprising SEQ ID NO: 255, and a second primer comprising SEQ ID NO: 274. In additional examples, the reaction mixture further comprises a probe, wherein the probe comprises: (I) where said first primer is selected from SEQ ID NOs: 206-220, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 221-235; (II) where said first primer comprises SEQ ID NO: 251, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 252-253; and (III) where said first primer comprises SEQ ID NO: 255, said probe comprises SEQ ID NO: 256. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 236-250, 254, and 257.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 690 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 275-289 and 324, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs:327-341. In additional examples, the reaction mixture further comprises a probe, wherein the probe comprises: (I) where said first primer is selected from SEQ ID NOs: 275-289, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 290-306; and (II) where said first primer comprises SEQ ID NO: 324, said probe comprises SEQ ID NO: 325. In further examples, the 3' end of the probe

is attached to the 5' end of the primer forming a primer-probe complex. In still further examples. the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 307-323 and 326.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 691 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 342-356, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 393-407. In additional examples, the reaction mixture further comprises a probe, wherein said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 357-374. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 375-392.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 692 comprises: (A) a first primer comprising SEQ ID NO: 456, and a second primer comprising SEQ ID NO: 480; (B) a first primer comprising SEQ ID NO: 459, and a second primer comprising SEQ ID NO: 481; or (C) a first primer comprising SEQ ID NO: 462, and a second primer comprising SEQ ID NO: 482. In additional examples, the reaction mixture further comprises a probe, wherein said probe comprises: (I) where said first primer comprises SEQ ID NO: 456, said probe comprises SEQ ID NO: 457; (II) where said first primer comprises SEQ ID NO: 459, said probe comprises of SEQ ID NO: 460; and (III) where said first primer comprises SEQ ID NO: 462, said probe comprises SEQ ID NO: 463. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide

comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 458, 461, and 464.

In certain examples, a said suitable primer pair for amplification of SEQ ID NO: 693 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 408-423, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 465-479. In additional examples, the reaction mixture further comprises a probe, wherein said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 424-439. In further examples, the 3' end of the probe is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 440-455.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 694 comprises: (A) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 483-497 and 537, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 540-554; (B) a first primer comprising SEQ ID NO: 528, and a second primer comprising SEQ ID NO: 555; (C) a first primer comprising SEQ ID NO: 531, and a second primer comprising SEQ ID NO: 556; or (D) a first primer comprising SEQ ID NO: 534, and a second primer comprising SEQ ID NO: 557. In additional examples, the reaction mixture further comprises a probe, wherein said probe comprises: (I) where said first primer is selected from SEQ ID NOs: 483-497, said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 498-512; (II) where said first primer comprises SEQ ID NO: 528, said probe comprises SEQ ID NO: 529; (III) where said first primer comprises SEQ ID NO: 531, said probe comprises SEQ ID NO: 532; (IV) where said first primer comprises SEQ ID NO: 534, said probe comprises SEQ ID NO: 535; (V) where said first primer comprises SEQ ID NO: 537, said probe comprises SEQ ID NO: 538. In further examples, the 3' end of the probe

is attached to the 5' end of the primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 513-527, 530, 533, 536, and 539.

In certain examples, a suitable primer pair for amplification of SEQ ID NO: 695 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 558-572, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 603-617. In additional examples, the reaction mixture further comprises a probe, wherein said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 573-587. In further examples, the 3' end of said probe is attached to the 5' end of said primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 588-602.

In certain examples, a said suitable primer pair for amplification of SEQ ID NO: 696 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 618-633, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 671-685. In additional examples, the reaction mixture further comprises a probe, wherein said probe comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 634-653. In further examples, the 3' end of said probe is attached to the 5' end of said primer forming a primer-probe complex. In still further examples, the primer-probe complex is detectably labeled. In additional examples, the reaction mixture further comprises a quencher oligonucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 654-670.

In one particular embodiment:

(A) a suitable primer pair for amplification of SEQ ID NO: 686 comprises

(I) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-15 and 48, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 58-72; or

(II) a first primer comprising SEQ ID NO: 55 and a second primer comprising SEQ ID NO: 73;

(B) a suitable primer pair for amplification of SEQ ID NO: 687 comprises

(I) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 74-88, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 128-142;

(II) a first primer comprising SEQ ID NO: 120 and a second primer comprising SEQ ID NO: 143; or

(III) a first primer comprising SEQ ID NO: 125 and a second primer comprising SEQ ID NO: 144;

(C) a suitable primer pair for amplification of SEQ ID NO: 688 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 145-159, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 191-205;

(D) a suitable primer pair for amplification of SEQ ID NO: 689 comprises

(I) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 206-220, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 258-272;

(II) a first primer comprising SEQ ID NO: 251, and a second primer comprising SEQ ID NO: 273; or

(III) a first primer comprising SEQ ID NO: 255, and a second primer comprising SEQ ID NO: 274;

(E) a suitable primer pair for amplification of SEQ ID NO: 690 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 275-289 and 324, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 327-341;

(F) a suitable primer pair for amplification of SEQ ID NO: 691 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 342-356, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 393-407;

(G) a suitable primer pair for amplification of SEQ ID NO: 692 comprises

(I) a first primer comprising SEQ ID NO: 456, and a second primer comprising SEQ ID NO: 480;

(II) a first primer comprising SEQ ID NO: 459, and a second primer comprising SEQ ID NO: 481; or

(III) a first primer comprising SEQ ID NO: 462, and a second primer comprising SEQ ID NO: 482;

(H) a suitable primer pair for amplification of SEQ ID NO: 693 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 408-423, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 465-479;

(I) a suitable primer pair for amplification of SEQ ID NO: 694 comprises

(I) a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 483-497 and 537, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 540-554;

(II) a first primer comprising SEQ ID NO: 528, and a second primer comprising SEQ ID NO: 555;

(III) a first primer comprising SEQ ID NO: 531, and a second primer comprising SEQ ID NO: 556; or

(IV) a first primer comprising SEQ ID NO: 534, and a second primer comprising SEQ ID NO: 557;

(J) a suitable primer pair for amplification of SEQ ID NO: 695 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 558-572, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 603-617; and

(K) a suitable primer pair for amplification of SEQ ID NO: 696 comprises a first primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 618-633, and a second primer comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 671-685.

In additional examples, the reaction mixture comprises suitable primer pairs for amplification of SEQ ID NOs: 686-688 and one or more of SEQ ID NOs: 689-696.

In particular examples, the sample comprises a food sample or a water sample.

In additional embodiments, the invention relates to an isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1-685.

In other embodiments, the invention relates to an isolated polynucleotide comprising a primer-probe complex, wherein said primer probe complex comprises:

(A) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-15, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 16-30;

(B) a primer region comprising SEQ ID NO: 48, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 49-52;

(C) a primer region comprising SEQ ID NO: 55, and a probe region comprising SEQ ID NO: 56;

(D) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 74-88, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 89-104;

(E) a primer region comprising SEQ ID NO: 120, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 121-122;

(F) a primer region comprising SEQ ID NO: 125, and a probe region comprising SEQ ID NO: 126;

(G) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 145-159, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 160-175;

(H) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 206-220, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 221-235;

(I) a primer region comprising SEQ ID NO: 251, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 252-253;

(J) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 255, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 256;

(K) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 275-289, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 290-306;

(L) a primer region comprising SEQ ID NO: 324, and a probe region comprising SEQ ID NO: 325;

(M) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 342-356, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 357-374;

(N) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 408-423, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 424-439;

(O) a primer region comprising SEQ ID NO: 456, and a probe region comprising SEQ ID NOs: 457;

(P) a primer region comprising SEQ ID NO: 459, and a probe region comprising SEQ ID NO: 460;

(Q) a primer region comprising SEQ ID NO: 462, and a probe region comprising SEQ ID NO: 463;

(R) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 483-497, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 498-512;

(S) a primer region comprising SEQ ID NO: 528, and a probe region comprising SEQ ID NO: 529;

(T) a primer region comprising SEQ ID NO: 531, and a probe region comprising SEQ ID NO: 532;

(U) a primer region comprising SEQ ID NO: 534, and a probe region comprising SEQ ID NO: 535;

(V) a primer region comprising SEQ ID NO: 537, and a probe region comprising SEQ ID NO: 538;

(W) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 558-572, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 573-587;

(X) a primer region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 618-633, and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 634-653.

In certain examples, the probe region and primer region each have a 5' and 3' terminus, wherein the 3' terminus of the probe region is attached to the 5' terminus of the primer region via a linker moiety. In other examples, the primer-probe complex further comprises a detectable label.

In still further embodiments, the invention relates to a kit for detection of STEC bacteria in a sample, comprising an isolated polynucleotide of the present application. In other embodiments, the invention relates to a reagent tablet comprising a replication composition of the present application.

SUMMARY OF THE SEQUENCES

SEQ ID NOs: 1-30 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* Stx1A gene target, such as the target sequence provided herein as SEQ ID NO: 686. Amplification using a primer selected from SEQ ID NOs: 1-30 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 58-72. In certain embodiments, one of SEQ ID NOs: 1-15 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 16-30. In certain other embodiments, the 3'

terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 31-47.

SEQ ID NOs: 48-52 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* Stx1A gene target, such as the target sequence provided herein as SEQ ID NO: 686. Amplification using a primer selected from SEQ ID NOs: 48-52 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 58-72. In certain embodiments, SEQ ID NO: 48 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 49-52. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 53-54.

SEQ ID NOs: 55-56 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* Stx1A gene target, such as the target sequence provided herein as SEQ ID NO: 686. Amplification using a primer selected from SEQ ID NOs: 55-56 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 73. In certain embodiments, SEQ ID NO: 55 is used as a primer in conjunction with a probe comprising SEQ ID NO: 56. In certain other embodiments, the 3' terminus of the SEQ ID NO: 56 probe is attached to the 5' terminus of the SEQ ID NO: 55 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 57.

SEQ ID NOs: 74-104 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* Stx2A gene target, such as the target sequence provided herein as SEQ ID NO: 687. Amplification using a primer selected from SEQ ID NOs: 74-104 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 128-142. In certain embodiments, one of SEQ ID NOs: 74-88 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 89-104. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 105-119.

SEQ ID NOs: 120-122 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* Stx2A gene target, such as the target sequence provided herein as SEQ ID NO: 687. Amplification using a primer selected from SEQ ID NOs: 120-122 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 143. In certain embodiments, SEQ ID NO: 120 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 121-122. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 123-124.

SEQ ID NOs: 125-126 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* Stx2A gene target, such as the target sequence provided herein as SEQ ID NO: 687. Amplification using a primer selected from SEQ ID NOs: 125-126 can be performed in conjunction with a

suitable reverse primer, such as SEQ ID NO: 144. In certain embodiments, SEQ ID NO: 125 is used as a primer in conjunction with a probe comprising SEQ ID NO: 126. In certain other embodiments, the 3' terminus of the SEQ ID NO: 126 probe is attached to the 5' terminus of the SEQ ID NO: 125 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 127.

SEQ ID NOs: 145-175 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* eae gene target, such as the target sequence provided herein as SEQ ID NO: 688. Amplification using a primer selected from SEQ ID NOs: 145-175 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 191-205. In certain embodiments, one of SEQ ID NOs: 145-159 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 160-175. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 176-190.

SEQ ID NOs: 206-235 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O26 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 689. Amplification using a primer selected from SEQ ID NOs: 206-235 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 258-272. In certain embodiments, one of SEQ ID NOs: 206-220 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 221-235. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5'

terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 236-250.

SEQ ID NOs: 251-253 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O26 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 689. Amplification using a primer selected from SEQ ID NOs: 251-253 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 273. In certain embodiments, SEQ ID NO: 251 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 252-253. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the SEQ ID NO: 251 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 254.

