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(54) **Title**: COMPOSITIONS AND METHODS FOR DETECTION OF PROPIONIBACTERIUM ACNES NUCLEIC ACID

(57) **Abstract**: Methods for amplifying and detecting Propionibacterium acnes nucleic acid by targeting specific sequences in 16S rRNA, 23S rRNA, or DNA encoding 16S rRNA or 23S rRNA are disclosed. Nucleic acid oligonucleotide sequence compositions specific for *P. acnes* nucleic acid sequences in 16S or 23S rRNA or DNA encoding 16S or 23S rRNA sequences are disclosed, which are useful for amplification oligonucletides, capture probes in sample preparation, and probes for detection of *P. acnes* nucleic acid sequences.

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COMPOSITIONS AND METHODS FOR DETECTION OF PROPIONIBACTERIUM ACNES NUCLEIC ACID

FIELD OF THE INVENTION

This invention relates to detection of bacteria in a sample by using molecular biological methods and more specifically relates to detection of the presence of *Propionibacterium acnes* in a sample by amplifying nucleic acids from *P. acnes* and detecting the amplified nucleic acid sequences.

BACKGROUND

Propionibacterium acnes is a gram positive, non-spore-forming, rod-shaped bacterium. *P. acnes* is part of the normal flora of the skin, oral cavity, large intestine and conjunctiva. *P. acnes* accounts for approximately half of the total skin microbiota with an estimated density of 10^2 to 10^6 organisms per square centimeter (*J. Clin. Microbiol.* 2005;43:326). Although *P. acnes* is mainly associated with acne vulgaris, it has also been associated with a number of inflammatory conditions, post operative disorders and opportunistic infections in immunosuppressed hosts (*Lett. Applied Microbiol.* 2006;42:185, *Anaerobe* 2003;9:5).

P. acnes is a fastidious organism that is difficult to culture, requiring special equipment and expertise for anaerobic culture. Routine cultures often miss *P. acnes*, especially when a less fastidious aerobic bacterium is present. Also, the presence of *P. acnes* in clinical samples is often dismissed as contamination when other medically important bacteria are present (*J. Med. Microbiol.* 2004;53:1247). *P. acnes* is slow growing (e.g., forming colonies less than one millimeter after four days) and, therefore, samples suspected of containing *P. acnes* should be cultured for up to two weeks.

Due to the abundance of *P. acnes* on human skin, it can become a contaminant in any process that requires the presence of people. In many industries, it is important to keep bacterial contaminants below a certain level. Materials, such as water, used to make products for the food, drugs, diagnostics and cosmetics industries are tested for contaminants throughout the manufacturing process. Water used in drug, device or diagnostic manufacturing for the USA must meet United States Pharmacopia (USP) specifications limiting the amount of chemical and microbial contaminants. The USP purity standards for drugs, devices and diagnostics are enforceable by the U.S. Food and Drug Administration. For example, the USP 23 standard

limits microbial contamination, measured as colony forming units (CFU), to less than or equal to 100 CFU per milliliter of purified water.

There is a need for a rapid, sensitive and accurate method to detect *P. acnes,* particularly in manufacturing samples so that a contaminating source is accurately detected and then eliminated. There is also a need for methods that allow rapid and accurate diagnosis of *P. acnes* infections in humans to allow prompt and effective treatment of infected individuals and minimize further infections.

SUMMARY

A composition comprising at least two oligomers specific for a *P. acnes* 16S rRNA sequence or DNA encoding 16S rRNA contained within SEQ ID NO:199 selected from the group consisting of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, the completely complementary sequences of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, and the RNA equivalent of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, wherein when a first oligomer of the at least two oligomers is selected from the group consisting of SEQ ID Nos. 16 to 26, 135 to 160, 184 and 210, then a second oligomer is selected from the group consisting of SEQ ID Nos. 35 to 54. One embodiment of the composition further comprises at least one third oligomer selected from the group consisting of SEQ ID Nos. 1 to 8, 75 to 98, 127 to 130, 181, 182, 195 to 198, and 211 to 226, the completely complementary sequences of SEQ ID Nos. 1 to 8, 75 to 98, 127 to 130, 181, 182, 195 to 198, and 211 to 226, and the RNA equivalents of SEQ ID Nos. 1 to 8, 75 to 98, 127 to 130, 181, 182, 195 to 198, and 211 to 226. Another embodiment of the composition of includes a promoter provider oligomer selected from the group consisting of SEQ ID Nos. 35 to 54 and 135 to 160. One embodiment of the composition that includes at least one third oligomer includes an oligomer selected from the group consisting of SEQ ID Nos. 1 to 8, the completely complementary sequences of SEQ ID Nos. 1 to 8, and the RNA equivalent of SEQ ID Nos. 1 to 8, for which the third oligomer selected from this group includes a 3' blocked end. In another embodiment, the at least one third oligomer is selected from the group consisting of SEQ ID Nos. 75 to 98, 181, and 182, the completely complementary sequences of SEQ ID Nos. 75 to 98, 181, and 182, and the RNA equivalent of SEQ ID Nos. 75 to 98, 181, and 182, for which the third oligomer selected from this group includes a label attached to the third oligomer.

A composition comprising at least two oligomers specific for a *P. acnes* 23S rRNA sequence or DNA encoding 23S rRNA contained within SEQ ID NO:202 selected from the

group consisting of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, the completely complementary sequences of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, and the RNA equivalent of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, wherein when a first oligomer of the at least two oligomers is selected from the group consisting of SEQ ID Nos. 27 to 34, 161 to 180, and 185, then a second oligomer is selected from the group consisting of SEQ ID Nos. 55 to 72, 186, and 187. In one embodiment, the composition further includes at least one third oligomer selected from the group consisting of SEQ ID Nos. 9 to 15, 99 to 126, 131 to 134, 183, and 188, the completely complementary sequences of SEQ ID Nos. 9 to 15, 99 to 126, 131 to 134, 183, and 188, and the RNA equivalent of SEQ ID Nos. 9 to 15, 99 to 126, 131 to 134, 183, and 188. In another embodiment of the composition, the at least two oligomers include a promoter provider oligomer selected from the group consisting of SEQ ID Nos. 55 to 72, 186, and 187, the completely complementary sequences of SEQ ID Nos. 55 to 72, 186, and 187, and the RNA equivalent of SEQ ID Nos. 55 to 72, 186, and 187. In an embodiment of the composition that includes at least one third oligomer, it is selected from the group consisting of SEQ ID Nos. 9 to 15 and 188, the completely complementary sequences of SEQ ID Nos. 9 to 15 and 188, and the RNA equivalent of SEQ ID Nos. 9 to 15 and 188, and the third oligomer selected from this group includes a 3' blocked end. In another embodiment, the at least one third oligomer is selected from the group consisting of SEQ ID Nos. 99 to 126 and 183, the completely complementary sequences of SEQ ID Nos. 99 to 126 and 183, and the RNA equivalent of SEQ ID Nos. 99 to 126 and 183, for which the third oligomer selected from this group includes a label attached to the third oligomer.

A method for specifically detecting a *P. acnes* in a sample by detecting a target nucleic acid sequence that is a *P. acnes* rRNA or DNA encoding a *P. acnes* rRNA comprising the steps of: providing a sample that contains a *P. acnes* target region in a 16S rRNA or DNA encoding 16S rRNA contained in SEQ ID NO:199, and/or a target region in a 23S rRNA or gene encoding 23S RNA contained in SEQ ID NO:202; amplifying a sequence in the *P. acnes* target region to make an amplified product by using at least two amplification oligomers in an *in vitro* amplification reaction that includes enzymatic elongation of at least one amplification oligomer, wherein when the target sequence is in the 16S rRNA or DNA encoding the 16S rRNA contained in SEQ ID NO:199, the at least two amplification oligomers are selected from the group consisting of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, the completely complementary sequences of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, and the

