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(54) **ASSAYS AND METHODS FOR DETERMINING MICROBIAL RESISTANCE**(71) Applicants: **STRECK LLC**, La Vista, NE (US); **CREIGHTON UNIVERSITY**, Omaha, NE (US)(72) Inventors: **Maria Torres-Gonzalez**, Omaha, NE (US); **Nancy Hanson**, Omaha, NE (US); **Joel Lechner**, Omaha, NE (US); **Stephanie Cossette**, Omaha, NE (US); **Cathy Scheer**, Omaha, NE (US); **Matthew R. Kreifels**, Elkhorn, NE (US); **Stacey Morrow**, Omaha, NE (US); **Christopher Connelly**, Gretna, NE (US); **Laura R. Porter**, Omaha, NE (US); **Randy Fowler**, Broomfield, CO (US)(73) Assignees: **STRECK, LLC**, La Vista, NE (US); **CREIGHTON UNIVERSITY**, Omaha, NE (US)

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CI2Q 1/6851	(2018.01)
CI2Q 1/686	(2018.01)

(52) **U.S. Cl.**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — Samuel C Woolwine*Assistant Examiner* — Carolyn L Greene(74) *Attorney, Agent, or Firm* — MARSHALL, GERSTEIN & BORUN LLP(57) **ABSTRACT**

Assays and methods for detecting resistance to beta-lactam antibiotics including detection of multiple β -lactamase family specific gene targets by polymerase chain reaction or microarray. One or more kits including primers and/or probes for identification of β -lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, ACT/MIR-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. A kit may also include one or more primers and/or probes for the identification a non-beta lactamase gene family which confers antibiotic resistance, such as the MCR-1 gene.

10 Claims, 9 Drawing Sheets**Specification includes a Sequence Listing.**

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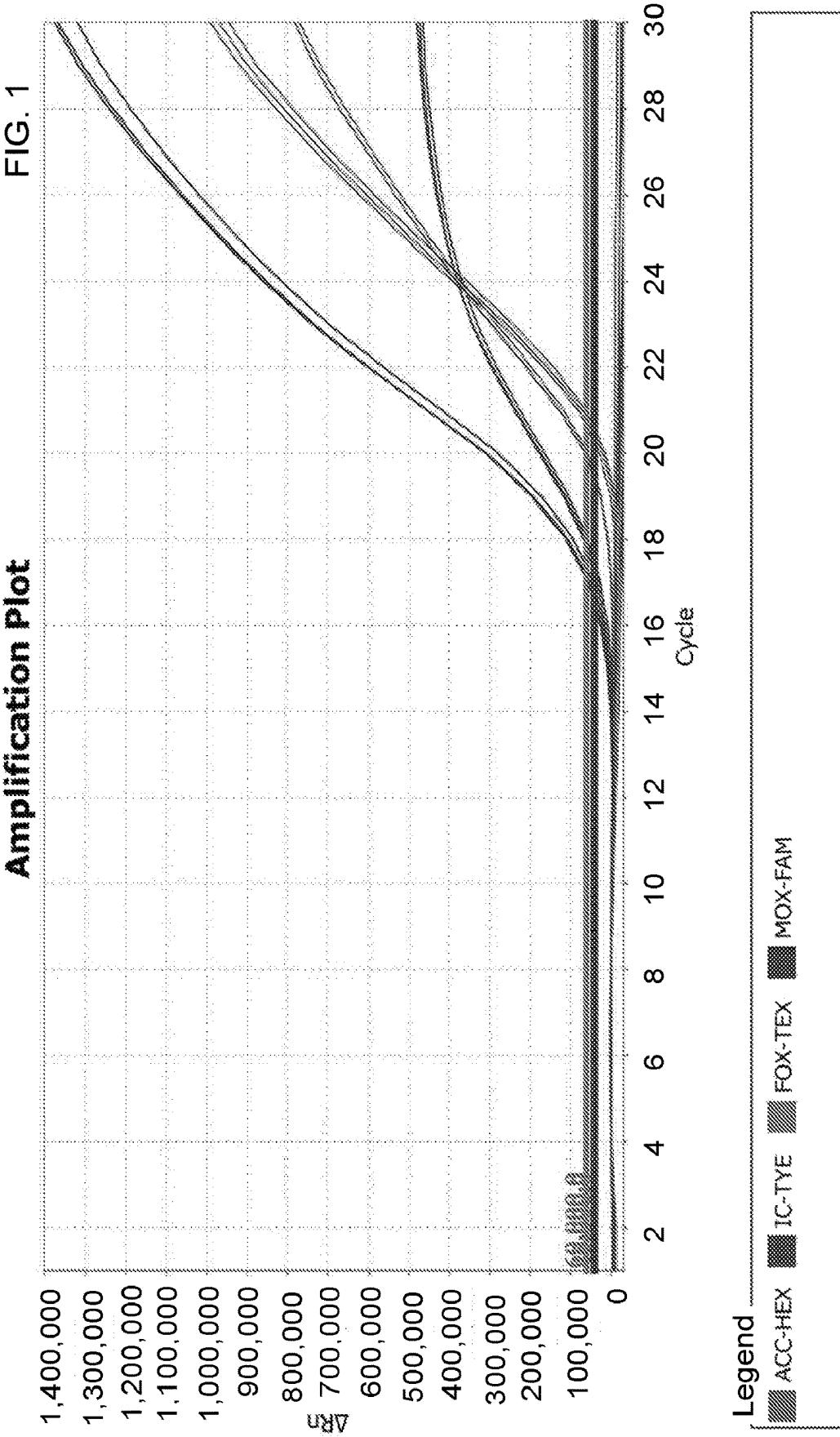
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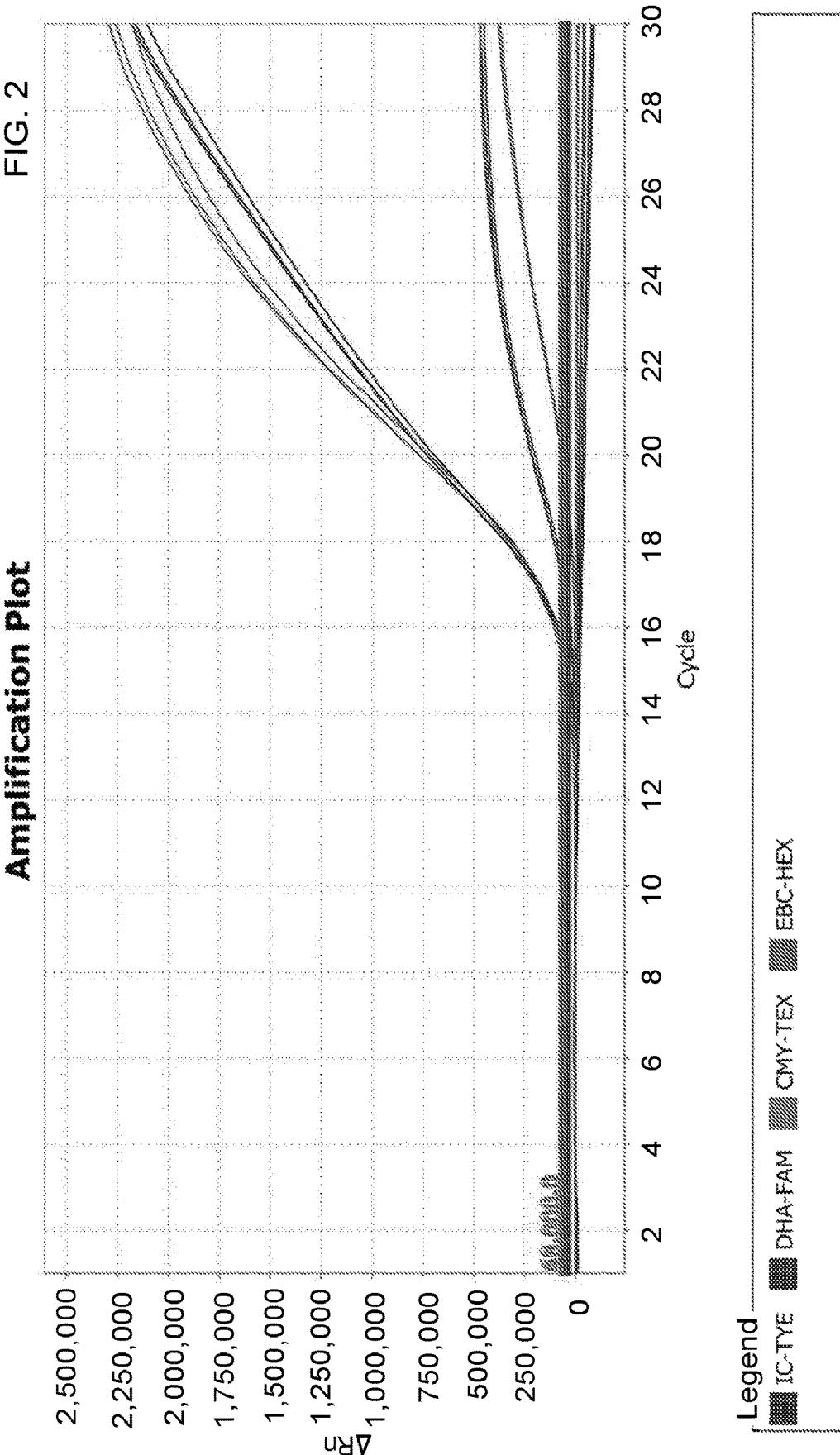
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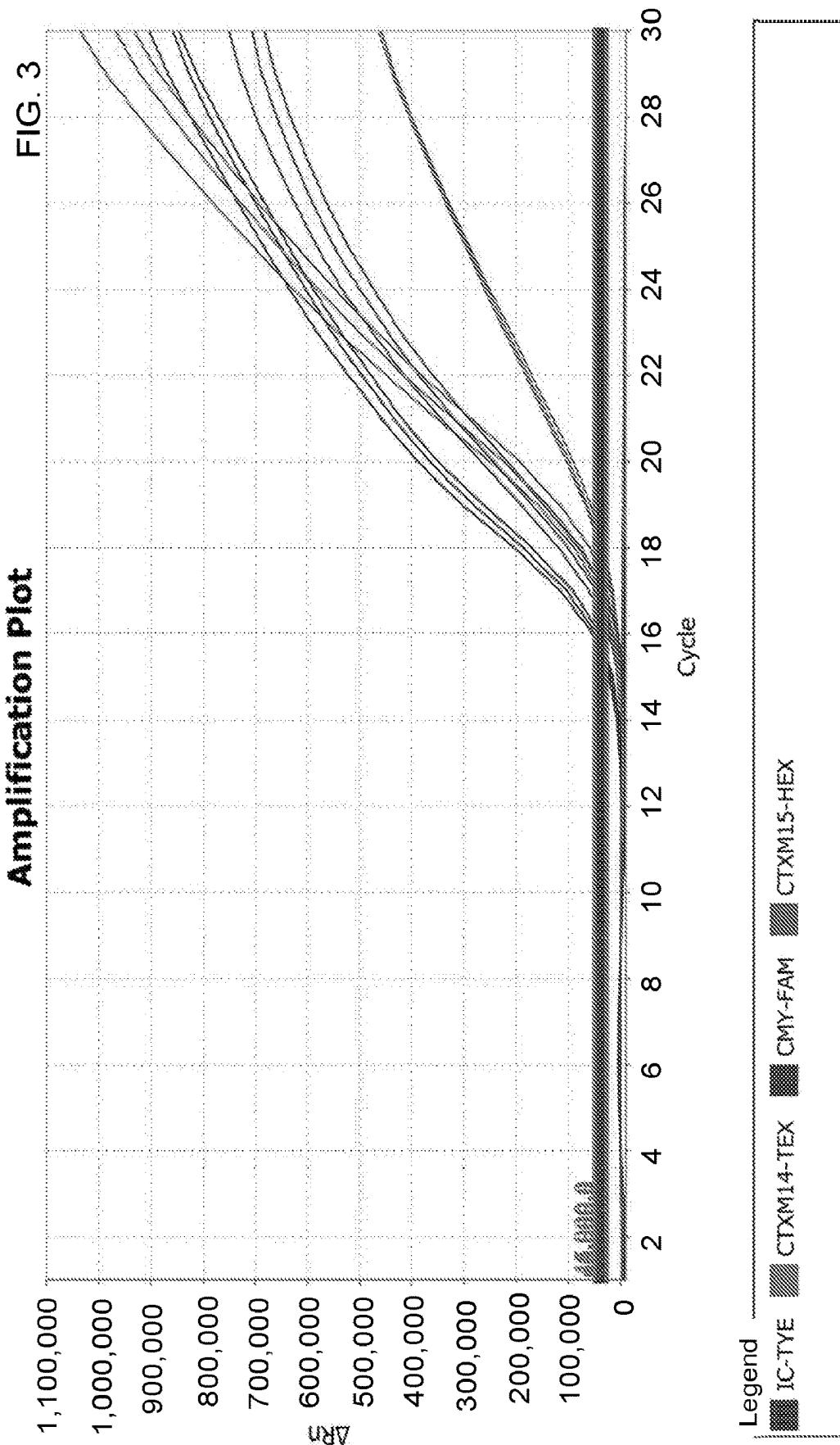
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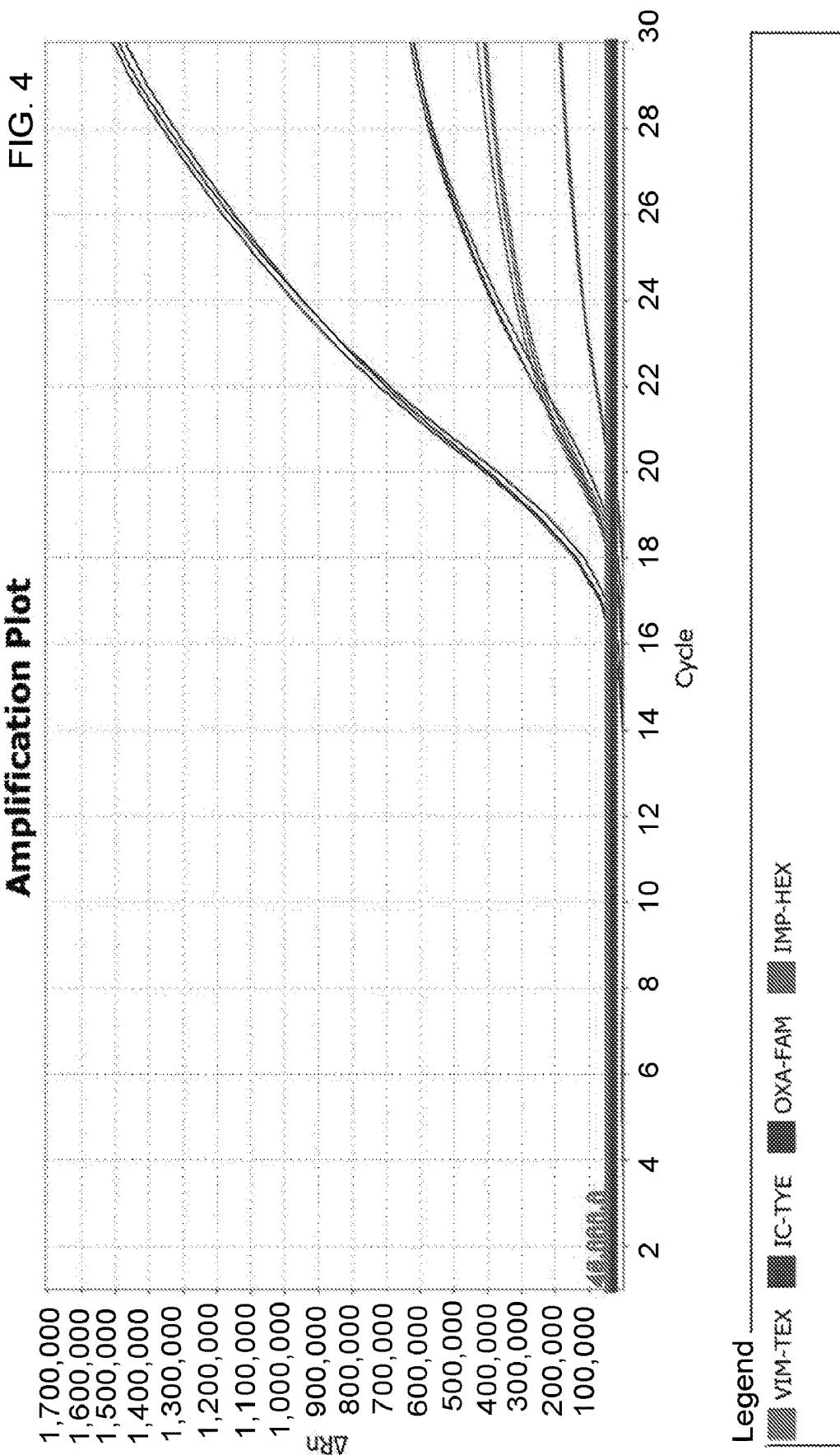
ampC kit-Mix 1