SEQ ID NOs: 255-256 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O26 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 689. Amplification using a primer selected from SEQ ID NOs: 255-256 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 274. In certain embodiments, SEQ ID NO: 255 is used as a primer in conjunction with a probe comprising SEQ ID NO: 256. In certain other embodiments, the 3' terminus of the SEQ ID NO: 256 probe is attached to the 5' terminus of the SEQ ID NO: 255 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 257.

SEQ ID NOs: 275-306 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* SO111 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 690. Amplification using a primer selected from SEQ ID NOs: 275-306 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 327-341. In certain embodiments, one of SEQ ID NOs: 275-289 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 290-306. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 307-323.

SEQ ID NOs: 324-325 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* SO111 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 690. Amplification using a primer selected from SEQ ID NOs: 324-325 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 327-341. In certain embodiments, SEQ ID NO: 324 is used as a primer in conjunction with a probe comprising SEQ ID NO: 325. In certain other embodiments, the 3' terminus of the SEQ ID NO: 325 probe is attached to the 5' terminus of the SEQ ID NO: 324 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 326.

SEQ ID NOs: 342-374 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O121 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 691. Amplification using a primer selected from SEQ ID NOs: 342-374 can be performed

in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 393-407. In certain embodiments, one of SEQ ID NOs: 342-356 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 357-374. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 375-392.

SEQ ID NOs: 408-439 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O45 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 693. Amplification using a primer selected from SEQ ID NOs: 408-439 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 465-479. In certain embodiments, one of SEQ ID NOs: 408-423 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 424-439. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 440-455.

SEQ ID NOs: 456-457 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O45 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 692. Amplification using a primer selected from SEQ ID NOs: 456-457 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 480. In certain embodiments, SEQ ID NO: 456 is used as a primer in conjunction with a probe comprising SEQ ID NO: 457. In certain other embodiments, the 3' terminus of the

SEQ ID NO: 457 probe is attached to the 5' terminus of the SEQ ID NO: 456 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 458.

SEQ ID NOs: 459-460 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O45 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 692. Amplification using a primer selected from SEQ ID NOs: 459-460 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 481. In certain embodiments, SEQ ID NO: 459 is used as a primer in conjunction with a probe comprising SEQ ID NO: 460. In certain other embodiments, the 3' terminus of the SEQ ID NO: 460 probe is attached to the 5' terminus of the SEQ ID NO: 459 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 461.

SEQ ID NOs: 462-464 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O45 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 692. Amplification using a primer selected from SEQ ID NOs: 464-464 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 482. In certain embodiments, SEQ ID NO: 462 is used as a primer in conjunction with a probe comprising SEQ ID NO: 463. In certain other embodiments, the 3' terminus of the SEQ ID NO: 463 probe is attached to the 5' terminus of the SEQ ID NO: 462 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 464.

SEQ ID NOs: 483-512 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O103 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 694. Amplification using a primer selected from SEQ ID NOs: 483-512 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 540-554. In certain embodiments, one of SEQ ID NOs: 483-497 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 498-512. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 513-527.

SEQ ID NOs: 528-529 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O103 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 694. Amplification using a primer selected from SEQ ID NOs: 528-529 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 555. In certain embodiments, SEQ ID NO: 528 is used as a primer in conjunction with a probe comprising SEQ ID NO: 529. In certain other embodiments, the 3' terminus of the SEQ ID NO: 529 probe is attached to the 5' terminus of the SEQ ID NO: 528 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 530.

SEQ ID NOs: 531-532 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O103 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 694. Amplification using a primer selected from SEQ ID NOs: 531-532 can be performed

in conjunction with a suitable reverse primer, such as SEQ ID NO: 556. In certain embodiments, SEQ ID NO: 531 is used as a primer in conjunction with a probe comprising SEQ ID NO: 532. In certain other embodiments, the 3' terminus of the SEQ ID NO: 532 probe is attached to the 5' terminus of the SEQ ID NO: 531 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 533.

SEQ ID NOs: 534-535 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O103 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 694. Amplification using a primer selected from SEQ ID NOs: 534-535 can be performed in conjunction with a suitable reverse primer, such as SEQ ID NO: 557. In certain embodiments, SEQ ID NO: 534 is used as a primer in conjunction with a probe comprising SEQ ID NO: 535. In certain other embodiments, the 3' terminus of the SEQ ID NO: 535 probe is attached to the 5' terminus of the SEQ ID NO: 534 primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 536.

SEQ ID NOs: 537-538 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O103 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 694. Amplification using a primer selected from SEQ ID NOs: 537-538 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 540-554. In certain embodiments, SEQ ID NO: 537 is used as a primer in conjunction with a probe comprising SEQ ID NO: 538. In certain other embodiments, the 3' terminus of the SEQ ID NO: 538 probe is attached to the 5' terminus of the SEQ ID NO: 537 primer via a suitable linker moiety, such as an 18-

carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as SEQ ID NO: 539.

SEQ ID NOs: 558-587 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O145 surface antigen gene target, such as the target sequence provided herein as SEQ ID NO: 695. Amplification using a primer selected from SEQ ID NOs: 558-587 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 603-617. In certain embodiments, one of SEQ ID NOs: 558-572 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 573-587. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 588-602.

SEQ ID NOs: 618-653 are nucleotide sequences capable of use as primers or probes for amplifying and detecting a portion of an *E. coli* O157 prophage pathogenicity factor gene target, such as the target sequence provided herein as SEQ ID NO: 696. Amplification using a primer selected from SEQ ID NOs: 618-653 can be performed in conjunction with a suitable reverse primer, such as one selected from SEQ ID NOs: 671-685. In certain embodiments, one of SEQ ID NOs: 618-633 is used as a primer in conjunction with a probe selected from SEQ ID NOs: 634-653. In certain other embodiments, the 3' terminus of the selected probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled and is used in conjunction with a separate quencher oligonucleotide capable of hybridizing to the probe and quenching its fluorescence, such as an oligonucleotide selected from SEQ ID NOs: 654-670.

SEQ ID NO: 686 is the nucleotide sequence of a portion of an *E. coli* Stx1A gene that is useful for detecting the presence of the Stx1A gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 686 include SEQ ID NOs: 1-30, 48-52, 55-56, and 58-73. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 1-15 and a reverse primer selected from SEQ ID NOs: 58-72, while detection is accomplished using a probe selected from SEQ ID NOs: 16-30 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 31-47. In other embodiments, amplification is performed using SEQ ID NO: 48 as a forward primer and a reverse primer selected from SEQ ID NOs: 58-72, while detection is accomplished using a probe selected from SEQ ID NOs: 49-52 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 53-54. In still further embodiments, amplification is performed using SEQ ID NO: 55 as a forward primer and SEQ ID NO: 73 as a reverse primer, while detection is accomplished using SEQ ID NO: 56 as a probe and, optionally, SEQ ID NO: 57 as a quencher oligonucleotide.

SEQ ID NO: 687 is the nucleotide sequence of a portion of an *E. coli* Stx2A gene that is useful for detecting the presence of the Stx2A gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 687 include SEQ ID NOs: 74-104, 120-122, 125-126, and 128-144. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 74-88 and a reverse primer selected from SEQ ID NOs: 128-142, while detection is accomplished using a probe selected from SEQ ID NOs: 89-104 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 105-119. In other embodiments, amplification is performed using SEQ ID NO: 120 as a forward primer and SEQ ID NO: 143 as a reverse primer, while detection is accomplished using a probe selected from SEQ ID NOs: 121-122 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 123-124. In still further embodiments, amplification is performed using SEQ ID NO: 125 as a forward primer and SEQ ID NO: 144 as a reverse primer, while detection is accomplished using

SEQ ID NO: 126 as a probe and, optionally, SEQ ID NO: 127 as a quencher oligonucleotide.

SEQ ID NO: 688 is the nucleotide sequence of a portion of an *E. coli* eae gene that is useful for detecting the presence of the eae gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 688 include SEQ ID NOs: 145-175. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 145-159 and a reverse primer selected from SEQ ID NOs: 191-205, while detection is accomplished using a probe selected from SEQ ID NOs: 160-175 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 176-190.

SEQ ID NO: 689 is the nucleotide sequence of a portion of an *E. coli* O26 surface antigen gene that is useful for detecting the presence of the O26 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 689 include SEQ ID NOs: 206-235, 251-253, 255-256, and 258-274. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 206-220 and a reverse primer selected from SEQ ID NOs: 258-272, while detection is accomplished using a probe selected from SEQ ID NOs: 221-235 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 236-250. In other embodiments, amplification is performed using SEQ ID NO: 251 as a forward primer and SEQ ID NO: 273 as a reverse primer, while detection is accomplished using a probe selected from SEQ ID NOs: 252-253 and, optionally, SEQ ID NO: 254 as a quencher oligonucleotide. In still further embodiments, amplification is performed using SEQ ID NO: 255 as a forward primer and SEQ ID NO: 274 as a reverse primer, while detection is accomplished using SEQ ID NO: 256 as a probe and, optionally, SEQ ID NO: 257 as a quencher oligonucleotide.

SEQ ID NO: 690 is the nucleotide sequence of a portion of an *E. coli* O111 surface antigen gene that is useful for detecting the presence of the O111 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a

sample. Suitable primers useful for amplification of SEQ ID NO: 690 include SEQ ID NOs: 275-306, 324-325, and 327-341. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 275-289 and a reverse primer selected from SEQ ID NOs: 327-341, while detection is accomplished using a probe selected from SEQ ID NOs: 290-306 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 307-323. In other embodiments, amplification is performed using SEQ ID NO: 324 as a forward primer and a reverse primer selected from SEQ ID NOs: 327-341, while detection is accomplished using a probe comprising SEQ ID NO: 325 and, optionally, a quencher oligonucleotide comprising SEQ ID NO: 326.

SEQ ID NO: 691 is the nucleotide sequence of a portion of an *E. coli* O121 surface antigen gene that is useful for detecting the presence of the O121 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 691 include SEQ ID NOs: 342-356 and 393-407. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 342-356 and a reverse primer selected from SEQ ID NOs: 393-407, while detection is accomplished using a probe selected from SEQ ID NOs: 357-374 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 375-392.

SEQ ID NO: 692 is the nucleotide sequence of a portion of an *E. coli* O45 surface antigen gene that is useful for detecting the presence of the O45 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 692 include SEQ ID NOs: 456-457, 459-460, 462-463, and 480-482. In certain embodiments, amplification is performed using SEQ ID NO: 456 as a forward primer and SEQ ID NO: 480 as a reverse primer, while detection is accomplished using a probe comprising SEQ ID NO: 457 and, optionally, a quencher oligonucleotide comprising SEQ ID NO: 458. In further embodiments, amplification is performed using SEQ ID NO: 459 as a forward primer and SEQ ID NO: 481 as a reverse primer, while

detection is accomplished using a probe comprising SEQ ID NO: 460 and, optionally, a quencher oligonucleotide comprising SEQ ID NO: 461. In still further embodiments, amplification is performed using SEQ ID NO: 462 as a forward primer and SEQ ID NO: 482 as a reverse primer, while detection is accomplished using a probe comprising SEQ ID NO: 463 and, optionally, a quencher oligonucleotide comprising SEQ ID NO: 464.

SEQ ID NO: 693 is the nucleotide sequence of a portion of an *E. coli* O45 surface antigen gene that is useful for detecting the presence of the O45 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 692 include SEQ ID NOs: 408-439 and 465-479. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 408-423 and a reverse primer selected from SEQ ID NOs: 465-479, while detection is accomplished using a probe selected from SEQ ID NOs: 424-439 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 44-455.