RNA equivalent of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, and wherein when the target sequence is in the 23S rRNA or DNA encoding 23S rRNA contained in SEQ ID NO:202, the at least two amplification oligomers are selected from the group consisting of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, the completely complementary sequences of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, and the RNA equivalent of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187; and detecting the amplified product. One embodiment of the method, in which the target sequence is in the 16S rRNA or DNA encoding the 16S rRNA , the amplification oligomers use a first amplification oligomer selected from the group consisting of SEQ ID Nos. 16 to 26, 135 to 160, 184 and 210, with a second amplification oligomer selected from the group consisting of SEQ ID Nos. 35 to 54. In an embodiment in which the amplifying step amplifies the target sequence in the 16S rRNA or DNA encoding the 16S rRNA, the method further includes a third oligomer selected from the group consisting of SEQ ID Nos. 1 to 8, the completely complementary sequences of SEQ ID Nos. 1 to 8, and the RNA equivalent of SEQ ID Nos. 1 to 8, for which the third oligomer selected from this group includes a 3' blocked end. In one embodiment, the detecting step for the amplified product made from the target sequence in the 16S rRNA or DNA encoding the 16S rRNA uses a detection probe selected from the group consisting of SEQ ID Nos. 75 to 98, 181, and 182, the completely complementary sequences of SEQ ID Nos. 75 to 98, 181, and 182, and the RNA equivalent of SEQ ID Nos. 75 to 98, 181, and 182. In another embodiment, the method further includes a target capture step to purify the 16S rRNA or DNA encoding the 16S rRNA from the sample by hybridizing to the 16S rRNA or DNA encoding the 16S rRNA a target capture probe selected from the group consisting of SEQ ID Nos. 127 to 130. In one embodiment of the method for which the target sequence is in the 23S rRNA or DNA encoding the 23S rRNA, the two amplification oligomers use a first amplification oligomer selected from the group consisting of SEQ ID Nos. 27 to 34, 161 to 180, and 185, with a second amplification oligomer selected from the group consisting of SEQ ID Nos. 55 to 72, 186, and 187. In another embodiment, in which the amplifying step amplifies the target sequence in the 23S rRNA or DNA encoding the 23S rRNA, the amplifying step further includes a third oligomer selected from the group consisting of SEQ ID Nos. 9 to 15 and 188, the completely complementary sequences of SEQ ID Nos. 9 to 15 and 188, and the RNA equivalent of SEQ ID Nos. 9 to 15 and 188, for which the third oligomer selected from this group includes a 3' blocked end. In one embodiment, the detecting step for the amplified product made from the

target sequence in the 23S rRNA or DNA encoding the 23S rRNA uses a detection probe selected from the group consisting of SEQ ID Nos. 99 to 126 and 183, the completely complementary sequences of SEQ ID Nos. 99 to 126 and 183, and the RNA equivalent of SEQ ID Nos. 99 to 126 and 183. One embodiment of the method further includes a target capture step to purify the 23S rRNA or DNA encoding the 23S rRNA from the sample by hybridizing to the 23S rRNA or DNA encoding the 23S rRNA a target capture probe selected from the group consisting of SEQ ID Nos. 131 to 134. In an embodiment of the method, the amplified product is detected by using a detection probe that includes a label.

DETAILED DESCRIPTION

Disclosed are methods for detecting the presence or absence of *P. acnes* in a sample, including food, water, industrial, environmental or biological specimens. These methods provide for the sensitive and specific detection of *P. acnes* nucleic acids. The methods include performing nucleic acid amplification of the 16S or 23S rRNA sequences specific for *P. acnes* and detecting the amplified product. In some embodiments, detection occurs by specifically hybridizing the amplified product with a nucleic acid probe that provides a signal to indicate the presence of *P. acnes* in the sample. Absence of *P. acnes* in the sample is detected in an embodiment of the method includes an internal control that is a non-*P. acnes* nucleic acid in the reaction mixture that treated for simultaneous amplification of a *P. acnes* target nucleic acid, but in which no amplified *P. acnes* product is made while an amplified product is made for the internal control in the reaction mixture. That is, amplification of the internal control in the reaction mixture indicates that the method steps were properly performed and the reaction conditions were functional for making amplified nucleic acid but no *P. acnes* amplified product was made because a *P. acnes* target nucleic acid was not present in the tested sample.

Amplification includes contacting the sample with a one or more amplification oligonucleotides specific for a target sequence in a ribosomal RNA (rRNA) or DNA encoding an rRNA, more specifically 16S or 23S rRNA or DNA encoding 16S or 23S rRNA, to produce an amplified product if the *P. acnes* target nucleic acid is is present in the sample. Amplification synthesizes additional copies of the target sequence or its complement by using at least one nucleic acid polymerase to extend the sequence from an amplification oligomer (e.g., a primer) by using a template strand that is part of the target nucleic acid or its complementary strand. One embodiment detects the amplified product by using a hybridizing step that includes contacting the amplified product with at least one probe specific for a sequence amplified by the

amplification oligonucleotides, e.g., a sequence contained in the target sequence flanked by a pair of selected amplification oligomers.

Detecting the amplified products may be performed after the amplification reaction is completed, or may be performed simultaneous with amplifying the target region, i.e., in real time. In one embodiment, the detection step allows homogeneous detection, i.e., detection of the hybridized probe to the amplified product without removal of unhybridized probe from the mixture (e.g., see US Pats. 5,639,604 and 5,283,174, Arnold Jr. et al.). In embodiments that detect the amplified product near or at the end of the amplification step, a linear probe may be used to provide a signal to indicate hybridization of the probe to the amplified product. In other embodiments that use real-time detection, the probe may be a hairpin probe, such as a molecular beacon, molecular torch, or hybridization switch probe, that is labeled with a reporter moiety that is detected when the probe binds to amplified product. For example, a hairpin probe may include a label, such as a fluorophore ("F"), attached to one end of the probe and an interacting compound, such as quencher ("Q"), attached to the other end the hairpin structure to inhibit signal production from the label when the hairpin structure is in the "closed" conformation and not hybridized to the amplified product, whereas a signal is detectable when the probe is in the "open" conformation because it is hybridized to a complementary sequence in the amplified product. Various forms of such probes are known (e.g., see US Pats. 5,118,801 and 5,312,728, Lizardi et al., US Pats. 5,925,517 and 6,150,097, Tyagi et al., US Pats. 6,849,412, 6,835,542, 6,534,274, and 6,361,945, Becker et al., and US2006-0068417A1, Becker et al., and US2006-0194240A1, Arnold Jr.).

To aid in understanding aspects of this disclosure, some terms used herein are described in more detail. All other scientific and technical terms used herein have the same meaning as commonly understood by those skilled in the relevant art, such as may be provided in *Dictionary of Microbiology and Molecular Biology*, 2nd ed. (Singleton et al., 1994, John Wiley & Sons, New York, NY), *The Harper Collins Dictionary of Biology* (Hale & Marham, 1991, Harper Perennial, New York, NY), and references cited herein. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methods well known to a person of ordinary skill in the art of molecular biology.

"Sample" includes any specimen that may contain *P. acnes* or components thereof, such as nucleic acids or fragments of nucleic acids. Samples may be obtained from environmental or manufacturing sources, e.g., water, soil, slurries, debris, biofilms from containers of aqueous

fluids, airborne particles or aerosols, and the like, which may include processed samples, such as obtained from passing samples over or through a filtering device, or following centrifugation, or by adherence to a medium, matrix, or support. Samples include "biological samples" which include any tissue or material derived from a living or dead mammal or organism which may contain *P. acnes* or target nucleic acid derived therefrom, including, e.g., respiratory tissue or exudates such as bronchoscopy, bronchoalveolar lavage, lung biopsy, sputum, peripheral blood, plasma, serum, lymph node, gastrointestinal tissue, feces, urine, or other body fluids or materials. A sample may be treated to physically or mechanically disrupt tissue aggregates or cells, thus releasing intracellular components, including nucleic acids, into a solution which may contain other components, such as enzymes, buffers, salts, detergents and the like.

"Nucleic acid" refers to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases, or base analogs, where the nucleosides are linked together by phosphodiester bonds or other linkages to form a polynucleotide. Nucleic acids include RNA, DNA, or chimeric DNA-RNA polymers or oligonucleotides, and analogs thereof. A nucleic acid "backbone" may be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds (in "peptide nucleic acids" or PNAs, see PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of the nucleic acid may be either ribose or deoxyribose, or similar compounds having known substitutions, e.g., 2' methoxy substitutions and 2' halide substitutions (e.g., 2'-F). Nitrogenous bases may be conventional bases (A, G, C, T, U), analogs thereof (e.g., inosine, 5-methylisocytosine, isoguanine ; *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992, Abraham et al., 2007, BioTechniques 43: 617-24), which include derivatives of purine or pyrimidine bases (e.g., N^4-methyl deoxygaunosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases having substituent groups at the 5 or 6 position, purine bases having an altered or replacement substituent at the 2, 6 and/or 8 position, such as 2-amino-6-methylaminopurine, O^6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O^4-alkyl-pyrimidines, and pyrazolo-compounds, such as unsubstituted or 3-substituted pyrazolo[3,4-d]pyrimidine; US Pats. 5,378,825, 6,949,367 and PCT No. WO 93/13121). Nucleic acids may include "abasic" residues in which the backbone does not include a nitrogenous base for one or more residues (US Pat. 5,585,481, Arnold et al.). A nucleic acid may comprise only conventional sugars, bases, and linkages as found in RNA and DNA, or may include conventional components and substitutions

(e.g., conventional bases linked by a 2' methoxy backbone, or a nucleic acid including a mixture of conventional bases and one or more base analogs). Nucleic acids may include "locked nucleic acids" (LNA), in which one or more nucleotide monomers have a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhances hybridization affinity toward complementary sequences in single-stranded RNA (ssRNA), single-stranded DNA (ssDNA), or double-stranded DNA (dsDNA) (Vester et al., 2004, *Biochemistry* 43(42):13233-41). Synthetic methods for making nucleic acids *in vitro* are well known in the art although nucleic acids may be purified from natural sources using routine techniques.