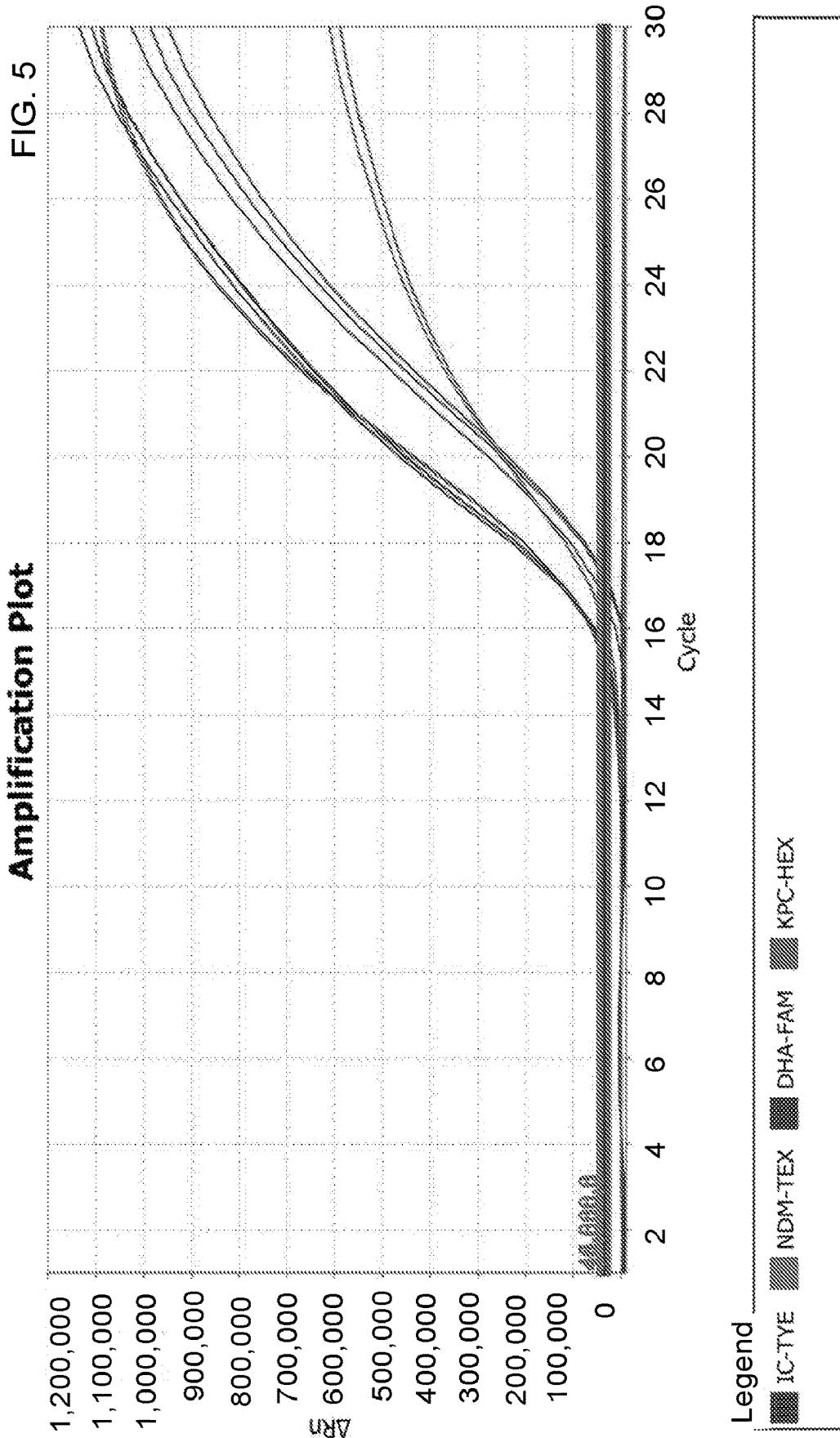
FIG. 1

ampC kit-Mix 2

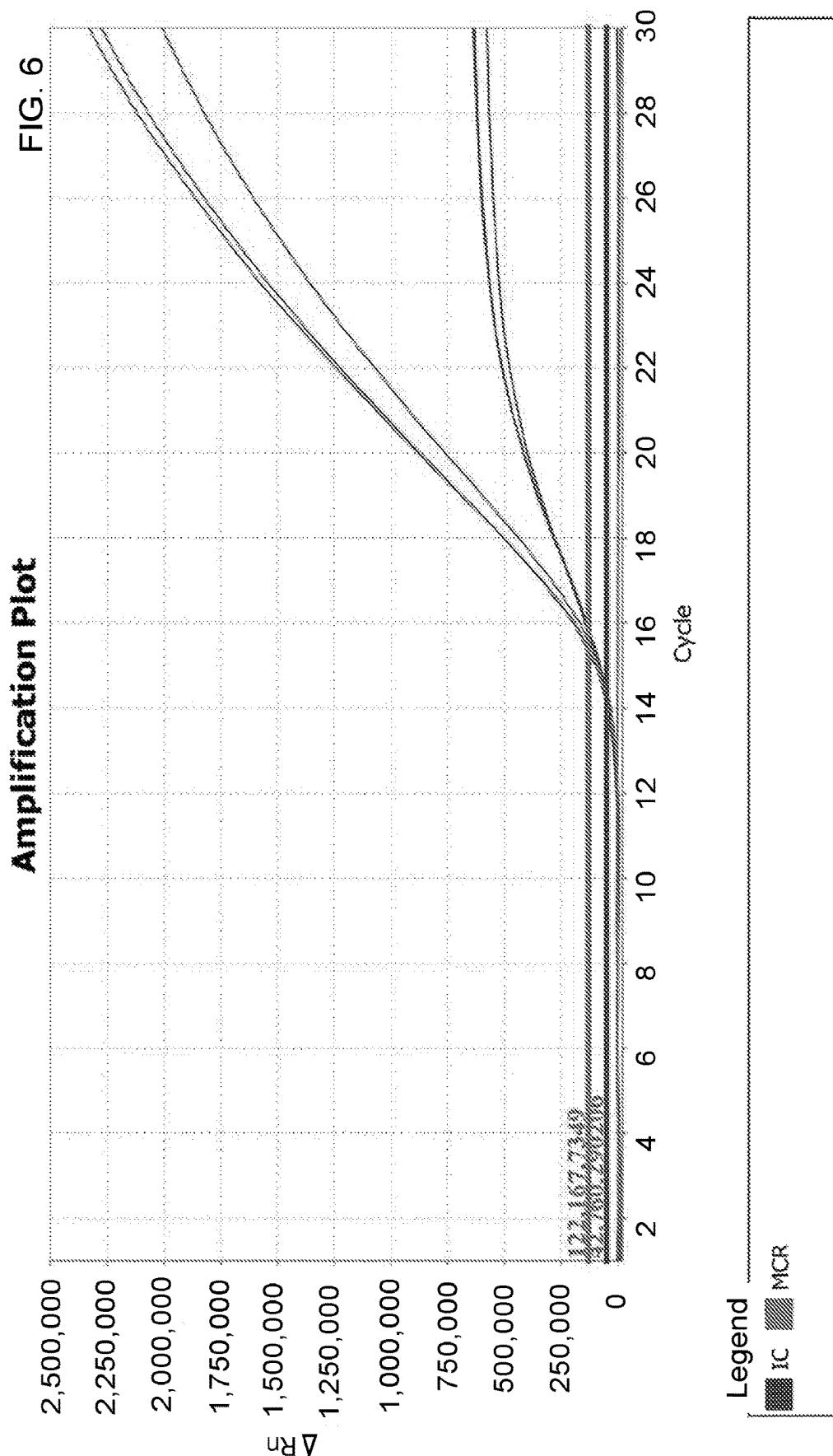
FIG. 2

β -lactamase Mix 1**FIG. 3**

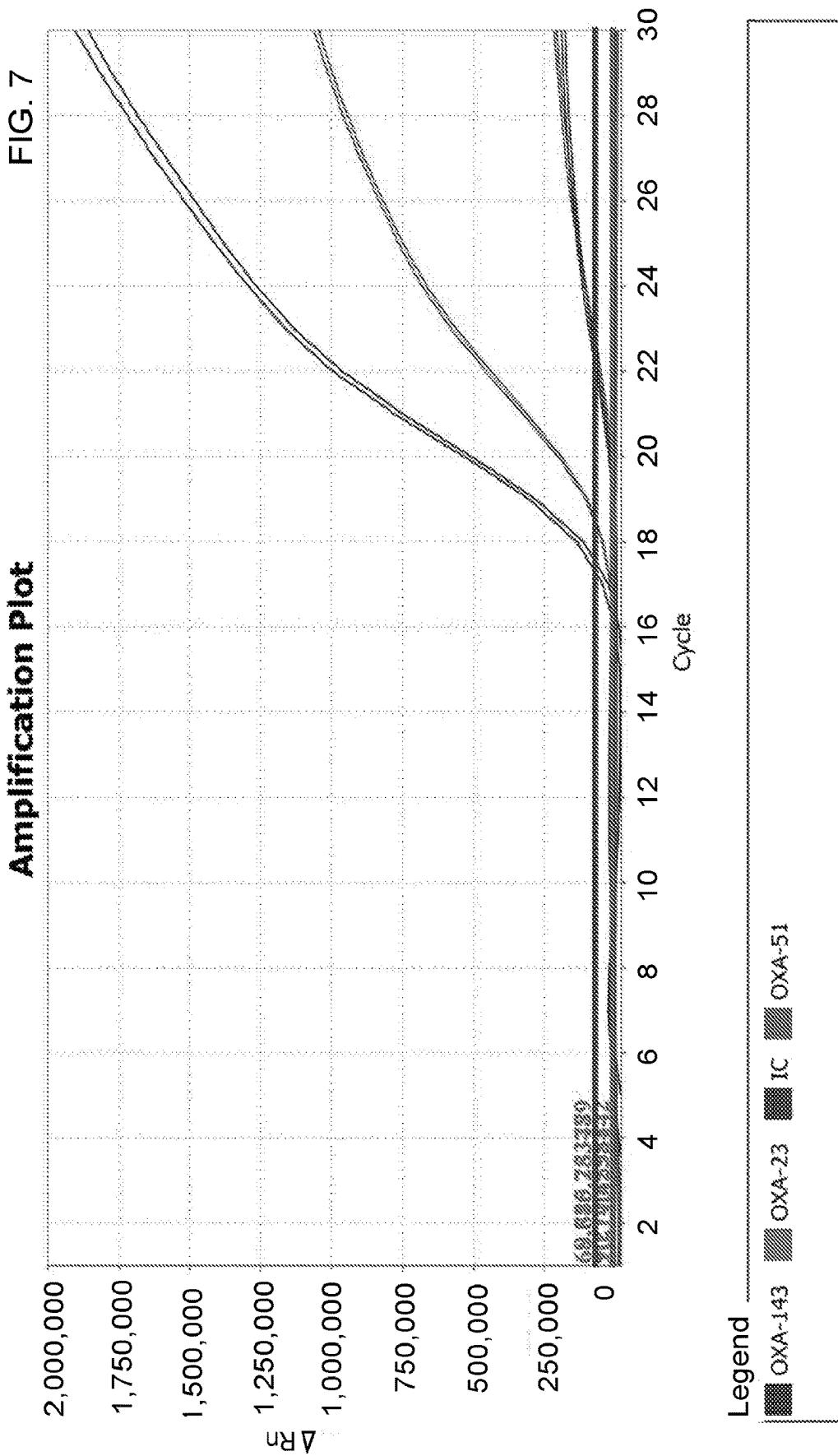
β-lactamase Mix 2**FIG. 4**

β -lactamase Mix 3**FIG. 5**

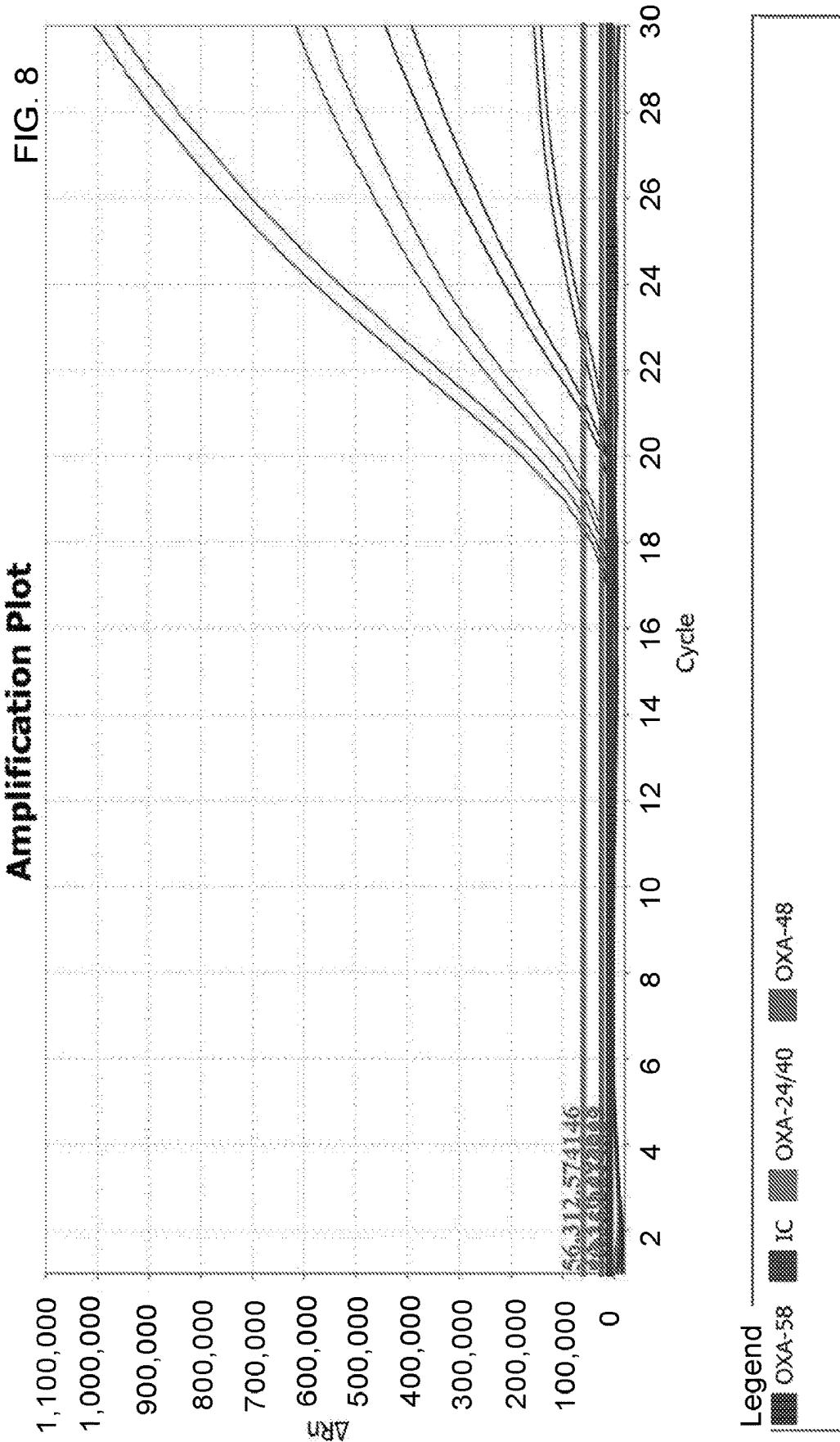
MCR-IC

FIG. 6

OXA kit-Mix 1

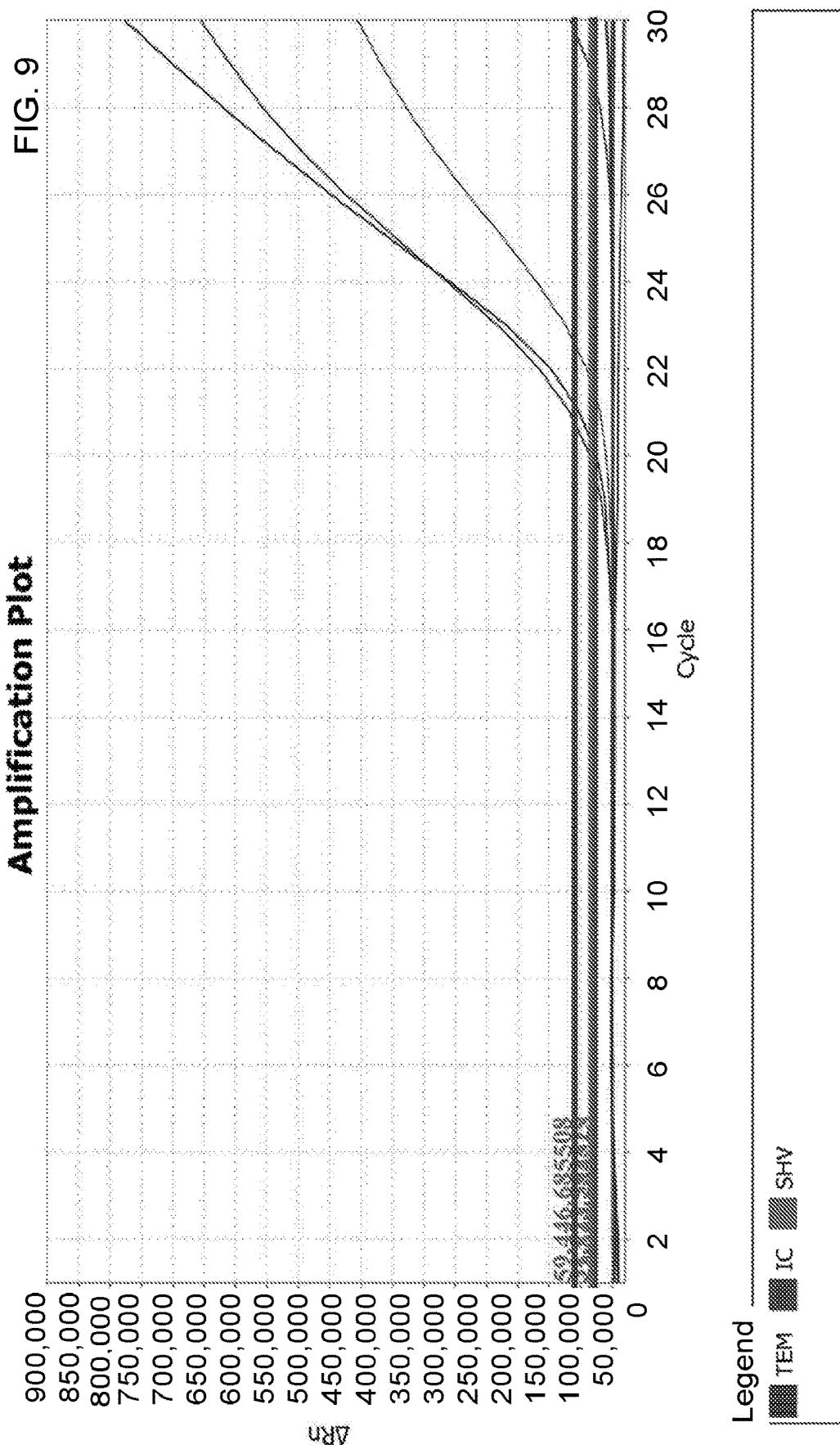
FIG. 7

OXA kit-Mix 2

FIG. 8

SHV-TEM-IC

FIG. 9



1**ASSAYS AND METHODS FOR DETERMINING MICROBIAL RESISTANCE****CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a Divisional of U.S. application Ser. No. 16/310,074, filed Dec. 14, 2018, now U.S. Pat. No. 11,708,614, issued Jul. 25, 2023, which is a U.S. National Phase of PCT/US17/37700, filed Jun. 15, 2017, which claims the priority benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 62/350,457, filed Jun. 15, 2016, which are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file. The name of the text file containing the Sequence Listing is “50035A_SubSeqListing.xml”, which was created on Jun. 8, 2023 and is 403,656 bytes in size. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.

FIELD

The present teachings relate to assays and methods for detecting resistance to antibiotics. The present teachings provide for the detection of family specific gene targets including AmpC β-lactamases, metallo-β-lactamases, carbapenemases, and extended-spectrum β-Lactamases by multiplex real-time polymerase chain reaction.

BACKGROUND

Bacterial resistance to antibiotics is a major public health issue. This resistance not only presents severe limitations to the ability to control and treat infection, but it also is difficult to identify and characterize in the laboratory. The significant increase in the resistance of pathogenic bacteria over the last 20 years, leads to extended periods of hospitalization, high morbidity and high mortality rates.

Enzymatic inactivation is the most common cause of resistance in terms of number of species and of antibiotics involved. As an example, β-lactamases are enzymes expressed by some bacteria. Such enzymes are capable of hydrolyzing the C—N bond of the β-lactam ring structure of a β-lactam antibiotic, effectively inactivating the antibiotic. Despite the existence of several β-lactamase inhibitors, the constant exposure of strains to antibiotics results in constant evolution of β-lactamases.

As a result, it becomes essential to be able to identify such resistant microorganisms and their resistance mechanisms as quickly as possible. Typically, biological samples can be tested for antibiotic resistance, but many test protocols are time consuming and/or limited in the types of resistance they are able to identify. It would therefore be beneficial to provide a test protocol for the simplified identification of resistance for all major β-lactamases.

One approach to the identification of β-lactamases has been to employ oligonucleotide primers specific for nucleic acid characteristic of certain β-lactamases with polymerase chain reaction to identify nucleic acid characteristics of family specific β-lactamase enzymes in samples. See for example, U.S. Pat. Nos. 6,893,846 and 7,476,520, incorpo-

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rated by reference herein. Another approach has been to employ oligonucleotide primers specific for nucleic acid characteristic of certain AmpC β-lactamases with multiplex polymerase chain reaction to detect the presence or absence of an AmpC β-lactamase gene and to identify nucleic acid characteristic of AmpC β-lactamase genes in samples. Multiplex polymerase chain reaction refers to the use of polymerase chain reaction to amplify several different DNA sequences simultaneously in single or multiple reactions. See for example, U.S. Pat. Nos. 7,045,291 and 7,521,547 incorporated by reference herein.

However, such primers have been limited with regards to the number of β-lactamase gene families or the number of gene targets that may be identified. Furthermore, such primers have been employed mainly with conventional polymerase chain reaction, which typically requires agarose gels to detect and analyze the PCR product(s). The use of agarose gel detection methods based on size discrimination may lead to poor resolution and difficulty in interpreting the data. Conventional polymerase chain reaction also lacks the sensitivity to detect endpoint variability from sample to sample and may not be automated. Real-time polymerase chain reaction allows for monitoring of reaction products as they are formed.

Detection of β-lactamases using real-time polymerase chain reaction and a single primer set may be limited to detection of a single β-lactamase gene family. See for example, United States Patent Application 2007/0248954 incorporated by reference herein. Multiplex real-time polymerase chain reaction has been designed for the identification of many AmpC β-lactamases simultaneously. See Geyer C N, Reisbig M D, Hanson N D. Development of a TaqMan® Multiplex PCR Assay for Detection of Plasmid-Mediated AmpC β-lactamase Genes. *Journal of clinical microbiology*. 2012 Aug. 15;JCM-02038. The primer/probe combinations in this study, however, have been directed only to AmpC β-lactamases and are limited in the number of gene targets that may be identified.

Multiple factors such as primer and probe design, reaction conditions, and enzyme selection must all be considered when designing a working polymerase chain reaction. This complexity is compounded in multiplex PCR, in which multiple targets are detected simultaneously in the same tube. Balancing the concentrations of primers, probes, and control vectors provided as composite “multiplex PCR” mixes for an assay is a challenging aspect. It is extremely difficult to balance these ratios, as a change of concentration for any of these reagents, corresponding to just one of the genetic targets, may adversely affect detection of any other multiplex target in the reaction mix. If these concentrations are not balanced, one could expect a reduction in efficiency, sensitivity, and specificity. This would reduce confidence in the effectiveness of the assay to correctly identify the gene families identified with the described kits.

Therefore, there is a significant amount of time and technical know-how required to develop these assays into a reliable method. For example, the PCR master mixture, with DNA polymerase, is a customized formulation that permits the final assay to work. Concentrations of DNA polymerase and magnesium may have to be adjusted. The specific concentrations and ranges surrounding DNA polymerase and magnesium are required for the assay to work successfully. In addition to determining concentrations for all reagents, a PCR cycling protocol must be identified that is compatible with all reaction conditions and facilitates real-time multiplex polymerase chain reaction.

Accurate and rapid detection of antibiotic resistance is essential for surveillance, epidemiologic tracking, patient therapy, and infection control. Thus, a multiplex PCR based diagnostic assay should provide comprehensive genotypic characterization of β -lactamases and be versatile as well as providing rapid results. The present teachings make it possible to test a sample for the presence of antibiotic resistant microorganisms by identifying any of the major β -lactamases in one test. The present teachings provide for the detection of multiple family-specific β -lactamase gene targets, including but not limited to metallo- β -lactamases, carbapenemases, extended-spectrum β -Lactamases, ampC chromosomal and/or plasmid-mediated AmpC β -lactamases, by multiplex real-time polymerase chain reaction.

The present teachings provide for a kit or kits including one or more primers and/or probes for identification of β -lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, ACT/MIR-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. The kits or kits of the present teachings may provide control material for the aforementioned β -lactamase genes. The present teachings provide one or more of the following: primers, probes, controls, assay process and detection strategy for one or more of the following β -lactamases: extended-spectrum β -lactamases (ESBLs), metallo- β -lactamases (MBLs), carbapenem-resistant enterobacteriaceae (CREs), and serine-dependent carbapenemases and plasmid-mediated ampC β -lactamases. A kit may also include one or more primers and/or probes for the identification of mobilized colistin-resistant (MCR) genes, a non-beta lactamase gene family that confers antibiotic resistance. The present teachings provide multiplex PCR assays which may test for any combination of these or are directed towards identification of a specific group. The present teachings provide assays with improved clinical sensitivity and analytical specificity of detection. The primer, probes, and control DNA sequences of the present teachings provide both an analytical and commercial advantage as they permit enhanced screening capabilities for detection of a larger number of genetic variants associated with genes conferring resistance to antibiotics in Gram-negative bacteria.

SUMMARY

The present teachings provide a kit including one or more primers and/or probes for the identification by polymerase chain reaction, microarray, NGS-based target enrichment, and/or mass spectrometric characterization of one or more β -lactamase genes selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, MOX, ACC, FOX, EBC, NDM, TEM, and SHV. The present teachings provide for one or more kits including primers and/or probes for identification of β -lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, EBC-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. A kit may also include one or more primers and/or probes for the identification of a non-beta lactamase gene family which confers antibiotic resistance. A kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of

MCR gene variants. Primers and probes may also be made compatible with next-generation sequencing and mass spectrometry.

5 BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 depicts an amplification plot of an exemplary mix 1 of a kit including ampC gene targets.

10 FIG. 2 depicts an amplification plot of an exemplary mix 2 of a kit including ampC gene targets.

FIG. 3 depicts an amplification plot of an exemplary mix 1 of a kit including β -lactamase gene targets.

15 FIG. 4 depicts an amplification plot of an exemplary mix 2 of a kit including β -lactamase gene targets.

FIG. 5 depicts an amplification plot of an exemplary mix 3 of a kit including β -lactamase gene targets.

20 FIG. 6 depicts an amplification plot of an exemplary internal control mix of a kit including MCR gene targets.

FIG. 7 depicts an amplification plot of an exemplary mix 1 of a kit including OXA gene targets.

25 FIG. 8 depicts an amplification plot of an exemplary mix 2 of a kit including OXA gene targets.

FIG. 9 depicts an amplification plot of an exemplary internal control mix of a kit including SHV-TEM gene targets.

DETAILED DESCRIPTION

The explanations and illustrations presented herein are 30 intended to acquaint others skilled in the art with the teachings, its principles, and its practical application. Those skilled in the art may adapt and apply the teachings in its numerous forms, as may be best suited to the requirements of a particular use. Accordingly, the specific embodiments of 35 the present teachings as set forth are not intended as being exhaustive or limiting of the teachings. The scope of the teachings should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the 40 full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated by reference for all purposes. Other combinations are also possible as will be gleaned from the following claims, which 45 are also hereby incorporated by reference into this written description.

Bacterial resistance to antibiotics poses a global threat to 50 public health and in recent years has shown an increase in mortality rates and the potential to spread through the population. Of these resistance mechanisms, β -Lactamases are enzymes that cleave β -Lactam rings rendering the β -Lactam family of antibiotics ineffective for treatment of 55 clinically-important Gram-negative bacterial infections. Specifically, β -Lactamases confer resistance to penicillins, cephamycins, and, in some cases, carbapenems. β -Lactam-resistant Gram-negative organisms, producing multiple or 60 plasmid-mediated β -lactamases, are difficult to identify phenotypically and necessitate more specific detection methods to identify clinically important β -lactamases. Genetic identification of these resistance mechanisms is critical for active 65 surveillance and infection control. Because these antibiotics are often selected for the management and prevention of infectious disease, the presence and characteristics of specific β -Lactamases play a critical role in selecting the appropriate antibiotic therapy.

AmpC β -lactamases are clinically important cephalosporinases that are resistant to most β -lactam antibiotics. AmpC

enzymes are chromosomally encoded in many bacterial species and can be inducible and overexpressed as a consequence of mutation. Overexpression can lead to resistance to most β -lactam antibiotics. The occurrence of transmissible plasmids with acquired genes for AmpC β -lactamases often result in increased β -lactamase production, compared to chromosomally-expressed ampC genes. Additionally, plasmid-mediated AmpC β -lactamases can appear in organisms lacking or having low-level expression of a chromosomal ampC gene. Resistance due to plasmid-mediated AmpC enzymes can be broad in spectrum and often hard to detect. As such, it is clinically useful to detect and discriminate between plasmid-mediated and chromosomally expressed AmpC β -lactamases.

The present teachings relate to assays and methods for detecting Gram-negative bacteria resistant to beta-lactam antibiotics from a biological sample. β -lactam antibiotics are all antibiotic agents that contain a β -lactam ring in their molecular structures. β -lactam antibiotics include penicillins, cephalosporins, carbapenems and monobactams. Antibiotic resistant organisms may produce one or more enzymes known as β -lactamases that provide resistance to β -lactam antibiotics. β -lactamases may confer resistance by the bacteria to antibiotics, which is plasmid-mediated and/or chromosomally expressed making detection difficult.

β -lactamases may be classified based on molecular structure. The four major classes include A to D. Class A, C and D β -lactamases are serine based. Class B β -lactamases, also known as metallo-beta-lactamases, are zinc based.

Extended spectrum β -lactamases (ESBLs) are enzymes that confer bacterial resistance to certain categories of antibiotics, such as third-generation cephalosporins and monobactams. The presence of an ESBL-producing organism in a clinical infection can cause treatment failure if one of the above classes of drugs is used. Detection of ESBLs can be difficult because they have different levels of activity against various cephalosporins. Thus genetic identification of the exact enzyme can facilitate selection of the optimal antimicrobial agent, which is critical to determine the most effective treatment response.

First-generation cephalosporins include cefalexin, cefalidine, cefalotin, cefazolin, cefadroxil, cefazidone, cefatrizine, cefapirin, cefradine, cefacetrile, cefrodxidine, ceftezole. Second-generation cephalosporins include cefoxitin, cefuroxime, cefamandole, cefaclor, cefotetan, cefonicide, cefotiam, loracarbef, cefmetazole, cefprozil, ceforanide. Third-generation cephalosporins include cefotaxime, ceftazidime, cefsulodine, ceftriaxone, cefmenoxime, latamoxef, ceftizoxime, cefixime, cefodizime, cefetamet, cefpiramide, cefoperazone, cefpodoxime, cefibuten, cefdinir, cefditoren, ceftriaxone, cefoperazone, cefuperazone. Fourth-generation cephalosporins include cefepime and cefpirome.

β -lactamase producing bacteria may include Gram-negative bacteria such as those found in the following genera: *Pseudomonas*, *Escherichia*, *Salmonella*, *Shigella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, *Campylobacter*, *Harmophilus*, *Morganella*, *Vibrio*, *Yersinia*, *Acinetobacter*, *Branhamella*, *Neisseria*, *Burkholderia*, *Citrobacter*, *Hafnia*, *Edwardsiella*, *Aeromonas*, *Moraxella*, *Pasteurella*, *Providencia* and *Legionella*.

Antibiotic resistance is intended to mean any type of mechanism which allows a microorganism to render a treatment partially or completely ineffective on the microorganism, guaranteeing its survival. β -lactam antibiotic resistance is intended to mean any type of β -lactamase-based mechanism which allows a microorganism to render a treatment partially or completely ineffective on the micro-

organism, guaranteeing its survival. For example, wherein the mechanism is related to the expression of an enzyme belonging to the β -lactamase group including extended-spectrum β -lactamase or of an enzyme belonging to the group of class C cephalosporinases.

Biological sample is intended to mean a clinical sample, derived from a specimen of biological fluid, or a food sample, derived from any type of food or drink, or from an agricultural source, such as animals, soil, water, or air, or from a surface such as with a biofilm. This sample may thus be liquid or solid. For example the biological sample may be a clinical sample of blood, plasma, urine or feces, or of rectal, nose, throat, skin, wound or cerebrospinal fluid specimens.

The present teachings relate to assays and methods for detecting resistance to beta-lactam antibiotics. The present teachings may detect β -lactamase gene targets which are chromosomally encoded and/or plasmid mediated. The present teachings provide for the detection of family specific gene targets relating to β -lactamase genes including AmpC β -lactamases. The β -lactamase genes detected with the present teachings may include those classified into molecular groups A through D. The β -lactamase genes detected with the present teachings may include those classified into functional groups 1 through 3.

The present teachings relate to assays and methods for detecting resistance of one or more gene beta lactamase gene families including like genes. A like gene may be a beta-lactamase that has one or more of the following: similar amino acid sequence, similar function and similar antibiotic susceptibility profiles. A like gene may be considered as like the target gene detected with the present teachings. For example, OXA-48-like enzymes may include: OXA-48, OXA-48b, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245 and OXA-24.

The present teachings provide one or more primers and/or probes for the identification of one or more β -lactamase genes selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, MOX, ACC, FOX, EBC, NDM, TEM, and SHV. The present teachings provide one or more primers and/or probes for the identification of β -lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, EBC-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. The present teachings provide one or more primers and/or probes for the identification of a non-beta lactamase gene family which confers antibiotic resistance. For example, one or more primers and/or probes for the identification of MCR gene variants. The primers and/or probes of the present teachings may be included in one or more kits. The one or more kits may be used for identification with any of the following: polymerase chain reaction, microarray, NGS-based target enrichment, and/or mass spectrometric characterization.

Exemplary sequences for primers and probes for of the present teachings are depicted in Table 1. [SEQ. ID NOS 67-260] Primers and/or probes may be degenerate at any nucleotide position. Primers and/or probes may not be degenerate at any nucleotide position. Any suitable fluorophore and/or quencher and nucleic acid sequence combination may be used. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, a probe may be labeled with a

fluorescent tag at one end and a fluorescent quencher at the other end. For example, two fluorescent quenchers may be included at one end or within the probe sequence. It is contemplated that the probe sequences of the present teach-

ings may be labeled with any suitable fluorophore and quencher combinations. For example, any fluorophore of the present teachings may be attached to any probe DNA sequence of the present teachings.

TABLE 1

Primer/Probe Sequence	
SEQ ID NO. 67	TGGCCAGAACTGACAGGGAAA
SEQ ID NO. 68	TTTCTCCTGAACGTGGCTGGC
SEQ ID NO. 69	56-FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/
SEQ ID NO. 70	CCGTCACGCTGTTAGG
SEQ ID NO. 71	GCTGTGTTAACATGCCACAC
SEQ ID NO. 72	5HEX/AACCTGCCG/ZEN/AATTAGAGCRGCACT/3IABkFQ
SEQ ID NO. 73	CGTTTCGTCTGGATCGCAC
SEQ ID NO. 74	GCTGGGTAATAGGTAC
SEQ ID NO. 75	5TEX615/TATCATTGGTGGCGTAGTCGC/3IAbRQSp
SEQ ID NO. 76	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 77	CGCCCATTGTSAAATTCC
SEQ ID NO. 78	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 79	AATCACAGGGCGTAGTTGTG
SEQ ID NO. 80	ACCCACCAGCCAATCTTAGG
SEQ ID NO. 81	56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTGGG/3IABkFQ/
SEQ ID NO. 82	GCGGAGTTAACTATTGGCTAG
SEQ ID NO. 83	GGCCAAGCTTCTATATTGCG
SEQ ID NO. 84	5HEX/TTRRTYGGT/ZEN/GGTTGYTTRTAA/3IABkFQ
SEQ ID NO. 85	GCGGAGTTARYTATTGGCTAG
SEQ ID NO. 86	GGCCAAGCYTCAWATTGCG
SEQ ID NO. 87	/5HEX/CCGGACGGT/ZEN/CTTGGTAATTGGG/3IABkFQ/
SEQ ID NO. 88	/5HEX/CCGTACGGT/ZEN/TTAGGCAATTGGG/3IABkFQ/
SEQ ID NO. 89	GGCGGCCTTGATGTCCTTCG
SEQ ID NO. 90	CCATTCCAGCCAGATCGGCATC
SEQ ID NO. 91	5TEX615/AGCTCTCTATCCTGGTGGCTGCG/3IAbRQSp
SEQ ID NO. 92	AACTTTCACAGGTGTGCTGGGT
SEQ ID NO. 93	CCGTACGCATACTGGCTTTGC
SEQ ID NO. 94	56-FAM/AAACGGGG/ZEN/GATATGCGTCTGTAT/3IABkFQ/
SEQ ID NO. 95	GTATCGCCGTCTAGTCTGC
SEQ ID NO. 96	CCTTGAATGAGCTGCACAGTGG
SEQ ID NO. 97	5HEX/TCGTCGCGG/ZEN/AACCATTGCTAAA/3IABkFQ/
SEQ ID NO. 98	GTGGATCGTCAGGGATGGC
SEQ ID NO. 99	GGCGAAAGTCAGGCTGTG
SEQ ID NO. 100	5TEX615/CATCAGGACAAGATGGCGGTATG/3IAbRQSp
SEQ ID NO. 101	GCTGCTCAAGGAGCACAGGAT
SEQ ID NO. 102	CACATTGACATAGGTGTGGTGC

TABLE 1-continued

Primer/Probe Sequence	
SEQ ID NO. 103	56-FAM/AGGATGGCA/ZEN/AGGCCACTATTC/3IABkFQ
SEQ ID NO. 104	AACAGCCTCAGCAGCCGGTTA
SEQ ID NO. 105	TTCGCCGCAATCATCCCTAGC
SEQ ID NO. 106	5HEX/AGCCATTAC/ZEN/GTCCAGAGTTGGCT/3IABkFQ
SEQ ID NO. 107	GCCGAGGCTTACGGGATCAAG
SEQ ID NO. 108	CAAAGCGCGTAACCGGATTGG
SEQ ID NO. 109	5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp
SEQ ID NO. 110	AACTTCACAGGTGTGCTGGGT
SEQ ID NO. 111	CCGTACGCATACTGGCTTGC
SEQ ID NO. 112	56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/
SEQ ID NO. 113	CTGGGTTCTATAAGTAAAACCTTCACCGG
SEQ ID NO. 114	CTTCCACTGGGGCTGCCAGTT
SEQ ID NO. 115	5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ
SEQ ID NO. 116	CCGAAGCCTATGGCGTGAAATCC
SEQ ID NO. 117	GCAATGCCCTGCTGGAGCG
SEQ ID NO. 118	5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp
SEQ ID NO. 119	AGCACATACAGAATATGTCCCTGC
SEQ ID NO. 120	ACCTGTTAACCAACCTACTTGAGGG
SEQ ID NO. 121	/56-FAM/TTGCAAGACGGACTGGCTTAGACC/3BHQ_1/
SEQ ID NO. 122	CCTGATCGGATTGGAGAACCC
SEQ ID NO. 123	CTACCTCTTGAATAGGCGTAACC
SEQ ID NO. 124	/5TEX615/ACGTCGCGAAGTTCTGATAGAC/3IAbRQSp/
SEQ ID NO. 125	TAGTGACTGCTAATCCAATCACAG
SEQ ID NO. 126	GCACGAGCAAGATCATTACATAGC
SEQ ID NO. 127	/5HEX/AGTTATCCAACAAGGCCAAACTCAACA/3BHQ_1/
SEQ ID NO. 128	AATCACAGGGCGTAGTTGTG
SEQ ID NO. 129	ACCCACCAGCCAATCTTAGG
SEQ ID NO. 130	/5HEX/TAGCTTGATCGCCCTCGATTTGGG/3BHQ_1/
SEQ ID NO. 131	GTGGGATGGAAAGCCACG
SEQ ID NO. 132	CACTTGGGGTCTACAGC
SEQ ID NO. 133	/56-FAM/TTACTTGGCGAAGCCATGCAAG/3BHQ_1/
SEQ ID NO. 134	CACCTATGGTAATGCTCTTGC
SEQ ID NO. 135	CTGGAACTGCTGACAATGCC
SEQ ID NO. 136	/5TEX615/TGGGAGAAAGATATGACTTTAGGTGAGGCA/3IAbRQSp/
SEQ ID NO. 137	CCGTGTATGTTAGCTAT
SEQ ID NO. 138	CTTATCCATCACGCCCTT
SEQ ID NO. 139	/5TEX615/TATGATGTCGATAACGCCAAATACCA/3IAbRQSp/
SEQ ID NO. 140	CTGTATGTCAGCGATCAT
SEQ ID NO. 141	GATGCCAGTTGCTTATCC

TABLE 1-continued

Primer/Probe Sequence	
SEQ ID NO. 142	/5FAM/AAGTCTGGG/ZEN/TGAGAACGGTGCTAT/3IABkFQ
SEQ ID NO. 143	CAGTCAGTATGCGAGTTTC
SEQ ID NO. 144	AAAATTGCCAAGCCATC,
SEQ ID NO. 145	/5HEX/TGCATAAGC/ZEN/CAGTGCCTTTTATAT/3IABkFQ
SEQ ID NO. 146	AGATCAGTTGGGTGACG
SEQ ID NO. 147	TGCTTAATCAGTGAGGCACC
SEQ ID NO. 148	/56-FAM/ATGAAGCCA/ZEN/TACCAAACGACGAGC/3IABkFQ/
SEQ ID NO. 149	CTGGAGCGAAAGATCCACTA
SEQ ID NO. 150	ATCGTCCACCATCCACTG
SEQ ID NO. 151	/5HEX/CCAGATCGG/ZEN/CGACAACGTCACC/3IABkFQ/
SEQ ID NO. 152	TGGCCAGAACTGACAGGCAA
SEQ ID NO. 153	TTTCTCCTGAACGTGGCTGGC
SEQ ID NO. 154	56-FAM/ACGCTAACT/ZEN/CCAGCATTGGCTGT/3IABkFQ/
SEQ ID NO. 155	CCGTCACGCTGTTGTTAGG
SEQ ID NO. 156	GCTGTGTTAACATGCCACAC
SEQ ID NO. 157	5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ
SEQ ID NO. 158	CGTTTCGTCCTGGATCGCAC
SEQ ID NO. 159	GCTGGGTAAAATAGGTCAAC
SEQ ID NO. 160	5TEX615/TATCATTGGTGGTGCCGTAGTCGC/3IAbRQSp
SEQ ID NO. 161	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 162	CGCCCATTGTSCAATATTCC
SEQ ID NO. 163	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 164	AATCACAGGGCGTAGTTGTG
SEQ ID NO. 165	ACCCACCAGCCAATCTTAGG
SEQ ID NO. 166	56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTGGG/13IABkFQ/
SEQ ID NO. 167	GCGGAGTTAACTATTGGCTAG
SEQ ID NO. 168	GGCCAAGCTTCTATATTGCG
SEQ ID NO. 169	5HEX/TTRTTYGGT/ZEN/GGTTGYTTRTTAA/3IABkFQ
SEQ ID NO. 170	GCGGAGTTARYTATTGGCTAG
SEQ ID NO. 171	GGCCAAGCYTCTAWATTGCG
SEQ ID NO. 172	/5HEX/CCGGACGGT/ZEN/CTTGGTAATTGGGT/3IABkFQ/
SEQ ID NO. 173	/5HEX/CCGTACGGT/ZEN/TTAGGCAATTGGGT/3IABkFQ
SEQ ID NO. 174	GGCGGCGTTGATGCTCTCG
SEQ ID NO. 175	CCATTCAAGCCAGATCGGCATC
SEQ ID NO. 176	5TEX615/AGCTCTCTATCCTGGTGCTGCG/3IAbRQSp
SEQ ID NO. 177	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 178	CGCCCATTGTSCAATATTCC
SEQ ID NO. 179	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 180	AACTTCAACAGGTGTGCTGGGT

TABLE 1-continued

Primer/Probe Sequence	
SEQ ID NO. 181	CCGTACGCATACTGGCTTTGC
SEQ ID NO. 182	56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/
SEQ ID NO. 183	GTATGCCGTCTAGTTCTGC
SEQ ID NO. 184	CCTTGAATGAGCTGCACAGTGG
SEQ ID NO. 185	5HEX/TCGTCGCGG/ZEN/AACCATTGCTAAA/3IABkFQ/
SEQ ID NO. 186	GTTTGATCGTCAGGGATGGC
SEQ ID NO. 187	GGCGAAAGTCAGGCTGTG
SEQ ID NO. 188	5TEX615/CATCAGGACAAGATGGCGGTATG/3IAbRQSp
SEQ ID NO. 189	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 190	CGCCCATTGTSAAATATTCC
SEQ ID NO. 191	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 192	GCTGCTCAAGGAGCACAGGAT
SEQ ID NO. 193	CACATTGACATAGGTGTGGTGC
SEQ ID NO. 194	56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTCA/3IABkFQ
SEQ ID NO. 195	AACAGCCTCAGCAGCCGGTTA
SEQ ID NO. 196	TTCGCCGCAATCATCCCTAGC
SEQ ID NO. 197	5H EX/AGCCATTAC/ZEN/GTCCAGAGTTGCGT/3IABkFQ
SEQ ID NO. 198	GCCGAGGCTTACGGGATCAAG
SEQ ID NO. 199	CAAAGCGCGTAACCGGATTGG
SEQ ID NO. 200	5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp
SEQ ID NO. 201	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 202	CGCCCATTGTSAAATATTCC
SEQ ID NO. 203	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 204	AACTTCACAGGTGTGCTGGGT
SEQ ID NO. 205	CCGTACGCATACTGGCTTTGC
SEQ ID NO. 206	56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ
SEQ ID NO. 207	CTGGGTTCTATAAGTAAACCTTCACCGG
SEQ ID NO. 208	CTTCCACTGCGGCTGCCAGTT
SEQ ID NO. 209	5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ
SEQ ID NO. 210	CCGAAGCCTATGGCGTGAAATCC
SEQ ID NO. 211	GCAATGCCCTGCTGGAGCG
SEQ ID NO. 212	5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp
SEQ ID NO. 213	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 214	CGCCCATTGTSAAATATTCC
SEQ ID NO. 215	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 216	AGCACACAGAACCTACTTGAGGG
SEQ ID NO. 217	ACCTGTTAACCAACCTACTTGAGGG
SEQ ID NO. 218	/56-FAM/TTGCAAGACGGACTGGCTTAGACCC/3BHQ_1/
SEQ ID NO. 219	CCTGATCGGATTGGAGAACCC