SEQ ID NO: 694 is the nucleotide sequence of a portion of an *E. coli* O103 surface antigen gene that is useful for detecting the presence of the O103 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 694 include SEQ ID NOs: 483-512, 528-529, 531-532, 534-535, 537-538, and 540-557. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 483-497 and a reverse primer selected from SEQ ID NOs: 550-554, while detection is accomplished using a probe selected from SEQ ID NOs: 498-512 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 513-527. In other embodiments, amplification is performed using SEQ ID NO: 528 as a forward primer and SEQ ID NO: 555 as a reverse primer, while detection is accomplished using SEQ ID NO: 529 as a probe and, optionally, a SEQ ID NO: 530 as a quencher oligonucleotide. In still further embodiments, amplification is performed using SEQ ID NO: 531 as a forward primer and SEQ ID NO: 556 as a reverse primer, while

detection is accomplished using SEQ ID NO: 532 as a probe and, optionally, SEQ ID NO: 533 as a quencher oligonucleotide. In additional embodiments, amplification is performed using SEQ ID NO: 534 as a forward primer and SEQ ID NO: 557 as a reverse primer, while detection is accomplished using SEQ ID NO: 535 as a probe and, optionally, SEQ ID NO: 536 as a quencher oligonucleotide. In yet further embodiments, amplification is performed using SEQ ID NO: 537 as a forward primer and a reverse primer selected from SEQ ID NOs: 550-554, while detection is accomplished using SEQ ID NO: 538 as a probe and, optionally, SEQ ID NO: 539 as a quencher oligonucleotide.

SEQ ID NO: 695 is the nucleotide sequence of a portion of an *E. coli* O145 surface antigen gene that is useful for detecting the presence of the O145 surface antigen gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 695 include SEQ ID NOs: 558-587 and 603-617. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 558-572 and a reverse primer selected from SEQ ID NOs: 603-617, while detection is accomplished using a probe selected from SEQ ID NOs: 573-587 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 588-602.

SEQ ID NO: 696 is the nucleotide sequence of a portion of an *E. coli* O157 prophage pathogenicity factor gene that is useful for detecting the presence of the O157 prophage pathogenicity factor gene in a sample, and, ultimately, the presence of STEC bacteria in a sample. Suitable primers useful for amplification of SEQ ID NO: 696 include SEQ ID NOs: 618-653 and 671-685. In certain embodiments, amplification is performed with a forward primer selected from SEQ ID NOs: 618-633 and a reverse primer selected from SEQ ID NOs: 671-685, while detection is accomplished using a probe selected from SEQ ID NOs: 634-653 and, optionally, a quencher oligonucleotide selected from SEQ ID NOs: 654-670.

SEQ ID NOs: 697-700 comprise nucleotide sequences useful for amplifying SV40 DNA, which, in some examples, is employed as a positive control reaction. In

certain examples, amplification is performed using SEQ ID NO: 697 or 698 as a forward primer in conjunction with SEQ ID NO: 700 as a reverse primer. In certain embodiments, one of SEQ ID NOs: 697 or 698 is used as a primer in conjunction with SEQ ID NO: 699 as a probe. In certain other embodiments, the 3' terminus the SEQ ID NO: 699 probe is attached to the 5' terminus of the selected primer via a suitable linker moiety, such as an 18-carbon spacer. In additional embodiments, the probe is detectably labeled with a reporter-quencher pair and possesses self-complementary sequences flanking the probe region, thereby allowing the nucleic acid molecule to self-hybridize into a stem-loop structure, thus allowing for quenching of the reporter signal by the quencher molecule.

The sequences conform with 37 C.F.R. §§ 1.821-1.825 ("Requirements for Patent Applications Containing Nucleotide Sequences and/or Amino Acid Sequence Disclosures - the Sequence Rules") and are consistent with World Intellectual Property Organization (WIPO) Standard ST.25 (1998) and the sequence listing requirements of the EPO and PCT (Rules 5.2 and 49.5(a-*bis*), and Section 208 and Annex C of the Administrative Instructions). The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. § 1.822.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

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 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 particular embodiments, a primer is at least about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 49, or 60 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 particular embodiments, a probe is at least about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 49, or 60 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 via its 3' terminus to a primer's 5' terminus. Such an attachment can be either direct or indirect, such as when the attachment is accomplished 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 a primer-probe complex 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."

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 which 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 which 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 are 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 which 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. 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 which 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 which 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.

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 STEC bacteria by detecting one or more gene targets selected from Stx1A, Stx2A, eae, O26, O111, O121, O45, O103, O145, and O157. In certain embodiments, the methods involve detecting one or both of Stx1A and Stx2A, as well as detecting eae, and, optionally, one or more of

O26, O111, O121, O45, O103, O145, and O157. In still further embodiments, the methods involve detecting one or more of SEQ ID NOs: 686-688. In certain embodiments, the method involves detecting one or both of SEQ ID NOs: 686-687, as well as detecting SEQ ID NO: 688, and, optionally, one or more of SEQ ID NOs: 689-696. Oligonucleotides have been developed for the detection of such nucleotide sequences and the subsequent detection and identification of STEC bacteria, including forward and reverse primers, probes, and quencher oligonucleotides. Oligonucleotides of the instant invention may be used as primers for PCR amplification. Exemplary primer pairs and their corresponding targets, blocking oligonucleotides, and probes are shown in Table 1.

TABLE 1 – Target sequences and the primers, probes, and quenchers related thereto

Target Gene Name	Target Gene SEQ ID NO.	5' (Forward) Primer(s) SEQ ID NO(s)	Probe(s) SEQ ID NO(s)	Quencher Oligo(s) SEQ ID NO(s)	3' (Reverse) Primer(s) SEQ ID NO(s)
Stx1A	686	1-15	16-30	41-47	58-72
		48	49-52	53-54	58-72
		55	56	57	73
Stx2A	687	74-88	89-104	105-119	128-142
		120	121-122	123-124	143
		125	126	127	144
Eae	688	145-159	160-175	176-190	191-205
O26	689	206-220	221-235	236-250	258-272
		251	252-253	254	273
		255	256	257	274
O111	690	275-289	290-306	307-323	327-341
		324	325	326	327-341
O121	691	342-356	357-374	375-392	393-407

O45	692	456	457	458	480
		459	460	461	481
		462	463	464	482
	693	408-423	424-439	440-455	465-479
O103	694	483-497	498-512	513-527	540-554
		528	529	530	555
		531	532	533	556
		534	535	536	557
		537	538	539	540-555
O145	695	558-572	573-587	588-602	603-617
O157	696	618-633	634-653	654-670	671-685

Each of these primers and probes was designed based on sequence analysis of its corresponding region of the *E. coli* genome.

In certain embodiments, a primer for amplifying and/or detecting SEQ ID NO: 686 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 1, 48, or 55, or a sequence complementary thereto. In other embodiments a probe for amplifying and/or detecting SEQ ID NO: 686 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 16, 49-52, or 56, or a sequence complementary thereto. In further embodiments, a quencher useful for quenching the signal of such a probe comprises at least about 15 contiguous nucleotides of SEQ ID NO: 41, 53, 54, or 57, or a sequence complementary thereto. In additional embodiments a second primer, or reverse primer, for amplifying and/or detecting SEQ ID NO: 686 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 58 or 73, or a sequence complementary thereto. In still further embodiments, at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides of these sequences could be used.

In certain embodiments, a primer for amplifying and/or detecting SEQ ID NO: 687 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 74, 120, or

125, or a sequence complementary thereto. In other embodiments a probe for amplifying and/or detecting SEQ ID NO: 687 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 89, 121, 122, or 126, or a sequence complementary thereto. In further embodiments, a quencher useful for quenching the signal of such a probe comprises at least about 15 contiguous nucleotides of SEQ ID NO: 105, 123, 124, or 127, or a sequence complementary thereto. In additional embodiments a second primer, or reverse primer, for amplifying and/or detecting SEQ ID NO: 687 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 128, 143, or 144, or a sequence complementary thereto. In still further embodiments, at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides of these sequences could be used.

In certain embodiments, a primer for amplifying and/or detecting SEQ ID NO: 688 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 145, or a sequence complementary thereto. In other embodiments a probe for amplifying and/or detecting SEQ ID NO: 688 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 160, or a sequence complementary thereto. In further embodiments, a quencher useful for quenching the signal of such a probe comprises at least about 15 contiguous nucleotides of SEQ ID NO: 176, or a sequence complementary thereto. In additional embodiments a second primer, or reverse primer, for amplifying and/or detecting SEQ ID NO: 688 comprises at least about 15 contiguous nucleotides of SEQ ID NO: 191, or a sequence complementary thereto. In still further embodiments, at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides of these sequences could be used.

These oligonucleotide primers 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).

The oligonucleotide primers of the present invention can also contain a detectable label, for example a 5' end label.

In addition, oligonucleotides of the present invention also may be used as hybridization probes. Some examples of useful probes are provided in Table 1. 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. The nucleic acid probes of the present invention can also possess a detectable label, such as a reporter-quencher combination as are employed in Scorpion probe assays or in 5'-exonuclease detection assays, such as the Taqman® assay.

The 3' terminal nucleotide of the nucleic acid probe may be rendered incapable of extension by a nucleic acid polymerase in one embodiment of the invention. Such blocking may be carried out, for example by the attachment of a replication inhibitor moiety, such as a reporter or quencher, to the terminal 3' carbon of the nucleic acid probe by a linking moiety, or by making the 3'-terminal nucleotide a dideoxynucleotide. Alternatively, the 3' end of the nucleic acid probe may be rendered impervious to the 3' to 5' extension activity of a polymerase by incorporating one or more modified internucleotide linkages onto the 3' end of the oligonucleotide. Minimally, the 3' terminal internucleotide linkage must be modified, however, additional internucleotide linkages may be modified. Internucleotide modifications which prevent elongation from the 3' end of the nucleic acid probe and/or which block the 3' to 5' exonuclease activity of the DNA polymerase during PCR may include phosphorothioate linkages, methylphosphonate linkages, boranophosphate linkages, and other similar polymerase-resistant internucleotide linkages. An alternative method to block 3' extension of the probe is to form an adduct at the 3' end of the probe using mitomycin C or other like antitumor antibiotics such as described in Basu et al., Biochemistry 32:4708-4718, 1993. Thus, the precise mechanism by which the

3' end of the nucleic acid probe is protected from cleavage is not essential so long as the quencher is not cleaved from the nucleic acid probe.

A nucleic acid probe sequence can also optionally be employed with the primer sequence pairs of the present invention in an amplification based detection technique, such as in the 3'-exonuclease assay. Preferred primer/probe combinations are indicated in Table 1.

In some embodiments a corresponding primer and probe are used together in a primer-probe complex. In such embodiments, the 3' terminus of the probe is typically attached to the 5' terminus of the primer. These primer probe complexes of the instant invention can 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 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 instances, a quencher oligonucleotide can be employed with a primer-probe complex, which quencher oligonucleotide is capable of hybridizing to the probe region of the primer-probe complex when the probe region is unbound from its target DNA. If the reporter is attached to the 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. Examples of corresponding primers, probes, and quenchers are provided in Table 1.

Assay Methods

Detection of the selected gene targets and/or the genomic DNA regions identified by SEQ ID NOs: 686-696, and subsequent detection of the presence of STEC bacteria 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 STEC bacteria 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 pair of the invention and optionally with a nucleic acid probe comprising a detectable label.

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 corresponding forward and reverse primer pairs listed in Table 1 may be used as primers for use in primer-directed nucleic acid amplification for the detection of the target genes of interest and/or SEQ ID NOs: 686-696, and, ultimately, the detection and identification of STEC bacteria.

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 gene targets and subsequently STEC bacteria in a sample comprises (a) performing PCR amplification of two or more of SEQ ID NOs: 686-696 using primer pairs listed in Table 1 to produce a PCR amplification result; and (b) detecting the amplification, whereby a positive detection of the amplification indicates the presence of STEC bacteria in the sample.

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 gene targets and the target STEC 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. Optimally, they achieve PCR amplification results from all of the intended specific targets while giving no PCR results for other, non-target species.