The interchangeable terms "oligomer" and "oligonucleotide" refer to a nucleic acid having generally less than 1,000 nucleotide (nt) residues, including polymers in a range having a lower limit of about 5 nt residues and an upper limit of about 500 to 900 nt residues. In some embodiments, oligonucleotides are in a size range having a lower limit of about 12 to 15 nt and an upper limit of about 50 to 600 nt, and other embodiments are in a range having a lower limit of about 15 to 20 nt and an upper limit of about 22 to 100 nt. Oligonucleotides may be purified from naturally occurring sources, but or may be synthesized using any of a variety of well known enzymatic or chemical methods.

An "amplification oligomer" is an oligomer that hybridizes to a target nucleic acid, or its complement, and participates in a nucleic acid amplification reaction. An example of an amplification oligomer is a "primer" that hybridizes to a template nucleic acid and contains a 3' OH end that is extended by a polymerase in an amplification process. Another example of an amplification oligomer is an oligomer that is not extended by a polymerase (e.g., because it has a 3' blocked end) but participates in or facilitates amplification. Size ranges for amplification oligonucleotides include those that are about 10 to about 70 nt long (not including the promoter sequence or poly-A tails) and contain at least about 10 contiguous bases, or even at least 12 contiguous bases that are complementary to a region of the target nucleic acid sequence (or a complementary strand thereof). The contiguous bases are at least 80%, or at least 90%, or completely complementary to the target sequence to which the amplification oligomer binds. An amplification oligomer may optionally include modified nucleotides or analogs, or additional nucleotides that participate in an amplification reaction but are not complementary to or contained in the target nucleic acid, or template sequence. For example, the 5' region of an amplification oligonucleotide may include a promoter sequence that is non-complementary to the target nucleic acid (which may be referred to as a "promoter-primer" or "promoter

provider"). Those skilled in the art will understand that an amplification oligomer that functions as a primer may be modified to include a 5' promoter sequence, and thus function as a promoter-primer. Similarly, a promoter-primer may be modified by removal of, or synthesis without, a promoter sequence and still function as a primer. In another example, an amplification oligomer that is 3' blocked but capable of hybridizing to a target nucleic acid and providing an upstream promoter sequence that serves to initiate transcription is referred to as a "promoter provider" oligomer.

Oligomers that are not intended to be extended by a nucleic acid polymerase may include a blocker group that replaces the 3'OH to prevent enzyme-mediated extension of the oligomer in an amplification reaction. For example, blocked amplification oligomers and/or detection probes present during amplification may not have functional 3'OH and instead include one or more blocking groups located at or near the 3' end. In some embodiments a blocking group near the 3' end and may be within five residues of the 3' end and is sufficiently large to limit binding of a polymerase to the oligomer. In other embodiments a blocking group is covalently attached to the 3' terminus. Many different chemical groups may be used to block the 3' end, e.g., alkyl groups, non-nucleotide linkers, alkane-diol dideoxynucleotide residues, and cordycepin.

"Amplification" refers to any known procedure for obtaining multiple copies of a target nucleic acid sequence or its complement or fragments thereof. Amplification of "fragments" refers to production of an amplified nucleic acid that contains less than the complete target nucleic acid or its complement, e.g., produced by using an amplification oligonucleotide that hybridizes to, and initiates polymerization from, an internal position of the target nucleic acid. Known amplification methods include, for example, replicase-mediated amplification, polymerase chain reaction (PCR), ligase chain reaction (LCR), strand-displacement amplification (SDA), and transcription-mediated or transcription-associated amplification. Replicase-mediated amplification uses self-replicating RNA molecules, and a replicase such as QB-replicase (e.g., US Pat. 4,786,600, Kramer et al.). PCR amplification uses a DNA polymerase, pairs of primers, and thermal cycling to synthesize multiple copies of two complementary strands of dsDNA or from a cDNA (e.g., US Pats. 4,683,195, 4,683,202, and 4,800,159, Mullis et al.). LCR amplification uses four or more different oligonucleotides to amplify a target and its complementary strand by using multiple cycles of hybridization, ligation, and denaturation (e.g., US Pat. 5,427,930, Birkenmeyer et al., US Pat. 5,516,663, Backman et al.). SDA uses a primer that contains a recognition site for a restriction

endonuclease and an endonuclease that nicks one strand of a hemimodified DNA duplex that includes the target sequence, whereby amplification occurs in a series of primer extension and strand displacement steps (e.g., see US Pats. 5,422,252, Walker et al., 5,547,861, Nadeau et al., and 5,648,211, Fraiser et al.).

"Transcription associated amplification" or "transcription mediated amplification" (TMA) refer to nucleic acid amplification that uses an RNA polymerase to produce multiple RNA transcripts from a nucleic acid template. These methods generally employ an RNA polymerase, a DNA polymerase, deoxyribonucleoside triphosphates, ribonucleoside triphosphates, and a template complementary oligonucleotide that includes a promoter sequence, and optionally may include one or more other oligonucleotides. Variations of transcription associated amplification are well known in the art as disclosed in detail previously (US Pats. 5,399,491 and 5,554,516, Kacian et al.; US Pat. 5,437,990, Burg et al.; PCT Nos. WO 88/01302 and WO 88/10315, Gingeras et al.; US Pat. 5,130,238, Malek et al.; US Pats. 4,868,105 and 5,124,246, Urdea et al.; PCT No. WO 95/03430, Ryder et al.; and US 2006-0046265 A1, Becker et al.). TMA methods (e.g., US Pats. 5,399,491 and 5,554,516) and single-primer transcription associated amplification method (US 2006-0046265 A1) are embodiments of amplification methods used for detection of *P. acnes* target sequences as described herein. The person of ordinary skill in the art will appreciate that the disclosed compositions may be used in amplification methods based on extension of oligomer sequences by a polymerase.

"Probe" refers to a nucleic acid oligomer that hybridizes specifically to a target sequence in a nucleic acid, or in an amplified nucleic acid, under conditions that promote hybridization to allow detection of the target sequence or amplified nucleic acid. Detection may either be direct (i.e., a probe hybridized directly to its target sequence) or indirect (i.e., a probe linked to its target via an intermediate molecular structure). A probe's "target sequence" generally refers to a sequence within a larger sequence (e.g., a subset of an amplified sequence) that hybridizes specifically to at least a portion of a probe oligomer by standard base pairing. A probe may comprise target-specific sequences and other sequences that contribute to the three-dimensional conformation of the probe (e.g., described in US Pats. 5,118,801 and 5,312,728, Lizardi et al.; and US Pats. 6,849,412, 6,835,542, 6,534,274, 6,361,945, and US 2006-006847 A1, Becker et al.).

Sequence that hybridize to each other may be completely complementary or partially complementary to the intended target sequence by standard nucleic acid base pairing (e.g. G:C,

A:T or A:U pairing). By "sufficiently complementary" is meant a contiguous sequence that is capable of hybridizing to another sequence by hydrogen bonding between a series of complementary bases, which may be complementary at each position in the sequence by standard base pairing or may contain one or more residues, including abasic residues, that are not complementary. Sufficiently complementary contiguous bases typically are at least 80%, or at least 90%, complementary to a sequence to which an oligomer is intended to specifically hybridize. Sequences that are "sufficiently complementary" allow stable hybridization of a nucleic acid oligomer with its target sequence under appropriate hybridization conditions, even if the sequences are not completely complementary. Appropriate hybridization conditions are well known in the art, may be predicted based on sequence composition, or can be determined by using routine testing methods (e.g., Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) at §§ 1.90-1.91, 7.37-7.57, 9.47-9.51 and 11.47-11.57, particularly §§ 9.50-9.51, 11.12-11.13, 11.45-11.47 and 11.55-11.57).