TABLE 1-continued

Primer/Probe Sequence	
SEQ ID NO. 220	CTACCTCTTGAATAGCGCTAACCC
SEQ ID NO. 221	/5TEX615/ACGTCGGCAAGTTCTGATAGAC/3IAbRQSp/
SEQ ID NO. 222	TAGTGAUTGCTAATCCAATCACAG
SEQ ID NO. 223	GCACGAGCAAGATCATTACCATAGC
SEQ ID NO. 224	/5HEX/AGTTATCCAACAAGGCCAAACTCAACA/3BHQ_1/
SEQ ID NO. 225	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 226	CGCCCATTTGTSAAATATTCC
SEQ ID NO. 227	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 228	AATCACAGGGCGTAGTTGTG
SEQ ID NO. 229	ACCCACCAGCCAATCTTAGG
SEQ ID NO. 230	/5HEX/TAGCTTGATCGCCCTCGATTTGGG/3BHQ_1/
SEQ ID NO. 231	GTGGGATGGAAAGCCACG
SEQ ID NO. 232	CACTTGCGGGTCTACAGC
SEQ ID NO. 233	/56-FAM/TTACTTGGCGAAGCCATGCAAG/3BHQ_1/
SEQ ID NO. 234	CACCTATGGTAATGCTCTTGC,
SEQ ID NO. 235	CTGGAUACTGCTGACAATGCC
SEQ ID NO. 236	/5TEX615/TGGGAGAAAGATATGACTTAGGTGAGGCA/3IAbRQSp/
SEQ ID NO. 237	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 238	CGCCCATTTGTSAAATATTCC
SEQ ID NO. 239	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 240	AGATCAGTTGGGTGCACG
SEQ ID NO. 241	TGCTTAATCAGTGAGGCACC
SEQ ID NO. 242	/56-FAM/ATGAAGCCA/ZEN/TACCAAACGACGAGC/3IABkFQ/
SEQ ID NO. 243	CTGGAGCGAAAGATCCACTA
SEQ ID NO. 244	ATCGTCCACCATCCACTG
SEQ ID NO. 245	/5HEX/CCAGATCGG/ZEN/CGACAACGTCACC/3IABkFQ/
SEQ ID NO. 246	GAGAGGATGAYCAGCCACAC
SEQ ID NO. 247	CGCCCATTTGTSAAATATTCC
SEQ ID NO. 248	5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp
SEQ ID NO. 249	CCGTGTATGTTCACTAT
SEQ ID NO. 250	CTTATCCATCACGCCCTT
SEQ ID NO. 251	/5TEX615/TATGATGTCGATACCGCCAAATACCA/3IAbRQSp/
SEQ ID NO. 252	CTGTATGTCAGCGATCAT
SEQ ID NO. 253	GATGCCAGTTGCTTATCC
SEQ ID NO. 254	/56FAM/AAGTCTGGG/ZEN/TGAGAACGGTGTCTAT/3IABkFQ/
SEQ ID NO. 255	CAGTCAGTATGCGAGTTTC
SEQ ID NO. 256	AAAATCGCCAAGCCATC
SEQ ID NO. 257	/5HEX/TGCATAAGC/ZEN/CAGTGCCTTTTATAT/3IABkFQ/
SEQ ID NO. 258	GAGAGGATGAYCAGCCACAC

TABLE 1-continued

Primer/Probe Sequence	
SEQ ID NO. 259	CGCCCATGTSCAATATTCC
SEQ ID NO. 260	5'TYE665/TGAGACACGGTCCAGACTCCTACG/3'IAbRQSP

The present teachings provide a molecular assay. The present teachings may provide a qualitative (i.e. end point) molecular assay for the detection of family-specific KPC, ESBL, MBL, and ampC gene targets. The present teachings may provide a qualitative (i.e. end point) molecular assay for the detection of family-specific plasmid-mediated ampC β-lactamase genes. The present teachings may provide a qualitative (i.e. end point) molecular assay for the detection of OXA gene targets. Fluorescently-labeled DNA probes may be used for detection. The assay of the present teachings may provide for differentiation between a plasmid-mediated ampC β-lactamase gene from a chromosomal ampC β-lactamase gene; provided the two genes are not from the same chromosomal origin. The assay may involve extraction of DNA from bacterial cells. The assay may include subsequent PCR amplification. The assay may include gel-based detection.

In contrast, to traditional phenotypic methods which require 24-48 hours for data, the present teachings may provide for data generation in just hours or one hour. The total time required for DNA extraction, PCR set-up, amplification, and analysis may be around about 2 hours to about 3 hours. The sensitivity of the assay may be about 100%. The specificity of the assay may be about 100%. Therefore, the present teachings provide for fast and reliable detection. Implementation of such rapid assays have a positive impact for infection control and patient care.

The present teachings allow for the detection of multiple β-lactamase gene families. The β-lactamases may include all major β-lactamases including ampC types. For example, the present teachings may allow for identification of up to six to nine β-lactamase gene families. The β-lactamase gene families may include CMY, CTX-Ms, DHA, IMP, KPC, NDM, OXA and VIM. The AmpC β-lactamases gene families may include MOX, ACC, FOX, DHA, CMY and EBC.

The present teachings provide for a kit which allows for identification of at least nine β-lactamase gene families. The gene families may include: IMP-1-like, NDM-like, OXA-48-like, CTX-M-14-like, CTX-M-15-like, CMY-2-like, DHA-like, VIM-like, and KPC-like. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each target group. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

The kit may include one or more multiplex primer-probe mixes containing one or more primers and one or more probes. The multiplex primer-probe mix may be a 10×PCR mix. In one example, the kit includes three multiplex primers-probes mix vials. The mix vials may provide for simultaneous real-time PCR amplification of all targets between three reaction tubes. PCR Mix 1 may amplify a first set of

three gene families. For example, CMY-2, CTX-M-14, and CTX-M-15. PCR Mix 2 may amplify a second set of three gene families. For example, OXA-48, IMP, and VIM. PCR mix 3 may amplify a third set of gene families. For example, DHA, KPC, and NDM. The multiplex mix may also include an internal control (IC) in each mix. The kit may include three external DNA control vials or first control mix vial, a second control mix vial and a third control mix vial. The DNA control mix vial may contain synthetic DNA templates of the corresponding multiplex targets. The DNA control mixes may serve as a positive control for each multiplex reaction. The DNA control mix may contain stabilized bacteria with chromosomal or transmissible genetic elements in a sample matrix similar to a patient sample.

The present teachings provide for a kit which allows for identification of at least six plasmid-mediated ampC gene families. The gene families may include: MOX-like, DHA-like, ACC-like, EBC-like, FOX-like, and CMY-2-like. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each family. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

The kit may include one or more multiplex primer-probe mixes containing one or more primers and one or more probes. The multiplex primer-probe mix may be a 10×PCR mix. In one example, the kit includes two multiplex primers-probes mix vials. The mix vials may provide for simultaneous real-time PCR amplification of all targets between two reaction tubes. PCR Mix 1 may amplify a first set of three gene families. For example, MOX, ACC and FOX. PCR Mix 2 may amplify a second set of three gene families. For example, DHA, EBC and CMY-2. The multiplex mix may also include an internal control (IC) in each mix. The kit may include two external DNA control vials or first control mix vial and a second control mix vial. The DNA control mix vial may contain synthetic DNA templates of the corresponding multiplex targets. The DNA control mixes may serve as a positive control for each multiplex reaction.

The present teachings provide for a kit which allows for identification of at least six OXA carbapenemase gene families. The gene families may include: OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143. The gene families may include like gene families. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer

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pairs for the PCR amplification of each family. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

The kit may include one or more multiplex primer-probe mixes containing one or more primers and one or more probes. The multiplex primer-probe mix may be a 10 \times PCR mix. In one example, the kit includes two multiplex primers-probes mix vials. The mix vials may provide for simultaneous real-time PCR amplification of all targets between two reaction tubes. PCR Mix 1 may amplify a first set of three gene families. For example, OXA 143, OXA 23 and OXA 51. PCR Mix 2 may amplify a second set of three gene families. For example, OXA 24/40, OXA-48 and OXA-58. The multiplex mix may also include an internal control (IC) in each mix. The kit may include two external DNA control vials or first control mix vial and a second control mix vial. The DNA control mix vial may contain synthetic DNA templates of the corresponding multiplex targets. The DNA control mixes may serve as a positive control for each multiplex reaction.

In addition, the present teachings contemplate that the kit or kits of the present teachings may provide for the detection of a non-beta lactamase gene family. The kit or kits may provide for detection of plasmid-mediated mechanisms of antibiotic resistance for one more types/categories of antibiotics. For example, the kit may also provide for the detection of the MCR-1 gene which confers polymixin resistance. The kit or kits may include primer sequences, probe sequences, and a control sequence for detection of one or more non-beta lactamase gene family in addition to beta-lactamase genes. For example, a kit may provide for the detection of ampC genes families and a MCR-1 gene family.

Furthermore, the present teachings allow for the expansion of the detection of other β -lactamase gene families including TEM and SHV. The gene families may include like gene families. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each family. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

The kit or kits of the present teachings may include synthetic DNA oligonucleotide primers, target-specific DNA probes and DNA controls for the specified gene targets suspended in TE buffer, pH 8.0. The contents of the kit may be enclosed in vials. For example, the one or more 10 \times PCR mixes may be comprised of 275 μ L. For example, the one or more control mixes may be comprised of 14 μ L. For example, the contents of the kit may be sufficient for about 100 reactions total and about 12 reactions of the control DNA mix.

Detection of each target is based on the optical fluorescence of the fluorophore conjugated to each target-specific DNA probe. Any suitable fluorophore and nucleic acid sequence combination may be used. For example, the fluorophores may be selected from the group consisting of: FAM (6-Carboxyfluorescein), HEX (Hexachlorofluorescein), TEX615 and TYE665.

The present teachings provide assays for the detection of β -lactamase gene families from a biological sample. The assays may be included in a kit or kits. The kit may provide for the detection of β -lactamase by various molecular biol-

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ogy technologies and platforms. The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of one or more β -lactamase genes selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, MOX, ACC, FOX, EBC, NDM, TEM, and SHV.

The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of a non-beta lactamase gene family which confers antibiotic resistance. The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of one or more MCR genes. The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of a MCR-1 gene.

The kit may provide for detection of specified targets from crude biological samples such as blood, urine, plasma, feces, sputum, etc. The kit may provide for detection of specified targets directly from or extracted directly from crude biological samples including but not limited to blood, blood cultures, urine, plasma, feces, fecal swabs, peri-rectal/peri-anal swabs, sputum, and bacterial cultures.

The kit may be used for detection of specified targets from purified nucleic acid samples. The kit may be used for any nucleic acid amplification methodology. The kit may be used with conventional polymerase chain reaction. The kit may be used with real-time polymerase chain reaction. The kit may be used with digital droplet polymerase chain reaction. The kit may be used with detection by microarray technology. The kit may be used with fluorescence and/or infra-red probe-based detection chemistries. The kit may be used with intercalating dye-based detection chemistries. The kit may be used for detection of nucleic acid polymerase chain reaction amplicons ranging from 25 base pairs to 2000 base pairs.

The kit may include various reagents. The various reagents may be contained in various vials. The kit may include a primer set or primer sets. The primer set or primer sets may be labeled or unlabeled with a tracking dye or fluorophore. The kit may include probes. The kit may include a primer-probe mix. The kit may include controls. The kit may include magnesium chloride. The kit may include dNTPs. The kit may include DNA polymerase. The kit may include a tracking dye. The kit may include a composition containing a tracking dye. The kit may include a written protocol. The kit may include a customized master mix in a single tube, two tubes, three tubes, or four tubes containing all chemicals and enzymes necessary to run the PCR assay described herein. The kit may include freeze-dried or lyophilized reagents in a single assay tube or multiple assay tubes. The kit may provide for detection of nucleic acid and the kit reagents may be provided in any liquid form, pooled reaction mix, or lyophilized, freeze dried, or cryo-preserved format.

The kit may include a primer set. The primer set may include at least one primer pair. A primer pair may include a forward primer and a reverse primer. The primer set may include one pair of primers. The primer set may include more than one pair of primers. The primer set may include two pairs of primers. The primer set may include three pairs of primers. The primer set may include one to six pairs of primers. The primer set may include one to ten pairs of primers. The primer set may include up to 30 pairs of primers. The primer set may include up to 50 pairs of primers. The primer set may include up to 100 pairs of primers.

The kit may include a primer-probe mix. The primer-probe mix may include a primer set. The primer-probe mix may include one or more probes. Each pair of primers of the primer set may include a probe or set of probes. The primer-probe mix may include a pair of internal control primers. The pair of internal control primers may include a forward primer and a reverse primer. The primer-probe mix may include an internal control probe.

For example, a primer-probe mix may include one or more pairs of primers, one associated probe per primer pair and internal controls including a pair of primers and a probe. Preferably, the primer-probe mix is a multiplex mix including more than one pair of primers, a probe for each primer pair and internal controls. The multiplex mix may be used for the identification of more than one β -lactamase gene family. Each primer pair and probe may detect a different β -lactamase gene family. For example, three primer pairs and their associated three probes may be used for detection of three different β -lactamase gene families.

The DNA concentration range of each primer set in a PCR may be about 1 nM to about 10 μ M (10,000 nM). One or more primers may be labeled with a fluorescent marker as a probe. The DNA concentration of each probe in a PCR may be about 1 nM to about 10,000 nM. The DNA concentration of each probe in a PCR may be about 10 to about 500 nM.

The kit may include at least one control. The kit may include one, two, three or four controls. The kit may include one or more negative controls. The negative control may include nucleic acid known to express a resistance gene other than the target gene of interest. The kit may include one or more positive controls. The one or more positive controls may be internal controls. The positive control may include nucleic acid known to express or contain the resistance gene. The kit may include an endogenous internal control to reduce false negatives due to PCR inhibition, DNA degradation, and/or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The endogenous internal control may target a conserved nucleotide sequence or sequences common to the Gram-negative bacteria genome. For example, the internal control may detect the 16S rRNA and/or 23S rRNA gene(s). The internal control may detect the 16S and/or 23S rRNA gene for *E. coli*, *Pseudomonas*, *Acinetobacter*, *Klebsiella* and *Salmonella*.

The kit may include control vector in the control vial. One or more μ ls of the vector control may be added to a 25 μ l reaction to get the working concentration. The DNA concentrations for each control vector may be equivalent to 0.1 copy to 2000 copies or 0.0000243 pg/uL to 0.0455 pg/uL. The DNA concentrations for each control vector may be equivalent to 10 copies to 5000 copies or 0.001 pg/uL to 0.5 pg/uL. Control vector concentrations may be as high as 1 \times 10(9) copies and any dilution thereof.

The assays of the present teachings may include the use of magnesium chloride. The kit may include magnesium chloride. The assay may be utilized with a concentration of about 2 mM to about 7 mM MgCl₂. Preferably, the concentration is about 3.0 mM to about 5.5 mM MgCl₂. More preferably, the concentration is 5.0 mM MgCl₂ for an assay for the detection of β -lactamase genes. More preferably the concentration is 5.0 mM MgCl₂ for an assay for the detection of ampC β -lactamase genes. More, preferably, the concentration is 5 mM MgCl₂ for an assay for the detection of OXA genes.

The assays of the present teachings may include the use of DNA polymerase. The kit may include DNA polymerase. The assay may be utilized with a concentration of about 0.25 U/25 μ l reaction to about 3 U/25 μ l reaction of DNA polymerase. Preferably, the concentration is 1.25 U/25 μ l reaction DNA polymerase for an assay for the detection of β -lactamase genes. Preferably the concentration is 1.25 U/25 μ l DNA polymerase for an assay for the detection of β -lactamase ampC genes. For example, the present teachings may utilize the PhilisaFAST® DNA polymerase.

The assays and methods of the present teachings may include a PCR cycling protocol. In one example, the cycling protocol comprises (1) 95° C. for 30 s; (2) 95° C. for 1 s; (3) 55° C. for 10 s; (4) 68° C. for 20 s; and repeating steps (2) to (4) for 40 cycles. In one example, the cycling protocol comprises (1) 95° C. for 30 s; (2) 95° C. for 6 s; (3) 66° C. for 10 s; and repeating steps (2) to (3) for 40 cycles. In one example, the cycling protocol includes a hot start of 98° C. for 30 s and 30 cycles of: 98° C. for 5 s, 60° C. for 10 s and 72° C. for 20 s. In one example, the cycling protocol includes using 98° C. for 30 s, followed by 30 cycles of 98° C. for 5 s, 60° C. for 10 s., and 72° C. for 25 s. In one example, the PCR protocols include a detection step where fluorescent signal is measured.

The kit may include one or more of the following: primer, probe and control. A mix of one or more of the following: primer, probe and internal control, may be enclosed in one container. A mix of one or more of the following: primer, probe and internal control, may be enclosed in more than one container. The container may be a vial. In one example, the kit includes 3 DNA control vials and 3 10 \times primer/probe mix vials. Nine antibiotic resistance gene families and one internal control may be identified with the vials. In one example, the kit includes 2 DNA control vials and 2 10 \times primer/probe mix vials. Six antibiotic resistance gene families and one internal control may be identified with the vials.

The present teachings allow for detection of the β -lactamase CMY-2 gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the CMY-2-like gene family. The biological sample may include Gram-negative bacteria such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Citrobacter freundii* and other *Citrobacter* species. The CMY-2-like genes detected may include CMY-2, CMY-4, CMY-6, CMY-7, CMY-12, CMY-14, CMY-15, CMY-16, CMY-18, CMY-21, CMY-22, CMY-23, CMY-24, CMY-25, CMY-26, CMY-27, CMY-28, CMY-29, CMY-30, CMY-31, CMY-32, CMY-33, CMY-34, CMY-35, CMY-37, CMY-38, CMY-39, CMY-40, CMY-41, CMY-42, CMY-43, CMY-44, CMY-45, CMY-46, CMY-47, CMY-48, CMY-49, CMY-50, CMY-51, CMY-53, CMY-54, CMY-55, CMY-56, CMY-57, CMY-58, CMY-59, CMY-60, CMY-61, CMY-62, CMY-63, CMY-64, CMY-65, CMY-66, CMY-67, CMY-68, CMY-69, CMY-71, CMY-72, CMY-73, CMY-75, CMY-76, CMY-77, CMY-78, CMY-79, CMY-80, CMY-81, CMY-84, CMY-85, CMY-86, CMY-87, CMY-89, CMY-90, CMY-96, CMY-97, CMY-99, CMY-102, CMY-103, CMY-104, CMY-105, CMY-107, CMY-108, CMY-110, CMY-111, CMY-112, CMY-113, CMY-114, CMY-115, CMY-116, CMY-117, CMY-118, CMY-119, CMY-121, CMY-122, CMY-124, CMY-125, CMY-126, CMY-127, CMY-128, CMY-129, CMY-130, CMY-131, CMY-132, CMY-133 and CMY-135.

The present teachings allow for the detection of the β -lactamase CTX-M gene family from a biological sample.

The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the CTX-M-14-like gene family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis* and *Shigella* species. The CTX-M-14-like genes detected may include CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-24, CTX-M-27, CTX-M-38, CTX-M-51, CTX-M-64, CTX-M-65, CTX-M-67, CTX-M-82, CTX-M-83, CTX-M-84, CTX-M-85, CTX-M-86, CTX-M-90, CTX-M-93, CTX-M-98, CTX-M-99, CTX-M-102, CTX-M-104, CTX-M-105, CTX-M-110, CTX-M-111, CTX-M-112, CTX-M-113, CTX-M-121, CTX-M-122, CTX-M-123, CTX-M-125, CTX-M-129, CTX-M-130, CTX-M-132, CTX-M-134, CTX-M-147, CTX-M-148 and CTX-M-159.

The present teachings allow for the detection of the β -lactamase CTX-M gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the CTX-M-15-like gene family. The biological sample may include Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Shigella* species and *Proteus mirabilis*. The CTX-M-15-like genes detected may include CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-15, CTX-M-22, CTX-M-28, CTX-M-29, CTX-M-30, CTX-M-32, CTX-M-37, CTX-M-55, CTX-M-64, CTX-M-71, CTX-M-103, CTX-M-117, CTX-M-123, CTX-M-132, CTX-M-136, CTX-M-138, CTX-M-142, CTX-M-144, CTX-M-155, CTX-M-156, CTX-M-157, CTX-M-158, CTX-M-163, CTX-M-164, CTX-M-166 and CTX-M-172.

The present teachings allow for the detection of the β -lactamase DHA gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the DHA-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Morganella morganii*, *Escherichia coli*, *Enterobacter cloacae*, *Proteus mirabilis* and *Citrobacter koseri*. The DHA-like genes detected may include DHA-1, DHA-2, DHA-5, DHA-6, DHA-7, DHA-9, DHA-10, DHA-12, DHA-13, DHA-14, DHA-15, DHA-16, DHA-17, DHA-18, DHA-19, DHA-20, DHA-21 and DHA-22.

The present teachings allow for the detection of the β -lactamase IMP gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the IMP-like family. The biological sample may include Gram-negative bacteria such as *Serratia marcescens*, *Escherichia coli* and *Pseudomonas aeruginosa*. The IMP-like genes detected may include IMP-1, IMP-2, IMP-3, IMP-4, IMP-5, IMP-6, IMP-7, IMP-8, IMP-9, IMP-10, IMP-13, IMP-14, IMP-15, IMP-16, IMP-18, IMP-19, IMP-20, IMP-22, IMP-24, IMP-25, IMP-26, IMP-27, IMP-28, IMP-30, IMP-32, IMP-33, IMP-34, IMP-37, IMP-38, IMP-40, IMP-42, IMP-45, IMP-48, IMP-49, IMP-51 and IMP-52.

The present teachings allow for the detection of the β -lactamase KPC gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex

real-time polymerase chain reaction of β -lactamase genes including the KPC-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* and other *Enterobacter* species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The KPC-like genes detected may include KPC-1, KPC-2, KPC-3, KPC-4, KPC-5, KPC-6 KPC-7, KPC-8, KPC-9, KPC-10, KPC-11, KPC-13, KPC-14, KPC-15, KPC-16, KPC-17, KPC-18, KPC-19, KPC-21, KPC-22, KPC-47, KPC-56, KPC-63, KPC-272, KPC-484, KPC-629, KPC-727, and KPC-860.

The present teachings allow for the detection of the β -lactamase NDM gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the NDM-like family. The biological sample may include Gram-negative bacteria such as *Escherichia coli*, *Acinetobacter baumannii*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. The NDM-like genes detected may include NDM-1, NDM-2, NDM-3, NDM-4, NDM-5, NDM-6, NDM-7, NDM-8, NDM-9, NDM-10, NDM-11, NDM-12, NDM-13, NDM-15, NDM-16 and NDM-32.

The present teachings allow for the detection of the β -lactamase OXA gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the OXA-48-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Shewanella xiamenensis*, *Escherichia coli* and *Serratia marcescens*. The OXA-48-like genes detected may include OXA-48, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245, OXA-247, OXA-370, OXA-405, OXA-416, OXA-438 and OXA-439.

The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including one or more of the following: OXA-143-like, OXA-23-like, OXA-51-like, OXA-48-like, OXA-58-like and OXA24/40-like. The OXA-143-like genes detected may include the following: OXA-143, OXA-182, OXA-231, OXA-253, and OXA-255. The OXA-23-like genes detected may include the following: OXA-23, OXA-27, OXA-49, OXA-73, OXA-102, OXA-103, OXA-105, OXA-133, OXA-134, OXA-146, OXA-165, OXA-166, OXA-167, OXA-168, OXA-169, OXA-170, OXA-171, OXA-225 and OXA-239. The OXA-51-like genes detected may include the following: OXA-51, OXA-64, OXA-65, OXA-66, OXA-67, OXA-68, OXA-69, OXA-70, OXA-71, OXA-75, OXA-76, OXA-77, OXA-78, OXA-79, OXA-80, OXA-82, OXA-83, OXA-84, OXA-86, OXA-87, OXA-88, OXA-89, OXA-90, OXA-91, OXA-92, OXA-93, OXA-94 OXA-95, OXA-98, OXA-99, OXA-100, OXA-104, OXA-106, OXA-107, OXA-108, OXA-109, OXA-110, OXA-111, OXA-112, OXA-113, OXA-115, OXA-116, OXA-117, OXA-120, OXA-121, OXA-122, OXA-123, OXA-124, OXA-125, OXA-126, OXA-127, OXA-128, OXA-130, OXA-131, OXA-132, OXA-138, OXA-144, OXA-148, OXA-149, OXA-150, OXA-172, OXA-173, OXA-174, OXA-175, OXA-176, OXA-177, OXA-178, OXA-179, OXA-180, OXA-194, OXA-195, OXA-196, OXA-197, OXA-200, OXA-201, OXA-202, OXA-203, OXA-206, OXA-208, OXA-216, OXA-217, OXA-219, OXA-223, OXA-241, OXA-242, OXA-248, OXA-249, OXA-250 and OXA-254. The OXA-48-like genes detected may include the following:

OXA-48, OXA-48b, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245 and OXA-247. The OXA-58-like genes may include the following: OXA-58, OXA-96, OXA-97 and OXA-164. The OXA-40-like genes may include the following: OXA-40, OXA-25, OXA-26, OXA-72, OXA-139, OXA-160 and OXA-207.

The present teachings allow for the detection of the β -lactamase VIM gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the VIM-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella oxytoca*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter cloacae*. The VIM-like genes detected may include VIM-1, VIM-2, VIM-3, VIM-4, VIM-5, VIM-6, VIM-8, VIM-9, VIM-10, VIM-11, VIM-12, VIM-13, VIM-14, VIM-15, VIM-16, VIM-17, VIM-18, VIM-19, VIM-20, VIM-23, VIM-24, VIM-25, VIM-26, VIM-27, VIM-28, VIM-31, VIM-33, VIM-34, VIM-35, VIM-36, VIM-37, VIM-38, VIM-39, VIM-40, VIM-41, VIM-42, VIM-43, VIM-44, VIM-45 and VIM-46.

The present teachings allow for the detection of the AmpC β -lactamase MOX gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the MOX-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Aeromonas punctata/Aeromonas caviae* and other *Aeromonas* species and *Escherichia coli*. The MOX-like genes detected may include MOX-1, MOX-2, MOX-3, MOX-4, MOX-5, MOX-6, MOX-7, MOX-8, MOX-10, CMY-1, CMY-8, CMY-9, CMY-10, CMY-11 and CMY-19.

The present teachings allow for the detection of the AmpC β -lactamase ACC gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the ACC-like family. The biological sample may include Gram-negative bacteria such as *Salmonella enterica*, *Escherichia coli*, *Hafnia alvei* and *Proteus mirabilis*. The ACC-like genes detected may include ACC-1, ACC-2, ACC-4, ACC-5 and ACC-6.

The present teachings allow for the detection of the AmpC β -lactamase FOX gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the FOX-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae* and *Aeromonas punctata*. The FOX-like genes detected may include FOX-1, FOX-2, FOX-3, FOX-4, FOX-5, FOX-6, FOX-7, FOX-8, FOX-9, FOX-10 and FOX-12.

The present teachings allow for the detection of the AmpC β -lactamase DHA gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the DHA-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Morganella morganii*, *Escherichia coli* and *Enterobacter cloacae*. The DHA-like genes detected may include DHA-1, DHA-2, DHA-5, DHA-6, DHA-7, DHA-9, DHA-10, DHA-12, DHA-13, DHA-14, DHA-15, DHA-16, DHA-17, DHA-18, DHA-19, DHA-20, DHA-21 and DHA-22.

The present teachings allow for the detection of the AmpC β -lactamase CMY-2 gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the CMY-2-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Morganella morganii*, *Escherichia coli* and *Enterobacter cloacae*. The CMY-2-like genes detected include CMY-2, CMY-4, CMY-6, CMY-7, CMY-12, CMY-14, CMY-15, CMY-16, CMY-18, CMY-21, CMY-22, CMY-23, CMY-24, CMY-25, CMY-26, CMY-27, CMY-28, CMY-29, CMY-30, CMY-31, CMY-32, CMY-33, CMY-34, CMY-35, CMY-37, CMY-38, CMY-39, CMY-40, CMY-41, CMY-42, CMY-43, CMY-44, CMY-45, CMY-46, CMY-47, CMY-48, CMY-49, CMY-50, CMY-51, CMY-53, CMY-54, CMY-55, CMY-56, CMY-57, CMY-58, CMY-59, CMY-60, CMY-61, CMY-62, CMY-63, CMY-64, CMY-65, CMY-66, CMY-67, CMY-68, CMY-69, CMY-71, CMY-72, CMY-73, CMY-75, CMY-76, CMY-77, CMY-78, CMY-79, CMY-80, CMY-81, CMY-84, CMY-85, CMY-86, CMY-87, CMY-89, CMY-90, CMY-96, CMY-97, CMY-99, CMY-102, CMY-103, CMY-104, CMY-105, CMY-107, CMY-108, CMY-110, CMY-111, CMY-112, CMY-113, CMY-114, CMY-115, CMY-116, CMY-117, CMY-118, CMY-119, CMY-121, CMY-122, CMY-124, CMY-125, CMY-126, CMY-127, CMY-128, CMY-129, CMY-130, CMY-131, CMY-132, CMY-133 and CMY-135.

The present teachings allow for the detection of the AmpC β -lactamase EBC gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the EBC-like family such as ACT and MIR. The biological sample may include Gram-negative bacteria such as *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Enterobacter asburiae*, *Enterobacter kobei*, and other *Enterobacter* species. The EBC-like genes detected may include ACT-1, ACT-2, ACT-5, ACT-8, ACT-13, ACT-14, ACT-15, ACT-16, ACT-17, ACT-18, ACT-20, ACT-21, ACT-23, ACT-24, ACT-25, ACT-27, ACT-29, ACT-30, ACT-31, ACT-32, ACT-33, ACT-34, ACT-35, ACT-36, ACT-37, ACT-38, MIR-1, MIR-2, MIR-3, MIR-4, MIR-6, MIR-7, MIR-8, MIR-9, MIR-10, MIR-11, MIR-12, MIR-13, MIR-14, MIR-15, MIR-16, MIR-17 and MIR-18.

The present teachings may allow for the detection of the β -lactamase TEM gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the TEM-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Shewanella xiamensis*, *Escherichia coli* and *Serratia marcescens*. The TEM-like genes detected may include TEM-1, TEM-2, TEM-3, TEM-15, TEM-20, TEM-32, TEM-40, TEM-52, TEM-88, TEM-91, TEM-97, TEM-98, TEM-106, TEM-107, TEM-112, TEM-120, TEM-126, TEM-135, TEM-141, TEM-150, TEM-153, TEM-163, TEM-168, TEM-170, TEM-171, TEM-206, TEM-214, and TEM-220.