Detection/Examination/Analysis:

Primer-directed amplification products of SEQ ID NOs: 686-696 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 pairs of the instant invention (e.g., "real-time" PCR and "real-time" RT-PCR). Preferred "real-time" methods are set forth in U.S. Patent Nos. 6,171,785, 5,994,056, 6,326,145, 5,804,375, 5,538,848, 5,487,972, and 5,210,015, each of which is hereby incorporated by reference in its entirety.

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 a different detectable label (e.g., reporter-quencher pair) attached, thus allowing each probe to be detected independently of the other probes.

In a certain embodiment, amplification and detection of two or more of SEQ ID NOs: 686-696 is performed using differentially labeled Scorpion probes. Examples of primers and probes, including the target sequences to which they are directed, are provided in Table 1. Additional specific examples of primers, probes, and quenchers which can be employed alone or in combination for detection of STEC bacteria are provided in Tables 2, 3, and 8.

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 which binds intermediate to or between the two members of the 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.

Another preferred method of homogenous detection involves the use of DNA melting curve analysis, particularly with the BAX® System hardware and reagent tablets from DuPont Qualicon Inc. The details of the system are given in U.S. Patent

No. 6,312,930 and PCT Publication Nos. WO 97/11197 and WO 00/66777, each of which is hereby incorporated by reference in its entirety.

Melting curve analysis detects and quantifies double stranded nucleic acid molecule ("dsDNA" or "target") by monitoring the fluorescence of the target amplification product ("target amplicon") during each amplification cycle at selected time points.

As is well known to the skilled artisan, the two strands of a dsDNA separate or melt, when the temperature is higher than its melting temperature. Melting of a dsDNA molecule is a process, and under a given solution condition, melting starts at a temperature (designated T_{MS} hereinafter), and completes at another temperature (designated T_{ME} hereinafter). The familiar term, T_m, designates the temperature at which melting is 50% complete.

A typical PCR cycle involves a denaturing phase where the target dsDNA is melted, a primer annealing phase where the temperature optimal for the primers to bind to the now-single-stranded target, and a chain elongation phase (at a temperature T_E) where the temperature is optimal for DNA polymerase to function.

According to the present invention, T_{MS} should be higher than T_E, and T_{ME} should be lower (often substantially lower) than the temperature at which the DNA polymerase is heat-inactivated. Melting characteristics are effected by the intrinsic properties of a given dsDNA molecule, such as deoxynucleotide composition and the length of the dsDNA.

Intercalating dyes will bind to double stranded DNA. The dye/dsDNA complex will fluoresce when exposed to the appropriate excitation wavelength of light, which is dye dependent, and the intensity of the fluorescence may be proportionate to concentration of the dsDNA. Methods taking advantage of the use of DNA intercalating dyes to detect and quantify dsDNA are known in the art. Many dyes are known and used in the art for these purposes. The instant methods also take advantage of such relationship.

Examples of such intercalating dyes include, but are not limited to, SYBR Green-I®, ethidium bromide, propidium iodide, TOTO®-1 {Quinolinium, 1-1'-[1,3-propanediylbis [(dimethyliminio)-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzothiazolylidene) methyl]]-, tetraiodide}, and YoPro® {Quinolinium, 4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]-1-[3-(trimethylammonio)-propyl]-,diiodide}. Most preferred for the instant invention is a non-asymmetrical cyanide dye such as SYBR Green-I®, manufactured by Molecular Probes, Inc. (Eugene, OR).

Melting curve analysis is achieved by monitoring the change in fluorescence while the temperature is increased. When the temperature reaches the T_{MS} specific for the target amplicon, the dsDNA begins to denature. When the dsDNA denatures, the intercalating dye dissociates from the DNA and fluorescence decreases. Mathematical analysis of the negative of the change of the log of fluorescence divided by the change in temperature plotted against the temperature results in the graphical peak known as a melting curve.

It should be understood that the present invention could be operated using a combination of these techniques, such as by having a Scorpion probe directed to one target region and a Taqman® probe directed to a second target region. It should also be understood that the invention is not limited to the above described techniques. Rather, one skilled in the art would recognize that other techniques for detecting amplification as known in the art may also be used. For example, techniques such as PCR-based quantitative sequence detection (QSD) may be performed using nucleic acid probes which, when present in the single-stranded state in solution, are configured such that the reporter and quencher are sufficiently close to substantially quench the reporter's emission. However, upon hybridization of the intact reporter-quencher nucleic acid probe with the amplified target nucleic acid sequence, the reporter and quenchers become sufficiently distant from each other. As a result, the quenching is substantially abated causing an increase in the fluorescence emission detected.

In addition to homogenous detection methods, a variety of other heterogeneous detection methods are known in the art which 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.

Denaturing Gradient Gel Electrophoresis (DGGE) is a separation method that detects differences in the denaturing behavior of small DNA fragments (200-700 bp). The principle of the separation is based on both fragment length and nucleotide sequence. In fragments that are the same length, a difference as little as one base pair can be detected. This is in contrast to non-denaturing gel electrophoresis, where DNA fragments are separated only by size. This limitation of non-denaturing gel electrophoresis results because the difference in charge density between DNA molecules is near neutral and plays little role in their separation. As the size of the DNA fragment increases, its velocity through the gel decreases.

DGGE is primarily used to separate DNA fragments of the same size based on their denaturing profiles and sequence. Using DGGE, two strands of a DNA molecule separate, or melt, when heat or a chemical denaturant is applied. The denaturation of a DNA duplex is influenced by two factors: 1) the hydrogen bonds formed between complimentary base pairs (since GC rich regions melt at higher denaturing conditions than regions that are AT rich); and 2) the attraction between neighboring bases of the same strand, or "stacking." Consequently, a DNA molecule may have several melting domains with each of their individual characteristic denaturing conditions determined by their nucleotide sequence. DGGE exploits the fact that otherwise identical DNA molecules having the same length and DNA sequence, with the exception of only one nucleotide within a specific denaturing domain, will denature at different temperatures or T*m*. Thus, when the double-stranded (ds) DNA fragment is electrophoresed through a gradient of increasing chemical denaturant it begins to denature and undergoes both a conformational and

mobility change. The dsDNA fragment will travel faster than a denatured single-stranded (ss) DNA fragment, since the branched structure of the single-stranded moiety of the molecule becomes entangled in the gel matrix. As the denaturing environment increases, the dsDNA fragment will completely dissociate and mobility of the molecule through the gel is retarded at the denaturant concentration at which the particular low denaturing domains of the DNA strand dissociate. In practice, the electrophoresis is conducted at a constant temperature (around 60°C) and chemical denaturants are used at concentrations that will result in 100% of the DNA molecules being denatured (i.e., 40% formamide and 7M urea). This variable denaturing gradient is created using a gradient maker, such that the composition of each DGGE gel gradually changes from 0% denaturant up to 100% denaturant. Of course, gradients containing a reduced range of denaturant (e.g., 35% to 60%) may also be poured for increased separation of DNA.

The principle used in DGGE can also be applied to a second method that uses a temperature gradient instead of a chemical denaturant gradient. This method is known as Temperature Gradient Gel Electrophoresis (TGGE). This method makes use of a temperature gradient to induce the conformational change of dsDNA to ssDNA to separate fragments of equal size with different sequences. As in DGGE, DNA fragments with different nucleotide sequences will become immobile at different positions in the gel. Variations in primer design can be used to advantage in increasing the usefulness of DGGE for characterization and identification of the PCR products. These methods and principles of using primer design variations are described in PCR Technology Principles and Applications, Henry A. Erlich Ed., M. Stockton Press, NY, pages 71 to 88 (1988).

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, dTTP, target specific primers 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.

A preferred replication composition of the instant invention comprises (a) at least one primer pair selected from Table 1, and (b) thermostable DNA polymerase. Another preferred replication composition comprises (a) at least two primer pairs selected from Table 1, each directed toward a different target DNA region; and (b) thermostable DNA polymerase. In some embodiments, primer pairs directed to three or more of SEQ ID NOs: 686-696 are included. In further embodiments, primer pairs directed to four or more of SEQ ID NOs: 686-696 are included. In still further embodiments, primer pairs directed to five or more of SEQ ID NOs: 686-696 are included.

A more preferred replication composition of the present invention comprises (a) at least two primer pairs and any corresponding probe or blocking oligonucleotide selected from Table 1, wherein each nucleic acid probe or primer-probe complex employed comprises a detectable label; and (b) thermostable DNA polymerase. Preferably the detectable label comprises a reporter capable of emitting a detectable signal and a quencher capable of substantially quenching the reporter and preventing

the emission of the detectable signal when the reporter and quencher are in sufficiently close proximity to one another.

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 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 the *E. coli* genome, or from another 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.

Nucleic Acid Hybridization Methods

In addition to primer-directed amplification assay methods, nucleic acid hybridization assay methods can be employed in the present invention for detection of STEC bacteria. The basic components of a nucleic acid hybridization test include a probe, a sample suspected of containing STEC bacteria, and a specific

hybridization method. Typically the probe length can vary from as few as 5 bases to the full length of the diagnostic sequence and will depend upon the specific test to be done. Only part of the probe molecule need be complementary to the nucleic acid sequence to be detected. In addition, the complementarity between the probe and the target sequence need not be perfect. Hybridization does occur between imperfectly complementary molecules with the result that a certain fraction of the bases in the hybridized region are not paired with the proper complementary base.

Probes particularly useful in nucleic acid hybridization methods are any of SEQ ID NOs: 1-696, or sequences derived therefrom.

The sample may or may not contain STEC bacteria. The sample may take a variety of forms, however will generally be extracted from an animal, environmental or food source suspected of contamination. The DNA may be detected directly but most preferably, the sample nucleic acid must be made available to contact the probe before any hybridization of probe and target molecule can occur. Thus the organism's DNA is preferably free from the cell and placed under the proper conditions before hybridization can occur. Methods of in-solution hybridization necessitate the purification of the DNA in order to be able to obtain hybridization of the sample DNA with the probe. This has meant that utilization of the in-solution method for detection of target sequences in a sample requires that the nucleic acids of the sample must first be purified to eliminate protein, lipids, and other cell components, and then contacted with the probe under hybridization conditions. Methods for the purification of the sample nucleic acid are common and well known in the art (Sambrook et al., *supra*).

In one preferred embodiment, hybridization assays may be conducted directly on cell lysates, without the need to extract the nucleic acids. This eliminates several steps from the sample-handling process and speeds up the assay. To perform such assays on crude cell lysates, a chaotropic agent is typically added to the cell lysates prepared as described above. The chaotropic agent stabilizes nucleic acids by inhibiting nuclease activity. Furthermore, the chaotropic agent allows sensitive and

stringent hybridization of short oligonucleotide probes to DNA at room temperature (Van Ness and Chen, *Nucl. Acids Res*. 19:5143-5151 (1991)). Suitable chaotropic agents include guanidinium chloride, guanidinium thiocyanate, sodium thiocyanate, lithium tetrachloroacetate, sodium perchlorate, rubidium tetrachloroacetate, potassium iodide, and cesium trifluoroacetate, among others. Typically, the chaotropic agent will be present at a final concentration of about 3M. If desired, one can add formamide to the hybridization mixture, typically 30-50% (v/v).

Alternatively, one can purify the sample nucleic acids prior to probe hybridization. A variety of methods are known to one of skill in the art (e.g., phenol-chloroform extraction, IsoQuick extraction (MicroProbe Corp., Bothell, WA), and others). Pre-hybridization purification is particularly useful for standard filter hybridization assays. Furthermore, purification facilitates measures to increase the assay sensitivity by incorporating *in vitro* RNA amplification methods such as self-sustained sequence replication (see for example Fahy et al., In PCR Methods and Applications, Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1991), pp. 25-33) or reverse transcriptase PCR (Kawasaki, In PCR Protocols: A Guide to Methods and Applications, M. A. Innis et al., Eds., (1990), pp. 21-27).