"Sample preparation" refers to any steps or method that treats a sample for subsequent amplification and/or detection of *P. acnes* nucleic acids present in the sample. Samples may be complex mixtures of components of which the target nucleic acid is a minority component. Sample preparation may include any known method of concentrating components, such as microbes or nucleic acids, from a larger sample volume, such as by filtration of airborne or waterborne particles from a larger volume sample or by isolation of microbes from a sample by using standard microbiology methods. Sample preparation may include physical disruption and/or chemical lysis of cellular components to release intracellular components into a substantially aqueous or organic phase and removal of debris, such as by using filtration, centrifugation or adsorption. Sample preparation may include use of nucleic acid oligonucleotide that selectively or non-specifically capture a target nucleic acid and separate it from other sample components (e.g., as described in US Pat. 6,110,678, Weisburg et al., and US 2008-0286775 A1, Becker et al.).

A "capture probe" or "capture oligomer" refers to at least one nucleic acid oligomer that joins a target sequence and an immobilized oligomer by using base pair hybridization. An embodiment of a capture oligomer includes two binding regions: a target sequence-binding region and an immobilized probe-binding region, usually on the same oligomer, although the two regions may be present on different oligonucleotides that are joined by one or more linkers.

For example, a first oligomer may include the immobilized probe-binding region and a second oligomer may include the target sequence-binding region, and the two different oligonucleotides are joined by hydrogen bonding with a linker that links the two sequences of the first and second oligonucleotides. Another embodiment of a capture oligomer uses a target-sequence binding region that includes random or non-random poly-GU, poly-GT, or poly U sequences to bind non-specifically to a target nucleic acid and link it to an immobilized probe on a support.

An "immobilized probe" or "immobilized nucleic acid" refers to a nucleic acid that joins, directly or indirectly, a capture oligomer to an immobilized support. One embodiment of an immobilized probe is an oligomer joined to a support that facilitates separation of bound target sequence from unbound material in a sample. Supports may include known materials, such as matrices and particles free in solution, which may be made of nitrocellulose, nylon, glass, polyacrylate, mixed polymers, polystyrene, silane, polypropylene, metal, or other compositions, of which one embodiment is magnetically attractable particles. Supports may be monodisperse magnetic spheres (e.g., uniform size ± 5%), to which an immobilized probe is joined directly (via covalent linkage, chelation, or ionic interaction), or indirectly (via one or more linkers), where the linkage or interaction between the probe and support is stable during hybridization conditions.

"Separating" or "purifying" means that one or more components of a sample are removed or separated from other sample components. Sample components include target nucleic acids usually in a generally aqueous solution phase, which may also include cellular fragments, proteins, carbohydrates, lipids, and other nucleic acids. Separating or purifying removes at least 70%, or at least 80%, or at least 95% of the target nucleic acid from other sample components.

A "label" refers to a molecular moiety or compound that can be detected or lead to a detectable response or signal. A label may be joined directly or indirectly to a nucleic acid probe. Direct labeling can occur through bonds or interactions that link the label to the probe, including covalent bonds or non-covalent interactions, e.g. hydrogen bonds, hydrophobic and ionic interactions, or formation of chelates or coordination complexes. Indirect labeling can occur through use of a bridging moiety or "linker" such as a binding pair member, an antibody or additional oligomer, which is either directly or indirectly labeled, and which may amplify the detectable signal. Labels include any detectable moiety, such as a radionuclide, ligand (e.g., biotin, avidin), enzyme or enzyme substrate, reactive group, or chromophore (e.g., dye, particle,

or bead that imparts detectable color), luminescent compound (e.g., bioluminescent, phosphorescent, or chemiluminescent labels), or fluorophore. Labels may be detectable in a homogeneous assay in which bound labeled probe in a mixture exhibits a detectable change different from that of an unbound labeled probe, e.g., instability or differential degradation properties. A "homogeneous detectable label" can be detected without physically removing bound from unbound forms of the label or labeled probe (e.g., US Pats. 5,283,174, 5,656,207, and 5,658,737). Labels include chemiluminescent compounds, e.g., acridinium ester ("AE") compounds that include standard AE and derivatives (e.g., US Pats. 5,656,207, 5,658,737, and 5,639,604). Synthesis and methods of attaching labels to nucleic acids and detecting labels are well known (e.g., Sambrook *et al., Molecular Cloning, A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Habor, NY, 1989), Chapter 10; US Pats. 5,658,737, 5,656,207, 5,547,842, 5,283,174, and 4,581,333).

"Consisting essentially of" is used to mean that additional component(s), composition(s) or method step(s) that do not materially change the basic and novel characteristics of the present invention may be included in the compositions or kits or methods of the present invention. Such characteristics include the ability to detect *P. acnes* nucleic acid in a biological sample at a copy number of about 10^4 copies of *P. acnes*. Other characteristics include limited cross-reactivity with other Bacteria or mammalian nucleic acid and targeting 16S or 23S rRNA. Any component(s), composition(s), or method step(s) that have a material effect on the basic and novel characteristics of the present invention would fall outside of this term.

Disclosed are methods for amplifying and detecting *P. acnes* nucleic acid, specifically sequences of *P. acnes* 16S and 23S rRNA or genes encoding 16S and 23S rRNA. The methods include oligonucleotide sequences that specifically recognize target sequences of *P. acnes* 16S and 23S rRNA or their complementary sequences, or genes encoding 16S and 23S rRNA or their complementary sequences. Such oligonucleotides may be used as amplification oligonucleotides, which may include primers, promoter primers, blocked oligonucleotides, and promoter provider oligonucleotides, whose functions have been described previously (e.g., US Pats. 5,399,491, 5,554,516 and 5,824,518, Kacian et al.; and US 2006-0046265 A1, Becker et al., and US Pats. 4,683,195, 4,683,202, and 4,800,159, Mullis et al.). Other oligonucleotides may be used as probes for detecting amplified sequences of *P. acnes*.

Amplification methods that use TMA amplification include the following steps (described previously in US Pats. 5,399,491, 5,554,516 and 5,824,518, Kacian et al.). Briefly,

the target nucleic acid that contains the sequence to be amplified is provided as single stranded nucleic acid (e.g., ssRNA or ssDNA). Those skilled in the art will appreciate that conventional melting of double stranded nucleic acid (e.g., dsDNA) may be used to provide single-stranded target nucleic acids. A promoter primer binds specifically to the target nucleic acid at its target sequence and a reverse transcriptase (RT) extends the 3' end of the promoter primer using the target strand as a template to create a cDNA copy of the target sequence strand, resulting in an RNA:DNA duplex. RNase (e.g., RnaseH of the RT) digests the RNA strand of the RNA:DNA duplex and a second primer binds specifically to its target sequence which is located on the cDNA strand downstream from the promoter-primer end. RT synthesizes a new DNA strand by extending the 3' end of the second primer using the first cDNA template to create a dsDNA that contains a functional promoter sequence. An RNA polymerase specific for the promoter sequence then initiates transcription to produce RNA transcripts that are about 100 to 1000 amplified copies ("amplicons") of the initial target strand in the reaction. Amplification continues when the second primer binds specifically to its target sequence in each of the amplicons and RT creates a DNA copy from the amplicon RNA template to produce an RNA:DNA duplex. RNase in the reaction mixture digests the amplicon RNA from the RNA:DNA duplex and the promoter primer binds specifically to its complementary sequence in the newly synthesized DNA. RT extends the 3' end of the promoter primer to create a dsDNA that contains a functional promoter to which the RNA polymerase binds to transcribe additional amplicons that are complementary to the target strand. The autocatalytic cycles of making more amplicon copies repeat during the course of the reaction resulting in about a billion-fold amplification of the target nucleic acid present in the sample. The amplified products may be detected during amplification, i.e., in real-time, or at the end of the amplification reaction by using a probe that binds specifically to a target sequence contained in the amplified products. Detection of a signal resulting from the bound probes indicates the presence of the target nucleic acid in the sample.