The present teachings may allow for the detection of the β -lactamase SHV gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β -lactamase genes including the SHV-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumo-*

niae, *Enterobacter cloacae*, *Shewanella xiamenensis*, *Escherichia coli* and *Serratia marcescens*. The SHV-like genes detected may include SHV-1, SHV-2, SHV-3, SHV-5, SHV-7, SHV-8, SHV-9, SHV-11, SHV-12, SHV-13, SHV-14, SHV-15, SHV-16, SHV-18, SHV-24, SHV-25, SHV-26, SHV-27, SHV-28, SHV-29, SHV-30, SHV-31, SHV-32, SHV-33, SHV-34, SHV-35, SHV-36, SHV-37, SHV-38, SHV-40, SHV-41, SHV-42, SHV-43, SHV-44, SHV-45, SHV-46, SHV-48, SHV-49, SHV-50, SHV-51, SHV-52, SHV-53, SHV-55, SHV-56, SHV-57, SHV-59, SHV-60, SHV-61, SHV-62, SHV-63, SHV-64, SHV-65, SHV-66, SHV-67, SHV-69, SHV-70, SHV-71, SHV-72, SHV-73, SHV-74, SHV-75, SHV-76, SHV-77, SHV-78, SHV-79, SHV-80, SHV-81, SHV-82, SHV-85, SHV-86, SHV-89, SHV-92, SHV-93, SHV-94, SHV-95, SHV-96, SHV-97, SHV-98, SHV-99, SHV-100, SHV-101, SHV-102, SHV-103, SHV-104, SHV-105, SHV-106, SHV-107, SHV-109, SHV-110, SHV-111, SHV-119, SHV-120, SHV-121, SHV-122, SHV-123, SHV-124, SHV-125, SHV-126, SHV-127, SHV-128, SHV-129, SHV-132, SHV-133, SHV-134, SHV-135, SHV-136, SHV-137, SHV-140, SHV-141, SHV-142, SHV-143, SHV-144, SHV-145, SHV-146, SHV-147, SHV-148, SHV-149, SHV-150, SHV-151, SHV-152, SHV-153, SHV-154, SHV-155, SHV-156, SHV-157, SHV-158, SHV-159, SHV-160, SHV-161, SHV-162, SHV-163, SHV-164, SHV-165, SHV-168, SHV-172, SHV-173, SHV-178, SHV-179, SHV-180, SHV-182, SHV-183, SHV-185, SHV-186, SHV-187, SHV-188, SHV-189, SHV-190, SHV-191, SHV-193, SHV-194, SHV-195, SHV-196, and SHV-197.

The present teachings may allow for the detection of the MCR gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of MCR genes including the MCR-like family. The MCR-like genes detected may include MCR-1, MCR-1.2, MCR-1.3, MCR-1.4, MCR-1.5, MCR-1.6, MCR-1.7, MCR-1.8, MCR-1.9 and MCR-2.

The kit of the present teachings may include a mix of at least one primer and/or at least one probe. Primers and/or probes may be degenerate at any nucleotide position. Primers and/or probes may not be degenerate at any nucleotide position. A hydrolysis and/or hybridization probe may be designed for the detection of a specific nucleic acid sequence. Multiple probes may be labeled with a different colored fluorophore. The probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. Two fluorescent quenchers may be included at one end or within the probe sequence. For example, the fluorophores may be selected from the group consisting of fluorescein, hexachlorofluorescein, TEX 615, and TYE™ 665. The fluorophores may excite between 450 nm and 763 nm and emit between 500 nm and 800 nm. For example, the quenchers may be selected from the group consisting of Iowa Black® quenchers and Black Hole Quenchers®. Peak absorbance of each quencher may be at 531 nm, 534 nm, 578 nm, or 656 nm.

Multiple hydrolysis and/or hybridization probes can be added to the same nucleic acid amplification reaction. The selection of the fluorescent labels may depend on the type of hydrolysis and/or hybridization probe used, the number of targets to be detected and the type of thermal cycler used. Preferable combinations of fluorophores and quenchers for multiplex reactions require appropriate excitation wavelengths and little to no overlap in their emission spectra as well as reduction of background fluorescence. It is contemplated that the probe sequences of the present teachings may be labeled with any suitable fluorophore and quencher

combinations. For example, any fluorophore of the present teachings may be attached to any probe DNA sequence of the present teachings.

The one or more primers and/or probes maybe selected from the group consisting of:
 5 TGGCCAGAACCTGACAGGGAAA, TTTCTCCT-GAACCGTGGCTGGC, 5-FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/, CCGT-CACGCTTGTAGTAGG,
 10 GCTGTGTTAATCAATGCCACAC, 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ, CGTTTCGTCTGGATCGCAC, GCTGGGTAAAATAGGTACC, 5TEX615/TATCAT-TGGTGGTGCCTAGTCGC/3IAbRQSp, GAGAGGAT-GAYCAGCCACAC, CGCCCATTGSCAATATTCC, 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp, AATCACAGGGCGTAGTTGTG, ACC-CACCAGCCAATCTTAGG, 5-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTGGG/3IABkFQ/
 20 GCGGAGTTAACATTGGCTAG, GGCCAAGCTTCTAT-ATTTGCG, 5HEX/TTRTTYGGT/ZEN/GGTTGYTTTR-TAA/3IABkFQ, GCGGAGTTARYTATTGGCTAG, GGC-CAAGCYTCAWATTGCG, /5HEX/CCGGACGGT/ZEN/CTTGGTAATTGGGT/3IABkFQ/, /5HEX/CCGTACGGT/ZEN/TTAGGCAATTGGGT/3IABkFQ/, GGCAGCGTTGATGTCCTTCG, CCATTCAAGGCA-GATCGGCATC, 5TEX615/AGCTCTCT-TATCTGGTGCCTGCG/3IAbRQSp, AACTT-CACAGGTGTGCTGGGT,
 30 CCGTACGCATACTGGTTTG, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/, GTATGCCGTCTAGTTCTGC, CCTTGAAT-GAGCTGCACAGTGG, 5HEX/TCGTCGCGG/ZEN/AACCATTGCTAAA/3IABkFQ/, GTTT-GATCGTCAGGGATGGC,
 35 GGCAGAAAGTCAGGCTGTG, 5TEX615/CATCAGGACAAGATGGGCGGTATG/3IAbRQSp, GCTGCTCAAGGAGCACAGGAT, CACATTGACAT-AGGTGTGGTGC, 5-FAM/AGGATGGCA/ZEN/40 AGGCCCACTATTCA/3IABkFQ, AACAGCCTCAGCAGCCGGTTA, TTCGCCGAAT-CATCCCTAGC, 5HEX/AGCCATTAC/ZEN/GTTCAGAGTTGCGT/3IABkFQ, GCCGAGGCT-TACGGGATCAAG, CAAAGCGCGTAACCGGATTGG,
 45 5TEX615/TCTGCTGAAGITTRYCGAGGCMAA/3IAbRQSp, AACITTCACAGGTGTGCTGGGT, CCGTACGCATACTGGTTTG, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/, CTGGGTTCTATAAGTAAACCTTCACCGG, CTTC-50 CACTCGGGCTGCCAGTT, 5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ, CCGAAGCC-TATGGCGTGAATCC, GCAATGCCCTGCTGGAGCG, 5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp.
 Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 55 67-118]

The kit may include one or more primer-probe multiplex mixes. The primer-probe multiplex mix may include one or more internal controls. The primer-probe multiplex mix and one or more internal controls may be enclosed in one container, such as a vial. The primer-probe multiplex mix and one or more internal controls may be enclosed in more than one container, such as vials.

A primer-probe mix may include sequences for detecting any combination of the following genes: CMY-2-like, CTX-M-14-like, CTX-M-15-like, IMP-like, VIM-like, DHA-like, KPC-like, NDM-like, MOX-like, ACC-like, FOX-like,

DHA-like, EBC-like, OXA-143-like, OXA-23-like, OXA-51-like, OXA-48-like, OXA-58-like and OXA-24/40-like.

For example, the kit may include a first primer-probe mix and one or more internal controls in a first vial and a second primer-probe mix and one or more internal controls in a second vial. For example, the kit may include a first primer-probe mix and one or more internal controls in a first vial, a second primer-probe mix and one or more internal controls in a second vial and a third primer-probe mix and one or more internal controls in a third vial. Each vial may contain different mixtures. Each vial may contain the same mixture.

The kit may include at least one control DNA mix. The kit may include one or more DNA control mixes. The kit may include exactly two control DNA mixes. The kit may include exactly three control DNA mixes. The DNA control mix may include at least one DNA sequence corresponding to at least one gene family and at least one internal control DNA sequence. The DNA control mix may be enclosed in one container, such as a vial. The DNA control mix may be enclosed in more than one container, such as vials.

For example, the kit may include a first DNA control mix in a first vial and a second DNA control mix in a second vial. For example, the kit may include a first DNA control mix in a first vial, a second DNA control mix in a second vial and a third DNA control mix in a third vial. Each vial may contain different mixtures. Each vial may contain the same mixture.

In one example, the kit includes three primer-probe multiplex mix vials including internal controls and three DNA control mix vials. The three primer-probe multiplex mixes may provide for identification of up to nine antibiotic resistance genes and internal controls. A first primer-probe mix may include sequences for detecting gene families which are CMY-2-like, CTX-M-14-like, CTX-M-15-like and internal controls. A second primer-probe mix may include sequences for detecting gene families which are OXA-48-like, IMP-like, VIM-like and internal controls. A third primer-probe mix may include sequences for detecting gene families which are DHA-like, KPC-like, NDM-like and internal controls. The one or more DNA control mixes may be plasmid or vector controls. A first DNA control mix may include DNA sequences for CMY-2, CTX-M-14, CTX-M-15 and an internal control DNA sequence. A second DNA control mix may include DNA sequences for OXA-48, IMP, VIM and an internal control DNA sequence. A third DNA control mix may include DNA sequences for DHA, KPC, NDM and an internal control DNA sequence.

It is contemplated that the combination of gene families may vary. For example, a primer-probe mix may include sequences for detecting any combination of the following genes: CMY-2-like, CTX-M-14-like, CTX-M-15-like, and OXA-48-like, IMP-like, VIM-like, DHA-like, KPC-like and NDM-like. It is further contemplated that additional β -lactamase gene targets may be included in the primer-probe mix or mixes.

The first primer-probe mix may include one or more primers and/or probes selected from the group consisting of: TGGCCAGAACTGACAGGCCAA, TTTCTCCT-GAACGTGGCTGGC, 56FAM/ACGCTAACT/ZEN/CCAGCATGGCTGT/3IAbkFQ/, CCGT-CACGCTGTGTTAGG, GCTGTGTTAATCAATGCCACAC, 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IAbkFQ, CGTTTCGCTCTGGATCGCAC, GCTGGGTAAAATAGGTCACC and 5TEX615/TATCAT-TGGTGGCGTAGTCGC/3IAbRQSp. The first primer-probe mix may include one or more internal controls

selected from the group consisting of: GAGAGGATGAY-CAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 152-163]

The kit may include a first, second and third primer and/or probe mix, the first primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: TGGCCAGAACTGACAGGCCAA, TTTCTCCTGAACGTGGCTGGC, 56-FAM/ACGCTAACT/ZEN/CCAGCATGGCTGT/3IAbkFQ/, CCGTCACGCTGTGTTAGG, GCTGTGTTAAT-CAATGCCACAC, 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IAbkFQ, CGTTTCGCTCG-GATGCCAC, GCTGGGTAAAATAGGTCACC, 5TEX615/TATCAT-TGGTGGCGTAGTCGC/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and 5TYE665/TGAGACACGGTCAGACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 152-163]

The second primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AATCACAGGGCGTAGTTGTG, ACCCACAGC-CAATCTTAGG, 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTGGG/3IAbkFQ/, GCGGAGTTAACT-ATTGGCTAG, GGCCAAGCTCTATATTGCG, 5HEX/TTRRTTYGGT/ZEN/GGTTGYTTTRTAA/3IAbkFQ, GCGGAGTTARYTATTGGCTAG, GGCCAAGCYCTA-WATTGCG, /5HEX/CCGGACGGT/ZEN/CTTGGTAATTGGG/3IAbkFQ/, /5HEX/CCGTACGGT/ZEN/TAGGCAATTGGGT/3IAbkFQ, GCGGGCGTTGATGTCCTTCG, CCATTCAAGCCA-GATCGGCATC and 5TEX615/AGCTCTTC-TATCCTGGTGCTGCG/3IAbRQSp. The second primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAY-CAGCCACAC, CGCCCATTGTSCAATATTCC, and 5TYE665/TGAGACACGGTCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 164-179]

The kit may include a first, second, and third primer and/or probe mix, the second primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AATCACAGGGCGTAGTTGTG, ACCCACAGCCAATCTTAGG, 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTGGG/3IAbkFQ/, GCGGAGTTAACTATTGGCTAG, GGCCAAGCTCTATATTGCG, 5HEX/TTRRTTYGGT/ZEN/GGTTGYTTTRTAA/3IAbkFQ, GCGGAGTTARYTATTGGCTAG, GGCCAAGCYCTA-WATTGCG, /5HEX/CCGGACGGT/ZEN/CTTGGTAATTGGG/3IAbkFQ/, /5HEX/CCGTACGGT/ZEN/TAGGCAATTGGGT/3IAbkFQ, GCGGGCGTTGATGTCCTTCG, CCATTCAAGCCA-GATCGGCATC, 5TEX615/AGCTCTTC-TATCCTGGTGCTGCG/3IAbRQSp, GAGAGGATGAY-CAGCCACAC, CGCCCATTGTSCAATATTCC, and 5TYE665/TGAGACACGGTCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 164-179]

3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 164-179]

The third primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AACTTTCACAGGTGTGCTGGGT, CCGTACGCAT- ACTGGCTTGC, 56-FAM/AAACCGGGC/ZEN/GA-TATGCGTCTGTAT/3IAbFQ/, GTATGCCGTCTAGTTCTGC, CCTTGAA-T-GAGCTGCACAGTGG, 5HEX/TCGTCGCG/ZEN/AACCATTGCTAAA/3IAbFQ/, GTTT-GATCGTCAGGGATGGC, GGC GAAAGTCAGGCTGTG and 5TEX615/CATCAGGACAAGATGGCGGTATG/ 3IAbRQSp. The third primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCAITGTS-CAATATTCC and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 180-191]

The kit may include a first, second and third primer and/or probe mix, the third primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IAbFQ/, GTATGCCGTCTAGTTCTGC, CCTTGAA-T-GAGCTGCACAGTGG, 5HEX/TCGTCGCG/ZEN/AACCATTGCTAAA/3IAbFQ/, GTTT-GATCGTCAGGGATGGC, GGC GAAAGTCAGGCTGTG, 5TEX615/CATCAGGACAAGATGGCGGTATG/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCAITGTS-CAATATTCC, and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 180-191]

A first DNA control mix may include one or more sequences selected from the group consisting of: TGGCCAGAACTGACAGGCAAACAGTGGCAGGGTA TCCGCCTGTCGCACTTAGCCA CCTATACGGCAGGCCCTACCGCTGCAGATCCCC- GATGACGTAGGGATAAACGC CGCATTACTGCAT- TTTTAT- CAAAATGGCAGCCCAATGGACTCCGGCGCTA AGC GACTTTACGCTAACTCCAGCAT- TGGTCTGTITGGCGCGCTGGCGGTGAAACCTC AGGAATGAGTTACGAAGAGGCAATGACCA- GACGCGTCTGCAACCATTAAACTG GCGCAT- ACCTGGATTACGGITCCGCAGAACAGAACAAAAA- GATTATGCCCTGGGCT ATCGCGAAGGGAAGGCCGTA- CACGTTTCTCCGGGACAACCTGACGCCGAAGCCTA TGGCGTGAATCCAGCGTTATGTA- TATGGCCCGCTGGGTTCAGGCCAACATGGAT GCCAGCCACGTTCAGGAGAAA, CCGT- CACGCTGTGTTAG- GAAGTGTGCCGCTGTATGCGAAACGGCGGACGTA- CA GCAAAAAACTTGCCTGAATTAGAGCGGCAGTCGG- GAGGCAGACTGGGTGTGGCATT GATTAACACAGC, and CGTTCTGCTGGATCGCACTGAACCTACGCT- GAATACCGCCATTCCCGCGACCC GAGAGACAC- CACCACGCCGCGGGCGATGGCGCA- GACGTTGCGTCAGCTACGCT

GGGT- CATGCGCTGGCGAAACCCAGCGGGCGCAGTTGG TGACGTGGCTCAAAGG CAATACGACCGGGCGCAGCCAGCAT- TCGGGCCGGCTTACCGACGTCGTGGACTGT GGGT- GATAAGACCGGCAGCGGCAGTACGGCACCAC- CAATGATATTGCGGTGATC TGGCCGAGGGCTGCGCCGCTGGTCTGGTGA CCTATTTACCCAGC. The first DNA control mix may include the following internal control sequence: GAGAG- GATGACCGAGCCACACTGGAACTGA- GACACGGTCCAGACTCTACGGGAG GCAGCAGTGGGAATATTGCAAAATGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix, the third control mix and the internal control sequence. [SEQ. ID NOS 261-264]

A second DNA control mix may include one or more sequences selected from the group consisting of: AAT- CACAGGGCGTAGTTGTGCTCTGGAAAT- GAGAATAAGCAGCAAGGATTACCAAT AATCT- TAAACGGGCGAACCAAGCATTTCACCGCATTCA CCTTAAATTCCTAAAT AGCCTGATCGCCCTCGAT- TTGGCGTGGTTAAGGATGAACACCAAGTCTT- TAAGTG GGATGGACAGACGCGCGATATCGC- CACTTGAATCGCATTAACTAATCACC GCGATGAAATATTCACTAGTTGTGCCTGTTTAT- CAAGAATTGCCCACAAATTGGCGAGGCACGTAT- GAGCAAGATGCTACATGCTTCGATTATGGTAAT- GAGGACATTTCGG GCAATGTAGACAGTTCTGGCTCGACGGTGGTAT- TCGAATTTGCCGACGGAGCA AATCAGCTTT- TAAGAAAAGCTGTATCACATAAGTTACACGTATCG- GAGCGCAGCC 35 AGCGTATTGTCAAACAAGCCATGCTGACCGAAGC- CAATGGTACTATATTATTTCGG GCTAAAACGGTACTCGAATCGAACCTAAGAT- TGGCTGGTGGGT GCGGAGTTAGTTATGGCTAGTTAAAAATAAAATT- 40 GAAGTTTTTATCCGGCCCG GGCACACTCAA- GATAACGTAGTGGTTGGTACCTGAAAAGAAAAT- TTTATTCGGT GGTGTTTGTAAACCGGACGGTCTGGTAAT- TTGGGTGACGCAAATTAGAAGC TTGGCC and 45 GCGCGCCTT- GATGCTTCGGCGCGCTGGGTGGCAACGTACG- CATCACCGTCG ACACGCCGGCTAGCCGAGGTAGAGGG- GAACGAGATTCCCACGCACTCTAGAA 50 GGACTCT- CATCGAGCGGGGACGCAGTGCCTCGGTCCAGTA GAACTCTCTATC CTGGTGTGCGCAT- TCGACCGACAACT- TAGTTGTGTACGTCGGCTGCGAGTGT GCTC- 55 TATGGTGGTTGCGATTCACTGAGTTGTCACGCAC GTCTGCGGGGAACGTG GCGGATGCGCATCTGCT- GAATGG. The second DNA control mix may include the following internal control sequence: GAGAG- GATGACCGACCAACTGGAACTGA- GACACGGTCCAGACTCTACGGGAG GCAGCAGTGGGAATATTGCAAAATGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix, the third control mix and the internal control sequence. [SEQ. ID NOS 265-268]

A third DNA control mix may include one or more sequences selected from the group consisting of: AACTT-CACAGGTGTGCTGGGTGCGGTTCTGTGGCGAAAAA AAGAGATGGCGCTG AAT-GATCCGGCGCAAAATACCAGCCG-GAGCTGGCTCTGCCGCAGTGGAAAGGGG ATCACAT-TGCTGGATCTGGCTACCTATAACCGCAGGCGGACTG CCGTTACAGGTGC CGGATGCGTAAAAAGCCGTGCGGATCTGCTGAAT-TTCTATCAGCAGTGGCAGCC GTCCCG-GAAACCGGGCGA-TATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTT GGTGCTCTGACCGCAAACGCGCGGG-GATGCCGTATGAGCAGTTGCTGACTGCA CGGATCCTGGCACCGCTGGGGTTATCTCACACCTT-TATTACTGTGCCGGAAAGTG CGCAAAGCCAGTATGCGTACGG, GTATGCCGTCTAGTCTGCTGTCTGTCT-CATGGCCGCTGGCTGGCTTTCTG CCACCGCGCTGACCAACCTCGTCGCGGAACCAT-TCGCTAAACTCGAACAGGACTT TGGCGGCTC-CATCGGTGTGACCGATGGA-TACCGGCTCAGGCACACTGTAAGT TACCGCGCTGAGGAGCGCTCCCCACTGTGCAGCT-CATTGCGAAG and GTTGATCGTCAGG-GATGGCGGCCGCTGCTGGTGGTCGA-TACCGCCTGGACCG ATGACCAGACCGCCCAGATCCTCAACTGGAT-CAAGCAGGAGATCAAACCTGCCGT CGCGCTGGCGGTGGTGAECTACCGCG-CATCAGGACAAGATGGCGGTATGGACGC GCTG-CATCGGGGGGATTGCGACTTATGC-CAATGCGTTGCGAACCAGCTTGC CCGCAAGAGGG-GATGGTTGCGCGCAACACAGCCTGACTTCGCC. The third DNA control mix may include the following internal control sequence: GAGAGGATGACCAGC-CACACTGGAACTGAGACACGGTCCAGACTCC-TACGGGAG GCAGCAGTGGGAATAT-TGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix, the third control mix and the internal control sequence. [SEQ. ID NOS 269-272]

In one example, the kit includes two primer-probe multiplex mix vials including internal controls and two DNA control mix vials. The two primer-probe multiplex mixes may provide for identification of up to six antibiotic resistance genes and internal controls. A first primer-probe mix may include sequences for detecting gene families which are MOX-like, ACC-like, FOX-like and internal controls. A second primer-probe mix may include sequences for detecting gene families which are DHA-like, ACT/MIR-like, CMY-2-like and internal controls. A first DNA control mix may include DNA sequences for MOX, ACC, FOX and an internal control DNA sequence. A second DNA control mix may include DNA sequences for DHA, ACT/MIR, CMY-2 and an internal control DNA sequence.

It is contemplated that the combination of gene families may vary. For example, a primer-probe mix may include sequences for detecting any combination of the following genes: MOX-like, ACC-like, FOX-like, DHA-like, ACT/MIR-like and CMY-2-like. It is further contemplated that additional β -lactamase gene targets may be included in the primer-probe mix or mixes.

The first primer-probe mix may include one or more primers and/or probes selected from the group consisting of:

GCTGCTCAAGGAGCACAGGAT, CACATTGACAT-AGGTGTGGTGC, 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTCA/3IAbkFQ, AACAGCCTCAGCAGCCGGTTA, TTGCGCGCAAT-CATCCCTAGC, 5HEX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IAbkFQ, GCCGAGGCT-TACGGGATCAAG, CAAAGCGCGTAACCGGATTGG and 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp. The first primer-probe mix may include one or 10 more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCAATTGTS-CAATATTCC and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of 15 primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 192-203]

The second primer-probe mix may include one or more primers and/or probes selected from the group consisting of: 20 AACTTACAGGTGTGCTGGGT, CCGTACGCAT-ACTGGCTTGC, 56-FAM/AAACCGGGC/ZEN/GAT-TATGCGTCTGTAT/3IAbkFQ, CTGGGTTCT-TATAAGTAAAACCTTCACCGG, CTTCCACTGCGGCTGCCAGTT, 5HEX/GATGCCATT/25 ZEN/GCYCGSGGTGAAAT/3IAbkFQ, CGGAAGCC-TATGGCGTAAATCC, GCAATGCCCTGCTGGAGCG, and 5TEX615/ATGTTGCCCTGAACCCAGCG/3IAbRQSp. The second primer-probe mix may include one or more internal controls selected from the group consisting of: 30 GAGAGGATGAYCAGCCACAC, CGCCCAATTGTS-CAATATTCC and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 204-215]

The kit may include exactly two primer and/or probe mixes, a first primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: 40 GCTGCTCAAGGAGCACAGGAT, CACATTGACAT-AGGTGTGGTGC, 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTCA/3IAbkFQ, AACAGCCTCAGCAGCCGGTTA, TTGCGCGCAAT-CATCCCTAGC, 5HEX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IAbkFQ, GCCGAGGCT-TACGGGATCAAG, CAAAGCGCGTAACCGGATTGG, 45 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCC-CATTGTS-CAATATTCC, and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp; and a second primer and/or probe mix including one or more 50 primers and/or probes selected from the group consisting of: AACTTACAGGTGTGCTGGGT, CCGTACGCAT-ACTGGCTTGC, 56-FAM/AAACCGGGC/ZEN/GAT-TATGCGTCTGTAT/3IAbkFQ, CTGGGTTCT-TATAAGTAAAACCTTCACCGG, CTTCCACTGCGGCTGCCAGTT, 5HEX/GATGCCATT/55 ZEN/GCYCGSGGTGAAAT/3IAbkFQ, CGGAAGCC-TATGGCGTAAATCC, GCAATGCCCTGCTGGAGCG, and 5TEX615/ATGTTGCCCTGAACCCAGCG/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCAATTGTS-CAATATTCC, and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any 60 nucleotide position. [SEQ. ID NOS 192-215]

The first DNA control mix may include one or more sequences selected from the group consisting of: GCTGCT

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CAAGGAGCACAGGAATCCCAGG-
 CATGGCGGTGGCCGTGCTCAAGGATGG CAAGGCC-
 CACTATTCAATTACGGGGTGGCCAACCGGGAGAG
 CGGGGCCAGCGT CAGCGAGCA-
 GACCTGTTCGAGATAGGATCCGTGAGCAA-
 GACCTGTACTGCGACC CTGGGGGC-
 TATGCGGTGGTCAAGGGAGCGATGCAGCTGGATG
 ACAAGGCGAGC CGGCACGCCCTGGCTCAAGG-
 GATCCGTCTTGACAGCATCACCATGGGGAG
 CTTGCCACCTACAGCGCCGGAGGCCCTGC-
 CACTGCAATTCCCCGAGGAGGGTGGATT
 CATCCGAGAAGATGCGCCTAC-
 TACCGCCAGTGGGCCCTGTCTATTGCCGGG
 CTCCCATGCCAGTACTCCAACCCCAGCAT-
 AGGGCTTCTGGCCACCTGGCGCG AGCAGCCT-
 GAAGCAGCCAATTGCCAGTTGATGGAGCA-
 GACCTGTGCCGGG
 CTCGGCATGCACCACACCTATGCAATGTG,
 AACAGCCTCAGCAGCCGGTACGGAAAATACGTT-
 ATTTGAAGTGGGTTGCTGAGT
 AAAACGTTGCTGCCACCTTGGCGTCC-
 TATGCGCAGGTGAGCGGTAAGCTGTCTTT GGAT-
 CAAAGCGTTAGCCAT-
 TACGTTCCAGAGTTGCGTGGCAGCAGCTTGACCA
 C GTTAGCGTACTCAATGTGGCACGCAT-
 ACCTCAGGCCCTACAGCTATTATGCCGG A AGATAT-
 TAAAAATACCAACACAGCTGATGGCT-
 TATCTAAAAGCATGGAAACCTGCCG
 ATGCGGCTGGAACCCATCGCGTTATTCA-
 CAATATCGTACTGGTTGCTAGGGATG
 ATTGCGGCGAA and GCCGAGGCTTACGGGATCAA-
 GACCGGCTCGCGGATCTGCTGAAGTTACCGAG
 GCCAACATGGGTATCAGG-
 GAGATGCCCGCTAAAACGCCGATCGCGCTGACC
 CATACCGGTTCTACTCGGTGGAGA-
 CATGACTCAGGGCTGGGTTGGGAGAGCT ACGCC-
 TATCCGTTGACCGAGCAGGCCGCTGCTGGGGCAA
 CTCCCCGGCGGTGA GCTTCCAGGCCATCCGGT-
 TAGCGCTTTG. The first DNA control mix may include
 the following internal control sequence: GAGAG-
 GATGACCGGCCACACTGGAAGTGA-
 GACACGGTCCAGACTCCTACGGGAG
 GCAGCAGTGGGAATATTGCACATGGCG. A DNA
 control mix may include a combination of the one or more
 said group of sequences and the said internal control
 sequence. A DNA control mix may include any combination
 of sequences from the first control mix, the second control
 mix and the internal control sequence. [SEQ. ID NOS
 273-276]

A second DNA control mix may include one or more
 sequences selected from the group consisting of: AACTT-
 CACAGGTGTGCTGGGTGGCTTCTGTGGCGAAA
 AAAGAGATGGCGCTG AAT-
 GATCCGGCGCAAAATACCAGCCG-
 GAGCTGGCTCTGCCGAGTGGAAAGGGG ATCACAT-
 TGCTGGATCTGGCTACCTATACCGCAGGCCGACTG
 CCGTTACAGGTGC
 CGGATGCGTAAAAAGCCGTGCGGATCTGCTGAAT-
 TTCTATCAGCAGTGGCAGCC GTCCCG-
 GAAACCGGGCGA-
 TATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTT
 T GGTGCTCTGACCGCAAACGCCGGGG-
 GATGCCGTATGAGCAGTTGCTGACTGCA
 CGGATCCTGGCACCGCTGGGGTATCTCACACCTT-
 TATTACTGTGCCGGAAAGTG
 CGCAAAGCCAGTATGCGTACGG, TCGGTAAAGCC-
 GATGTTGCCGGCAACAAACCCGT-

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CACCCCGCAAACCCCTGTTGA GCTGGGCTC-
 TATAAGTAAAACCTTCACCGCGTACTGGGCGCG
 ATGCCATTGCC CGGGGTGAAATAGCGCTGGGC-
 GATCCGGTAGCAAATACTGGCCTGAGCTCACG
 5 GGCAGCAGTGGCAGGGCATTGCGATGCTG-
 GATCTGGCAACCTATACCGCAGGC GGTCTGCCGT-
 TACAGGTGCCGATGAGGTACCGGA-
 TACCGCCTCTGCTGCGCT
 TTTATCAAAACTGGCAGCCGAGTGGAAAG and
 10 CGGAAGCCTATGGCGTGAATCCAGCGTTATTGA-
 TATGCCCGCTGGGTTCAGGC CAACATG-
 GATGCCAGCCACGTTCAAG
 GAGAAAACGCTCCAGCAGGGCATTGC. The second
 15 DNA control mix may include the following internal control
 sequence: GAGAGGATGACCAGCCACACTGGAACT-
 GAGACACGGTCCAGACTCCTACGGGAG
 GCAGCAGTGGGAATATTGCACAATGGCG. A DNA
 control mix may include a combination of the one or more
 20 said group of sequences and the said internal control
 sequence. A DNA control mix may include any combination
 of sequences from the first control mix, the second control
 mix and the internal control sequence. [SEQ. ID NOS
 277-280]

In one example, the kit includes two primer-probe multiplex mix vials including internal controls and two DNA control mix vials. The two primer-probe multiplex mixes may provide for identification of up to six antibiotic resistance genes and internal controls. A first primer-probe mix may include sequences for detecting gene families which are OXA-143-like, OXA-23-like, OXA-51-like and internal controls. A second primer-probe mix may include sequences for detecting gene families which are OXA-48-like, OXA-58-like, OXA-24/40-like and internal controls. A first DNA control mix may include DNA sequences for OXA-143, OXA-23, OXA-51 and an internal control DNA sequence. A 35 second DNA control mix may include DNA sequences for OXA-48, OXA-58 and OXA 24/40 and an internal control DNA sequence.