Once the DNA is released, it can be detected by any of a variety of methods. However, the most useful embodiments have at least some characteristics of speed, convenience, sensitivity, and specificity.

Hybridization methods are well known in the art. Typically the probe and sample must be mixed under conditions which will permit nucleic acid hybridization. This involves contacting the probe and sample in the presence of an inorganic or organic salt under the proper concentration and temperature conditions. The probe and sample nucleic acids must be in contact for a long enough time that any possible hybridization between the probe and sample nucleic acid may occur. The concentration of probe or target in the mixture will determine the time necessary for hybridization to occur. The higher the probe or target concentration, the shorter the hybridization incubation time needed.

Various hybridization solutions can be employed. Typically, these comprise from about 20 to 60% volume, preferably 30%, of a polar organic solvent. A common hybridization solution employs about 30-50% v/v formamide, about 0.15 to 1M sodium chloride, about 0.05 to 0.1M buffers, such as sodium citrate, Tris-HCl, PIPES or HEPES (pH range about 6-9), about 0.05 to 0.2% detergent, such as sodium dodecylsulfate, or between 0.5-20 mM EDTA, FICOLL (Pharmacia Inc.) (about 300-500 kilodaltons), polyvinylpyrrolidone (about 250-500 kdal), and serum albumin. Also included in the typical hybridization solution will be unlabeled carrier nucleic acids from about 0.1 to 5 mg/mL, fragmented nucleic DNA (e.g., calf thymus or salmon sperm DNA, or yeast RNA), and optionally from about 0.5 to 2% wt/vol glycine. Other additives may also be included, such as volume exclusion agents which include a variety of polar water-soluble or swellable agents (e.g., polyethylene glycol), anionic polymers (e.g., polyacrylate or polymethylacrylate), and anionic saccharidic polymers (e.g., dextran sulfate).

Nucleic acid hybridization is adaptable to a variety of assay formats. One of the most suitable is the sandwich assay format. The sandwich assay is particularly adaptable to hybridization under non-denaturing conditions. A primary component of a sandwich-type assay is a solid support. The solid support has adsorbed to it or covalently coupled to it immobilized nucleic acid probe that is unlabeled and complementary to one portion of the DNA sequence.

The sandwich assay may be encompassed in an assay kit. This kit would include a first component for the collection of samples suspected of contamination and buffers for the disbursement and lysis of the sample. A second component would include media in either dry or liquid form for the hybridization of target and probe polynucleotides, as well as for the removal of undesirable and nonduplexed forms by washing. A third component includes a solid support (dipstick) upon which is fixed (or to which is conjugated) unlabeled nucleic acid probe(s) that is (are) complementary to one or more targets of interest, for istance one or more of SEQ ID NOs: 686-696. A fourth component would contain labeled probe that is

complementary to a second and different region of the same DNA strand to which the immobilized, unlabeled nucleic acid probe of the third component is hybridized.

In a preferred embodiment, SEQ ID NOs: 1-685 or derivations thereof may be used as 3' blocked detection probes in either a homogeneous or heterogeneous assay format. For example, a probe generated from these sequences may be 3' blocked or non-participatory and will not be extended by, or participate in, a nucleic acid amplification reaction. Additionally, the probe incorporates a label that can serve as a reactive ligand that acts as a point of attachment for the immobilization of the probe/analyte hybrid or as a reporter to produce detectable signal. Accordingly, genomic or cDNA isolated from a sample suspected of *E. coli* contamination is amplified by standard primer-directed amplification protocols in the presence of an excess of the 3' blocked detection probe to produce amplification products. Because the probe is 3' blocked, it does not participate or interfere with the amplification of the target. After the final amplification cycle, the detection probe anneals to the relevant portion of the amplified DNA and the annealed complex is then captured on a support through the reactive ligand.

In some instances it is desirable to incorporate a ligand labeled dNTP, with the label probe in the replication composition to facilitate immobilization of the PCR reaction product on a support and then detection of the immobilized product by means of the labeled probe reagent. For example a biotin, digoxigenin, or digoxin labeled dNTP could be added to PCR reaction composition. The biotin, digoxigenin, or digoxin incorporated in the PCR product could then be immobilized respectively on to a strepavidin, anti-dixogin or antidigoxigenin antibody support. The immobilized PCR product could then be detected by the presence of the probe 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 *stx*, *eae*, O157, O45, O103, and O145 primers and probes

Samples of organisms were analyzed to establish inclusivity and exclusivity of Scorpion® probes of the present invention directed toward stx1/2, eae, O157, O45, O103, and O145 targets. For inclusivity, independent, bona fide STEC isolates appropriate for each assay (O157:H7, O45, O103 and O145, and various other organisms containing stx_1, stx_2, and *eae*) were used. For exclusivity, closely related non-target organisms, including other STEC organisms that were not targets of the assays, 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 were grown overnight to cell densities of approximately $1X10^9$ cfu/ml. For exclusivity, non-diluted overnight cultures were tested. For inclusivity, overnight cultures were diluted approximately 1:10,000 into TSB. 20 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

5-30 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 included lyophilized reagent tablets containing APTATaq DNA Polymerase (Roche, Mannheim, Germany), deoxynucleotides (Roche Diagnostics, Indianapolis, IN), BSA, surfactamps (Sigma-Aldrich, St. Louis, MO), and positive

control DNA. In addition, the primers, quenchers, and Scorpions® listed in Tables 2 and 3 were included in the amounts provided. For the Scorpion® probes, the 5' end label and linker are also provided in Tables 2 and 3. Each of these Scorpion® probes was designed as a bi-molecular Scorpion®, such that its structure includes (in 5' to 3' order) a 5' fluorescent end label, a probe sequence, an 18-carbon non-amplifiable linker, and a primer sequence. Tables 2 and 3 also list the quenching label that was attached to the 3' terminus of the corresponding quencher oligonucleotides.

TABLE 2 – Nucleic acid primers, probes, and quenchers used for inclusivity and exclusivity testing

					For Scorpions		
Nucleotide Name	**Target**	**Amt. Per Reaction**	**SEQ ID NO:**	**End Label**	**Primer SEQ ID NO:**	**Internal Label / Linker**	**Probe SEQ ID NO:**
Scorpion O45A-807B	O45	20-250 nM		Cal Fluor Red 610	415	18-Carbon Spacer	431
Forward primer O45A807-24	O45	100-500 nM	423				
Quencher O45A-807B	O45	100-1300 nM	447	BHQ2			
Reverse Primer O45Arc901-24	O45	100-250 nM	472				
Scorpion O103A-rc851	O103	20-250 nM		Cal Fluor Gold 540	490	18-Carbon Spacer	505
Forward Primer O103Arc851-22	O103	100-500 nM	490				
Quencher O103A-rc851	O103	100-1300 nM	520	BHQ1			
Reverse Primer O103A707-24	O103	100-250 nM	547				

Scorpion O145A-rc713	O145	20-250 nM		Quasar 670	565	18-Carbon Spacer	580
Forward Primer O145Arc713-22	O145	100-500 nM	565				
Quencher O145A-rc713	O145	100-1300 nM	595	BHQ2			
Reverse Primer O145A620-22	O145	100-250 nM	610				
Scorpion (Unimolecular) SV40 scorpion 1	Pos. Control	10-100 nM		Tamra	697	BHQ2 / 18-Carbon Linker	699
Forward Primer SV4312	Pos. Control	25-150 nM	698				
Reverse Primer SV4222	Pos. Control	100-400 nM	700				

TABLE 3 - Nucleic acid primers, probes, and quenchers used for inclusivity and exclusivity testing

					For Scorpions		
Nucleotide Name	**Target**	**Amt. Per reaction**	**SEQ ID NO:**	**End Label**	**Primer SEQ ID NO:**	**Internal Label / Linker**	**Probe SEQ ID NO:**
Scorpion O157i-rc478	O157	20-250 nM		Cal Fluor Gold 540	625	18-Carbon Spacer	641
Quencher #3 O157i rc478	O157	100-1300 nM	661	BHQ1			
Forward Primer O157irc478-26	O157	100-500 nM	625				
Reverse Primer O157i346-23	O157	100-500 nM	678				
Scorpion Stx1A-rc203B	Stx1A	20-250 nM		Cal Fluor Red 610	8	18-Carbon Spacer	23

Quencher Stx1A-rc203B	Stx1A	100-1300 nM	38	BHQ2			
Forward Primer Stx1A-rc203	Stx1A	100-500 nM	8				
Reverse Primer Stx1A114-27	Stx1A	100-500 nM	65				
Scorpion stx2A-rc650	Stx2A	20-250 nM		Cal Fluor Red 610	81	18-Carbon Spacer	96
Quencher stx2A-rc650	Stx2A	100-1300 nM	112	BHQ2			
Forward Primer stx2Arc650-24	Stx2A	100-500 nM	81				
Reverse Primer Stx2A594-26	Stx2A	100-500 nM	135				
Scorpion EAE-rc745	EAE	20-250 nM		Quasar 670	152	18-Carbon Spacer	167
Quencher EAE-rc745	EAE	100-1300 nM	183	BHQ2			
Forward Primer EAErc745-24	EAE	100-500 nM	152				
Reverse Primer EAE685-29	EAE	100-500 nM	198				
Scorpion (Unimolecular) SV40 scorpion 1	Pos. Control	10-100 nM		Tamra	697	BHQ2 / 18-Carbon Linker	699
Forward Primer SV4312	Pos. Control	25-150 nM	698				
Reverse Primer SV4222	Pos. Control	100-400 nM	700				

Amplification and testing were performed on the BAX® Q7 instrument (DuPont Qualicon, Wilmington, DE). The thermal cycling conditions were as follows: 2 minutes at 94 °C, followed by 43 cycles of 94 °C for 10 seconds and 63 °C for 40 seconds, with the fluorescent signal captured during the 63 °C step at each cycle.

Results

As can be seen in Tables 4 through 7, the individual Scorpion® probes were each able to distinguish the various targets from non-targets.

TABLE 4 – O45, O103, and O145 inclusivity

			BAX® System Result		
Sample ID	**Source**	**O-Type**	**O45**	**O103**	**O145**
R62	MSU	O45	Positive	Negative	Negative
R63	MSU	O45	Positive	Negative	Negative
R64	MSU	O45	Positive	Negative	Negative
R66	MSU	O103	Negative	Positive	Negative
R67	MSU	O103	Negative	Positive	Negative
R68	MSU	O103	Negative	Positive	Negative
R77	MSU	O145	Negative	Negative	Positive
R78	MSU	O145	Negative	Negative	Positive
R79	MSU	O145	Negative	Negative	Positive
R80	MSU	O145	Negative	Negative	Positive
R163	USDA-MARC	O103	Negative	Positive	Negative
R164	USDA-MARC	O103	Negative	Positive	Negative
R165	USDA-MARC	O103	Negative	Positive	Negative
R166	USDA-MARC	O103	Negative	Positive	Negative
R167	USDA-MARC	O103	Negative	Positive	Negative
R168	USDA-MARC	O103	Negative	Positive	Negative
R198	USDA-MARC	O145	Negative	Negative	Positive
DD2439	DuPont	O145	Negative	Negative	Positive
DD2450	DuPont	O45	Positive	Negative	Negative
DD2483	DuPont	O145	Negative	Negative	Positive
DD2521	DuPont	O103	Negative	Positive	Negative
DD2526	DuPont	O145	Negative	Negative	Positive
DD2530	DuPont	O103	Negative	Positive	Negative
DD13349	USDA-ARS	O45	Positive	Negative	Negative
DD13350	USDA-ARS	O45	Positive	Negative	Negative
DD13351	USDA-ARS	O45	Positive	Negative	Negative
DD13352	USDA-ARS	O45	Positive	Negative	Negative
DD13353	USDA-ARS	O45	Positive	Negative	Negative