One method of transcription associated amplification uses one primer and one or more additional amplification oligonucleotides to amplify nucleic acids *in vitro*, making transcripts (amplicons) that indicate the presence of the target sequence in a sample (see US2006-0046265 A1 and US Pat. 7,374,885, Becker et al.). Briefly, the single primer amplification method uses a primer (or "priming oligomer"), a modified promoter oligomer (or "promoter provider") that is modified to prevent the initiation of DNA synthesis from its 3' end (e.g., by including a 3'-

blocking moiety) and, optionally, a binding molecule (e.g., a 3'-blocked extender oligomer) to terminate elongation of a cDNA from the target strand. This method synthesizes multiple copies of a target sequence. It includes treating a target RNA that contains a target sequence with primer and a binding molecule, where the primer hybridizes to the 3' end of the target strand. RT initiates primer extension from the primer's 3' end to produce a cDNA in a RNA:cDNA duplex with the target strand. When a binding molecule, such as a 3' blocked extender oligomer, is used in the reaction, it binds to the target strand next to the 5' end of the target sequence to be amplified. When the primer is extended by DNA polymerase activity of RT to produce cDNA, the 3' end of the cDNA is determined by the position of the binding molecule because polymerization stops when the primer extension product reaches the binding molecule bound to the target strand. Thus, the 3' end of the cDNA is complementary to the 5' end of the target sequence. The RNA:cDNA duplex is separated when RNase (e.g., RNase H of RT) degrades the RNA strand, although those skilled in the art will appreciate that any form of strand separation may be used. Then, the promoter provider oligomer hybridizes to the cDNA near the 3' end of the cDNA strand. The promoter provider oligomer includes a 5' promoter sequence for an RNA polymerase and a 3' region complementary to a sequence in the 3' sequence of the cDNA. The promoter provider has a modified 3' end that includes a blocking moiety to prevent initiation of DNA synthesis from the 3' end of the promoter provider. In the promoter provider:cDNA duplex, the 3'-end of the cDNA is extended by DNA polymerase activity of RT using the promoter oligomer as a template to add a promoter sequence to the cDNA and create a double-stranded functional promoter. RNA polymerase specific for the promoter sequence then binds to the functional promoter and transcribes multiple RNA transcripts complementary to the cDNA, which are amplified RNA identical to the target region sequence that was amplified from the initial target strand. The amplified RNA can cycle through the process again by binding the primer and serving as a template for further cDNA production, ultimately producing many amplified products or amplicons from the initial target nucleic acid present in the sample. Some embodiments of the single primer amplification method do not include the binding molecule and, therefore, the cDNA product made from the primer has an indeterminate 3' end, but the amplification steps proceed substantially as described above.

Detection of the amplified products may be accomplished by a variety of methods. The nucleic acids may be associated with a surface that results in a physical change, such as a detectable electrical change. Amplified nucleic acids may be detected by concentrating them in

or on a matrix and detecting the nucleic acids or dyes associated with them (e.g., an intercalating agent such as ethidium bromide or cyber green), or detecting an increase in dye associated with nucleic acid in solution phase. Other detection methods may use nucleic acid probes that are complementary to a sequence in the amplified product and detecting the presence of the probe:product complex, or by using a complex of probes that may amplify the detectable signal associated with the amplified products (e.g., US Pats. 5,424,413 and 5,451,503, Hogan et al., and 5,849,481, Urdea et al.). Directly or indirectly labeled probes that specifically associate with the amplified product provide a detectable signal that indicates the presence of the target nucleic acid in the sample. For example, if the target nucleic acid is *P. acnes* 16S rRNA, the amplified product will contain a sequence in or complementary to a *P. acnes* 16S rRNA sequence and a probe will bind directly or indirectly to a sequence contained in the amplified product to indicate the presence of *P. acnes* in the tested sample.

Embodiments of probes that hybridize to the amplified sequences may be DNA or RNA oligonucleotides, or oligonucleotides that contain a combination of DNA and RNA nucleotides, or oligonucleotides synthesized with a modified backbone, e.g., includes one or more 2'-methoxy substituted ribonucleotides. Probes used for detection of the amplified *P. acnes* rRNA sequences may be unlabeled and detected indirectly (e.g., by binding of another binding partner to a moiety on the probe) or may be labeled with a variety of detectable labels. Label embodiments include compounds that emit a detectable light signal, e.g., fluorophores or luminescent compounds that can be detected in a homogeneous mixture. More than one label, and more than one type of label, may be present on a particular probe, or detection may use a mixture of probes in which each probe is labeled with a compound that produces a detectable signal (e.g., US Pats. 6,180,340 and 6,350,579, Nelson). Labels may be attached to a probe by various means including covalent linkages, chelation, and ionic interactions. Probes may be linear oligonucleotides that substantially do not form conformations held by intramolecular bonds. Other probes may form conformations by using intramolecular hybridization, e.g. hairpins. Linear probe embodiments include a chemiluminescent compound as the label, e.g. an AE compound.

Hairpin probes may be labeled with any of a variety of different types of interacting labels, where one interacting member is usually attached to the 5' end of the hairpin probe and the other interacting member is attached to the 3' end of the hairpin probe. Such interacting members, which may be generally referred to as a reporter dye and a quencher, include a

luminescent/quencher pair, luminescent/adduct pair, Forrester energy transfer pair, or a dye dimer. A luminescent/quencher pair may be made up of one or more luminescent labels, such as chemiluminescent or fluorescent labels, and one or more quenchers. In one embodiment, a hairpin probe is labeled at one end with a fluorescent label ("F") that absorbs light of a particular wavelength or range and emits light another emission wavelength or range and at the other end with a quencher ("Q") that dampens, partially or completely, signal emitted from the excited F when Q is in proximity with the fluorophore. Such a hairpin probe may be referred to as labeled with a fluorescent/quencher (F/Q) pair. Fluorophores are well known compounds that include, e.g., acridine and its derivatives such as 2-methoxy-6-chloro-9-amino-acridine, coumarin 500, ethidium, fluorescein and its derivatives such as 5 carboxy-fluorescein (FMA), tetrachloro-6-carboxyfluorescein, and 2,7-dimethyl-4,5-dichloro-6-carboxy-fluorescein (JOE), rhodamine and its derivatives such as sulforhodamine 101, N,N,N',N'-tetramethyl-6-carboxy-rhodamine (TAMRA™), 6-carboxy-X-rhodamine (ROX), HEX, TET, IRD40, IRD41, 5-(2'-aminoethyl)aminoaphthaline-1-sulfonic acid (EDANS™), Texas Red, RedX, eosine and its derivatives, ALEXA dyes such as ALEXA-488, ALEXA-532, ALEXA-546, ALEXA-568 and ALEXA-594, BODIPY dyes such as BODIPY-FL, BODIPY-TMR, BODIPY-TR, BODIPY-630/650 and BODIPY-650-670, cyamine dyes such as Cy3, Cy3.5 and Cy5, oxazine fluorophores such as MR121, lucifer yellow and other dyes that are well known in the art, or fluorescent bases such as 2-aminopurine and pyrrolo-dC (Tyagi et al., 1998, Nature Biotechnol. 16:49-53, Vet et al., 2002, Expert Rev. Mol. Diagn.2(1):77-86, Marti et al, 2006, Nucl. Acids Res. 34(6):50). Quenchers are also well known and include, e.g., 4-(4'-dimethyl-amino-phenylaxo) benzoic acid (DABCYL™), thallium, cesium, p-xylene-bis-pyridinium bromide, and intrinsic properties of nucleotides in nucleic acids, such as deoxyguanosine residues. Different F/Q combinations are well known and many combinations may function together, e.g., DABCYL™ with fluorescein, rhodamine, or EDANS compounds. Other combinations of labels for hairpin probes include a reporter dye, e.g., FAM™, TET™, JOE™, VIC™ combined with a quencher such as TAMRA™ or a non-fluorescent quencher.

One embodiment of a hairpin probe is a "molecular torch" that detects an amplified product to indicate whether a target *P. acnes* sequence is present in the sample after the amplification step. A molecular torch probe contains a target binding domain to hybridize to the target sequence, if present, resulting in an "open" conformation; a target torch closing domain that hybridizes to the target binding domain in the absence of the target sequence resulting in a

"closed" conformation; and a joining region that joins the two domains (e.g., see US Pats. 6,849,412, 6,835,542, 6,534,274, and 6,361,945, Becker et al.). The target binding domain forms a more stable hybrid with the target sequence than with the target closing domain under the same hybridization conditions so that in the presence of the amplified target sequence, it is in open conformation that produces an amount of signal that is distinguishable from that of the closed conformation. Another hairpin probe embodiment is a "molecular beacon" that includes a label on one arm of the hairpin sequence, a quencher on the other arm, and a loop region joining the two arms (see Tyagi et al., 1998, Nature Biotechnol. 16:49-53, US Pats. 5,118,801 and 5,312,728, Lizardi et al.). Methods for using such hairpin probes to detect the presence of a target sequence are well known in the art.