It is contemplated that the combination of gene families may vary. For example, a primer-probe mix may include sequences for detecting any combination of the following genes: OXA-143-like, OXA-23-like, OXA-51-like, OXA-48-like, OXA-58-like and OXA-24/40-like. It is further contemplated that additional β -lactamase gene targets may be included in the primer-probe mix or mixes.

The first primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AGCACATACAGAATATGCTCTGC, ACCTGTTAAC-
 CAACCTACTTGAGGG, /56-FAM/TTGCAA-
 GACGGACTGGCTTAGACC/3HQ_1/, CCTGATCG-
 GATTGGAGAAC, CTACCTCTGAATAGGCGTAACC, /5TEX615/
 ACGTCGCGCAAGTTCTGATAGAC/3IAbRQSp/,
 TAGTGAATGCTAATCCAAATCACAG, GCACGAGCAAGATCATTACCATAGC, /5HEX/AGT-
 55 TATCCAACAAGGCCAAACTCAACA/3HQ_1/. [SEQ. ID NOS 119-127] The first primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC (SEQ ID NO: 201), CGCCCATTGTSCAATATTCC (SEQ ID NO: 202) and 5TYE665/TGAGACACGGTCCAGACTCC-TACG/3IAbRQSp (SEQ ID NO: 203). A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix.

The second primer-probe mix may include one or more primers and/or probes selected from the group consisting of:

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AATCACAGGGCGTAGTTGTG, ACCCACCCAGC-
CAATCTTAGG, /5HEX/TAGCTTGATGCCCTCGAT-
TTGGG/3BHQ_1/, GTGGGATGGAAGGCCACG,
CACTTGCAGGTCTACAGC, /56-FAM/
TTACTTGGGGGAAGCCATGCAAG/3BHQ_1/, CACC-
TATGGTAATGCTCTTC, CTG-
GAACTGCTGACAATGCC, /5TEX615/
TGGGAGAAAGATATGACTTAGGTGAGGCA/
3IAbRQSp/. [SEQ. ID NOS 128-136] The second primer-
probe mix may include one or more internal controls
selected from the group consisting of: GAGAGGATGAY-
CAGCCACAC (SEQ ID NO: 201), CGCCCATTGTS-
CAATATTCC (SEQ ID NO: 202) and 5TYE665/TGA-
GACACGGTCCAGACTCCTACG/3IAbRQSp (SEQ ID
NO: 203). A primer-probe mix may include a combination of
the one or more said group of primers and/or probes and the
one or more said group of internal controls. The primer-
probe mix including internal controls may be a multiplex
mix.

The kit may include exactly two primer and/or probe
mixes, a first primer and/or probe mix including one or more
primers and/or probes selected from the group consisting of:
AGCACATACAGAATATGTCCTGC, ACCTGTTAAC-
CAACCTACTTGAGGG, /56-FAM/TTGCAA-
GACGGACTGGCTTAGACC/3BHQ_1/, CCTGATCG-
GATTGGAGAAC, CTACCTTGAATAGGCGTAACC, /5TEX615/
ACGTCGCGCAAGTTCTGATAGAC/3IAbRQSp/,
TAGTGACTGCTAATCCAATCACAG, GCACGAGCAAGATCATTACCATAGC, /5HEX/AGT-
TATCCAACAAAGGCCAAACTCAACA/3BHQ_1/,
GAGAGGATGAYCAGCCACAC, CGCCCATTGTS-
CAATATTCC and 5TYE665/TGAGACACGGTCCA-
GACTCCTACG/3IAbRQSp; and a second primer and/or
probe mix including one or more primers and/or probes
selected from the group consisting of: AAT-
CACAGGGCGTAGTTGTG, ACCCACCCAGCAATCT-
TAGG, /5HEX/TAGCTTGATGCCCTCGATTGGG/
3BHQ_1/, GTGGGATGGAAGGCCACG,
CACTTGCAGGTCTACAGC, /56-FAM/
TTACTTGGGGGAAGCCATGCAAG/3BHQ_1/, CACC-
TATGGTAATGCTCTTC, CTG-
GAACTGCTGACAATGCC, /5TEX615/
TGGGAGAAAGATATGACTTAGGTGAGGCA/
3IAbRQSp/, GAGAGGATGAYCAGCCACAC,
CGCCCATTGTSAAATATTCC and 5TYE665/TGA-
GACACGGTCCAGACTCCTACG/3IAbRQSp. Primers
and/or probes included in this group may or may not be
degenerate at any nucleotide position. [SEQ. ID NOS 216-
239]

A first DNA control mix may include one or more
sequences selected from the group consisting of: AGCA-
CATACAGAATATGTCCTGCATCAACATTAA-
GATGCTAAATGCCATTAAITGG ACTAGAAAATCAT-
AAAGCTACACAACTGAGATTITCAAATGGGACGG
AAAAAGA GATCTTATCCCATTGTTGGGAAAAAGA-
TATGACTTATGGTGTGATGCCATTGCACTTCA
GCAGTTCTGTATATAAGAAACTTGCAA-
GACGGACTGGCTTAGACCTAATGCAAA AGAAGT-
TAAACGGGGTTGGTTTGGTAATGAACATTG-
GAACACAAGTTGATAACTT
CTGGTTGGTTGGCCCCCTCAAGATTACACCAATA-
CAAGAGGTTAATTTGCCATG
ATTGCAAAATATGCAATTACCTTTAAATTAGA-
GACTCAAGAAGAAGTTAAAAAAAT GCTTCTGAT-
TAAAGAATTCAATGGTAGTAAAATT-
TATGCAAAAGCGGCTGGGAA

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TGGATGTAACCCCTCAAGTAGGTTGGTTAACAGGT,
CCTGATCGGATTGGAGAACAGAAAACGGATAT-
TAATGAAATATTAAATGGAAGG
GCGAGAAAAGGTCAATTACCGCTTGGGAAAAAGA-
5 CATGACACTAGGAGAACGCAT
GAAGCTTCTGCAGTCCCAGTCTATCAG-
GAACCTGCGCAGCTATCGGTCTTGTATC
TCATGCAAAAGAAGTAAACGTAT-
TGGTTCGGTAATGCTGAAATTGGACAGCAG GTT-
10 GATAATTCTGGTTGGTAGGACCAATTAAAGGT-
TACGCCATTCAAGAGGTAG and
TAGTGACTGCTAATCCAATCACAGCGCTT-
CAAAATCTGATGAAAAGCAGAGAAA
15 ATTAAAAATTATTAACGAAGTACACAC-
TACGGGTGTTTAGTTATCCAACAAAGGC CAAACT-
CAACAAAGCTATGGTAATGATCTTGTGCTGTGC. The first DNA control mix may include the following internal
control sequence: GAGAGGATGACCAGCCACACTG-
GAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGAATATTGCAACATGGCG. A DNA
control mix may include a combination of the one or more
said group of sequences and the said internal control
sequence. A DNA control mix may include any combination
of sequences from the first control mix, the second control
mix and the internal control sequence. [SEQ. ID NOS
281-284]

A second DNA control mix may include one or more
sequences selected from the group consisting of: AAT-
CACAGGGCGTAGTTGTGCTCTGGAAAT-
GAGAATAAGCAGCAAGGATTACCAAT AATCT-
TAAACGGGCGAACCAAGCATTTCACCGCATCTA-
CCTTAAATTCCTAAAT AGCTTGATGCCCTCGAT-
TTGGCGTGGTTAAGGATGAACACCAAGTCTT-
TAAGTG GGATGGACAGACGCGCGATATCGC-
CACTTGAATCGCGATCATAATCTAACACC
GCGATGAAATATTCTGTTGCGCTGTTAT-
CAAGAATTGCCCCGCAAATTGGCGA GGCACGTAT-
40 GAGCAAGATGCTACATGCTTCGATTATGGTAAT-
GAGGACATTCTCG
GCAATGTAGACAGTTCTGGCTGACGGTGGTAT-
TCGAATTTCGGGCCACGGAGCA AATCAGCTTT-
TAAGAAAAGCTGTATCACAATAAGTTACACGTATCG-
GAGCGCAGCC
45 AGCGTATTGTCAAACAAAGCCATGCTGACCGAACG-
CAATGGTACTATATTATTCCGG GCTAAAAGCTGGA-
TACTCGACTAGAACCTAACAGAT-
TGGCTGGTGGGT,
50 GTGGGATGGAAGCCACGTTTTT-
TAAAGCATGGGACAAAGATTTACTTTGGCG
AAGCCATGCAAGCATCTACAGTGCCTGTATAT-
CAAGAATTGGCACGTCGTATGGT CCAAGCT-
TAATGCAAAGTGAATTGCAACGTATTGGT-
55 TATGCAATATGCAAAATAGG
CACCGAAGTTGATCAATTGGTT-
GAAAGGGCCTTGACAATTACACCTATACAAG
AAGTAAAGTTGTGATGTTAGCC-
CAAGGGCAATTGCCCTTAAACCTGAAGTTC
60 AGCAACAAAGT-
GAAAGAGATGTTGATGAGCGCAGAGGG-
GAGAATCGTCTATA TGCTAAAAGTGGCTGGG-
GAATGGCTGTAGACCCGCAAGTG,
CACTTGCAGGTCTACAGCCATTCCCCAGCCACTTT-
65 TAGCATATAGACGATTCTCCC CTCTGCGCTCTA-
CATACAACATCTTTCACTTGTGCT-
GAACTTCAGGTTAAAAG

39

GCAATTGCCCTGGGCTAAATCATACACAAACTT-
TACTTCTGTATAGGTGAATTG TCAAAGGCCCTT-
CAACCAAAATTGATCAACTTCCGTGCCTATTGCAT-
ATTGCCAT
AACCAATACGTTGCAATTCACTTTGCAT-
TAAGCTTGGACCAATACGACGTGCCAATT CTTGA-
TATACAGGCACTGTAGATGCTTGCATGGCTTCGCC-
CAAAGTAAAATCTTGT
CCCATGCTTAAAAAAACGTGGCTTCCATCCCAC,
and CACC-
TATGGTAATGCTCTGCACGAGCAAATAAAGAATAT
GTCCCTGCATCAACATT TAAGATGCTAAATGCTT-
TAATCGGGCTAGAAAATCATAAAGCAACAACAAAT-
GAGAT TTTCAAATGGGATGGTAAAAAAAAGAACT-
TATCCTATGTGGGAGAAAGATATGACTTT
AGGTGAGGCAATGGCATTGTCAGCAGTCCAG. The
second DNA control mix may include the following internal
control sequence: GAGAGGATGACCAGCACACTG-
GAACTGAGACACGGTCCAGACTCCTACGGAG
GCAGCAGTGGGAATATTGCAACATGGCG. A DNA
control mix may include a combination of the one or more
said group of sequences and the said internal control
sequence additional β -lactamase. A DNA control mix may
include any combination of sequences from the first control
mix, the second control mix and the internal control
sequence. [SEQ. ID NOS 285-289]

In one example, the kit includes one primer-probe multiplex mix vials including internal control and one DNA control mix vial. A primer-probe mix may include sequences for detecting MCR gene families and internal control.

The primer-probe mix may include primers and/or probes selected from the group consisting of: CGGTGTATGTTCAGCTAT, CTTATCCATCACGCCTT, /5TEX615/TATGATGTCGATACCGCCAAATACCA/ 3IAbRQSp/, CTGTATGTCAGCGATCAT, GATGCCAGTTGCTTATCC, /56-FAM/AAGTCTGGG/ZEN/TGAGAACGGTGTCTAT/3IABkFQ/, CAGTCAGTATGCGAGTTTC, AAAATTGCCAAGC-CATC, and /5HEX/TGCATAAGC/ZEN/CAGTGCCTTT-TATAT/3IABkFQ/. The primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCCATTGTS-CAATATTTC and 5TYE665/TGAGACACGGTCCA-GACTCCTACG/3IAbRQSp. The primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 137-145]

A DNA control mix may include one or more sequences selected from the group consisting of

ATGATGCAGCATACTTCTGTGGTACCGACGCTCGGTAGCTCGTTG
TCTTGTGGAGTGTGCCGTTCTTGACCGCGACGCCAATCTTACCTT
TTTGATAAAATCAGCCAAACCTATCCCATCGCGACAATCTGGCTTG
TGCTGACGATCGCTGTCGTCTTGGCGCATGCTACTGATCACACAC
CTGTTATCATCGTATCGTATGCTAAAGCCTGTGTTGATTTGCTATT
AATCATGGGCCGGTGACCAGTTATTTACTGACACTATGGCACGGTCT
ATGATACGACCATGCTAAAAATGCCCTACAGACCGACCAAGCCGAGACC
AAGGATCTATTAACGCAGCGTTTATCATGCGTATCATTGGTTGGGTGT
GCTACCAAGTTGCTGTGGCTTTGTTAAAGTGGATTATCCGACTGGG

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-continued

GCAAGGGTTTGTGCGCCGATTGGCTTGTCAGCAGTCATTATGCCAGTTCTGCCTG
5 CGTGATAAGCCGCTGCCAGTATGTCATGCCATGCCAATCTACT
CGGTGGGTAAGCTGCCAGTATTGAGTATAAAAAGCCAGTGCGCCAAA
10 GATACCATTTATCACGCCAAAGACGCCAGTACAAGCAACCAAGCCTGATAT
GCGTAAGGCCACGCCAGTGGTGTGTCGTCGCGTGAGACGCCAGCAGC
15 ATCATGTCAGCTCAATGGCTATGAGCCGATACTTCCCACAGCCTGCC
AAGATCGATGGCGTGACCAATTAGCAATGTCACATGTCGGGCCACATC
19 GACGGCGTATTCTGTGCCGTGTGTCAGCTATCTGGCGCGATGAGT
ATGATGTCGATACGCCAAATACCAAGAAAATGTCGCGGATACGCTGGG
CGCTGGCGTAAGTATCTGTGCCGTGATAATAATTGGACTCAAAGG
20 CGTGATGGATAAGCTGCCAAAGCGCAATTGCGATTATAATCCCGCA
CCAACAAAGCCATCTGCAACACCAATCCTTATAACGAATGCCCGATGTC
GGTATGTCGTTGGCTTAGATGACTTGTGCGCTGCCAATACGCCAAAG
25 TATGCTGATCATGTCGACCAAATGGCAATCACGGCGTGTGATTGTC
AGCGATATGTAAGGTTGCCAAATTACGCCAGTGTGAGGTTAAT
GAGCTTGCAAGTGCACATCAGCCTGATCAATGCTTATGACAATGC
30 CTTGCTTGCCACCAGTGTGCTCAAAGTATCCAGTGGCTGCAGA
CGCACAGCAATGCCATGTCGCTAACGCTGTATGTCAGCGATCATGG
GAAAGTCTGGGTGAGAACGGTGTCTATCAGTGTGCTAACGCGGAA
35 TGACCAAAAGAACAGCGCAGTGTGCCCTGCATTTCTGGACGGATAAGC
AAACTGGCATCACGCCATTGGCAACCGATACCGCTCTGACCCATGACGCC
ATCACGCCACATTAAAGCTGTTGATGTCACCGGGACAAAGTCAA
40 AGACCGCACCGCATTCCGCTGA
and
ATGACATCACATCACTTGGTATCGCTATTCTATCAATCCTTTGTGCT
GATGGGTTTGGTGGCTTATTTGGCAGCGACAGCGAACCTGACATT
45 TTGAAAAAGCGATGGCGGTCTATCTGTATCGATAACTAGGCTTATC
ATCTCAATGGCGGTGGCGGTGATGGGTGCTATGCTACTGATTGTCGTC
GTTATCCTATCGCTATGCTAAAGCTGCCCTGATTTGCTACTGATTA
50 TGGGTGCGGTGACGAGCTATTTACCGATACTATGGCACGGCTATGAC
ACCACCATGCTCCAAATGCCATGCAACCGACCAAGCCGAGTCTAAGGA
CTTGATGAATTGGCTTTGTCGAAATTATGGCTTGGCGTGTGTTGC
55 CAAGTGTGTTGGTCGAGTGCAGTTGCCAAAGTCATTATCCAACATGGGG
GGTCTGATTAGCGCTGCGATGACATGGGTGTCAGCCTGTCGTTGCT
TGTGCCGATTGGACTATTTAGCAGTCAGTGCAGTTCTTCGGGTGC
60 ATAAGCCAGTGCCTTATATCAACCCGATTACGCCATTATTCGGTG
GGTAAGCTTGCCAGTATCGAGTACAAAAAGCCACTGCCAACAGACAC
CATCTATCATGCCAAAGACGCCAGTGCAGACCCACCAAGCCGAGCG
AGCCACGCCAGTGGTGTGTCGTCGGTGAGACGCCAGCAGC
65 GTGCAGTCAATGGCTATGGCGTGAGACTTCCCGCAGCTTGCCAAAGT

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TGATGGCTTGGCGAATTAGCCAAGTGACATCGTGTGGCACATCGACGG
 CGTATTCTGTGGCGTGTATGTTAGCTATTGGGTAAGTGACTATGAT
 GTCGATAACGCCAAATACCAAGAAAATGTGCTAGATACGCTTGACCGCTT
 GGGTGTGGGTATCTGTGGCGTGTATAAATTCAAGACTCAAAGCGTGA
 TGGATAAGCTACCTGCCACCGAGTATTTGATTATAAACAGCAACCAAC
 AATACCATCTGTAAACACCAATCCCTATAACGAATGCCGTATGCGGTAT
 GCTTGTGGGCTAGATGACTATGTCAGCGCAATAATGGCAAAGATATGC
 TCATCATGCTACACCAATGGCAATCATGGGCCGCTACTTAAAGCGT
 TATGATGAGCAATTGCAAATTCAACCCCGTGTGCGAAGGCAACGAGCT
 TGCCAAATGCGAACACCAATCACTCATCAATGCCATTGACAATGCCGTAC
 TTGCGACTGATGATTTATGCCAAAGCATCGATTGGCTAAAACGCAT
 GAAGCGAACTACGATGTCGCCATGCTCTATGTCAGTGACCACCGCGAGAG
 CTTGGCGAAAATGGTGTCTATCTGCATGGTATGCCAAATGCCCTTGCAC
 CAAAAGAACAGCAGGAGCTGTGCCCTGCGTTTTGGTCAAATAACGACA
 TTCAAGCCAATGCCAGCGATACTGTGCTGACGCATGATGCCATTAGCC
 AACACTGCTTAAGCTGTTGATGTCACAGCGGGCAAGGTCAAAGACCGCG
 CGGCATTTATCCAGTAA.

The DNA control mix may include the following internal control sequence: GAGAGGATGACCAGCCACACTG-GAACTGAGACACGGTCCAGACTCCTACGGGAG GCAGCAGTGGGAATATTGCAACATGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. [SEQ. ID NOS 290-292]

In one example, the kit includes one primer-probe multiplex mix vial including internal control and one DNA control mix vial. A primer-probe mix may include sequences for detecting TEM-like and SHV-like gene families and internal control.

The primer-probe mix may include primers and/or probes selected from the group consisting of: AGATCAGTGGGTGCACG, TGCTTAATCAGT-GAGGCACC, /5'-FAM/ATGAAGCCA/ZEN/TAC-CAAACGACGAGC/3'IBkFQ/, CTGGAGCGAAA-GATCCACTA, ATCGTCCACCATCCACTG, and /5'HEX/CCAGATCGG/ZEN/CGACAACGTCAAC/3'IBkFQ/. The primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAG-GATGAYCAGCCACAC, CGCCCAITGTSCAATATTCC and 5'TYE665/TGAGACACGGTCCAGACTCCTACG/3'IBkRQSp. The primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 240-248]

A DNA control mix may include one or more sequences selected from the group consisting of: AGATCAGTGGGTGCACGAGTGGGTAAG-CATCGAACTGGATCTAACACAGCGGTAAAG ATCCTT-GAGAGTTTCGCCCCGAAAGAACGTTTCAATGAT-GAGCACTTTAAAGTT-CTGCTATGTGGTGCAGGTAT-TATCCCGTGTGACGCCGGCAAGAGCAACTCGGT-C GCCGCATACACTATTCTCAGAATGACTTGGTT-GAGTACTCACCAGTCACAGAAAAG CATCTTACG-

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GATGGCATGACAGTAAGAGAATTATGCAGTGCTGC-CATAACCATGAG
 TGATAACACTGCGGCCAACCTACTCTGACAAC-GATCGGAGGACCGAAGGAGCTA
 ACCGCTTTTGCAACACATGGGGAT-CATGTAACTCGCCTTGATCGTGGGAACC GGAGCT-GAATGAAGCCATACCAAAACGACGAGCGTGACAC-CACGACGCCGTGAGC
 AATGGCAACAAACGTTGCCAAACTAT-TAACTGGCGAACTACTTACTCTAGCTTCCC-
 10 GGCAACAATTAAATAGACTGGATGGAGGCGGA-TAAAGTTGCGAGGACACTCTCG
 CTCGGCCCTCCCGCTGGCTGGTTATTGCTGA-TAAATCTGGAGGCCAGTGA
 15 GATGGTAAGCCCTCCCGTATCG TAGTTATCTACACGACGGGAGTCAGGCAACTATGGAT-GAACGAAATAGACAGATC
 GCTGAGATAGGTGCCTCACTGATTAAGCA and CTG-GAGCGAAAGATCCACTATGCCAGCAG-
 20 GATCTGGTGGACTACTGCCGGTCA GCGAAAACACCTTGCCGACGG-CATGACGGTGCAGCAACTCTGCCGCCGCC
 TTACCATGAGCGATAACAGCGCCGC-CAATCTGCTGCCACCGTCGGCGGCC
 25 CCGCAGGATTGACTGCCCTTGCAG-GATCGGCACACGTCAACCGCCITGA CCGCTGG-GAACCGAACTGAAT-GAGGCCTCCGGCAGCAGCCCCGCACACCAC
 TACCCCGGCCAG-
 30 CATGGCCCGCACCTGCCAGCTGCTGACCAGC-CAGCGTCT
 GAGGCCCGTTCGCAACGGCAGCTGCTGCAGTG-GATGGTGGACGAT. The DNA control mix may include the following internal control sequence: GAGAG-
 35 GATGACCAGCCACACTGGAACACTGA-GACACGGTCCAGACTCCTACGGGAG
 GCAGCAGTGGGAATATTGCAACATGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. [SEQ. ID NOS 293-295]

The primer-probe multiplex mix may comprise different oligonucleotide sequences. An oligonucleotide sequence may be utilized as a primer. An oligonucleotide sequence may be utilized as a probe. An oligonucleotide sequence may be utilized as an internal control sequence. The oligonucleotide concentration of a primer and/or probe sequence may range from 0.05 μM to 60 μM. For example, the oligonucleotide concentration of a primer and/or probe sequence may range from 3 μM to 8 μM. For example, the oligonucleotide concentration of an internal control sequence may range from 2 μM to 6 μM. For example, the oligonucleotide concentration of an internal control sequence may range from 2 μM to 8 μM. The vial oligonucleotide concentrations may be prepared as a 10x stock solution.

The synthetic gene size of a DNA control sequence may be from about 84 bp to about 533 bp. The concentration of a DNA control sequence may be about 25 ng/μl. The concentration of a DNA control sequence may be from 0.033 ng/μL to about 0.5 ng/μL.

The present teachings provide methods for detection of β-lactamase gene families from a biological sample. Preferably, the sample includes Gram-negative bacteria. The method may include sample processing. The method may include extracting DNA from the sample. The method may include extracting RNA from the sample. The method may include the use of assays of the present teachings. The assays

may be included in a kit or kits. The method may include employing the kit of the present teachings for the detection of multiple β -lactamase gene families from a biological sample.

The method may include employing the kit for analysis of nucleic acid contained in a clinical sample. The method may include employing the kit for analysis of DNA extracted from a clinical sample. The method may include employing the kit for analysis of DNA extracted from an overnight bacterial culture of a clinical sample.

The method may include amplifying a targeted DNA sequence by real-time polymerase reaction. The method may include amplifying several targeted DNA sequences by multiplex real-time polymerase reaction. The method may include analyzing the amplified sequences or amplicons. The method may include detecting the presence or absence of β -lactamase genes. The method may include detecting the presence or absence of *ampC* β -lactamase genes. The method may include identifying up to six β -lactamase gene families. The method may include identifying up to nine β -lactamase gene families. The method may include identifying up to fifteen β -lactamase gene families. The method may include identifying up to twenty β -lactamase gene families. The method may include identifying from about six to about thirty β -lactamase gene families. The method may include analyzing collected data.

Examples of real-time PCR amplification curves obtained on the ABI QS7 Flex-Real-Time System for some of the multiplex mixes described herein are shown in FIGS. 1-9. FIG. 1 depicts an amplification plot of an exemplary mix 1 including *ampC* gene targets. FIG. 2 depicts an amplification plot of an exemplary mix 2 including *ampC* gene targets. FIG. 3 depicts an amplification plot of an exemplary mix 1 including β -lactamase gene targets. FIG. 4 depicts an amplification plot of an exemplary mix 2 including β -lactamase gene targets. FIG. 5 depicts an amplification plot of an exemplary mix 3 including β -lactamase gene targets. FIG. 6 depicts an amplification plot of an exemplary internal control mix including MCR gene targets. FIG. 7 depicts an amplification plot of an exemplary mix 1 including OXA gene targets. FIG. 8 depicts an amplification plot of an exemplary mix 2 including OXA gene targets. FIG. 9 depicts an amplification plot of an exemplary internal control mix including SHV-TEM gene targets.

The method may include using one or more oligonucleotide primers that are complementary to at least a portion of the nucleic acid sequence of interest. The method may include annealing several pairs of primers to different target DNA sequences. The method may include annealing primer/probe sequences to bacterial nucleic acid sequences comprising targeted antibiotic resistant gene family variants of β -lactamases. The primer and/or probe sequences may anneal with 100% specificity to the target gene variants. The primer and/or probe sequences may anneal with about 95% specificity to the target gene variants. The primer and/or probe sequences may anneal with about 90% to about 100% specificity to the target gene variants. The primer and/or probe sequences may anneal with about 80% to about 100% specificity to the target gene variants.

The method may include using temperature mediated DNA polymerase. The method may include using fluorescent dyes. The method may include the using sequence specific DNA probes including oligonucleotides labeled with a reporter. The method may include using a microarray.

The method may include using a thermal cycler. For example, the kit of the present teachings may be utilized with the following PCR systems: Streck ZULU RTTM PCR

System, Applied Biosystems (ABI) QuantStudio 7 (QS7) Flex Real-Time System, ABI 7500 Real-Time PCR System, QIAGEN Rotor-Gene[®] Q, and CFX96 Touch[™] Real-Time PCR Detection System, Applied Biosystems[™] 7500 Fast Dx Real-Time PCR Instrument, Roche LightCycler[®] 480 I and II, and Cepheid SmartCycler[®]. It is contemplated that any detection system capable of detecting the multiplex fluorescent signal provided in the kit of the present teachings may be suitable.

The method may include real-time monitoring of qPCR reaction products. The probes may generate a signal when hydrolyzed by the DNA polymerase causing liberation of a detectable fluorescent signal. The real-time monitoring method may employ fluorescence at different wavelengths. The method may include the use of DNA-intercalating fluorescent dyes. The method may include the use of a target specific nucleotide probe labeled with a fluorescent tag at one end. The other end of the hybridization probe may be labeled with a fluorescent quencher. Fluorescent hybridization probes generate a fluorescence signal only when they bind to their target and enable real-time of monitoring of nucleic acid amplification assays.

Surprisingly, some DNA targets detected with these kits, allow for amplification of regions of DNA much larger than the conventional wisdom within the real-time PCR field. For example, most amplicons would traditionally be between 50 to 150 base pairs in size. The present teachings allow for successfully amplified amplicons up to 553 base pairs by real-time PCR.

There may be one or more benefits to detecting larger amplicons. Larger amplicons may, in some cases, provide greater specificity for a specific antibiotic resistance gene family. Detection of larger amplicons may permit detection of an increased number of gene variants within a given resistance gene family. Detection of larger amplicons may also allow confirmation by agarose gel electrophoresis since the molecular sizes of each gene that is detected can be resolved from one another.

The efficiency of detection for each target in a dilution series may be measured for amplicons between 25 base pairs and 2000 base pairs. The efficiency of the PCR for amplicons within this size range may be from 80% to 110%. More specifically, the efficiency of the reactions may be from 90% to 105%. The coefficient of determination may be from 0.98 to 1.1. More specifically, the coefficient of determination may be from 0.99 to 1.0. The limit of detection may be from 0.1 copies to 1×10^{10} copies.

Alternate sequences for primer, probes, and DNA controls for β -lactamase gene targets of the present teachings are depicted in Table 2 and Table 3. [SEQ. ID NOS 1-48 and SEQ. ID NOS 49-66]

Primers and/or probes may be degenerate at any nucleotide position. Primers and/or probes may not be degenerate at any nucleotide position. Any suitable fluorophore and/or quencher and nucleic acid sequence combination may be used. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, two fluorescent quenchers may be included at one end or within the probe sequence. It is contemplated that the probe sequences of the present teachings may be labeled with any suitable fluorophore and quencher combinations. For example, any fluorophore of the present teachings may be attached to any probe DNA sequence of the present teachings.