DD13354	USDA-ARS	O45	Positive	Negative	Negative
DD13355	USDA-ARS	O45	Positive	Negative	Negative
DD13358	USDA-ARS	O45	Positive	Negative	Negative
DD13359	USDA-ARS	O45	Positive	Negative	Negative
DD13360	USDA-ARS	O45	Positive	Negative	Negative
DD13361	USDA-ARS	O45	Positive	Negative	Negative
DD13373	USDA-ARS	O103	Negative	Positive	Negative
DD13374	USDA-ARS	O103	Negative	Positive	Negative
DD13375	USDA-ARS	O103	Negative	Positive	Negative
DD13376	USDA-ARS	O103	Negative	Positive	Negative
DD13377	USDA-ARS	O103	Negative	Positive	Negative
DD13378	USDA-ARS	O103	Negative	Positive	Negative
DD13379	USDA-ARS	O103	Negative	Positive	Negative
DD13380	USDA-ARS	O103	Negative	Positive	Negative
DD13381	USDA-ARS	O103	Negative	Positive	Negative
DD13382	USDA-ARS	O103	Negative	Positive	Negative
DD13383	USDA-ARS	O103	Negative	Positive	Negative
DD13384	USDA-ARS	O103	Negative	Positive	Negative
DD13385	USDA-ARS	O103	Negative	Positive	Negative
DD13386	USDA-ARS	O103	Negative	Positive	Negative
DD13387	USDA-ARS	O103	Negative	Positive	Negative
DD13388	USDA-ARS	O103	Negative	Positive	Negative
DD13389	USDA-ARS	O103	Negative	Positive	Negative
DD13390	USDA-ARS	O145	Negative	Negative	Positive
DD13391	USDA-ARS	O145	Negative	Negative	Positive
DD13392	USDA-ARS	O145	Negative	Negative	Positive
DD13393	USDA-ARS	O145	Negative	Negative	Positive
DD13394	USDA-ARS	O145	Negative	Negative	Positive
DD13395	USDA-ARS	O145	Negative	Negative	Positive
DD13397	USDA-ARS	O145	Negative	Negative	Positive
DD13398	USDA-ARS	O145	Negative	Negative	Positive

TABLE 5 – O157:H7, *stx*, and *eae* inclusivity

			BAX® System Result	
Sample ID	**Source**	**O-Type**	***stx*$_1$ and/or stx$_2$**	***eae***
TD8136	DuPont	O157:H7	Positive	Positive
MA6	DuPont	O157:H7	Positive	Positive
493/89	DuPont	O157:H7	Positive	Positive
R81	MDP04-01392	O-:H52	Positive	Positive
R88	MDP06-	O141:H38	Positive	Positive

	00245			
DD1450	DuPont	O157:H7	Positive	Positive
DD2530	DuPont	O103:H2	Positive	Positive
DD12787	DuPont	O157:H7	Negative	Positive
DD13040	DuPont	O157:H7	Negative	Positive
DD13469	DuPont	O157:H7	Positive	Positive
DD640	DuPont	O157:H7	Positive	Positive
DD642	DuPont	O157:H7	Positive	Positive
DD1385	DuPont	O157:H7	Positive	Positive
DD12797	DuPont	O157:H7	Positive	Positive
DD1452	DuPont	O157:H7	Positive	Positive
DD1460	DuPont	O157:H7	Positive	Positive
DD1461	DuPont	O157:H7	Positive	Positive
DD1972	DuPont	O157:H7	Positive	Positive
DD1976	DuPont	O157:H7	Positive	Positive
DD1977	DuPont	O157:H7	Positive	Positive
DD1979	DuPont	O157:H7	Positive	Positive
DD1982	DuPont	O157:H7	Positive	Positive
DD1987	DuPont	O157:H7	Positive	Positive
DD1988	DuPont	O157:H7	Positive	Positive
DD1989	DuPont	O157:H7	Positive	Positive
DD1991	DuPont	O157:H7	Positive	Positive
DD5896	DuPont	O157:H7	Positive	Positive
DD7101	DuPont	O157:H7	Positive	Positive
DD8295	DuPont	O157:H7	Positive	Positive
DD8300	DuPont	O157:H7	Positive	Positive
DD8856	DuPont	O157:H7	Positive	Positive
DD8865	DuPont	O157:H7	Positive	Positive
DD8872	DuPont	O157:H7	Positive	Positive
DD8873	DuPont	O157:H7	Positive	Positive
DD10133	DuPont	O157:H7	Positive	Positive
DD10901	DuPont	O157:H7	Positive	Positive
DD10911	DuPont	O157:H7	Positive	Positive
DD12817	DuPont	O157:H7	Positive	Positive
DD12901	DuPont	O157:H7	Positive	Positive
DD13038	DuPont	O157:H7	Positive	Positive
DD10920	DuPont	O157:H7	Positive	Positive
DD10921	DuPont	O157:H7	Positive	Positive
DD12807	DuPont	O157:H7	Positive	Positive
DD13054	DuPont	O157:H7	Positive	Positive
DD13055	DuPont	O157:H7	Positive	Positive
DD13072	DuPont	O157:H7	Positive	Positive
DD13077	DuPont	O157:H7	Positive	Positive
DD13078	DuPont	O157:H7	Positive	Positive
DD13174	DuPont	O157:H7	Positive	Positive

DD13175	DuPont	O157:H7	Positive	Positive
DD13176	DuPont	O157:H7	Positive	Positive
DD13182	DuPont	O157:H7	Positive	Positive
DD13189	DuPont	O157:H7	Positive	Positive
DD13190	DuPont	O157:H7	Positive	Positive
DD13196	DuPont	O157:H7	Positive	Positive
DD13197	DuPont	O157:H7	Positive	Positive
DD13199	DuPont	O157:H7	Positive	Positive
DD13241	DuPont	O157:H7	Positive	Positive
DD13262	DuPont	O157:H7	Positive	Positive

TABLE 6 – Non-*E. coli* exclusivity

			BAX® System Result					
Sample ID	**Source**	**Organism**	**O45**	**O103**	**O145**	***stx*$_1$ and/or stx$_2$**	***eae***	**O157: H7**
DD373	DuPont	*Klebsiella pneumoniae*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD374	DuPont	*Proteus mirabilis*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD383	DuPont	*Citrobacter freundii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD657	DuPont	*Klebsiella ozaenae*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD658	DuPont	*Klebsiella oxytoca*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2389	DuPont	*Hafnia alvei*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2417	DuPont	*Serratia liquefaciens*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2558	DuPont	*Citrobacter freundii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD3064	DuPont	*Morganella morganii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD3982	DuPont	*Pseudomonas aeruginosa*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD5588	DuPont	*Hafnia alvei*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD6121	DuPont	*Gram negative rod*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD7083	DuPont	*Unknown*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13142	DuPont	*Morganella morganii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13147	DuPont	*Providencia rettgeri*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13148	DuPont	*Pseudomonas aeruginosa*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13186	DuPont	*Enterobacter*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

		amnigenus						
DD13187	DuPont	*Enterobacter amnigenus*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13094	DuPont	*Enterobacter sakazakii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13099	DuPont	*Enterobacter sakazakii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13134	DuPont	*Enterobacter sakazakii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13135	DuPont	*Enterobacter cloacae*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13151	DuPont	*Escherichia hermanii*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13162	DuPont	*Enterobacter hormaechei*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13163	DuPont	*Enterobacter turicensis*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD13161	DuPont	*Enterobacter asburiae*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1535	DuPont	*Salmonella*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1774	DuPont	*Salmonella diarazoniae*	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

TABLE 7 – *E. coli* exclusivity

			BAX® System Results					
Sample ID	**Location**	**O type**	**O45**	**O103**	**O145**	***stx*$_1$ and/or stx$_2$**	***eae***	**O157:H7**
R61	MSU	O45	Pos.	Neg.	Neg.	nt	nt	nt
R65	MSU	O103	Neg.	Pos.	Neg.	nt	nt	nt
R77	MSU	O145	Neg.	Neg.	Pos.	nt	nt	nt
DD640	DuPont	O157	Neg.	Neg.	Neg.	nt	nt	nt
DD641	DuPont	O157	Neg.	Neg.	Neg.	nt	nt	nt
DD1718	DuPont	O128	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.
DD1721	DuPont	O114	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1730	DuPont	O86	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1732	DuPont	O143	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1762	DuPont	O164	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1769	DuPont	O139	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD1770	DuPont	O115	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1803	DuPont	O25	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1814	DuPont	O6	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD1821	DuPont	O55	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

DD1827	DuPont	O20	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1835	DuPont	O127	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1836	DuPont	O125	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1842	DuPont	O78	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1861	DuPont	O126	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1889	DuPont	O152	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD1915	DuPont	O28	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2432	DuPont	O165	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2434	DuPont	O1	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2438	DuPont	O118	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2441	DuPont	O117	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2445	USDA	O113	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD2480	USDA	O4	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD3124	USDA	O2	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD3130	USDA	O8	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
DD5884	USDA	O91	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13415	USDA	O165	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13417	USDA	O4	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13418	USDA	O14	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13419	USDA	O22	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13420	USDA	O28	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13421	USDA	O38	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13422	USDA	O48	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13423	USDA	O79	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13424	USDA	O83	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13425	USDA	O88	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13426	USDA	O93	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13427	USDA	O104	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13428	USDA	O117	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13429	USDA	O119	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13430	USDA	O125	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13431	USDA	O126	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13432	USDA	O128	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13433	USDA	O137	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13434	USDA	O146	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13435	USDA	O165	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13436	USDA	OX3	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13437	USDA	O113	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13438	USDA	O165	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13439	USDA	O5	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13440	USDA	O55	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13441	USDA	O91	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13442	USDA	O2	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13443	USDA	O2	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13444	USDA	O2	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.

DD13445	USDA	O128	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13446	USDA	O128	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13447	USDA	O63	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.
DD13448	USDA	O63	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13449	USDA	O63	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13450	USDA	O113	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13451	USDA	O113	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13452	USDA	O113	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13453	USDA	O91	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13454	USDA	O91	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13455	USDA	O2	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.
DD13456	USDA	O2	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13457	USDA	O2	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13458	USDA	O174	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13459	USDA	O55	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13460	USDA	O128	Neg.	Neg.	Neg.	Neg.	Pos.	Neg.
DD13461	USDA	O177	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13462	USDA	O111	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13463	USDA	O113	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.
DD13464	USDA	O103	Neg.	Pos.	Neg.	Pos.	Pos.	Neg.
DD13465	USDA	O26	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.
DD13466	USDA	O41	Neg.	Neg.	Neg.	Pos.	Pos.	Neg.

nt=not tested

EXAMPLE 2

Determination of inclusivity/exclusivity of the O26, O111, and O121 primers and probes

As O26, O111, and O121 are additional strains of STEC bacteria, samples of organisms were analyzed to establish inclusivity and exclusivity of a multiplex of O26, O111, and O121 Scorpion® probes of the present invention. For inclusivity, independent, bona fide *E. coli* O26, O111, and O121 isolates were used. For exclusivity, non-*E. coli* species and *E. coli* that did not belong to any of these three serotypes were used to ensure that the assay would discriminate the target organisms (O26, O111, and O121) from other bacteria.

DNA lysate preparation

DNA lysates were prepare using the methodology described in Example 1

PCR conditions

30 µl of the DNA lysate was used to hydrate lyophilized PCR reaction components to achieve a DNA lysate/PCR reaction component mixture as described in Example 1. The primers and probes listed in Table 8 were included in the amounts provided. For the Scorpion® probes, the 5' end label, internal label, and linker are also provided in Table 8. Each of these Scorpion® probes was designed as a unimolecular Scorpion®, such that its structure includes (in 5' to 3' order) a 5' fluorescent end label, a 5' stem sequence, a probe sequence, a 3' stem sequence, an internal quencher, an 18-carbon non-amplifiable linker, and a primer sequence, wherein the 5' and 3' stem sequences are reverse complements of one another such that they form a stem-loop structure that results in quenching of the 5' end label when the probe is not bound to its target.