One embodiment of amethod for detection of *P. acnes* sequences uses a transcription associated amplification with a hairpin probe, e.g. molecular torch or molecular beacon, which probe may be added before or during amplification, and detection is carried out without subsequent reagent addition. For example, a probe is designed so that the Tm of the hybridized arms of the hairpin probe (e.g., target binding domain:target closing domain complex of a molecular torch) is higher than the amplification reaction temperature to prevent the probe from prematurely binding to amplified target sequences. After an amplification interval, the mixture is heated to open the torch probe arms and allow the target binding domain to hybridize to its target sequence in the amplified product. Then, the solution is cooled to close probes not bound to amplified products, which brings the label/quencher (e.g., F/Q) pair together, allowing detection of signals from probes hybridized to the amplified target sequences in a homogeneous manner. The mixture containing the F/Q labeled hairpin probe is irradiated with the appropriate excitation light and the emission signal is measured.

In other embodiments, the hairpin detection probe is designed so that the amplified products hybridize to the target binding domain of the probe during amplification, resulting in changing the hairpin to its open conformation during amplification, and the amplification reaction mixture is irradiated at intervals to detect the emitted signal from the open probes in real time during amplification.

Preparation of samples for amplification and detection of *P. acnes* sequences may include methods of separating and/or concentrating organisms contained in a sample from other sample components, e.g., filtration of particulate matter from air, water or other types of samples. Sample preparation may include routine methods of disrupting cells or lysing bacteria

to release intracellular contents, including *P. acnes* 16S or 23S rRNA or genetic sequences encoding 16S or 23S rRNA. Sample preparation before amplification may include an optional step of target capture to specifically or non-specifically separate the target nucleic acids from other sample components. Nonspecific target capture methods may involve selective precipitation of nucleic acids from a substantially aqueous mixture, adherence of nucleic acids to a support that is washed to remove other sample components, other methods of physically separating nucleic acids from a mixture that contains *P. acnes* nucleic acid and other sample components.

In one embodiment, *P. acnes* rRNA or genes encoding rRNA are selectively separated from other sample components by specifically hybridizing the *P. acnes* nucleic acid to a capture oligomer specific for a *P. acnes* target sequence to form a target sequence:capture probe complex that is separated from sample components by binding the *P. acnes* target:capture probe complex to an immobilized probe, and separating the *P. acnes* target:capture probe:immobilized probe complex from the sample, as previously described (US Pats. 6,110,678, 6,280,952, and 6,534,273, Weisburg et al.). Briefly, the capture probe includes a sequence that specifically binds to the *P. acnes* target sequence in 16S or 23S rRNA or in a gene encoding 16S or 23S rRNA and also includes a specific binding partner that attaches the capture probe with its bound target sequence to a support, to facilitate separating the target sequence from the sample components. In one embodiment, the specific binding partner of the capture probe is a 3' "tail" sequence that is not complementary to the *P. acnes* target sequence but that hybridizes to a complementary sequence on an immobilized probe attached to a support. Any sequence may be used in a tail region, which is generally about 5 to 50 nt long, and other embodiments include a substantially homopolymeric tail of about 10 to 40 nt (e.g., A_{10} to A_{40}), or about 14 to 33 nt (e.g., A_{14} to A_{30} or T_3A_{14} to T_3A_{30}), that bind to a complementary immobilized sequence (e.g., poly-T) attached to the support, e.g., a matrix or particle. Target capture may occur in a solution phase mixture that contains one or more capture oligonucleotides that hybridize specifically to a *P. acnes* rRNA or gene target sequence under hybridizing conditions, usually at a temperature higher than the Tm of the tail sequence:immobilized probe sequence duplex. The *P. acnes* target:capture probe complex is captured by adjusting the hybridization conditions so that the capture probe tail hybridizes to the immobilized probe, and the entire complex on the support is then separated from other sample components. The support with the attached immobilized probe:capture probe:*P. acnes* target sequence may be washed one or more times to further

remove other sample components. Other embodiments use a particulate support, such as paramagnetic beads, so that particles with the attached *P. acnes* target:capture probe:immobilized probe complex may be suspended in a washing solution and retrieved from the washing solution, by using magnetic attraction. To limit the number of handling steps, *P. acnes* target nucleic acid may be amplified by mixing the target sequence in the complex on the support with amplification oligonucleotides and performing the amplification steps.

Another target capture method uses a capture probe that binds non-specifically to the *P. acnes* nucleic acid and captures the *P. acnes* target:capture probe complex to a support, such as by using an immobilized probe or binding pair member on the support. Non-specific capture probes may be poly-U, poly-GU, poly-GT, or random sequences (as described in detail in US 2008-0286775 A1, Becker et al).

Assays for detection of *P. acnes* nucleic acid may optionally include a non-*P. acnes* internal control (IC) nucleic acid that is amplified and detected in the same assay reaction mixtures by using amplification and detection oligomers specific for the IC sequence. Amplification and detection of a signal from the amplified IC sequence demonstrates that the assay reagents, conditions, and performance of assay steps were properly used in the assay if no signal is obtained for the intended target *P. acnes* nucleic acid (i.e., samples that test negative for *P. acnes*). An IC may be used as an internal calibrator for the assay when a quantitative result is desired, i.e., the signal obtained from IC amplification and detection is used to set a parameter used in an algorithm for quantitating the amount of *P. acnes* nucleic acid in a sample based on the signal obtained for an amplified a *P. acnes* target sequence. One embodiment of an IC is a randomized sequence that has been derived from a naturally occurring source (e.g., an HIV sequence that has been rearranged randomly). An IC may be an RNA transcript isolated from a naturally occurring source or synthesized *in vitro*, such as by making transcripts from a cloned randomized sequence such that the number of IC copies in an assay is accurately determined. Primers and probe for the IC target sequence are designed and synthesized by using any well known method provided that the primers and probe function for amplification of the IC target sequence and detection of the amplified IC sequence using substantially the same assay conditions used to amplify and detect the *P. acnes* target sequence. In embodiments that include a target capture-based purification step, capture of the IC target added to the sample may be included in the assay so that the IC is treated in all of the assay steps similar to the intended *P. acnes* analyte. Sequences that may be used for amplifying and detecting an internal control

include SEQ ID Nos. 205-209.

Amplification and Detection of 16S rRNA sequences of *P. acnes*

For amplification and detection of sequences found in the 16S rRNA sequences (which include 16S rRNA and genes encoding 16S rRNA) of *P. acnes*, oligonucleotides were designed that act as amplification oligomers and detection probes by comparing known sequences of 16S rRNA or gene sequences encoding 16S rRNA and selecting sequences that are common to *P. acnes* isolates, but may not be completely shared with 16S rRNA sequences of other *Propionibacterium* species or other bacteria. Sequence comparisons were conducted by using known 16S rRNA sequences (rRNA or genes) of *Propionibacterium* species (e.g., *P. theonii, P. avidum, P. jensenii, P. cyclohexanicum, P. granulosum, P. freudenreichii, P. acidipropionici,* and *P. propionicus*) and of other bacterial species (e.g., *Corynebacterium, Fusobacterium, Actinobacterium, Mycobacterium,* and *Nocardioides*). Specific sequences were selected, synthesized *in vitro*, and the oligomers were characterized by determining the Tm and hybridization characteristics of the *P. acnes* oligomers with complementary target sequences (synthetic or purified rRNA from bacteria) by using standard laboratory methods. Then, selected oligomer sequences were further tested by making different combinations of amplification oligomers (selected from those shown in Table 1) in amplification reactions with synthetic 16S RNA target sequences or 16S rRNA purified from various *Propionibacterium* species grown in culture and performing amplification reactions to determine the efficiency of amplification of the 16S rRNA target sequences. The relative efficiencies of different combinations of amplification oligomers were monitored by detecting the amplified products of the amplification reactions, generally by binding a labeled probe (Table 2) to the amplified products and detecting the relative amount of signal that indicated the amount of amplified product made.

Specific oligonucleotides were designed to amplify and detect target sequences in *P. acnes* 16S rRNA or DNA encoding 16S rRNA, namely within SEQ ID NO:199, which is based on a *P. acnes* 16S rRNA gene sequence (contained in GenBank accession number AY642048). Two sets of amplification and detection oligomers were designed that are specific for the 16S rRNA target sequences within a first subset target region, which is SEQ ID NO:200, and the second set of oligomers are specific for the 16S rRNA target sequences within SEQ ID NO. 201.