TABLE 2

Primer/ Probe	Sequence
SEQ ID NO. 1	MOX F'
	AGA CCC TGT TCG AGA TAG
SEQ ID NO. 2	MOX R'
	ATG GTG ATG CTG TCA AAG
SEQ ID NO. 3	MOX-FAM
	5'-56-FAM-CGT GAG CAA GAC CCT GAC TG-3'BHQ1
SEQ ID NO. 4	FOX F'
	ACT ATT TCA ACT ATG GGG TT
SEQ ID NO. 5	FOX R'
	TTG TCA TCC AGC TCA AAG
SEQ ID NO. 6	FOX-TEX
	5'-Tex615-TGA CCG CAG CAT AGG CAC-3'BHQ_2
SEQ ID NO. 7	EBC F'
	GTG GCG GTG ATT TAT GAG
SEQ ID NO. 8	EBC R'
	CGG TGA AGG TTT TAC TTA TAG AA
SEQ ID NO. 9	EBC-HEX
	5'-5HEX/CAGCCGCAC/ZEN/TACTTCACCT/-3'BHQ_1
SEQ ID NO. 10	DHA F'
	TGCGTACGGTTATGAGAACAA
SEQ ID NO. 11	DHA R'
	CCCAGCGCAGCATATCTT
SEQ ID NO. 12	DHA-FAM
	ATGCGGAATCTTACGGCGTGGAT
SEQ ID NO. 13	CMY F'
	TCC AGC GTT ATT GAT ATG G
SEQ ID NO. 14	CMY R'
	CAT CTC CCA GCC TAA TCC
SEQ ID NO. 15	CMY-TEX
	5'TexRd-XN/ACATATGCCAATACGCCAGT/3IAPRQSp/-3'
SEQ ID NO. 16	ACC F'
	GCCGCTGATGCAGAAGAATA
SEQ ID NO. 17	ACC R'
	TTT GCC GCT AAC CCA TAG TT
SEQ ID NO. 18	ACC-HEX
	5'-5HEX/TCA CTG CGA/ZEN/CCG ACA TAC CG/3IABkFQ/-3'
SEQ ID NO. 19	IC F'
	GAG AGG ATG ACC AGC CAC AC
SEQ ID NO. 20	IC R'
	AGT ACT TTA CAA CCC GAA GGC
SEQ ID NO. 21	IC-TYE
	5'/5TYE665/TGA GAC ACG GTC CAG ACT CCT ACG G/3BHQ_2/-3'
SEQ ID NO. 22	CTX-M-14 F'
	5'-TTGGTGACGTGGCTAAA-3'
SEQ ID NO. 23	CTX-M-14 R'
	5'-ATATCATTGGTGGTGCCGTAG-3'
SEQ ID NO. 24	CTX-M-14-FAM
	5'-56-FAM/CGTGGACTG/ZEN/TGGGTGATAAGACCG/3IABkFQ/-3'
SEQ ID NO. 25	CTX-M-15 F'
	5'-GTCACGCTGTTGTTAGGAAGT-3'
SEQ ID NO. 26	CTX-M-15 R'
	5'-TAATCAATGCCACACCCAGTC-3'
SEQ ID NO. 27	CTX-M-15-TEX615
	5'-5TEX615/AAC TTGCCATTAGAGCGGCAGT/3BHQ_2/-3'
SEQ ID NO. 28	OXA48-F'
	5'-AGCAGCAAGGATTACCAATAATC-3'
SEQ ID NO. 29	OXA48-R'
	5'-CGTCTGCCATCCACTTAAA-3'
SEQ ID NO. 30	OXA48-HEX
	5'-5HEX/TAGCTTGAT/ZEN/CGCCCTCGATTGGG/3IABkFQ/-3'
SEQ ID NO. 31	CMY F'
	5'-TCCAGCGTTATTGATATGG-3'
SEQ ID NO. 32	CMY R'
	5'-CATCTCCCAGCCTAATCC-3'
SEQ ID NO. 33	CMY-TxR
	5-/5TexRd-XN/ACATATGCCAATACGCCAGT/3IAbRQSp/-3'
SEQ ID NO. 34	NDM F'
	5'-TTTGATCGTCAGGGATGGC-3'
SEQ ID NO. 35	NDM R'
	5'-CAGGTTGATCTCCTGCTTGAT-3'
SEQ ID NO. 36	NDM-HEX
	5-/5HEX/AGACCGCCC/ZEN/AGATCCTCAACTG/3IABkFQ/-3'

TABLE 2-continued

Primer/ Probe	Sequence
SEQ ID NO. 37	KPC F'
	5' -CGCTAAACTCGAACAGGACTT-3'
SEQ ID NO. 38	KPC R'
	5' -TAACTTACAGTTGCGCCTGAG-3'
SEQ ID NO. 39	KPC-FAM
	5' -/5TYE665/ATCGGTGTACCGCATGGATACC/3HQ_2/-3'
SEQ ID NO. 40	VIM F'
	5' -CATTGACCGACAACCTAG-3'
SEQ ID NO. 41	VIM R'
	5' -CGTGCCTGACAACCTCAT-3'
SEQ ID NO. 42	VIM-TEX
	5' 45TEX615/TGTGCTCTATGGTGGTTGTGCGAT/3HQ_2/-3'
SEQ ID NO. 43	DHA F'
	5' -TGCACGGTTATGAGAACAA-3'
SEQ ID NO. 44	DHA R'
	5' -CCAGCGCAGCATATCTT-3'
SEQ ID NO. 45	DHA-FAM
	5' -/5-FAM/ATGCGGAAT/ZEN/CTTACGGCGTGAAT/3IABkFQ-3'
SEQ ID NO. 46	IMP F'
	5' -ACGTAGGGTTGGTTACCTG-3'
SEQ ID NO. 47	IMP R'
	5' -AAGCTTCTAAATTGCGTCACC-3'
SEQ ID NO. 48	IMP- TYE705
	5' -/5HEX/TTGTTAAA/ZEN/CCGGACGGCTTGTT/3IABkFQ/-3'

TABLE 3

DNA Control	Sequence
SEQ ID NO. 49	MOX
	AACCGGGAGAGCGGGGCCAGCGTCAGCGAGCAGACCTGTTGAGATAG GATCCGTGAGCAAGACCCCTGACTCGGACCTGGGGCTATGCGGTGGTC AAGGGAGCGATCGAGCTGGATGACAAGGGCAGGCCACGCCCTGGC TCAAGGGATCGTCTTGACAGCATCACCATGGGGAGCTTGCCACCTAC AGC
SEQ ID NO. 50	FOX
	GGGGATGGGGTGCCTGCTGAAAGATGCAAGGCCCCTATTTCACCT ATGGGGTTGCCAACCGCGAGAGTGCTCAGCGCTCAGCGAGCACCT GTTGAGATTGGCTCGGTCAAGACCCCTGACCGCGACCCCTGGTGCCT ATGCTGCGGTCAGGGGGCTTTGAGCTGGATGACAAGGTGAGCCAGCA CGCCCCCTGGCTCAAAGGTTCCGCTTGTGGTGTGACCAT
SEQ ID NO. 51	EBC
	GGACCGTTACGCCGCTGATGAAAGCGCAGGCCATTCCGGGTATGGCGGT GGCGGTGATTATGAGGGTCAGCCGCACTACTTCACCTTCGGTAAAGCCG ATGTTGCGCGAACAAACCTGTCACTCCACAAACCTTGTGAACTGGGTT CTATAAGTAAACCTTCACCGCGTACTCGGTGGCAGTGCCTGGCG GTGAAATATCGTGGCGA
SEQ ID NO. 52	DHA
	GACTGCACGGATCTGGCACCGCTGGGGTTATCTCACACCTTATTACTGT GCCGAAAGTGCAGAACGGCAGTATGCGTACGGTATGAGAACAAAAAA CCGGTCCGGTGTGCCGGACAGCTTGATGCGGAATCTACGGCGTGG ATCCGCCTAAAGATATGCTGCGCTGGCGGAATGAATATGGAGCCGT CACGGCCGGTAATGCGGAT
SEQ ID NO. 53	CMY
	GCCTGTACAGTTCTCGGGACAACCTGACGCCAGCCTATGGCGTGA AATCCAGCGTTATTGATATGGCCGCTGGGTTAGGTCAACATGGACGCC AGCCGGCTCAGGAGAAAACGCTCCAGCAGGGCATTGGCTTGGCAGTC TCGCTACTGGCGTATTGGCGATATGACCAAGGATTAGGCTGGGAGATGC TGAACGGCGCTGAAAGCTGATTGCGATCATCACCGTAGCGACAGCAA GTGGCATTGG
SEQ ID NO. 54	ACC
	GAGAGCAAATAAAGACACCGTTGATGACCTGATCCAGCCGCTGATGCA GAAGAATAATATTCCGGTATGCGTCAGTGACCGTCAACGGTAAAG ACTACATTTATAACTATGGGTTAGCGGCAAAACAGCCTCAGCAGCCGGTT
SEQ ID NO. 55	IC
	AGCTTGGTGGGGTAACGGCTCACCAAGGCAGATCCCTAGCTGGTC TGAGAGGATGACCAAGCCACACTGGAACTGAGACACGGTCAGACTCTAC GGGAGGCAGCAGTGGGAATTGCGACATGGGCGAAGCCTGATGCG CCATGCCGGTGTATGAGAAGGCTTCGGGTTGAAAGTACTTCAGCG GGGAGGAAGGGAGTAAAGTTAACCTTGTGCTATTGACGTTACCCGAG AGAACGACCGGCTAACCTCG

TABLE 3 - continued

	DNA Control	Sequence
SEQ ID NO. 56	CTX-M-14	CGTTTCGTCGGATCGCACTGAACCTACCGTGAATACCCGATTCCGGCGACCCGAGAGACACCAACGCCGGCGATGGCGCAGACGTTGGCTA GCTTACGCTGGGTATGCCGTGGCGAAACCCAGCGGGCGCAGTTGGTG ACGTGGCTAAAGCCAATACGACCGGCAGCCAGCATTGGCCGGCTT ACCGACGCTGGACTGTGGTGATAAGACCGGCAGCGGCAGTACGGC ACCACAAATGATATTGCGGTGATCTGGCCAGGGTCGCGCGCTGGT TCTGGTGACCTATTTACCCAGC
SEQ ID NO. 57	CTX-M-15	CCGTACACGCTGTTAGGAAGTGTGCCGTGTATGCGCAAACGGCGAC GTACAGCAAAAATTGCGCAATTAGAGCGCAGTCGGGAGGCAAGACTGG GTGTGGCATTGATTAACACAGC
SEQ ID NO. 58	OXA	AATCACAGGGCGTAGTTGCTCTGGAATGAGAATAAGCAGCAAGGATT ACCAATAATCTAACGGCGAACCAAGCATTTACCGCATCTACCTTA AAATTCCAATAGCTTGTATGCCCTCGATTGGCGTGGTTAAGGATGAAC ACCAAGTCTTAAGTGGGATGGCAGACCGCGCATATCCCACCTTGAAT CGCGATCATATACTAACTACCGCGATGAATATTCACTGGCTGTGTTAT CAAGAATTGGCCGCCAATTGGCGAGGCAAGCTATGAGCAAGATGCTACA TGCTTCGATTATGGAATGAGGACATTGCGGAATGTAGACAGTTCTG GCTGACGCTGGATTCAAAATAGTTACAGTATCGGAGCGCAAATCAGTTTTAA GAAAGCTGATCAAAATAGTTACAGTATCGGAGCGCAAGCAGCTATT GTCAAAACAGCCATGCTGACCGAACGGTACTAATTATTCGGGCT AAAACTGGTACTCGACTAGAATCGAACCTAAGATTGGCTGGCTGGGT
SEQ ID NO. 59	IC	CGGAGTTAGCCGGTGTCTCTCGGGTAACGTCAATGAGCAAAGGTAT TAATTTACTCCCTCCCTCCCGCTGAAAGTACTTACAACCCGAAAGCCTT CTTCATACAGCGGCATGGCTGATCAGGCTTGCGCCATTGCAATT ATTCCCCACTGTCGCTCCCGTAGGAGTCTGGACCGTGTCAAGTCCAGTGTG GCTGTCATCCTCTCAGACAGCTAGGGATCGTCGCTTGGTGAAGCCGTTA CCCCCACCAACAAGCT
SEQ ID NO. 60	CMY	GCCTGTACAGTTCTCCGGACAACCTGACGCCAGCCTATGGCGTGA AATTCAGCGCTTATTGATATGGCCCGCTGGGTTCAGGTCAACATGGACGCC AGCCGCTTCAGGAGAAAACGCTCCAGCAGGGCATTGCGCTTGCGCAGTC TCGCTACTGGCTATTGGCGATATGACCTAGGGATTAGGCTGGAGATGC TGAACGGCGCTGAAAGCTGATTGATCATCAACGGTAGCGACAGCAA GTGGCATTGG
SEQ ID NO. 61	NDM	GGCAGAAAGTCAGGCTGTGTTGCGCCGCAACCATCCCTCTGGGGGCAA GCTGGTCGACAACGCTTGGCATAAGTCGAATCCCCGCCGATGCGAC GCGCTCATCCGGCCATCTGTCTGATGCGCTGAGTCACCCACGCCAGC CGGACCCGGCAGGTTGATCTCTGCTTGTACCTCAAGTTGAGGATCTGGGGGT CTGGTCATCGTCCAGGCGGTATCGACCAACAGCACGGCCCATCCC TGACGATCAAAC
SEQ ID NO. 62	KPC	GTATCGCCGCTAGTTCTGCTGTTGTCCTCATGGCCGCTGGCTGGCTTT TCTGCCACCCGCTGACCAACCTGTCGGGAACATTGCTAAACTCGAA CAGGACTTTGGCGGCTCATCGGTGTACGCGATGGATAACCGGCTCAGG CGCAACTGTAAGTTACCGCCTGAGGAGCGCTTCCACTGTGAGCTATT CAAGG
SEQ ID NO. 63	VIM	CCATTCAAGGAGATCGGCATGGCACGTTCCCGCAGACGTGCGTGACA ACTCATGAGTCGCAACACCCATAGAGCACACTCGCAGACGGGACGTAC ACAACTAAGTGTGGTCAATGCGCAGCACCGGGATAGAAGAGTTCTAC TGGACCGAAGCGCACTGCTCCCGCTCGAGTCTTAGAGAGTGGCTG GGAATCTCGTCCCTCTACCTCGGCTAGCCGGTGTGACGGTGATGC GTACGTTGCCACCCAGCCCGAAGGACATCAACGCC
SEQ ID NO. 64	DHA	GACTGCACCGATCTGGCACCGCTGGGTTATCTCACACCTTATTACTGT GCGGAAAGTGCAGAACGGCAGTATGCGTACGGTTATGAGAACAAAAAA CCGGTCCGGCTGCGGGACAGCTTGTATGCGGAATCTACGGCTGAA ATCCGCTCTAAAGATATGCTGCGCTGGCGGAATGAATATGGAGCGT CACGGCCCGTAACTGGGT
SEQ ID NO. 65	IC	CGGAGTTAGCCGGTGTCTCTCGGGTAACGTCAATGAGCAAAGGTAT TAATTTACTCCCTCCCTCCCGCTGAAAGTACTTACAACCCGAAAGGCTT CTTCATACAGCGGCATGGCTGATCAGGCTTGCGCCATTGCAATT ATTCCCCACTGTCGCTCCCGTAGGAGTCTGGACCGTGTCAAGTCCAGTGTG GCTGTCATCCTCTCAGACAGCTAGGGATCGTCGCTTGGTGAAGCCGTTA CCCCCACCAACAAGCT
SEQ ID NO. 66	IMP	GGGGAGTTAGTTATTGGCTAGTTAAAATAAAATTGAAGTTTTATCCG GCCGGGGACACTCAAGATAACGCTAGTGTGTTGTTACTGAAAAGAAA ATTTTATTGGTGGTTGTTGTTAAACCGGACGGTCTTGGTAAATTGGGT GACGCAAAATTAGAAGCTTGGC

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The sequence listing including SEQ ID NOS 1-295 is hereby incorporated by reference for all purposes.

Unless otherwise stated, any numerical values recited herein include all values from the lower value to the upper value in increments of one unit provided that there is a separation of at least 2 units between any lower value and any higher value. As an example, if it is stated that the amount of a component, a property, or a value of a process variable such as, for example, temperature, pressure, time and the like is, for example, from 1 to 90, preferably from 20 to 80, more preferably from 30 to 70, it is intended that intermediate range values such as (for example, 15 to 85, 22 to 68, 43 to 51, 30 to 32 etc.) are within the teachings of this specification. Likewise, individual intermediate values are also within the present teachings. For values which are less than one, one unit is considered to be 0.0001, 0.001, 0.01, or 0.1 as appropriate. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner. As can be seen, the teaching of amounts expressed as "parts by weight" herein also contemplates the same ranges expressed in terms of percent by weight. Thus, an expression in the of a range in terms of "at least 'x' parts by weight of the resulting composition" also contemplates a teaching of ranges of same recited amount of "x" in percent by weight of the resulting composition."

Unless otherwise stated, all ranges include both endpoints and all numbers between the endpoints. The use of "about" or "approximately" in connection with a range applies to both ends of the range. Thus, "about 20 to 30" is intended to cover "about 20 to about 30", inclusive of at least the specified endpoints.

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The disclosures of all articles and references, including patent applications and publications, are incorporated by reference for all purposes. The term "consisting essentially of to describe a combination shall include the elements, ingredients, components or steps identified, and such other elements ingredients, components or steps that do not materially affect the basic and novel characteristics of the combination. The use of the terms "comprising" or "including" to describe combinations of elements, ingredients, components or steps herein also contemplates embodiments that consist of, or consist essentially of the elements, ingredients, components or steps.

Plural elements, ingredients, components or steps can be provided by a single integrated element, ingredient, component or step. Alternatively, a single integrated element, ingredient, component or step might be divided into separate plural elements, ingredients, components or steps. The disclosure of "a" or "one" to describe an element, ingredient, component or step is not intended to foreclose additional elements, ingredients, components or steps.

It is understood that the above description is intended to be illustrative and not restrictive. Many embodiments as well as many applications besides the examples provided will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated by reference for all purposes. The omission in the following claims of any aspect of subject matter that is disclosed herein is not a disclaimer of such subject matter, nor should it be regarded that the inventors did not consider such subject matter to be part of the disclosed inventive subject matter.