TABLE 8 - Nucleic acid primers, probes, and quenchers used for inclusivity and exclusivity testing

				For Scorpions			
Nucleotide Name	**Target**	**Amt. Per reaction**	**SEQ ID NO:**	**End Label**	**Probe SEQ ID NO:**	**Internal Label / Linker**	**Primer SEQ ID NO:**
Scorpion (Unimolecular) O26A-944 UM02	O26	0.75 pMole		Cal Fluor Red 610	228	BHQ2 / 18-Carbon Linker	213
Forward Primer O26A944-29	O26	5.25 pMole	213				
Reverse Primer O26Arc1070-28	O26	6.0 pMole	265				
Scorpion (Unimolecular) O111-rc320 UM02	O111	1.5 pMole		Cal Fluor Gold 540	297	BHQ1 / 18-Carbon Linker	282
Forward Primer O111Arc320-29	O111	4.5 pMole	282				

Reverse Primer O111A158-22	O111	6.0 pMole	334				
Scorpion (Unimolecular) O121A-rc214B UM01	O121	1.5 pMole		Quasar 670	364	BHQ2 / 18-Carbon Linker	349
Forward Primer O121A-rc214-22	O121	4.5 pMole	349				
Reverse Primer O121A90-27	O121	6.0 pMole	400				
Scorpion (Unimolecular) SV40 scorpion 1	Pos. Control	1.05 pMole		Tamra	697	BHQ2 / 18-Carbon Linker	699
Forward Primer SV4312	Pos. Control	2.25 pMole	698				
Reverse Primer SV4222	Pos. Control	4.5 pMole	700				

Amplification and testing were performed on the BAX® Q7 machine (DuPont Qualicon, Wilmington, DE). The thermal cycling conditions were as follows: 2 minutes at 94 °C, followed by 40 cycles of 94 °C for 10 seconds and 63 °C for 40 seconds, with the fluorescent signal captured during the 63 °C step at each cycle.

Results

As is shown in Tables 9-11, using a multiplex of Scorpion® probes, the method of the present invention was able to discriminate O26, O111, and O121 serotypes from non-target strains, including other O-type *E. coli* strains and non-*E. coli* strains (blank results indicate a negative result).

TABLE 9 – O26, O111, and O121 inclusivity

			BAX result		
Sample ID	**Location**	**O type**	**O26**	**O111**	**O121**
R58	MSU	O26	Pos.		
R59	MSU	O26	Pos.		
R60	MSU	O26	Pos.		
R70	MSU	O111		Pos.	

R71	MSU	O111		Pos.	
R72	MSU	O111		Pos.	
R75	MSU	O121			Pos.
R76	MSU	O121			Pos.
R84	MDP	O121			Pos.
R144	MARC	O26	Pos.		
R184	MARC	O121			Pos.
R185	MARC	O121			Pos.
R186	MARC	O121			Pos.
R187	MARC	O121			Pos.
R188	MARC	O121			Pos.
DD1720	DD collection	O26	Pos.		
DD1729	DD collection	O111		Pos.	
DD1807	DD collection	O26	Pos.		
DD1808	DD collection	O111		Pos.	
DD1809	DD collection	O111		Pos.	
DD1831	DD collection	O26	Pos.		
DD1858	DD collection	O111		Pos.	
DD1913	DD collection	O26	Pos.		
DD1927	DD collection	O111		Pos.	
DD2440	DD collection	O121			Pos.
DD2460	DD collection	O121			Pos.
DD5902	DD collection	O26	Pos.		
DD5903	DD collection	O26	Pos.		
DD5904	DD collection	O26	Pos.		
DD5905	DD collection	O26	Pos.		
DD9704	DD collection	O26	Pos.		
DD9705	DD collection	O26	Pos.		
DD9706	DD collection	O26	Pos.		
DD9707	DD collection	O26	Pos.		
DD13362	USDA	O121			Pos.
DD13363	USDA	O121			Pos.
DD13364	USDA	O121			Pos.
DD13365	USDA	O121			Pos.
DD13366	USDA	O121			Pos.
DD13367	USDA	O121			Pos.
DD13368	USDA	O121			Pos.
DD13370	USDA	O121			Pos.
DD133400	USDA	O111	Pos.	Pos.	
DD133401	USDA	O111		Pos.	

DD133402	USDA	O111		Pos.	
DD133403	USDA	O111		Pos.	

TABLE 10 – Exclusivity to non-*E. coli* species

			BAX result		
Sample ID	**Location**	**Species**	**O26**	**O111**	**O121**
DD373	DD collection	Klebsiella pneumoniae	Negative	Negative	Negative
DD374	DD collection	Proteus mirabilis	Negative	Negative	Negative
DD383	DD collection	Citrobacter freundii	Negative	Negative	Negative
DD657	DD collection	Klebsiella ozaenae	Negative	Negative	Negative
DD658	DD collection	Klebsiella oxytoca	Negative	Negative	Negative
DD2389	DD collection	Hafnia alvei	Negative	Negative	Negative
DD2417	DD collection	Serratia liquefaciens	Negative	Negative	Negative
DD2558	DD collection	Citrobacter freundii	Negative	Negative	Negative
DD3064	DD collection	Morganella morganii	Negative	Negative	Negative
DD3982	DD collection	Pseudomonas aeruginosa	Negative	Negative	Negative
DD5588	DD collection	Hafnia alvei	Negative	Negative	Negative
DD6121	DD collection	Gram negative rod	Negative	Negative	Negative
DD13142	DD collection	Morganella morganii	Negative	Negative	Negative
DD13147	DD collection	Providencia rettgeri	Negative	Negative	Negative
DD13148	DD collection	Pseudomonas aeruginosa	Negative	Negative	Negative
DD13186	DD collection	Enterobacter amnigenus	Negative	Negative	Negative
DD13187	DD collection	Enterobacter amnigenus	Negative	Negative	Negative
DD13094	DD collection	Enterobacter sakazakii	Negative	Negative	Negative
DD13099	DD collection	Enterobacter sakazakii	Negative	Negative	Negative
DD13134	DD collection	Enterobacter sakazakii	Negative	Negative	Negative
DD13135	DD collection	Enterobacter cloacae	Negative	Negative	Negative
DD13151	DD collection	Escherichia hermanii	Negative	Negative	Negative
DD13162	DD collection	Enterobacter hormaechei	Negative	Negative	Negative
DD13163	DD collection	Enterobacter turicensis	Negative	Negative	Negative
DD13161	DD collection	Enterobacter asburiae	Negative	Negative	Negative
DD1535	DD collection	Salmonella	Negative	Negative	Negative
DD1774	DD collection	Salmonella diarazoniae	Negative	Negative	Negative

TABLE 11 – Exclusivity versus other *E. coli* o-types

			BAX result		
Sample ID	**Location**	**O type**	**O26**	**O111**	**O121**
R61	MSU	O45	Negative	Negative	Negative
R65	MSU	O103	Negative	Negative	Negative
R77	MSU	O145	Negative	Negative	Negative
DD640	DD collection	O157	Negative	Negative	Negative
DD641	DD collection	O157	Negative	Negative	Negative
DD1718	DD collection	O128	Negative	Negative	Negative
DD1721	DD collection	O114	Negative	Negative	Negative
DD1730	DD collection	O86	Negative	Negative	Negative
DD1732	DD collection	O143	Negative	Negative	Negative
DD1762	DD collection	O164	Negative	Negative	Negative
DD1769	DD collection	O139	Negative	Negative	Negative
DD1770	DD collection	O115	Negative	Negative	Negative
DD1803	DD collection	O25	Negative	Negative	Negative
DD1814	DD collection	O6	Negative	Negative	Negative
DD1821	DD collection	O55	Negative	Negative	Negative
DD1827	DD collection	O20	Negative	Negative	Negative
DD1835	DD collection	O127	Negative	Negative	Negative
DD1836	DD collection	O125	Negative	Negative	Negative
DD1842	DD collection	O78	Negative	Negative	Negative
DD1861	DD collection	O126	Negative	Negative	Negative
DD1889	DD collection	O152	Negative	Negative	Negative
DD1915	DD collection	O28	Negative	Negative	Negative
DD2432	DD collection	O165	Negative	Negative	Negative
DD2434	DD collection	O1	Negative	Negative	Negative
DD2438	DD collection	O118	Negative	Negative	Negative
DD2441	DD collection	O117	Negative	Negative	Negative
DD2445	USDA	O113	Negative	Negative	Negative
DD2480	USDA	O4	Negative	Negative	Negative
DD3124	USDA	O2	Negative	Negative	Negative
DD3130	USDA	O8	Negative	Negative	Negative
DD5884	USDA	O91	Negative	Negative	Negative
DD13415	USDA	O165	Negative	Negative	Negative
DD13417	USDA	O4	Negative	Negative	Negative
DD13418	USDA	O14	Negative	Negative	Negative
DD13419	USDA	O22	Negative	Negative	Negative
DD13420	USDA	O28	Negative	Negative	Negative
DD13421	USDA	O38	Negative	Negative	Negative
DD13422	USDA	O48	Negative	Negative	Negative
DD13423	USDA	O79	Negative	Negative	Negative
DD13424	USDA	O83	Positive	Negative	Negative

DD13425	USDA	O88	Negative	Negative	Negative
DD13426	USDA	O93	Negative	Negative	Negative
DD13427	USDA	O104	Negative	Negative	Negative
DD13428	USDA	O117	Negative	Negative	Negative
DD13429	USDA	O119	Negative	Negative	Negative
DD13430	USDA	O125	Negative	Negative	Negative
DD13431	USDA	O126	Negative	Negative	Negative
DD13432	USDA	O128	Negative	Negative	Negative
DD13433	USDA	O137	Negative	Negative	Negative
DD13434	USDA	O146	Negative	Negative	Negative
DD13435	USDA	O165	Negative	Negative	Negative
DD13436	USDA	OX3	Negative	Negative	Negative
DD13437	USDA	O113	Negative	Negative	Negative
DD13438	USDA	O165	Negative	Negative	Negative
DD13439	USDA	O5	Negative	Negative	Negative
DD13440	USDA	O55	Negative	Negative	Negative
DD13441	USDA	O91	Negative	Negative	Negative
DD13442	USDA	O2	Negative	Negative	Negative
DD13443	USDA	O2	Negative	Negative	Negative
DD13444	USDA	O2	Negative	Negative	Negative
DD13445	USDA	O128	Negative	Negative	Negative
DD13446	USDA	O128	Negative	Negative	Negative
DD13447	USDA	O63	Negative	Negative	Negative
DD13448	USDA	O63	Negative	Negative	Negative
DD13449	USDA	O63	Negative	Negative	Negative
DD13450	USDA	O113	Negative	Negative	Negative
DD13451	USDA	O113	Negative	Negative	Negative
DD13452	USDA	O113	Negative	Negative	Negative
DD13453	USDA	O91	Negative	Negative	Negative
DD13454	USDA	O91	Negative	Negative	Negative
DD13455	USDA	O2	Negative	Negative	Negative
DD13456	USDA	O2	Negative	Negative	Negative
DD13457	USDA	O2	Negative	Negative	Negative
DD13458	USDA	O174	Negative	Negative	Negative
DD13459	USDA	O55	Negative	Negative	Negative
DD13460	USDA	O128	Negative	Negative	Negative
DD13461	USDA	O177	Negative	Negative	Negative
DD13462	USDA	O111	Negative	Positive	Negative
DD13463	USDA	O113	Negative	Negative	Negative
DD13464	USDA	O103	Negative	Negative	Negative
DD13465	USDA	O26	Positive	Negative	Negative
DD13466	USDA	O41	Negative	Negative	Negative

CLAIMS

What is claimed is:

1. A method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising

(a) providing a reaction mixture comprising a first primer, a second primer, and a probe for amplification and detection of at least a portion of SEQ ID NO: 688; wherein each of said first primer, second primer, and probe comprises a 5' end and a 3' end; wherein said first primer comprises at least 15 contiguous nucleotides of SEQ ID NO: 145 or a sequence complementary thereto; and wherein said probe comprises at least 15 contiguous nucleotides of SEQ ID NO: 160 or a sequence complementary thereto;

(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 second primer comprises a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 191 or a sequence complementary thereto.