Embodiments of amplification oligomers for *P. acnes* 16S rRNA sequences include those shown in Table 1. Amplification oligomers include those that may function as primers,

promoter primers, and promoter provider oligomers, with promoter sequences shown in lower case in Table 1. Some embodiments are the target-specific sequence of a promoter primer oligomer listed in Table 1, which optionally may be attached to the 3' end of any known promoter sequence. Examples of promoter sequences specific for the RNA polymerase of bacteriophage T7 include those of SEQ ID Nos. 73 and 74.

Amplification oligomers may be modified by synthesizing the oligomer with a 3' blocked end. The blocked oligomers may be used in a single primer transcription associated amplification reaction, i.e., functioning as blocking molecules or promoter provider oligomers. Embodiments of 3' blocked oligomers include those of SEQ ID Nos. 1-8 and 35-54 where an additional blocked C is added to the 3' end of the sequence.

Table 1: 16S rRNA Amplification Oligonucleotides

SEQ ID NO	Sequence
1	CGCCGCCAGCGUUCGU
2	AAGCACGCCGCCAGCGUU
3	UUAAGCACGCCGCCAGCG
4	UGUGCAAUAUUCCCCACUGC
5	AUUCCCCACUGCUGCC
6	ACUGCUGCCUCCCGUA
7	GUUCGUCCUGAGCCAGGAU
8	AGCGUUCGUCCUGAGCC
16	CCCAAAGTCAAGGGCAGGTTACTC
17	CCCAAAGTCAAGGGCAGGTTACT
18	CCCAAAGTCAAGGGCAGGTTAC
19	GTTATCCCAAAGTCAAGGGCAGGTTAC
20	CCTGAAGTTATCCCAAAGTCAAGGGCAGGT
21	CGGTGCTTCTTTACCCATTACCG
22	CGTAGTTAGCCGGTGCTTCTTTACCCATT
23	GCACGTAGTTAGCCGGTGCTTCTTTACC
24	GCCGGTGCTTCTTTACCCATTACCG
25	GCTTCTTTACCCATTACCGTCACTCACGC
26	CGTCACTCACGCTTCGTCACAGGC
184	CTGCTTTTGTGGGGTGCTC
35	aatttaatacgactcactatagggagaCGAACGCTGGCGGCGTGCTTAACACATGC
36	CGAACGCTGGCGGCGTGCTTAACACATGC
37	aatttaatacgactcactatagggagaACGCTGGCGGCGTGCTTAACACATGC
38	ACGCTGGCGGCGTGCTTAACACATGC
39	aatttaatacgactcactatagggagaCGGCGTGCTTAACACATGCAAGTCG
40	CGGCGTGCTTAACACATGCAAGTCG
41	aatttaatacgactcactatagggagaCGTGCTTAACACATGCAAGTCGAACGG
42	CGTGCTTAACACATGCAAGTCGAACGG
43	aatttaatacgactcactatagggagaCTTAACACATGCAAGTCGAACGGAAAGG

44	CTTAACACATGCAAGTCGAACGGAAAGG
45	aatttaatacgactcactatagggagaGCACAATGGGCGGAAGCCTGATGCAGCAA CG
46	GCACAATGGGCGGAAGCCTGATGCAGCAACG
47	aatttaatacgactcactatagggagaGCACAATGGGCGGAAGCCTGATGCAGC
48	GCACAATGGGCGGAAGCCTGATGCAGC
49	aatttaatacgactcactatagggagaGCACAATGGGCGGAAGCCTGATGC
50	GCACAATGGGCGGAAGCCTGATGC
51	aatttaatacgactcactatagggagaGGAATATTGCACAATGGGCGGAAGC
52	GGAATATTGCACAATGGGCGGAAGC
53	aatttaatacgactcactatagggagaGCAGTGGGGAATATTGCACAATGGGCG
54	GCAGTGGGGAATATTGCACAATGGGCG
73	aatttaatacgactcactatagggaga
74	agagggatatcactcagcataatttaa

Embodiments of detection probe oligomers for detection of amplified products of 16S rRNA sequences or genes encoding 16S rRNA are shown in Table 2. Detection probe embodiments include oligomers that can form hairpin configurations by intramolecular hybridization of the probe sequence, which include those of SEQ ID Nos. 75-98. For the sequences listed in Table 2, the lowercase letters indicate the sequences that hold a hairpin oligomer in the closed configuration. Embodiments of the hairpin probe oligomers were synthesized with a fluorescent label attached at one end of the sequence and a quencher compound attached at the other end of the sequence. Embodiments of hairpin probes may be labeled with a 5' fluorophore and a 3' quencher, for example a 5' fluorescein label and a 3' DABCYL quencher. Some embodiments of hairpin oligomers also include a non-nucleotide linker moiety at selected positions within the sequence. Examples of such embodiments include those that include an abasic 9-carbon ("C9") linker between residues 16 and 17 of SEQ ID Nos.: 75-84, between residues 17 and 18 of SEQ ID Nos.: 85-88, between residues 18 and 19 of SEQ ID Nos.: 89-92, between residues 19 and 20 of SEQ ID Nos.: 93 and 94, between residues 20 and 21 of SEQ ID Nos.: 95 and 96, and between residues 21 and 22 of SEQ ID Nos.: 97 and 98.

Another detection probe embodiment is a linear detection probe oligomers labeled with a chemiluminescent AE compound which is attached to the probe sequence via a linker (substantially as described in US Pats. 5,585,481 and 5,639,604, particularly at column 10, line 6 to column 11, line 3, and in Example 8). Examples of labeling positions are a central region of the probe oligomer and near a region of A:T base pairing, at a 3' or 5' terminus of the oligomer, and at or near a mismatch site with a known sequence that is not the desired target sequence.

The detection probes may be used with helper probes that are unlabeled and facilitate binding of the labeled probe to its target (see US Pat. 5,030,557, Hogan et al.).

Table 2: 16S rRNA Probes

SEQ ID NO	Sequence
75	CGAGCACCCCACAAAAGCucg
76	CGAGCACCCCACAAAAGC
77	CGAGCACCCCACAAAAGgcucg
78	CGAGCACCCCACAAAAG
79	GCACCCCACAAAAGCAGGugc
80	GCACCCCACAAAAGCAGG
81	CCGUCACUCACGCUUCGacgg
82	CCGUCACUCACGCUUCG
83	GCGGUUUACAACCCGAAccgc
84	GCGGUUUACAACCCGAA
85	GCCACUCGAGCACCCCAuggcg
86	GCCACUCGAGCACCCCA
87	CCCGAAGGCCGUCAUCCucggg
88	CCCGAAGGCCGUCAUCC
89	CGCCACUCGAGCACCCCAuggcg
90	CGCCACUCGAGCACCCCA
91	cCGUCACAGGCGAAAGCGGacgg
92	CGUCACAGGCGAAAGCGG
93	CGCCACUCGAGCACCCCACuggcg
94	CGCCACUCGAGCACCCCAC
95	CGCCACUCGAGCACCCCACAuggcg
96	CGCCACUCGAGCACCCCACA
97	CACGCUUCGUCACAGGCGAAAGCgug
98	CACGCUUCGUCACAGGCGAAAGC
181	cCAGGGCCUUUCCGUUCGccugg
182	CAGGGCCUUUCCGUUCG

Capture probe oligomers may be used in sample preparation to separate *P. acnes* 16S rRNA target nucleic acids from other sample components include those that contain the target-specific sequences of SEQ ID Nos. 120 and 122. Embodiments of the capture probes include a 3' tail region covalently attached to the target-specific sequence to serve as a binding partner that binds a hybridization complex made up of the target nucleic acid and the capture probe to an immobilized probe on a support. Embodiments of capture probes that include the target-specific sequences of SEQ ID Nos. 128 and 130, further include 3' tail regions made up of substantially homopolymeric sequences, such as dT_3A_{30} polymers (SEQ ID Nos. 127 and 129). Examples of target capture probes are listed in Table 3.