SEQUENCE LISTING

```

Sequence total quantity: 302
SEQ ID NO: 1      moltype = DNA length = 18
FEATURE           Location/Qualifiers
misc_feature      1..18
source            note = MOX F'
                   1..18
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 1
agacctgtt cgagatag

SEQ ID NO: 2      moltype = DNA length = 18
FEATURE           Location/Qualifiers
misc_feature      1..18
source            note = MOX R'
                   1..18
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 2
atggtgatgc tgtcaaag

SEQ ID NO: 3      moltype = DNA length = 20
FEATURE           Location/Qualifiers
misc_feature      1..20
source            note = MOX-FAM
                   1..20
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 3
cgtgagcaag accctgactg

SEQ ID NO: 4      moltype = DNA length = 20
FEATURE           Location/Qualifiers

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18

18

20

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misc_feature	1..20
source	note = FOX F'
	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 4	
actatcca ctatgggtt	20
SEQ ID NO: 5	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
source	note = TTG TCA TCC AGC TCA AAG
	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 5	
ttgtcatcca gctcaaag	18
SEQ ID NO: 6	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
source	note = FOX-TEX
	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 6	
tgaccgcagc ataggcac	18
SEQ ID NO: 7	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
source	note = EBC F'
	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 7	
gtggcggtga tttatgag	18
SEQ ID NO: 8	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
source	note = EBC R'
	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 8	
cggtaaggt tttacttata gaa	23
SEQ ID NO: 9	moltype = DNA length = 19
FEATURE	Location/Qualifiers
misc_feature	1..19
source	note = EBC-HEX
	1..19
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 9	
cagccgact acttcacct	19
SEQ ID NO: 10	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
source	note = DHA F'
	1..21
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 10	
tgcgtacggt tatgagaaca a	21
SEQ ID NO: 11	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
source	note = DHA R'
	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 11	
cccagcgcag catatctt	18
SEQ ID NO: 12	moltype = DNA length = 24

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FEATURE	Location/Qualifiers
misc_feature	1..24
	note = DHA-FAM
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 12	
atgcggaatc ttacggcggtg gaat	24
SEQ ID NO: 13	moltype = DNA length = 19
FEATURE	Location/Qualifiers
misc_feature	1..19
	note = CMY F'
source	1..19
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 13	
tccagcgtta ttgatatgg	19
SEQ ID NO: 14	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
	note = CMY R'
source	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 14	
catctccca g cctaattcc	18
SEQ ID NO: 15	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = CMY-TEX
source	1..21
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 15	
acatatcgcc aatacgccag t	21
SEQ ID NO: 16	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = ACC F'
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 16	
gccgctgtatc cagaagaata	20
SEQ ID NO: 17	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = ACC R'
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 17	
tttgcgcata acccatagtt	20
SEQ ID NO: 18	moltype = DNA length = 11
FEATURE	Location/Qualifiers
misc_feature	1..11
	note = ACC-HEX
source	1..11
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 18	
ccgacataacc g	11
SEQ ID NO: 19	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = IC F'
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 19	
gagaggatga ccagccacac	20

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SEQ ID NO: 20      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
misc_feature      1..21
source            note = IC R'
                  1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 20      agtactttac aacccgaagg c               21

SEQ ID NO: 21      moltype = DNA  length = 25
FEATURE           Location/Qualifiers
misc_feature      1..25
source            note = IC-TYE
                  1..25
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 21      ttagacacgg tccagactcc tacgg             25

SEQ ID NO: 22      moltype = DNA  length = 18
FEATURE           Location/Qualifiers
misc_feature      1..18
source            note = CTX-M-14 F'
                  1..18
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 22      ttgggtgacgt ggctcaaa                         18

SEQ ID NO: 23      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
misc_feature      1..21
source            note = CTX-M-14 R'
                  1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 23      atatcatgg tggtgccgta g               21

SEQ ID NO: 24      moltype = DNA  length = 24
FEATURE           Location/Qualifiers
misc_feature      1..24
source            note = CTX-M-14-FAM
                  1..24
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 24      cgtggactgt gggtgataag accg              24

SEQ ID NO: 25      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
misc_feature      1..21
source            note = CTX-M-15 F'
                  1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 25      gtcacgctgt tgtaggaag t               21

SEQ ID NO: 26      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
misc_feature      1..21
source            note = CTX-M-15 R'
                  1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 26      taatcaatgc cacacccagt c               21

SEQ ID NO: 27      moltype = DNA  length = 24
FEATURE           Location/Qualifiers
misc_feature      1..24
source            note = CTX-M-15-TEX615
                  1..24
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 27      aacttgcga attagagcgg cagt             24

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SEQ ID NO: 28      moltype = DNA length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
source           note = OXA48-F'
                1..24
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 28
agcagcaagg atttaccaat aatc                                24

SEQ ID NO: 29      moltype = DNA length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
source           note = OXA48-R'
                1..21
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 29
cgtctgtcca tccccacttaa a                                21

SEQ ID NO: 30      moltype = DNA length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
source           note = OXA48-HEX
                1..24
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 30
tagcttgatc gcccctcgatt tggg                                24

SEQ ID NO: 31      moltype = DNA length = 19
FEATURE          Location/Qualifiers
misc_feature     1..19
source           note = CMY F'
                1..19
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 31
tccagcgtta ttgatatatgg                                19

SEQ ID NO: 32      moltype = DNA length = 18
FEATURE          Location/Qualifiers
misc_feature     1..18
source           note = CMY R'
                1..18
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 32
catctccca g cctaattcc                                18

SEQ ID NO: 33      moltype = DNA length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
source           note = CMY-TxR
                1..21
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 33
acatatacgcc aatacgccag t                                21

SEQ ID NO: 34      moltype = DNA length = 19
FEATURE          Location/Qualifiers
misc_feature     1..19
source           note = NDM F'
                1..19
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 34
tttgatcgtc agggatggc                                19

SEQ ID NO: 35      moltype = DNA length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
source           note = NDM R'
                1..21
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 35

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caggttgate tcctgcttga t	21
SEQ ID NO: 36 moltype = DNA length = 22	
FEATURE Location/Qualifiers	
misc_feature 1..22	
source note = NDM-HEX	
SEQUENCE: 36 1..22	
mol_type = other DNA	
organism = synthetic construct	
agaccgcca gatcctcaac tg	22
SEQ ID NO: 37 moltype = DNA length = 21	
FEATURE Location/Qualifiers	
misc_feature 1..21	
source note = KPC F'	
SEQUENCE: 37 1..21	
mol_type = other DNA	
organism = synthetic construct	
cgctaaactc gaacaggact t	21
SEQ ID NO: 38 moltype = DNA length = 21	
FEATURE Location/Qualifiers	
misc_feature 1..21	
source note = KPC R'	
SEQUENCE: 38 1..21	
mol_type = other DNA	
organism = synthetic construct	
taacttagag ttgcgcctga g	21
SEQ ID NO: 39 moltype = DNA length = 24	
FEATURE Location/Qualifiers	
misc_feature 1..24	
source note = KPC-FAM	
SEQUENCE: 39 1..24	
mol_type = other DNA	
organism = synthetic construct	
atcgggtgt acgcgatgga tacc	24
SEQ ID NO: 40 moltype = DNA length = 19	
FEATURE Location/Qualifiers	
misc_feature 1..19	
source note = VIM F'	
SEQUENCE: 40 1..19	
mol_type = other DNA	
organism = synthetic construct	
cattcgaccg acaacttag	19
SEQ ID NO: 41 moltype = DNA length = 17	
FEATURE Location/Qualifiers	
misc_feature 1..17	
source note = VIM R'	
SEQUENCE: 41 1..17	
mol_type = other DNA	
organism = synthetic construct	
cgtgcgtgac aactcat	17
SEQ ID NO: 42 moltype = DNA length = 24	
FEATURE Location/Qualifiers	
misc_feature 1..24	
source note = VIM-TEX	
SEQUENCE: 42 1..24	
mol_type = other DNA	
organism = synthetic construct	
tgtgcttat ggtgggttgat cgat	24
SEQ ID NO: 43 moltype = DNA length = 21	
FEATURE Location/Qualifiers	
misc_feature 1..21	
source note = DHA F'	
SEQUENCE: 43 1..21	
mol_type = other DNA	
organism = synthetic construct	

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SEQUENCE: 43
tgcgtacggt tatgagaaca a 21

SEQ ID NO: 44      moltype = DNA length = 18
FEATURE          Location/Qualifiers
misc_feature    1..18
note = DHA R'
source          1..18
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 44
cccagcgcag catatctt 18

SEQ ID NO: 45      moltype = DNA length = 24
FEATURE          Location/Qualifiers
misc_feature    1..24
note = DHA-FAM
source          1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 45
atgcggaatc ttacggcggt aaat 24

SEQ ID NO: 46      moltype = DNA length = 21
FEATURE          Location/Qualifiers
misc_feature    1..21
note = IMP F'
source          1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 46
acgtatggt ttggttacct g 21

SEQ ID NO: 47      moltype = DNA length = 22
FEATURE          Location/Qualifiers
misc_feature    1..22
note = IMP R'
source          1..22
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 47
aagcttctaa atttgcgtca cc 22

SEQ ID NO: 48      moltype = DNA length = 24
FEATURE          Location/Qualifiers
misc_feature    1..24
note = IMP-TYE705
source          1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 48
tttgttaaac cggacggctc ttgt 24

SEQ ID NO: 49      moltype = DNA length = 201
FEATURE          Location/Qualifiers
misc_feature    1..201
note = MOX
source          1..201
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 49
aaccgggaga gcccccccgag cgtcagcagc agacccctgt tcgagatagg atccgtgagc 60
aagaccctga ctgcgacccct gggggctat gccgtggtc aagggagcgat gcagctggat 120
gacaaggcgcg gccggcacgc gcccggctc aagggtatccg tctttgacag cataccatg 180
ggggagcttg ccacctacag c 201

SEQ ID NO: 50      moltype = DNA length = 240
FEATURE          Location/Qualifiers
misc_feature    1..240
note = FOX
source          1..240
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 50
ggggatggcg gtgcggcgtgc tgaaagatgg caaggccac tatttcaact atgggggtgc 60
caaccgcgag agtggtcagc gcgtcagcga gcagacccctg ttcgagatgg gctcggtcag 120
caagaccctg accgcgaccc tcggtgccata tgctgcggtc aaggggggctt tgagctgg 180
tgacaaggtg agccagcacg cccctggct caaagggtcc gcctttgatg gtgtgaccat 240

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SEQ ID NO: 51 moltype = DNA length = 220
 FEATURE Location/Qualifiers
 misc_feature 1..220
 note = EBC
 source 1..220
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 51
 ggaccggtac ggcgtatgg aaaggcagg ccattccgg tatggcggt gcggtgatt 60
 atgagggtca ggcgaactac ttacactcg ttaagccga ttttgcggc aacaacctg 120
 tcactccaca aaccttgcgaaactgggtt ctataagtaa aacccaccg ggcgtactcg 180
 gtggcgatgc cattgtcgc ggtgaaatat cgctggcga 220

SEQ ID NO: 52 moltype = DNA length = 220
 FEATURE Location/Qualifiers
 misc_feature 1..220
 note = DHA
 source 1..220
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 52
 gactgcacgg atccgtggcac cgctgggtt atctcacacc ttttactcg tgccggaaag 60
 tgcgc当地 cagttatga gaacaaaaaa cccgtccggc tgccgggg 120
 acagcttgcgat gggatctt acggcgatggc atccgcctca aaagatatgc tgccgtggc 180
 gggaaatgaat atggagccgt cacggggccgg taatgcggat 220

SEQ ID NO: 53 moltype = DNA length = 260
 FEATURE Location/Qualifiers
 misc_feature 1..260
 note = CMY
 source 1..260
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 53
 gctgtacac gtttctccgg gacaacttga cggcgaaggc tatggcgta aatccagcgt 60
 tattatgtatg gcccgtggg ttcaagtcgaa catggacgccc agccgcgttc aggagaaaac 120
 gctccagcag ggcattgcgc ttgcgcagtc tcgcgtactgg cgtattggcg atatgtacca 180
 gggatttaggc tggggatgc tgaactggcc gctgaaagct gattcgatca tcaacggtag 240
 cgacagcaaa gtggcatttg 260

SEQ ID NO: 54 moltype = DNA length = 150
 FEATURE Location/Qualifiers
 misc_feature 1..150
 note = ACC
 source 1..150
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 54
 gagagcaaaa tttaagacac cggttatgac ctgtatccgc cgctgtatgc gaagaataat 60
 attcccgta tgcgtatgcg agtgcgtcc aacgtaaaaa actacattta taactatggg 120
 tttagcccaa aacagccctca gcagccgtt 150

SEQ ID NO: 55 moltype = DNA length = 270
 FEATURE Location/Qualifiers
 misc_feature 1..270
 note = IC
 source 1..270
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 55
 agtttgcgtgg tggggtaacg gtcaccaag ggcgtatcc ctagctggc tgagaggatg 60
 accagccaca ctggacta gacacgtcc agactccatc gggaggcgc agtggggat 120
 attgcacaat gggcgcaagc ctgtatgcgc catggcgatc gtatgaagaa ggccttcgg 180
 ttgtaaatgtt ctttcggcgg gggaggagg agttaatgtt atacccatgc tcattgcgt 240
 taccgcaga agaaggcaccg gctaactccg 270

SEQ ID NO: 56 moltype = DNA length = 321
 FEATURE Location/Qualifiers
 misc_feature 1..321
 note = CTX-M-14
 source 1..321
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 56
 cgtttcgtct ggatcgact gaacctacgc tgaataccgc cattccggc gacccgagag 60
 acaccaccac gccgcggcgc atggcgcaga cgttgcgtca gcttacgcgt ggtcatgcgc 120
 tggcgaaac ccagcggcgc cagttggcgtca gcttgcgtcaa aggcaatacg accggcgcag 180
 ccagcattcg ggccggctta cccgcgtcgt ggactgtggg tgataagacc ggcagcggcg 240
 actacggcac caccatgtt attcggcgtca tctggccgc gggcgtcgtc cccgtgggttc 300

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tggtgacctt ttttacccag c                                321
SEQ ID NO: 57      moltype = DNA  length = 121
FEATURE           Location/Qualifiers
misc_feature      1..121
                  note = CTX-M-15
source            1..121
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 57
ccgtcacgct gttgttaga agtgtgcgc tgtatgcgc aacggggac gtacagcaaa 60
aacttgcga attagagcgg cagtcggag gcagactggg tgtggcatg attaacacag 120
c                                         121

SEQ ID NO: 58      moltype = DNA  length = 553
FEATURE           Location/Qualifiers
misc_feature      1..553
                  note = OXA
source            1..553
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 58
aatcacagg cgtagttgtc ctcttggatg agaataagca gcaaggattt accaataatc 60
ttaaacgggc gaaccaagca tttttacccg catctacctt taaaattccc aatagctga 120
tcgcccctcgta ttggggctgtg gttaaaggatg aacaccagaat cttaaagtgg gatggacaga 180
cgcgcgatatacgccacttgg aatcgcgatc ataatctaatacccgatg aaatatttcgg 240
ttgtgcctgt ttatcaagaa ttggccgcgaa atttggcgca ggcacgtatg agcaagatgc 300
tacatgtttt cgattatggt aatgaggaca ttccggcaaa tgtagacagt ttctggctcg 360
acgggtgttat tcgaatttcg gccacggagc aaatcagttt tttaaagaaag ctgtatcaca 420
ataagttaca cgtatccggag cgcacggcgcgtt gttttttttt acaaggccatg ctgaccgaaag 480
ccatggcgtt ctaatttttccggccggctaaac tggatactcg actagaatcg aacctaagat 540
tggctggctg ggt                                         553

SEQ ID NO: 59      moltype = DNA  length = 270
FEATURE           Location/Qualifiers
misc_feature      1..270
                  note = IC
source            1..270
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 59
cgaggatgtc cggtgtttct tctggggta acgtcaatg gcaaaaggat taactttact 60
cccttcctcc cccgtgaaag tactttacaa cccgaaggcc ttcttcatac acggggatg 120
gtctgcatacg gtttgcggcc atttgtcaat attccccact gtcgcctccc gtggagatct 180
ggaccgtgtc tcagttccag tggctggctgtt catccctctca gaccagctggatcg 240
cttggtgacg cgttacccca ccaacaagct                                         270

SEQ ID NO: 60      moltype = DNA  length = 260
FEATURE           Location/Qualifiers
misc_feature      1..260
                  note = CMY
source            1..260
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 60
gcctgtacac gtttctccgg gacaacttga cggccgaaggcc tatggcgta aatccagcg 60
tattgtatg gcccgcgtggg ttccaggtaa catggacgcg agccgcgttc aggagaaaaac 120
gtcccgacg ggcatttgcgc ttgcgcgttc tgcgtactgg cgtattggcg atatgtacca 180
gggatttaggc tgggagatgc tgaactggcc gctgaaagct gattcgatca tcaacggtag 240
cgacagcaaa gtggcattgg                                         260

SEQ ID NO: 61      moltype = DNA  length = 263
FEATURE           Location/Qualifiers
misc_feature      1..263
                  note = NDM
source            1..263
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 61
ggcgaaagtc aggctgtgtt gcgccgcacatccctct tgcggggcaa gctggttcg 60
caacgcatttgcgataagtcg caatccccgcg cgcattgcgc gcttccatc cggccatctt 120
gttctgtatgc gctgtgatgc ccacccgcg cgcgcacccgc aggttgatct cctgttgc 180
ccagttgagg atctggccgg tctggatcgc ggtccaggcg gtatcgacca ccagcacgcg 240
ggccgcattcc ctgacgtatca aac                                         263

SEQ ID NO: 62      moltype = DNA  length = 209
FEATURE           Location/Qualifiers
misc_feature      1..209
                  note = KPC

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source          1..209
               mol_type = other DNA
               organism = synthetic construct

SEQUENCE: 62
gtatcgccgt ctatgttcgc tggcttgcgtc tttctgcac 60
cgcgctgacc aacctcgatcg cggaaaccatt cgctaaactc gaaggact ttggcggtc 120
catcggtgtg tacgcgatgg atacccgctc aggccaaact gtaagttacc gcgctgagga 180
gcgcgttccca ctgtgcagct cattcaagg 209

SEQ ID NO: 63      moltype = DNA  length = 292
FEATURE
misc_feature       1..292
note = VIM
source             1..292
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 63
ccattcagcc agatcgccat cgccacgtt ccccgacagc gtgcgtgaca actcatgaat 60
cgcacaaccca ccatacagca cactcgacgaa cgggacgtac acaactaaat tgtcggcg 120
atgcgcacga ccaggataga agaggatctac tggaccgaag cgcaactcgat ccccgctcg 180
gtcccttcgt agagtgcgtg ggaatctcgat tcccctctac ctccgtatgc cgcgctgtcg 240
acgggtatgc gtacgttgcc accccagccg cccgaaggac atcaacgcgc 292

SEQ ID NO: 64      moltype = DNA  length = 220
FEATURE
misc_feature       1..220
note = DHA
source             1..220
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 64
gactgcacgg atcctggcac cgctgggggtt atctcacacc tttattactg tgccggaaag 60
tgcgcaaaacg cagtagatcgat acggttatga aaacaaaaaa cccggcccg tgtcggccgg 120
acagctgtat gggaaatctt acggcgtgaa atccgcctca aaagatatgc tgcgctggc 180
ggaatataat atggagccgt cacggggccg taatcggtat 220

SEQ ID NO: 65      moltype = DNA  length = 270
FEATURE
misc_feature       1..270
note = IC
source             1..270
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 65
cgaggatgtac cgggtgtttct tctggggta acgtcaatga gcaaaggat taactttact 60
cccttcctcc cccgtgaaat tactttacaa cccgaaggcc ttcttcatac acgcggcatg 120
gtgtcatcgat gtttgcgtcc atttgcaat attttttact gtgtcctccc gttaggatct 180
ggaccgtgtc tcagttccat tttggctgtt catcctctca gaccagatgt ggatcgctcg 240
cttggtgagc cgttacccca ccaacaagct 270

SEQ ID NO: 66      moltype = DNA  length = 177
FEATURE
misc_feature       1..177
note = IMP
source             1..177
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 66
gcggagttat tattggctta gttaaaaata aaatttgaatg tttttatccc ggccggggc 60
acactcaaga taacgtatgt gtttggatc ctgaaaagaa aattttatc ggtgggttgtt 120
ttgttaaacc ggacggttt grvgtatattt gggtgacgca aattttagaaat cttggcc 177

SEQ ID NO: 67      moltype = DNA  length = 21
FEATURE
misc_feature       1..21
note = PRIMER/PROBE
source             1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 67
tggccagaac tgacaggcaa a 21

SEQ ID NO: 68      moltype = DNA  length = 21
FEATURE
misc_feature       1..21
note = PRIMER/PROBE
source             1..21
mol_type = other DNA
organism = synthetic construct

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SEQUENCE: 68
tttctcctga acgtggctgg c                                21

SEQ ID NO: 69      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 69
acgctaactc cagcattggc ctgt                                24

SEQ ID NO: 70      moltype = DNA  length = 19
FEATURE          Location/Qualifiers
misc_feature     1..19
note = PRIMER/PROBE
source           1..19
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 70
ccgtcacgct gttgtttagg                                19

SEQ ID NO: 71      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE
source           1..22
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 71
gctgtgttaa tcaatgccac ac                                22

SEQ ID NO: 72      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 72
aacttgccga attagagcrg cagt                                24

SEQ ID NO: 73      moltype = DNA  length = 19
FEATURE          Location/Qualifiers
misc_feature     1..19
note = PRIMER/PROBE
source           1..19
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 73
cgtttcgtct ggatcgacac                                19

SEQ ID NO: 74      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 74
gctgggtaaa ataggtcacc                                20

SEQ ID NO: 75      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 75
tatcatttgt ggtgcgttag tcgc                                24

SEQ ID NO: 76      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA

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SEQUENCE: 76	organism = synthetic construct	
gagaggatga ycagccacac		20
SEQ ID NO: 77	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
	note = PRIMER/PROBE	
source	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 77		
cggccattgt scaatattcc		20
SEQ ID NO: 78	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
	note = PRIMER/PROBE	
source	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 78		
tgagacacgg tccagactcc tacg		24
SEQ ID NO: 79	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
	note = PRIMER/PROBE	
source	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 79		
aatcacaggg cgttagttgt		20
SEQ ID NO: 80	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
	note = PRIMER/PROBE	
source	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 80		
acccaccaggc caatctttagg		20
SEQ ID NO: 81	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
	note = PRIMER/PROBE	
source	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 81		
tagcttgcatt gccctcgatt tggg		24
SEQ ID NO: 82	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	
misc_feature	1..21	
	note = PRIMER/PROBE	
source	1..21	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 82		
gcggagttaa ctattggcta g		21
SEQ ID NO: 83	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	
misc_feature	1..21	
	note = PRIMER/PROBE	
source	1..21	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 83		
ggccaagttt ctatatttgc g		21
SEQ ID NO: 84	moltype = DNA length = 23	
FEATURE	Location/Qualifiers	
misc_feature	1..23	
	note = PRIMER/PROBE	
source	1..23	

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mol_type = other DNA
organism = synthetic construct

SEQUENCE: 84
ttrttygggt gttgyttt taa                                23

SEQ ID NO: 85      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 85
gcggagttt ytattggcta g                                21

SEQ ID NO: 86      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 86
ggccaagcyt ctawatttgc g                                21

SEQ ID NO: 87      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 87
ccggacggtc ttggtaattt gggt                                24

SEQ ID NO: 88      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 88
ccgtacggtt taggcaattt gggt                                24

SEQ ID NO: 89      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 89
ggcggcggtt atgtccttcg                                20

SEQ ID NO: 90      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 90
ccattcagcc agatcggtat c                                21

SEQ ID NO: 91      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
misc_feature     1..23
note = PRIMER/PROBE
source           1..23
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 91
agcttctata tcctgggtgc gcg                                23

SEQ ID NO: 92      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE

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source          1..22
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 92
aacttcaca ggtgtgctgg gt                                22

SEQ ID NO: 93      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 93
ccgtacgcat actggcttgc c                                21

SEQ ID NO: 94      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 94
aaaccggggcg atatgcgtct gtat                                24

SEQ ID NO: 95      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 95
gtatcgccgt ctatgttctgc                                20

SEQ ID NO: 96      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE
source           1..22
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 96
ccttgaatga gctgcacagt gg                                22

SEQ ID NO: 97      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
misc_feature     1..23
note = PRIMER/PROBE
source           1..23
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 97
tcgtcgccga accattcgct aaa                                23

SEQ ID NO: 98      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 98
gtttgatcgat cagggatggc                                20

SEQ ID NO: 99      moltype = DNA  length = 18
FEATURE          Location/Qualifiers
misc_feature     1..18
note = PRIMER/PROBE
source           1..18
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 99
ggcgaaaatgc aggctgtg                                18

SEQ ID NO: 100     moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24

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source          note = PRIMER/PROBE
               1..24
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 100
catcaggaca agatggcggtatg                                24

SEQ ID NO: 101      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 101
gctgctcaag gaggcacaggat                                     21

SEQ ID NO: 102      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE
source           1..22
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 102
cacattgaca taggtgtggt gc                                    22

SEQ ID NO: 103      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 103
aggatggcaa ggcccaactat ttca                                24

SEQ ID NO: 104      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 104
aacagcctca gcagccgggtta a                                    21

SEQ ID NO: 105      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 105
ttccgcgccaa tcatccctag c                                    21

SEQ ID NO: 106      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 106
agccattacg ttccagagtt gcgt                                24

SEQ ID NO: 107      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 107
gccgaggctt acgggatcaa g                                     21

SEQ ID NO: 108      moltype = DNA  length = 21
FEATURE          Location/Qualifiers

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misc_feature      1..21
source           note = PRIMER/PROBE
                1..21
                mol_type = other DNA
                organism = synthetic construct
SEQUENCE: 108
caaagcgcgt aaccggattg g                                21

SEQ ID NO: 109      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 109
tctgctgaag tttrycgagg cmaa                            24

SEQ ID NO: 110      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE
source           1..22
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 110
aactttcaca ggtgtgctgg gt                               22

SEQ ID NO: 111      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 111
ccgtacgcat actggctttc c                                21

SEQ ID NO: 112      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 112
aaaccggggcg atatgcgtct gtat                            24

SEQ ID NO: 113      moltype = DNA  length = 29
FEATURE          Location/Qualifiers
misc_feature     1..29
note = PRIMER/PROBE
source           1..29
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 113
ctgggttcta taagtaaac cttcacccgg                         29

SEQ ID NO: 114      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 114
ttccactgc ggctgccagt t                                21

SEQ ID NO: 115      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
misc_feature     1..23
note = PRIMER/PROBE
source           1..23
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 115
gatgccattg cycgsggtga aat                             23

SEQ ID NO: 116      moltype = DNA  length = 23

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FEATURE	Location/Qualifiers
misc_feature	1..23
	note = PRIMER/PROBE
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 116	
ccgaaggcta tggcgtaaaa tcc	23
SEQ ID NO: 117	moltype = DNA length = 19
FEATURE	Location/Qualifiers
misc_feature	1..19
	note = PRIMER/PROBE
source	1..19
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 117	
gcaatgcctt gctggagcg	19
SEQ ID NO: 118	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = PRIMER/PROBE
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 118	
atgttggctt gaacccagcg	20
SEQ ID NO: 119	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = PRIMER/PROBE
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 119	
agcacataca gaatatgtcc ctgc	24
SEQ ID NO: 120	moltype = DNA length = 25
FEATURE	Location/Qualifiers
misc_feature	1..25
	note = PRIMER/PROBE
source	1..25
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 120	
acctgttaac caacctactt gaggg	25
SEQ ID NO: 121	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = PRIMER/PROBE
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 121	
ttgcaagacg gactggctta gacc	24
SEQ ID NO: 122	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = PRIMER/PROBE
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 122	
cctgatcgaa ttggagaacc	20
SEQ ID NO: 123	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = PRIMER/PROBE
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 123	
ctacctcttg aataggcgta acc	23

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SEQ ID NO: 124      moltype = DNA  length = 24
FEATURE
misc_feature        Location/Qualifiers
1..24
note = PRIMER/PROBE
source
1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 124
acgtcgcgca agttcctgtat agac                                24

SEQ ID NO: 125      moltype = DNA  length = 25
FEATURE
misc_feature        Location/Qualifiers
1..25
note = PRIMER/PROBE
source
1..25
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 125
tagtgactgc taatccaaat cacag                                25

SEQ ID NO: 126      moltype = DNA  length = 25
FEATURE
misc_feature        Location/Qualifiers
1..25
note = PRIMER/PROBE
source
1..25
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 126
gcacgagcaa gatcattacc atagc                                25

SEQ ID NO: 127      moltype = DNA  length = 27
FEATURE
misc_feature        Location/Qualifiers
1..27
note = PRIMER/PROBE
source
1..27
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 127
agttagtccaa caaggccaaa ctcaaca                                27

SEQ ID NO: 128      moltype = DNA  length = 20
FEATURE
misc_feature        Location/Qualifiers
1..20
note = PRIMER/PROBE
source
1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 128
aatcacaggg cgtagttgt                                20

SEQ ID NO: 129      moltype = DNA  length = 20
FEATURE
misc_feature        Location/Qualifiers
1..20
note = PRIMER/PROBE
source
1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 129
acccaccaggc caatctttagg                                20

SEQ ID NO: 130      moltype = DNA  length = 24
FEATURE
misc_feature        Location/Qualifiers
1..24
note = PRIMER/PROBE
source
1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 130
tagcttgatc gccctcgatt tggg                                24

SEQ ID NO: 131      moltype = DNA  length = 18
FEATURE
misc_feature        Location/Qualifiers
1..18
note = PRIMER/PROBE
source
1..18
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 131
gtggggatgga aagccacg                                18

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SEQ ID NO: 132      moltype = DNA length = 18
FEATURE
misc_feature
source
SEQUENCE: 132      cacttgcggg tctacagc          18

SEQ ID NO: 133      moltype = DNA length = 24
FEATURE
misc_feature
source
SEQUENCE: 133      ttactttggg cgaaggccatg caag          24

SEQ ID NO: 134      moltype = DNA length = 21
FEATURE
misc_feature
source
SEQUENCE: 134      cacctatggt aatgcttgc c          21

SEQ ID NO: 135      moltype = DNA length = 20
FEATURE
misc_feature
source
SEQUENCE: 135      ctggaactgc tgacaatgcc          20

SEQ ID NO: 136      moltype = DNA length = 30
FEATURE
misc_feature
source
SEQUENCE: 136      tgggagaaag atatgacttt aggtgaggca          30

SEQ ID NO: 137      moltype = DNA length = 18
FEATURE
misc_feature
source
SEQUENCE: 137      ccgtgtatgt tcagctat          18

SEQ ID NO: 138      moltype = DNA length = 18
FEATURE
misc_feature
source
SEQUENCE: 138      ctttatccatc acgccttt          18

SEQ ID NO: 139      moltype = DNA length = 26
FEATURE
misc_feature
source
SEQUENCE: 139

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tatgatgtcg ataccggccaa atacca	26
SEQ ID NO: 140 moltype = DNA length = 18	
FEATURE Location/Qualifiers	
misc_feature 1..18	
source note = PRIMER/PROBE	
SEQUENCE: 140 1..18	
mol_type = other DNA	
organism = synthetic construct	
ctgtatgtca gcgcgtatcat	18
SEQ ID NO: 141 moltype = DNA length = 19	
FEATURE Location/Qualifiers	
misc_feature 1..19	
source note = PRIMER/PROBE	
SEQUENCE: 141 1..19	
mol_type = other DNA	
organism = synthetic construct	
SEQUENCE: 142 gatgccagg tgcttatcc	19
SEQ ID NO: 142 moltype = DNA length = 25	
FEATURE Location/Qualifiers	
misc_feature 1..25	
source note = PRIMER/PROBE	
SEQUENCE: 142 1..25	
mol_type = other DNA	
organism = synthetic construct	
aagtctgggt gagaacgggtc tctat	25
SEQ ID NO: 143 moltype = DNA length = 19	
FEATURE Location/Qualifiers	
misc_feature 1..19	
source note = PRIMER/PROBE	
SEQUENCE: 143 1..19	
mol_type = other DNA	
organism = synthetic construct	
cagtcagttat gcgagtttc	19
SEQ ID NO: 144 moltype = DNA length = 18	
FEATURE Location/Qualifiers	
misc_feature 1..18	
source note = PRIMER/PROBE	
SEQUENCE: 144 1..18	
mol_type = other DNA	
organism = synthetic construct	
aaaattcgcc aagccatc	18
SEQ ID NO: 145 moltype = DNA length = 25	
FEATURE Location/Qualifiers	
misc_feature 1..25	
source note = PRIMER/PROBE	
SEQUENCE: 145 1..25	
mol_type = other DNA	
organism = synthetic construct	
tgcataagcc agtgcgtttt tataat	25
SEQ ID NO: 146 moltype = DNA length = 18	
FEATURE Location/Qualifiers	
misc_feature 1..18	
source note = PRIMER/PROBE	
SEQUENCE: 146 1..18	
mol_type = other DNA	
organism = synthetic construct	
agatcagttg gggtgcacg	18
SEQ ID NO: 147 moltype = DNA length = 20	
FEATURE Location/Qualifiers	
misc_feature 1..20	
source note = PRIMER/PROBE	
SEQUENCE: 147 1..20	
mol_type = other DNA	
organism = synthetic construct	

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SEQUENCE: 147
tgcttaatca gtgaggcacc                                         20

SEQ ID NO: 148          moltype = DNA  length = 24
FEATURE               Location/Qualifiers
misc_feature          1..24
note = PRIMER/PROBE
source                1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 148
atgaagccat accaaacgac gaga                                         24

SEQ ID NO: 149          moltype = DNA  length = 20
FEATURE               Location/Qualifiers
misc_feature          1..20
note = PRIMER/PROBE
source                1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 149
ctggagcgaa agatccacta                                         20

SEQ ID NO: 150          moltype = DNA  length = 18
FEATURE               Location/Qualifiers
misc_feature          1..18
note = PRIMER/PROBE
source                1..18
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 150
atcgtccacc atccactg                                         18

SEQ ID NO: 151          moltype = DNA  length = 22
FEATURE               Location/Qualifiers
misc_feature          1..22
note = PRIMER/PROBE
source                1..22
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 151
ccagatcggc gacaacgtca cc                                         22

SEQ ID NO: 152          moltype = DNA  length = 21
FEATURE               Location/Qualifiers
misc_feature          1..21
note = PRIMER/PROBE
source                1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 152
tggccagaac tgacaggcaa a                                         21

SEQ ID NO: 153          moltype = DNA  length = 21
FEATURE               Location/Qualifiers
misc_feature          1..21
note = PRIMER/PROBE
source                1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 153
tttcctctga acgtggctgg c                                         21

SEQ ID NO: 154          moltype = DNA  length = 24
FEATURE               Location/Qualifiers
misc_feature          1..24
note = PRIMER/PROBE
source                1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 154
acgctaactc cagcattggc ctgt                                         24

SEQ ID NO: 155          moltype = DNA  length = 19
FEATURE               Location/Qualifiers
misc_feature          1..19
note = PRIMER/PROBE
source                1..19
mol_type = other DNA

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SEQUENCE: 155 ccgtcacgct gttgttagg	organism = synthetic construct	
SEQ ID NO: 156 FEATURE misc_feature source	moltype = DNA length = 22 Location/Qualifiers 1..22 note = PRIMER/PROBE 1..22 mol_type = other DNA organism = synthetic construct	19
SEQUENCE: 156 gctgtgttaa tcaatgccac ac		22
SEQ ID NO: 157 FEATURE misc_feature source	moltype = DNA length = 24 Location/Qualifiers 1..24 note = PRIMER/PROBE 1..24 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 157 aacttgcga attagagcrg cagt		24
SEQ ID NO: 158 FEATURE misc_feature source	moltype = DNA length = 19 Location/Qualifiers 1..19 note = PRIMER/PROBE 1..19 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 158 cgtttcgtct ggatcgcac		19
SEQ ID NO: 159 FEATURE misc_feature source	moltype = DNA length = 20 Location/Qualifiers 1..20 note = PRIMER/PROBE 1..20 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 159 gctgggtaaa ataggtcacc		20
SEQ ID NO: 160 FEATURE misc_feature source	moltype = DNA length = 24 Location/Qualifiers 1..24 note = PRIMER/PROBE 1..24 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 160 tatcatttgt ggtgccgtag tcgc		24
SEQ ID NO: 161 FEATURE misc_feature source	moltype = DNA length = 20 Location/Qualifiers 1..20 note = PRIMER/PROBE 1..20 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 161 gagaggatga ycagccacac		20
SEQ ID NO: 162 FEATURE misc_feature source	moltype = DNA length = 20 Location/Qualifiers 1..20 note = PRIMER/PROBE 1..20 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 162 cgccccattgt scaatattcc		20
SEQ ID NO: 163 FEATURE misc_feature source	moltype = DNA length = 24 Location/Qualifiers 1..24 note = PRIMER/PROBE 1..24	

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mol_type = other DNA
organism = synthetic construct

SEQUENCE: 163
tgagacacgg tccagactcc tacg                                24
moltype = DNA length = 20
Location/Qualifiers
1..20
note = PRIMER/PROBE
1..20
mol_type = other DNA
organism = synthetic construct

SEQ ID NO: 164      moltype = DNA length = 20
FEATURE
misc_feature        Location/Qualifiers
1..20
note = PRIMER/PROBE
1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 164
aatcacaggc cgttagttgtg                                         20
moltype = DNA length = 20
Location/Qualifiers
1..20
note = PRIMER/PROBE
1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 165
acccaccaggc caatctttagg                                         20
moltype = DNA length = 24
Location/Qualifiers
1..24
note = PRIMER/PROBE