3. The method of claim 2, wherein said first primer comprises a sequence selected from the group consisting of SEQ ID NOs: 146-159, said second primer comprises a sequence selected from the group consisting of SEQ ID NOs: 192-205, and said probe comprises a sequence selected from the group consisting of SEQ ID NOs: 161-175.

4. The method of any of claims 1-3, wherein the 3' end of said probe is directly or indirectly attached to the 5' end of said first primer forming a primer-probe complex, and wherein said primer-probe complex is detectably labeled.

5. The method of claim 4, wherein said reaction mixture further comprises a quencher oligonucleotide comprising at least 15 contiguous nucleotides of SEQ ID NO: 176.

6. A method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising

(a) providing a reaction mixture comprising a first primer, a second primer, and a probe for amplification and detection of at least a portion of SEQ ID NO: 686; wherein each of said first primer, second primer, and probe comprises a 5' end and a 3' end; and wherein said first primer and probe are selected from the group consisting of:

(I) a first primer comprising at least 15 contiguous nucleotides of SEQ ID NO: 1 or a sequence complementary thereto and a probe comprising at least 15 contiguous nucleotides of SEQ ID NO: 16 or a sequence complementary thereto;

(II) a first primer comprising at least 15 contiguous nucleotides of SEQ ID NO: 1 or a sequence complementary thereto and a probe comprising at least 15 contiguous nucleotides of SEQ ID NO: 49 or a sequence complementary thereto; and

(III) a first primer comprising SEQ ID NO: 55 or a sequence complementary thereto and probe comprising SEQ ID NO: 56 or a sequence complementary thereto;

(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).

7. The method of claim 6, wherein said second primer comprises a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 58 or comprising SEQ ID NO: 73.

8. The method of claim 7, wherein said first primer comprises a sequence selected from the group consisting of SEQ ID NOs: 2-15, 48; said second primer comprises a sequence selected from the group consisting of SEQ ID NOs: 59-72; and said probe comprises a sequence selected from the group consisting of SEQ ID NOs: 17-30, 49-52.

9. The method of any of claims 6-8, wherein the 3' end of said probe is directly or indirectly attached to the 5' end of said first primer forming a primer-probe complex, and wherein said primer-probe complex is detectably labeled.

10. The method of claim 9, wherein said reaction mixture further comprises a quencher oligonucleotide comprising SEQ ID NO: 53, 54, or 57, or comprising at least 15 contiguous nucleotides of SEQ ID NO: 31.

11. A method for detecting the presence of STEC bacteria in a sample, said sample comprising nucleic acids, said method comprising

(a) providing a reaction mixture comprising a first primer, a second primer, and a probe for amplification and detection of at least a portion of SEQ ID NO: 687; wherein each of said first primer, second primer, and probe comprises a 5' end and a 3' end; and wherein said first primer and probe are selected from the group consisting of:

(I) a first primer comprising at least 15 contiguous nucleotides of SEQ ID NO: 74 or a sequence complementary thereto and a

probe comprising at least 15 contiguous nucleotides of SEQ ID NO: 89 or a sequence complementary thereto;

(II) a first primer comprising SEQ ID NO: 120 or a sequence complementary thereto and a probe comprising SEQ ID NO: 121 or 122 or a sequence complementary thereto; and

(III) a first primer comprising SEQ ID NO: 125 or a sequence complementary thereto and probe comprising SEQ ID NO: 126 or a sequence complementary thereto;

(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).

12. The method of claim 11, wherein said second primer comprises a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 128 or comprising SEQ ID NO: 143 or 144.

13. The method of claim 12, wherein said first primer comprises a sequence selected from the group consisting of SEQ ID NOs: 75-88; said second primer comprises a sequence selected from the group consisting of SEQ ID NOs: 129-144; and said probe comprises a sequence selected from the group consisting of SEQ ID NOs: 90-104.

14. The method of any of claims 11-13, wherein the 3' end of said probe is directly or indirectly attached to the 5' end of said first primer forming a primer-probe complex, and wherein said primer-probe complex is detectably labeled.

15. The method of claim 14, wherein said reaction mixture further comprises a quencher oligonucleotide comprising SEQ ID NO: 123, 124, or 127, or comprising at least 15 contiguous nucleotides of SEQ ID NO: 105.

16. The method of claim 1, 6, or 11, wherein the sample comprises a food sample or a water sample.

17. An isolated polynucleotide comprising a primer-probe complex, wherein said primer probe complex comprises:

(A) a primer region comprising a nucleic acid sequence comprising at least 15 contiguous nucleotides of SEQ ID NO: 1 and a probe region comprising at least 15 contiguous nucleotides of SEQ ID NO: 16;

(B) a primer region comprising SEQ ID NO: 48 and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 49-52;

(C) a primer region comprising SEQ ID NO: 55, and a probe region comprising SEQ ID NO: 56;

(D) a primer region comprising at least 15 contiguous nucleotides of SEQ ID NO: 74 and a probe region comprising at least 15 contiguous nucleotides of SEQ ID NO: 89;

(E) a primer region comprising SEQ ID NO: 120 and a probe region comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 121-122;

(F) a primer region comprising SEQ ID NO: 125 and a probe region comprising SEQ ID NO: 126; and

(G) a primer region comprising at least 15 contiguous nucleotides of SEQ ID NO: 145 and a probe region comprising at least 15 contiguous nucleotides of SEQ ID NO: 160;

wherein said probe region and said primer region each have a 5' and 3' terminus, wherein said 3' terminus of said probe region is attached to said 5' terminus of said primer region via a linker moiety, and wherein said primer-probe complex further comprises a detectable label.

18. A kit for detection of STEC bacteria in a sample, comprising an isolated polynucleotide of claim 17.

19. A reagent tablet comprising the replication composition of claim 18.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/057795

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/48046 A2 (BAVARIAN NORDIC RES INST AS [DE]; PFEFFER KLAUS [DE]) 29 October 1998 (1998-10-29)	1-3
Y	claims; example 1, page 27; page 5, lines 28-21; page 6, lines 28-29; page 8, lines 16-19; page 10, lines 13-15	4,5, 17-19

X	WO 2005/005659 A1 (STATENS SERUMINSTITUT [DK]; PERSSON SOEREN [DK]; SCHEUTZ FLEMMING [DK]) 20 January 2005 (2005-01-20)	1-3
Y	page 41, example 5	4,5, 17-19

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2012	15/03/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hennard, Christophe

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 292 799 A1 (AGENCE FRANCAISE DE SECURITE SANITAIRE DES ALIMENTS [FR]) 9 March 2011 (2011-03-09)	1-3
Y	claims; page 3, [0014]; page 4, [0020]; table 1	4,5, 17-19

X	J. MADIC ET AL: "Simplex and multiplex real-time PCR assays for the detection of flagellar (H-antigen) fliC alleles and intimin (eae) variants associated with enterohaemorrhagic Escherichia coli (EHEC) serotypes O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7", JOURNAL OF APPLIED MICROBIOLOGY, vol. 109, 1 July 2010 (2010-07-01), pages 1696-1705, XP055048359, ISSN: 1364-5072, DOI: 10.1111/j.1365-2672.2010.04798.x	1-3
Y	abstract; table 1; "Materials and methods"	4,5, 17-19

X	SIYA RAM: "Computing TaqMan Probes for Multiplex PCR Detection of E. coli O157 Serotypes in Water", IN SILICO BIOLOGY, vol. 5, 1 January 2005 (2005-01-01), pages 499-504, XP055048360,	1-3
Y	table 3	4,5, 17-19

X	CHING-FANG HSU: "Use of the Duplex TaqMan PCR System for Detection of Shiga-Like Toxin-Producing Escherichia coli O157", JOURNAL OF CLINICAL MICROBIOLOGY, vol. 43, no. 6, 1 January 2005 (2005-01-01), pages 2668-2673, XP055048361, DOI: 10.1128/JCM.43.6.2668-2673.2005	1-3
Y	page 2669, last paragraph; abstract	4,5, 17-19

Y	SINGH JITENDER ET AL: "A scorpion probe-based real-time PCR assay for detection of E. coli O157:H7 in dairy products", FOODBORNE PATHOGENS AND DISEASE, MARY ANN LIEBERT, INC. PUBLISHERS, US, vol. 6, no. 3, 1 April 2009 (2009-04-01), pages 395-400, XP002603978, ISSN: 1535-3141, DOI: 10.1089/FPD.2008.0178 [retrieved on 2009-03-09] abstract; page 396, column 2	17-19

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/057795

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/020823 A1 (BURNS FRANK R [US]) 27 January 2011 (2011-01-27) claims; examples -----	17-19

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Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/057795

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5(completely); 17-19(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5(completely); 17-19(partially)

   Concern a method for detecting STEC bacteria in a sample comprising providing a probe and a pair of primers for detecting a nucleic acid, performing a PCR amplification and detecting the amplification product; an isolated primer-probe complex; a kit for detection of STEC bacteria and a reagent tablet characterised in that the primers and probe used for amplification or in the product claims are specific for amplifying and detecting a portion of the eae gene of the shiga toxin producing E. coli bacteria.

   ---

2. claims: 6-10(completely); 17-19(partially)

   Concern a method for detecting STEC bacteria in a sample comprising providing a probe and a pair of primers for detecting a nucleic acid, performing a PCR amplification and detecting the amplification product; an isolated primer-probe complex; a kit for detection of STEC bacteria and a reagent tablet characterised in that the primers and probe used for amplification or in the product claims are specific for amplifying and detecting a portion of the STX1A gene of the shiga toxin producing E. coli bacteria.

   ---

3. claims: 11-16(completely); 17-19(partially)

   Concern a method for detecting STEC bacteria in a sample comprising providing a probe and a pair of primers for detecting a nucleic acid, performing a PCR amplification and detecting the amplification product; an isolated primer-probe complex; a kit for detection of STEC bacteria and a reagent tablet characterised in that the primers and probe used for amplification or in the product claims are specific for amplifying and detecting a portion of the STX2A gene of the shiga toxin producing E. coli bacteria.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/057795

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9848046 A2	29-10-1998	AU 753277 B2	10-10-2002
		AU 8014498 A	13-11-1998
		BR 9808963 A	01-08-2000
		CA 2286310 A1	29-10-1998
		CN 1258320 A	28-06-2000
		EP 0994965 A2	26-04-2000
		HK 1026002 A1	31-12-2004
		JP 2001521394 A	06-11-2001
		NZ 337506 A	31-08-2001
		US 6664080 B1	16-12-2003
		US 2004175723 A1	09-09-2004
		WO 9848046 A2	29-10-1998
WO 2005005659 A1	20-01-2005	EP 1651773 A1	03-05-2006
		US 2006194206 A1	31-08-2006
		WO 2005005659 A1	20-01-2005
EP 2292799 A1	09-03-2011	AU 2010283452 A1	01-03-2012
		CA 2770275 A1	17-02-2011
		EP 2292799 A1	09-03-2011
		EP 2464746 A1	20-06-2012
		JP 2013501516 A	17-01-2013
		KR 20120100890 A	12-09-2012
		US 2012202204 A1	09-08-2012
		WO 2011018762 A1	17-02-2011
US 2011020823 A1	27-01-2011	AU 2010276352 A1	02-02-2012
		CA 2768794 A1	27-01-2011
		EP 2456884 A1	30-05-2012
		JP 2013500008 A	07-01-2013
		US 2011020823 A1	27-01-2011
		WO 2011011391 A1	27-01-2011