1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 166
tagcttgcatt gccctcgatt tggg                                 24
moltype = DNA length = 21
Location/Qualifiers
1..21
note = PRIMER/PROBE
1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 167
gcggagttaa ctatggcta g                                         21
moltype = DNA length = 21
Location/Qualifiers
1..21
note = PRIMER/PROBE
1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 168
ggccaagctt ctatatttgc g                                         21
moltype = DNA length = 23
Location/Qualifiers
1..23
note = PRIMER/PROBE
1..23
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 169
tttttygggt gttgyttttaa                                         23
moltype = DNA length = 21
Location/Qualifiers
1..21
note = PRIMER/PROBE
1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 170
gcggagttar ytattggcta g                                         21
moltype = DNA length = 21
Location/Qualifiers
1..21
note = PRIMER/PROBE
1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 171

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source          1..21
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 171
ggccaagcyt ctawattgc g                                21

SEQ ID NO: 172      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source          1..24
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 172
ccggacggtc ttggtaattt gggt                                24

SEQ ID NO: 173      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source          1..24
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 173
ccgtacggtt taggcaattt gggt                                24

SEQ ID NO: 174      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source          1..20
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 174
ggcgccgttg atgtccttcg                                20

SEQ ID NO: 175      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source          1..21
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 175
ccattcagcc agatccggcat c                                21

SEQ ID NO: 176      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
misc_feature     1..23
note = PRIMER/PROBE
source          1..23
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 176
agctttcta tcctgggtct gcg                                23

SEQ ID NO: 177      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source          1..20
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 177
gagaggatga ycagccacac                                20

SEQ ID NO: 178      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source          1..20
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 178
cgccccattgt scaatattcc                                20

SEQ ID NO: 179      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24

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source          note = PRIMER/PROBE
               1..24
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 179
tgagacacgg tccagactcc tacg                                24

SEQ ID NO: 180      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE
source           1..22
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 180
aactttcaca ggtgtgctgg gt                                22

SEQ ID NO: 181      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature     1..21
note = PRIMER/PROBE
source           1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 181
ccgtacgcat actgggttttgc                                21

SEQ ID NO: 182      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 182
aaaccggggc atatcggtct gtat                                24

SEQ ID NO: 183      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 183
gtatcgccgt cttagttctgc                                20

SEQ ID NO: 184      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
misc_feature     1..22
note = PRIMER/PROBE
source           1..22
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 184
ccttgaatga gctgcacagt gg                                22

SEQ ID NO: 185      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
misc_feature     1..23
note = PRIMER/PROBE
source           1..23
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 185
tcgtcgccga accattcgct aaa                                23

SEQ ID NO: 186      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 186
gtttgatcgt cagggatggc                                20

SEQ ID NO: 187      moltype = DNA  length = 18
FEATURE          Location/Qualifiers

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misc_feature	1..18	
source	note = PRIMER/PROBE	
	1..18	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 187		
ggcgaaagtc aggctgtg		18
SEQ ID NO: 188	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
source	note = PRIMER/PROBE	
	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 188		
catcaggaca agatggccgg tatg		24
SEQ ID NO: 189	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
source	note = PRIMER/PROBE	
	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 189		
gagaggatga ycagccacac		20
SEQ ID NO: 190	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
source	note = PRIMER/PROBE	
	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 190		
cggccattgt scaatattcc		20
SEQ ID NO: 191	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
source	note = PRIMER/PROBE	
	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 191		
ttagagacacgg tccagactcc tacg		24
SEQ ID NO: 192	moltype = DNA length = 21	
FEATURE	Location/Qualifiers	
misc_feature	1..21	
source	note = PRIMER/PROBE	
	1..21	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 192		
gctgctcaag gagcacacagg t		21
SEQ ID NO: 193	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
misc_feature	1..22	
source	note = PRIMER/PROBE	
	1..22	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 193		
cacattgaca taggtgtggc gc		22
SEQ ID NO: 194	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
source	note = PRIMER/PROBE	
	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 194		
aggatggcaa ggcccaactat ttca		24
SEQ ID NO: 195	moltype = DNA length = 21	

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FEATURE          Location/Qualifiers
misc_feature    1..21
                  note = PRIMER/PROBE
source          1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 195
aacagcctca gcagccgggtt a                                21

SEQ ID NO: 196      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature    1..21
                  note = PRIMER/PROBE
source          1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 196
ttcgccgcaa tcatccctag c                                21

SEQ ID NO: 197      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature    1..24
                  note = PRIMER/PROBE
source          1..24
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 197
agccattacg ttccagagtt gcgt                                24

SEQ ID NO: 198      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature    1..21
                  note = PRIMER/PROBE
source          1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 198
gccgaggctt acgggatcaa g                                21

SEQ ID NO: 199      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
misc_feature    1..21
                  note = PRIMER/PROBE
source          1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 199
caaagcgcgt aaccggatttg g                                21

SEQ ID NO: 200      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature    1..24
                  note = PRIMER/PROBE
source          1..24
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 200
tctgctgaag tttrycagg cmaa                                24

SEQ ID NO: 201      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature    1..20
                  note = PRIMER/PROBE
source          1..20
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 201
gagaggatga ycagccacac                                20

SEQ ID NO: 202      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature    1..20
                  note = PRIMER/PROBE
source          1..20
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 202
cgccccattgt scaatattcc                                20

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SEQ ID NO: 203      moltype = DNA  length = 24
FEATURE           Location/Qualifiers
misc_feature      1..24
source            note = PRIMER/PROBE
                  1..24
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 203
ttagacacgg tccagactcc tacg                                24

SEQ ID NO: 204      moltype = DNA  length = 22
FEATURE           Location/Qualifiers
misc_feature      1..22
source            note = PRIMER/PROBE
                  1..22
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 204
aactttcaca ggtgtgtctgg gt                                22

SEQ ID NO: 205      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
misc_feature      1..21
source            note = PRIMER/PROBE
                  1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 205
ccgtacgcat actggctttc c                                21

SEQ ID NO: 206      moltype = DNA  length = 24
FEATURE           Location/Qualifiers
misc_feature      1..24
source            note = PRIMER/PROBE
                  1..24
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 206
aaaccggggcg atatgcgtct gtat                                24

SEQ ID NO: 207      moltype = DNA  length = 29
FEATURE           Location/Qualifiers
misc_feature      1..29
source            note = PRIMER/PROBE
                  1..29
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 207
ctgggttcta taagtaaaac cttcacccg                                29

SEQ ID NO: 208      moltype = DNA  length = 21
FEATURE           Location/Qualifiers
misc_feature      1..21
source            note = PRIMER/PROBE
                  1..21
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 208
cttccactgc ggctgccagt t                                21

SEQ ID NO: 209      moltype = DNA  length = 23
FEATURE           Location/Qualifiers
misc_feature      1..23
source            note = PRIMER/PROBE
                  1..23
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 209
gatgccattg cycgsaggta aat                                23

SEQ ID NO: 210      moltype = DNA  length = 23
FEATURE           Location/Qualifiers
misc_feature      1..23
source            note = PRIMER/PROBE
                  1..23
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 210
ccgaaggccta tggcgtgaaa tcc                                23

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SEQ ID NO: 211      moltype = DNA  length = 19
FEATURE
misc_feature        Location/Qualifiers
1..19
note = PRIMER/PROBE
1..19
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 211
gcaatgcctt gctggagcg                                         19

SEQ ID NO: 212      moltype = DNA  length = 20
FEATURE
misc_feature        Location/Qualifiers
1..20
note = PRIMER/PROBE
1..20
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 212
atgttggcctt gaacccagcg                                         20

SEQ ID NO: 213      moltype = DNA  length = 20
FEATURE
misc_feature        Location/Qualifiers
1..20
note = PRIMER/PROBE
1..20
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 213
gagaggatgtt ycagccacac                                         20

SEQ ID NO: 214      moltype = DNA  length = 20
FEATURE
misc_feature        Location/Qualifiers
1..20
note = PRIMER/PROBE
1..20
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 214
cgccccattttt scaatattcc                                         20

SEQ ID NO: 215      moltype = DNA  length = 24
FEATURE
misc_feature        Location/Qualifiers
1..24
note = PRIMER/PROBE
1..24
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 215
tgagacacgg tccagactcc tacg                                         24

SEQ ID NO: 216      moltype = DNA  length = 24
FEATURE
misc_feature        Location/Qualifiers
1..24
note = PRIMER/PROBE
1..24
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 216
agcacataca gaatatgtcc ctgc                                         24

SEQ ID NO: 217      moltype = DNA  length = 25
FEATURE
misc_feature        Location/Qualifiers
1..25
note = PRIMER/PROBE
1..25
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 217
acctgttaac caaccttactt gaggg                                         25

SEQ ID NO: 218      moltype = DNA  length = 24
FEATURE
misc_feature        Location/Qualifiers
1..24
note = PRIMER/PROBE
1..24
source
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 218

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ttgcaagacg gactggctta gacc	24
SEQ ID NO: 219 moltype = DNA length = 20	
FEATURE Location/Qualifiers	
misc_feature 1..20	
source note = PRIMER/PROBE	
SEQUENCE: 219 1..20	
ctctgatcgga ttggagaacc	20
SEQ ID NO: 220 moltype = DNA length = 23	
FEATURE Location/Qualifiers	
misc_feature 1..23	
source note = PRIMER/PROBE	
SEQUENCE: 220 1..23	
cttacaccttg aataggcgta acc	23
SEQ ID NO: 221 moltype = DNA length = 24	
FEATURE Location/Qualifiers	
misc_feature 1..24	
source note = PRIMER/PROBE	
SEQUENCE: 221 1..24	
acgttcgcga agttcctgat agac	24
SEQ ID NO: 222 moltype = DNA length = 25	
FEATURE Location/Qualifiers	
misc_feature 1..25	
source note = PRIMER/PROBE	
SEQUENCE: 222 1..25	
tagtgactgc taatccaaat cacag	25
SEQ ID NO: 223 moltype = DNA length = 25	
FEATURE Location/Qualifiers	
misc_feature 1..25	
source note = PRIMER/PROBE	
SEQUENCE: 223 1..25	
gcacgagcaa gatcattacc atagc	25
SEQ ID NO: 224 moltype = DNA length = 27	
FEATURE Location/Qualifiers	
misc_feature 1..27	
source note = PRIMER/PROBE	
SEQUENCE: 224 1..27	
agtttatccaa caaggccaaa ctcaaca	27
SEQ ID NO: 225 moltype = DNA length = 20	
FEATURE Location/Qualifiers	
misc_feature 1..20	
source note = PRIMER/PROBE	
SEQUENCE: 225 1..20	
gagaggatga ycagccacac	20
SEQ ID NO: 226 moltype = DNA length = 20	
FEATURE Location/Qualifiers	
misc_feature 1..20	
source note = PRIMER/PROBE	
SEQUENCE: 226 1..20	
moltype = other DNA	
organism = synthetic construct	

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SEQUENCE: 226	
cggccattgt scaatattcc	20
SEQ ID NO: 227	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = PRIMER/PROBE
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 227	
ttagacacgg tccagactcc tacg	24
SEQ ID NO: 228	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = PRIMER/PROBE
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 228	
aatcacaggg cgttagttgtg	20
SEQ ID NO: 229	moltype = DNA length = 20
FEATURE	Location/Qualifiers
misc_feature	1..20
	note = PRIMER/PROBE
source	1..20
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 229	
accaccaggc caatctttagg	20
SEQ ID NO: 230	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = PRIMER/PROBE
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 230	
tagcttgcatt gccctcgatt tggg	24
SEQ ID NO: 231	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
	note = PRIMER/PROBE
source	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 231	
gtgggatgga aagccacg	18
SEQ ID NO: 232	moltype = DNA length = 18
FEATURE	Location/Qualifiers
misc_feature	1..18
	note = PRIMER/PROBE
source	1..18
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 232	
cacttgcggg tctacagc	18
SEQ ID NO: 233	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = PRIMER/PROBE
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 233	
ttactttggg cgaagccatg caag	24
SEQ ID NO: 234	moltype = DNA length = 21
FEATURE	Location/Qualifiers
misc_feature	1..21
	note = PRIMER/PROBE
source	1..21
	mol_type = other DNA

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organism = synthetic construct
SEQUENCE: 234
cacctatggt aatgctttc c                                21
SEQ ID NO: 235      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 235
ctggaaactgc tgacaatgcc                                20
SEQ ID NO: 236      moltype = DNA  length = 30
FEATURE          Location/Qualifiers
misc_feature     1..30
note = PRIMER/PROBE
source           1..30
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 236
tgggagaaaag atatgacttt aggtgaggca                                30
SEQ ID NO: 237      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 237
gagaggatga ycagccacac                                20
SEQ ID NO: 238      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 238
cgccccattgt scaatattcc                                20
SEQ ID NO: 239      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 239
tgagacacgg tccagactcc tacg                                24
SEQ ID NO: 240      moltype = DNA  length = 18
FEATURE          Location/Qualifiers
misc_feature     1..18
note = PRIMER/PROBE
source           1..18
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 240
agatcagtgc ggtgcacg                                18
SEQ ID NO: 241      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source           1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 241
tgcttaatca gtgaggcacc                                20
SEQ ID NO: 242      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source           1..24

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	mol_type = other DNA organism = synthetic construct	
SEQUENCE: 242		
atgaagccat accaaacgac gagc		24
SEQ ID NO: 243	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
source	note = PRIMER/PROBE	
	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 243		
ctggagcgaa agatccacta		20
SEQ ID NO: 244	moltype = DNA length = 18	
FEATURE	Location/Qualifiers	
misc_feature	1..18	
source	note = PRIMER/PROBE	
	1..18	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 244		
atcgtccacc atccactg		18
SEQ ID NO: 245	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
misc_feature	1..22	
source	note = PRIMER/PROBE	
	1..22	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 245		
ccagatccgc gacaacgtca cc		22
SEQ ID NO: 246	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
source	note = PRIMER/PROBE	
	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 246		
gagaggatgtg ycagccacac		20
SEQ ID NO: 247	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	
source	note = PRIMER/PROBE	
	1..20	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 247		
cggccatttg scaatattcc		20
SEQ ID NO: 248	moltype = DNA length = 24	
FEATURE	Location/Qualifiers	
misc_feature	1..24	
source	note = PRIMER/PROBE	
	1..24	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 248		
tgagacacggg tccagactcc tacg		24
SEQ ID NO: 249	moltype = DNA length = 18	
FEATURE	Location/Qualifiers	
misc_feature	1..18	
source	note = PRIMER/PROBE	
	1..18	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 249		
ccgtgtatgt tcagctat		18
SEQ ID NO: 250	moltype = DNA length = 18	
FEATURE	Location/Qualifiers	
misc_feature	1..18	
source	note = PRIMER/PROBE	

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source	1..18	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 250		
cttataccatc acgccttt		18
SEQ ID NO: 251	moltype = DNA length = 26	
FEATURE	Location/Qualifiers	
misc_feature	1..26	
	note = PRIMER/PROBE	
source	1..26	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 251		
atatgtatcg ataccgccaa atacca		26
SEQ ID NO: 252	moltype = DNA length = 18	
FEATURE	Location/Qualifiers	
misc_feature	1..18	
	note = PRIMER/PROBE	
source	1..18	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 252		
ctgttatgtca gcgatcat		18
SEQ ID NO: 253	moltype = DNA length = 19	
FEATURE	Location/Qualifiers	
misc_feature	1..19	
	note = PRIMER/PROBE	
source	1..19	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 253		
gatgccagtt tgcttatacc		19
SEQ ID NO: 254	moltype = DNA length = 25	
FEATURE	Location/Qualifiers	
misc_feature	1..25	
	note = PRIMER/PROBE	
source	1..25	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 254		
aagtctgggt gagaacgggt tctat		25
SEQ ID NO: 255	moltype = DNA length = 19	
FEATURE	Location/Qualifiers	
misc_feature	1..19	
	note = PRIMER/PROBE	
source	1..19	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 255		
cagtcaagtat gcgagtttc		19
SEQ ID NO: 256	moltype = DNA length = 18	
FEATURE	Location/Qualifiers	
misc_feature	1..18	
	note = PRIMER/PROBE	
source	1..18	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 256		
aaaatttcgccc aagccatc		18
SEQ ID NO: 257	moltype = DNA length = 25	
FEATURE	Location/Qualifiers	
misc_feature	1..25	
	note = PRIMER/PROBE	
source	1..25	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 257		
tgcataaagcc agtgcgtttt tatat		25
SEQ ID NO: 258	moltype = DNA length = 20	
FEATURE	Location/Qualifiers	
misc_feature	1..20	

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source          note = PRIMER/PROBE
               1..20
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 258
gagaggatga ycagccacac                                20

SEQ ID NO: 259      moltype = DNA  length = 20
FEATURE          Location/Qualifiers
misc_feature     1..20
note = PRIMER/PROBE
source          1..20
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 259
cgccccatttgt scaatatattcc                                20

SEQ ID NO: 260      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = PRIMER/PROBE
source          1..24
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 260
tgagacacgg tccagactcc tacg                                24

SEQ ID NO: 261      moltype = DNA  length = 462
FEATURE          Location/Qualifiers
misc_feature     1..462
note = CONTROL MIX
source          1..462
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 261
tggcccagaac tgacaggcaa acagtggcag ggtatccgcg tgctgcacct agccacctat 60
acggcaggcg gcctaccgcgct gcagatcccc gatgacgta gggataaagc cgcattactg 120
catttttatc aaaaactggca gcccgaatgg actccggggcg ctaagcgact ttacgctaac 180
tccagcattg tgctgtttgg cgcgcgtggcg gtgaaaccct caggaatgag ttacgaagag 240
gcaatgacca gacgcgtccct gcaaccatta aaactggcgc atacctggat tacggttccg 300
cagaacgaaac aaaaagatta tgcctggggc tatcgcaag ggaagcccgt acacgttct 360
ccgggacaac ttgacgcccga agcctatggc gtgaaatcca gcgttattga tatggccgc 420
tgggttcagg ccaacatggc tgccagccac gttcaggaga aa                                462

SEQ ID NO: 262      moltype = DNA  length = 121
FEATURE          Location/Qualifiers
misc_feature     1..121
note = CONTROL MIX
source          1..121
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 262
ccgtcacgct gttgttagga agtgtgccgc tggatgcgcg aacggccgac gtacagcaaa 60
aacttgcgcg attagagccgg cagtcgggag cagactggg tggatgcgcg attaacacag 120
c                                121

SEQ ID NO: 263      moltype = DNA  length = 321
FEATURE          Location/Qualifiers
misc_feature     1..321
note = CONTROL MIX
source          1..321
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 263
cgtttcgtct ggatgcact gaacctacgc tgaataccgc cattccggc gacccgagag 60
acaccaccac gccgcgggccc atggcgcaga cgttgcgtca gcttacgcgt ggtcatgcgc 120
tgggcgaaac ccagcggggcg cagttggcg cgttgcgtca aggcaatggc accggccgc 180
ccagcattcg ggcggccgt cccagtcgt ggactgtggg tgataagacc ggcagcggcg 240
actacggcacc cacaatgtat attggcgat tctggccgcg gggctgtgcg ccgtgtttc 300
tgggtgaccta ttttacccag c                                321

SEQ ID NO: 264      moltype = DNA  length = 84
FEATURE          Location/Qualifiers
misc_feature     1..84
note = CONTROL MIX
source          1..84
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 264

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gagaggatga ccagccacac tggactgtag acacggtcca gactcctacg ggaggcagca
 gtgggaaata ttgcacaatg ggcg 60
 84

SEQ ID NO: 265 moltype = DNA length = 553
FEATURE Location/Qualifiers
misc_feature 1..553
source note = CONTROL MIX
 1..553
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 265
 aatcacaggc cgtagttgtg ctctggaatg agaataagca gcaaggattt accaataatc
 taaaacgggc gaaccaagca ttttacccg catctaccc taaaattccc aatagtttg
 tcggccctoga ttggggcggt gttaggatg aacaccaagt cttaagtgg gatggacaga
 cggcgatata cgccacttgg aatcgcgatc ataatctaatac caccggatg aaatattcg
 ttgtgcgtgt ttatcaagaa ttgtccggcc aaattggga ggcacatgtt agcaatgtc
 tacatcggtt cgattatgtt aatggaggaca ttccgggcaaa ttgtacatgtt ttctggctcg
 acgggtgtat tcgaatttcg gccacgggaa aatcatgtt tttaagaag ctgtatcaca
 ataagttaca cgtatcgag cgcagccagc gtattgtcaa acaaggatcg ctgaccgaag
 ccaatgggttataattttt cgggttaaaa ctggataactt gactagaatc gaaccttaaga
 ttggcgttgtt ggt 553

SEQ ID NO: 266 moltype = DNA length = 175
FEATURE Location/Qualifiers
misc_feature 1..175
source note = CONTROL MIX
 1..175
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 266
 gcggaggttag ttattggctta gttaaaaata aaattgaagt tttttatccc gggccccggc
 acactcaaga taacgtatgtt gttttgttac ctgaaaagaa aattttatcc ggtgggttgg
 ttgtttaacc ggacggttt ggttaatttgg gtgacgcaaa tttagaaagct tggcc 60
 120
 175

SEQ ID NO: 267 moltype = DNA length = 297
FEATURE Location/Qualifiers
misc_feature 1..297
source note = CONTROL MIX
 1..297
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 267
 ggccgcgtt atgtcccttcg ggcggctggg gtggcaacgt acgcacatcacc gtcgcacacgc
 cggctagccg aggttagaggg gaacgagatt cccacgcact ctctagaagg actctcatcg
 agccggggac cagtgcgtt cgggtccagta gaacttctt atccctgttgc tgccatcgat
 accgcacaact tagtgtgtat cgtcccgatc gcgagtgatc tctatgttgc ttgtgcgtt
 catgagttt cgcgcacgtc tgcggggaaat gtcggccatg cgcatcgatc tgaatgg 60
 120
 180
 240
 297

SEQ ID NO: 268 moltype = DNA length = 84
FEATURE Location/Qualifiers
misc_feature 1..84
source note = CONTROL MIX
 1..84
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 268
 gagaggatga ccagccacac tggactgtag acacggtcca gactcctacg ggaggcagca
 gtgggaaata ttgcacaatg ggcg 60
 84

SEQ ID NO: 269 moltype = DNA length = 405
FEATURE Location/Qualifiers
misc_feature 1..405
source note = CONTROL MIX
 1..405
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 269
 aactttcaca ggtgtgttgcggttcc tggggggaaa aaagagatgg cgctgtatgaa
 tcggccggca aaataccagc cggagctggc tctggcccgat tggaaaggaa tcacattgt
 ggtatctgttcc acctatacccg caggggact gccgttacag gtggccatgtt cggtaaaaag
 cccgtcgatgt ctgtatgttcc tctatcggca gtggcagccg tcccgaaac cggccatgt
 ggttctgtat gcaaacagca gtatccgtt gtttgggttgc ctgaccggaa acggccgggg
 gtatccgtat gaggcgttgc tgatcgacg gatccgttca cccgtgggtt tatctcacac
 ctttattact gtggccggaaa gtgcgcacatc ccgtatcgatc tacgg 60
 120
 180
 240
 300
 360
 420
 480
 540
 553

SEQ ID NO: 270 moltype = DNA length = 209
FEATURE Location/Qualifiers
misc_feature 1..209
source note = CONTROL MIX

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cgctttg                                         247

SEQ ID NO: 276      moltype = DNA length = 84
FEATURE
misc_feature        Location/Qualifiers
1..84
note = CONTROL MIX
source              1..84
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 276
gaagaggatga ccagccacac ttgaaacttag acacggtcca gactcctacg ggaggcagca 60
gtggggata ttgcacaatg ggcg 84

SEQ ID NO: 277      moltype = DNA length = 405
FEATURE
misc_feature        Location/Qualifiers
1..405
note = CONTROL MIX
source              1..405
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 277
aacttcaca ggtgtgtcgg gtgcgggttc tggggcggaaa aaagagatgg cgctgaatga 60
tccggcgccaa aataaccaggc cggagctggc tctggcgacgg tggaaaggggatc acatggatc 120
ggatctggctt acctataccg caggcgact gcccgttacag gtggcgatg cggtaaaaag 180
ccgtgcggat ctgtcgatt tctatcggca gtggcagccg tcccgaaac cgggcgatat 240
gctgtctgtat gcaaacacgca gtatggcctt gtttgggtgtc ctgaccggaa acggcgccgg 300
gtatgcgtat gacgttgc tgactgcacg gatcctggca cccgtgggt tatctcacac 360
ctttattact gtggggaaa gtgcgcggaa ag ccagtatgcg tacgg 405

SEQ ID NO: 278      moltype = DNA length = 302
FEATURE
misc_feature        Location/Qualifiers
1..302
note = CONTROL MIX
source              1..302
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 278
tcggtaaacgc cgatgttgcg gcaacaaccc cccgttcccccc gcaaaacctg tttgagctgg 60
gctctataacg taaaaccccttc accggcgatc tggggcgccgat tgccattggcc cgggggtggaaa 120
tagcgcgtggg cgatccggta gcaaaataact ggcttgcgtt caccggcaag cagtggcagg 180
gcattcgcattt gctggatctg gcaacctata ccgcaggccg tctggcgatc caggtgcgg 240
atggggatc ggataccggcc tctctgtcgc gcttttatca aaactggcag ccgcagtgaa 300
ag 302

SEQ ID NO: 279      moltype = DNA length = 106
FEATURE
misc_feature        Location/Qualifiers
1..106
note = CONTROL MIX
source              1..106
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 279
ccgaaggcta tggcggtaaa tccagcgatc ttgatatggc ccgtgggtt caggccaaca 60
tggatgcgcac acgttgcg gaaaaacgc tccagcaggg catatgc 106

SEQ ID NO: 280      moltype = DNA length = 84
FEATURE
misc_feature        Location/Qualifiers
1..84
note = CONTROL MIX
source              1..84
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 280
gaagaggatga ccagccacac ttgaaacttag acacggtcca gactcctacg ggaggcagca 60
gtggggata ttgcacaatg ggcg 84

SEQ ID NO: 281      moltype = DNA length = 487
FEATURE
misc_feature        Location/Qualifiers
1..487
note = CONTROL MIX
source              1..487
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 281
agcacatata gaatatgtcc ctgcataac attaatggat cttaaatgcgt taattggact 60
agaaaaatcat aaagctacaa caactggat tttcaatgg gacggtaaaa agatctta 120
tcccatgtgg gaaaaatggat tgactttggat tgatgcgtt gacacttcgt cagttccgt 180
atatacaagaa ctgcacacg ggactggctt agacctaatacg caaaaagaag taaaacgggt 240
tgggttttgtt aatatacata ttggaaacaca agttgataac ttctgggttgg tggccccct 300

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caagattaca ccaataacaag aggttaattt tgccgatgat tttgcaaata atcgattacc 360
 cttaaatta gagactcaag aagaaggtaaaaatgctt ctgattaaag aattcatgg 420
 tagtaaaattt tatgcaaaaa gccccgtgggg aatggatgta acccctcaag taggttggtt 480
 aacaggt 487

SEQ ID NO: 282 moltype = DNA length = 278
 FEATURE Location/Qualifiers
 misc_feature 1..278
 note = CONTROL MIX
 source 1..278
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 282
 ctgtatcgga ttggagaacc agaaaaacgga tattaatgaa atatttaat ggaagggcga 60
 gaaaaaggta tttaccgctt gggaaaaga catgacacta ggagaagcca tgaagcttc 120
 tgcagtccca gtctatcagg aacttgcgcg acgtatcggtt ctgtatctca tgcaaaaaga 180
 agtaaaaacgtt atgggttcg gtaatgctga aattggacag caggttgata atttctgggtt 240
 ggttaggacca ttaaaggta cgcctattca agaggtag 278

SEQ ID NO: 283 moltype = DNA length = 151
 FEATURE Location/Qualifiers
 misc_feature 1..151
 note = CONTROL MIX
 source 1..151
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 283
 tagtgactgc taatccaaat cacagcgctt caaaatctga tgaaaaagca gagaaaattta 60
 aaaattttttt taacgaagta cacactacgg gtgttttagt tatccaacaa ggc当地actc 120
 aacaaggcta tggtaatgat cttgtcgtg c 151

SEQ ID NO: 284 moltype = DNA length = 84
 FEATURE Location/Qualifiers
 misc_feature 1..84
 note = CONTROL MIX
 source 1..84
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 284
 gagaggatgta ccagccacac tggactgag acacggtcca gactcctacg ggaggcagca 60
 gtgggaaata ttgcacaatg ggcg 84

SEQ ID NO: 285 moltype = DNA length = 553
 FEATURE Location/Qualifiers
 misc_feature 1..553
 note = CONTROL MIX
 source 1..553
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 285
 aatcacaggc cgtagtgtt ctctggatg agaataagca gcaaggattt accaataatc 60
 ttaaacgggc gaaccaagca ttttacccg catctaccc taaaattccc aatagctga 120
 tcgcctcgta ttggcggtg gttaggatg aacaccaagt cttaatggatg gatggacaga 180
 cggcgcgatcgccacttgg aatcgccgatc ataattctaat caccgcgtatg aaatattcg 240
 ttgtgcctgtt ttatcaagaa aatggcgca ggcacgtatg agcaagatgc 300
 tacatgtttt cgattatggat aatgggaca ttccggcata ttttagacatg ttctggctcg 360
 acgggtgtat tcgaatttcg gccacggago aaatcagttt ttaagaaag ctgtatcaca 420
 ataagttaca cgtatccggat cgcagccatg gtattgtca acaagccatg ctgaccgaag 480
 ccaatggtga ctatattttt cggctaaaa ctggatactc gactagaatc gaacctaaaga 540
 ttggctgggtt ggt 553

SEQ ID NO: 286 moltype = DNA length = 376
 FEATURE Location/Qualifiers
 misc_feature 1..376
 note = CONTROL MIX
 source 1..376
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 286
 gtgggatgga aagccacgtt tttttaaagc atgggacaaa gattttactt tgggcaagc 60
 catgcaagca tctacagtgc ctgtatataca agaattggca cgtcgatattt gtccaaagctt 120
 aatgc当地gtt gatggcaac gtattggta tggcaatatg caaatagca cggaaatgtga 180
 tcaattttgg tggaaaggcc ctttgacat tacacccata caagaaggtaa agtttggta 240
 tgatggatcc caagggcaat tgccctttaa acctgaaatg cagcaacaag tgaaagagat 300
 gttgtatgta gagcgcagag gggagaatcg tcttatatgct aaaagtggct ggggaatggc 360
 tgttagacccg caagt 376

SEQ ID NO: 287 moltype = DNA length = 376
 FEATURE Location/Qualifiers

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misc_feature 1..376
 note = CONTROL MIX
source 1..376
 mol_type = other DNA
 organism = synthetic construct
SEQUENCE: 287
 cacttgcggg tctacagcca ttccccagec acttttagca tatagacgtatctccccc 60
 ggcgtctaca tacaacatct ctttcacttg ttgtgtaaact tcaggtaatc aaggcaattg 120
 cccttgggct aaatcataca caaactttac ttcttgtata ggtgttaatc tcaaaggccc 180
 ttcaaccaa aattgtatcaa cttecggtcc tatttgcataa cttccataac caatacgtt 240
 caattcactt tgcatcataa acgacgtgc aattcttgc atacaggcac 300
 tgttagatgt tgcgtatggc ttggaccat aaaaatcttgc tccatgtttaaaaaa 360
 tggcttccca tccccac 376

SEQ ID NO: 288 moltype = DNA length = 203
FEATURE Location/Qualifiers
misc_feature 1..203
 note = CONTROL MIX
source 1..203
 mol_type = other DNA
 organism = synthetic construct
SEQUENCE: 288
 caccttatgtt aatgccttgc cacgagcaaa taaaatgttccat caacattaa 60
 gatgctaat gcttaatcg ggctagaaaa tcataaaagca acaacaatg agatttca 120
 atgggatgtt aaaaaaaagaa ctatccat gtgggagaaaa gatatgactt taggtgaggc 180
 atggcatttgc ttagcgttc cag 203

SEQ ID NO: 289 moltype = DNA length = 84
FEATURE Location/Qualifiers
misc_feature 1..84
 note = CONTROL MIX
source 1..84
 mol_type = other DNA
 organism = synthetic construct
SEQUENCE: 289
 gagaggatgtt ccagccacac tggactgttgc acacggtcca gactccttgc ggaggcagca 60
 gtgggaaata ttgcacaatg ggcc 84

SEQ ID NO: 290 moltype = DNA length = 1625
FEATURE Location/Qualifiers
misc_feature 1..1625
 note = CONTROL MIX
source 1..1625
 mol_type = other DNA
 organism = synthetic construct
SEQUENCE: 290
 atgatgcgcg atacttctgt gtggtaccga cgctcggtca gtccgttgt tcttggg 60
 gtgttgcgt tttcttgcacc ggcacgcac atcttacat ttttgcataa atcagccaa 120
 cctatcccat cgccgacaat ctccgttttgc tgctgacgt cgctgtcg tcttggcg 180
 cgatgtactt gatcggcactcg ctgttcatcg ctgtatcgca tggctgttgc 240
 ttttgcattt aatcatggc ggggtgacca gttatatttc tgacacttgc ggcacggc 300
 atgatacgcg catgtccaa aatgcctac agaccgacca agccgagacc aaggatctat 360
 taaacgcgcg gtttgcattg cgtatcattt gtttgggtgt gcttcaatg ttgctgtgg 420
 cttttgcattt ggtgtttat ccgacttggg gcaagggtttt gatgcgcgcg ttggctgt 480
 tcgtggcagat tcttgcgtt atttactgc ctgtggcgtt gtcagcgtt cattatggca 540
 gtttcttgcgtt cgtgcataatg cgcgtgcgtat gctatgttca ctcgcattatgc ccaatctat 600
 cggtgggtaa gtttgcgtt atttgcattt aaaaaggccat tgcccaaaa gataccattt 660
 atcacgcacaa agacgcgcgta caagcaacca agccgtat gcttgcgcgcg cgcctagtgg 720
 ttttgcgtt ggtgtttat ccgacttggg gcaagggtttt gatgcgcgcg ttggctgt 780
 atacttccc acgatgttgc ggtatgttgc ttggcttgc gtttgcattatgc ctcacatcg 840
 ggggcacatc gacggcgtat tcttgcgtt gtttgcatttgc ttttgcattatgc ctcacatcg 900
 atgatgttgcg taccgcacaa taccatggaa atgttgcgttgc taccatggat cgcttggcg 960
 taagtatctt gtggcgtgtt aataatccg actcaaaaagg cgtatgttgc aagctgcacaa 1020
 aacgcgcattt aatccgcgcg ccaacacgcg catgttgcgcgcg ttttgcattatgc 1080
 ataacgcattt ggtatgttgc ttggcttgc gtttgcattatgc ctcacatcg 1140
 acggcaaaaatgatgttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1200
 agccatgttgc gtttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1260
 atgttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1320
 ttttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1380
 ttttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1440
 ctttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1500
 aacactggcat caccgcacaa gcaaccgcata ccgtccctgc acatgcgcgcg atcaccgcgc 1560
 cattattaaatgc taccatggcgc ttttgcattatgc ctcacatcg 1620
 gcttgcatttgc taccatggcgc ttttgcattatgc ctcacatcg 1625

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atgggtggacg at	372
SEQ ID NO: 295	moltype = DNA length = 84
FEATURE	Location/Qualifiers
misc_feature	1..84
	note = CONTROL MIX
source	1..84
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 295	
gagaggatga ccagccacac tggacttag acacggtcca gactcctacg ggaggcagca	60
gtgggaaata ttgcacaatg ggcg	84
SEQ ID NO: 296	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = Synthetic
misc_feature	1
	note = 6-FAM
misc_feature	9
	note = ZEN
misc_feature	24
	note = IABkFQ
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 296	
aggatggcaa ggccccactat ttca	24
SEQ ID NO: 297	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = Synthetic
misc_feature	1
	note = HEX
misc_feature	9
	note = ZEN
misc_feature	24
	note = IABkFQ
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 297	
agccattacg ttccagagtt gcgt	24
SEQ ID NO: 298	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = Synthetic
misc_feature	1
	note = TEX615
misc_feature	24
	note = 3IAbRQSp
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 298	
tctgctgaag tttrycgagg cmaa	24
SEQ ID NO: 299	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = Synthetic
misc_feature	1
	note = TYE665
misc_feature	24
	note = IAbRQSp
source	1..24
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 299	
tgagacacgg tccagactcc tacg	24
SEQ ID NO: 300	moltype = DNA length = 24
FEATURE	Location/Qualifiers
misc_feature	1..24
	note = Synthetic
misc_feature	1
	note = 6-FAM

-continued

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misc_feature      9
    note = ZEN
misc_feature     24
    note = IABkFQ
source          1..24
    mol_type = other DNA
    organism = synthetic construct
SEQUENCE: 300
aacaccggcg atatgcgtct gtat                               24

SEQ ID NO: 301      moltype = DNA  length = 23
FEATURE           Location/Qualifiers
misc_feature      1..23
    note = Synthetic
misc_feature      1
    note = HEX
misc_feature      9
    note = ZEN
misc_feature      23
    note = IABkFQ
source          1..23
    mol_type = other DNA
    organism = synthetic construct
SEQUENCE: 301
gatgccattg cycgsggtga aat                                23

SEQ ID NO: 302      moltype = DNA  length = 20
FEATURE           Location/Qualifiers
misc_feature      1..20
    note = Synthetic
misc_feature      1
    note = TEX615
misc_feature      20
    note = IAbRQSp
source          1..20
    mol_type = other DNA
    organism = synthetic construct
SEQUENCE: 302
atgttggcct gaacccagcg                                     20

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The invention claimed is:

1. A kit for identification of one or more β -lactamase genes, wherein the one or more β -lactamase genes are selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, and NDM genes, the kit comprising probes comprising the following sequences: ACGCTAACTCCAGCATTGGTCTGT (SEQ ID NO: 154), AACTTGCCTGAATAGAGCRGCACT (SEQ ID NO: 157), TATCATTGGTGGTGCCTAGTCGC (SEQ ID NO: 160), TGAGACACGGTCCAGACTCCTACG (SEQ ID NO: 163), TAGCTTGATGCCCTCGATTGGG (SEQ ID NO: 166), TTRRTYGGTGGTTGTTTAA (SEQ ID NO: 169), CCGGACGGTCTTGGTAATTGGGT (SEQ ID NO: 172), CCGTACGGTTAGGCAATTGGGT (SEQ ID NO: 173), AGCTCTTCTATCCTGGTGCTGCG (SEQ ID NO: 176), TGAGACACGGTCCAGACTCCTACG (SEQ ID NO: 179), AAACCGGGCGATATCGTCTGTAT (SEQ ID NO: 182), TCGTCGCGGAACCATTGCTAAA (SEQ ID NO: 185), CATCAGGACAAGATGGCGGTATG (SEQ ID NO: 188), and TGAGACACGGTCCAGACTCTACG (SEQ ID NO: 191), wherein each probe comprises a fluorophore and/or a fluorescent quencher.

2. The kit of claim 1, including an endogenous internal control.

3. The kit of claim 2, wherein the endogenous internal control targets a conserved region in Gram-negative bacteria.

4. The kit of claim 1, further comprising the following primers: TGGCCAGAACTGACAGGGCAA (SEQ ID NO: 152), TTTCTCCTGAACGTGGCTGGC (SEQ ID NO: 153), CCGTCACGCTGTTAGG (SEQ ID NO: 155),

40 GCTGTGTTAACATAATGCCACAC (SEQ ID NO: 156), CGTTTCGTCTGGATCGCAC (SEQ ID NO: 158), GCTGGGTAAATAGGTCAAC (SEQ ID NO: 159), GAGAGGATGAYCAGCCACAC (SEQ ID NO: 161), and CGCCCATTGTSCAATATTCC (SEQ ID NO: 162).

5. The kit of claim 4, further comprising the following primers: AATCACAGGGCGTAGTTGTG (SEQ ID NO: 164), ACCCACCAAGCCAATCTTAGG (SEQ ID NO: 165), 45 GCGGAGTTAACTAITGGCTAG (SEQ ID NO: 167), GGCAAGCTTCTATAATTGCG (SEQ ID NO: 168), GCGGAGTTARYTATITGGCTAG (SEQ ID NO: 170), GGCAAGCYTCTAWATTGCG (SEQ ID NO: 171), GGCAGCGTTGATGTCTTCG (SEQ ID NO: 174), 50 CCATTCAAGCCAGATCGGCATC (SEQ ID NO: 175), GAGAGGATGAYCAGCCACAC (SEQ ID NO: 177), and CGCCCATTGTSCAATATTCC (SEQ ID NO: 178).

6. The kit of claim 5, further comprising the following primers: AACTTTCACAGGGTGTGCTGGGT (SEQ ID NO:

55 180), CGTACGCTACTGGCTTCG (SEQ ID NO: 181), GTATCGCGTCTAGTTCTGC (SEQ ID NO: 183), CCTTGAATGAGCTGCACAGTGG (SEQ ID NO: 184), GTTTGATGCTCAGGGATGGC (SEQ ID NO: 186), GGCAGAAAGTCAGGCTGTG (SEQ ID NO: 187), 60 GAGAGGATGAYCAGCCACAC (SEQ ID NO: 189), and CGCCCATTGTSCAATATTCC (SEQ ID NO: 190).

7. The kit of claim 1, including at least one control DNA mix.

8. The kit of claim 1, including exactly two control DNA mixes.

9. The kit of claim 1, including exactly three control DNA mixes.

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10. The kit of claim 1, including a composition containing
a tracking dye.

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