Table 3: Target Capture Probes for *P. acnes* 16S rRNA

127	GCCUUGGUAAGCCACUAtttaaaaaaaaaaaaaaaaaaaaaaaaaaaa
128	GCCUUGGUAAGCCACUA
129	GCUGAUAAGCCGCGAGUtttaaaaaaaaaaaaaaaaaaaaaaaaaaaa
130	GCUGAUAAGCCGCGAGU

Amplification and Detection of 23S rRNA Sequences of *P. acnes*

For amplification and detection of *P. acnes* sequences found in 23S rRNA, which include 23S rRNA sequence or DNA encoding 23S rRNA, oligonucleotides were designed that act as amplification oligomers and detection probes by comparing known sequences of 23S rRNA or gene sequence encoding 23S rRNA and selecting sequences that are common to *P. acnes* isolates, but may not be completely shared with 23S rRNA sequences of other *Propionibacterium* species or other bacteria. Sequence comparisons were conducted by using known 23S rRNA sequences (RNA or genes) of *Propionibacterium* species (*P. granulosum, P. avidum, P. thoenii, P. freudenreichii, P. jensenii*, and *P. acidipropionici*) and of other bacterial species (*Campylobacter sp., Mycobacterium sp.*, and *Helicobacter sp.*). Specific sequences were selected, synthesized *in vitro*, and the oligomers were characterized by determining the hybridization characteristics of the *P. acnes* oligonucleotides with complementary target sequences (synthetic or purified rRNA from bacteria) by using standard laboratory methods. Then, selected oligonucleotide sequences were further tested by making different combinations of amplification oligomers (selected from those shown in Table 4) in amplification reactions with synthetic 23S RNA target sequences or 23S rRNA purified from various *Propionibacterium* species grown in culture and performing amplification reactions to determine the efficiency of amplification of the 23S rRNA target sequences. The relative efficiencies of different combinations of amplification oligomers were monitored by detecting the amplified products of the amplification reactions, generally by binding a labeled probe to the amplified products and detecting the relative amount of signal that indicated the amount of amplified product made.

Specific oligonucleotides were designed to amplify and detect target sequences in *P. acnes* 23S rRNA or DNA encoding 23S rRNA, namely within SEQ ID NO:202, which is based on a *P. acnes* 23S rRNA gene sequence (contained in GenBank accession number AE017283). Two sets of amplification and detection oligomers were designed that are specific for the 23S rRNA target sequences within a first subset target region, which is SEQ ID NO:203, and the

second set of oligomers are specific for the 23S rRNA target sequences within SEQ ID NO. 204.

Embodiments of amplification oligomers for *P. acnes* 23S rRNA sequences include those shown in Table 4, in which lower case letters are used for the promoter sequences in promoter primers. Other embodiments of promoter primers may include the target-specific sequence of an oligomer listed in Table 4 attached to the 3' end of any of a variety of promoter sequences.

Table 4: Amplification Oligonucleotides for 23S rRNA Target Sequences

SEQ ID NO	Sequence
9	ACCCAACCACACAGGTTGCC
10	CTACCCAACCACACAGGTTG
11	CACTGACACGACGCTCCCCT
12	CCTACCCAACCACACAGGTT
13	GCTCCCCTACCCAACCACAC
14	TAAGCCCCCTAGACCATCCA
15	TGCTCTACCTCCAACAAGAAC
188	ACUCCACAUCCUUUCCC
27	GGATTCCCACCCGTCACA
28	GGATTCCCACCCGTCACAC
29	GGATTCCCACCCGTCACACG
30	GATTCCCACCCGTCACACG
31	GATTCCCACCCGTCACAC
32	ATTCCCACCCGTCACAC
33	CCACATCCTTTCCCACTGAGTC
34	CTTATCCCCCGCCGTCTCACTGCC
185	GCTTCACCACTGACACGA
55	aatttaatacgactcactatagggagaTGGGTAGGGGAGCGTCGTGTCAGT
56	TGGGTAGGGGAGCGTCGTGTCAGT
57	aatttaatacgactcactatagggagaTGGGTAGGGGAGCGTCGTGTCAGTG
58	TGGGTAGGGGAGCGTCGTGTCAGTG
59	aatttaatacgactcactatagggagaCAGTGGTGAAGCTGCCGGGTGACT
60	CAGTGGTGAAGCTGCCGGGTGACT
61	aatttaatacgactcactatagggagaCAGTGGTGAAGCTGCCGGGTGACTG
62	CAGTGGTGAAGCTGCCGGGTGACTG
63	aatttaatacgactcactatagggagaGTAGGGGAGCGTCGTGTCAGTG
64	GTAGGGGAGCGTCGTGTCAGTG
65	aatttaatacgactcactatagggagaGGAGCGTCGTGTCAGTGGTGAAGCTG
66	GGAGCGTCGTGTCAGTGGTGAAGCTG
67	aatttaatacgactcactatagggagaGCTTATCGGCTTACCGAAATCAGCCAAAC
68	GCTTATCGGCTTACCGAAATCAGCCAAAC
69	aatttaatacgactcactatagggagaGCTTATCGGCTTACCGAAATCAGCC
70	GCTTATCGGCTTACCGAAATCAGCC
71	aatttaatacgactcactatagggagaGAGCACTGGATGGTCTAGGGGGCTTATC G

72	GAGCACTGGATGGTCTAGGGGGCTTATCG
186	aatttaatacgactcactatagggagaGGAGTTGCGTAGACAACCAGG
187	GGAGTTGCGTAGACAACCAGG

Embodiments of detection probe oligomers for amplified products of 23S rRNA sequences or genes encoding 23S rRNA are shown in Table 5. Detection probe embodiments are oligomers that can form hairpin configurations by intramolecular hybridization of the probe sequence, which include those of SEQ ID Nos. 99-126 and 183. Embodiments of the detection probes include a 5' fluorophore (e.g., fluorescein), a 3' quencher (e.g., DABCYL), and an abasic moiety (e.g., C9) between residues 5 and 6 of SEQ ID Nos. 121-126, or between residues16 and 17 of SEQ ID Nos. 99-100, or between residues 17 and 18 of SEQ ID Nos. 103-106, or between residues18 and 19 of SEQ ID Nos.111-112, or between residues 19 and 20 of SEQ ID Nos. 107-110 and 113-114, or between residues 20 and 21 of SEQ ID Nos.101-102 and 113-116. Another embodiment includes detection probes labeled with an AE attached to the oligomer by a non-nucleotide linker.

Table 5: 23S rRNA Probes

SEQ ID NO	Sequence
99	CUCGCAUCAACUCCACgcgAg
100	CUCGCAUCAACUCCAC
101	GCCUGCAUUCUCACUCGCAUCAggc
102	GCCUGCAUUCUCACUCGCAUCA
103	ccCACUCGCAUCAACUCguggg
104	CACUCGCAUCAACUC
105	CUCGCAUCAACUCCACCgcgag
106	CUCGCAUCAACUCCACC
107	CUCGCAUCAACUCCACCACgcgag
108	CUCGCAUCAACUCCACCAC
109	cgCUCACUCGCAUCAACUCgagcg
110	CUCACUCGCAUCAACUC
111	gCUCACUCGCAUCAACUCugagc
112	CUCACUCGCAUCAACUC
113	gCUCACUCGCAUCAACUCCugagc
114	CUCACUCGCAUCAACUCC
115	gCUCACUCGCAUCAACUCCAugagc
116	CUCACUCGCAUCAACUCC
117	CGCUACACUUGCCAGCAUUCagcg
118	CGCUACACUUGCCAGCAUUC
119	CUGCCACGCUACACUUGCCAGggcag
120	CUGCCACGCUACACUUGCCAG
121	gguggCACUCGCAUCAACUCCACC

122	CACUCGCAUCAACUCCACC
123	gguggCUCGCAUCAACUCCACC
124	CUCGCAUCAACUCCACC
125	gguggACUCGCAUCAACUCCACC
126	ACUCGCAUCAACUCCACC
183	CCGCUACAUUAUCGGCGagcgg

Embodiments of capture probe oligomers for use in sample preparation to separate *P. acnes* 23S rRNA target nucleic acids from other sample components include those that contain a target-specific sequence that hybridizes to a 23S rRNA sequence or gene encoding 23S rRNA, such as the target-specific sequence of SEQ ID Nos. 132 and 134. Embodiments of capture probes include a 3' tail region covalently attached to the target-specific sequence to serve as a binding partner that binds the hybridization complex made up of the target nucleic acid and the capture probe to an immobilized probe on a support. Embodiment of the covalently linked 3' tail region is a substantially homopolymeric sequence, such as dT_3A_{30} linked to the 3' end as shown in SEQ ID Nos. 131 and 133. Sequences of target capture probe embodiments are in Table 6.

Table 6: Target Capture Probes for *P. acnes* 23S rRNA

131	CAAACUUGACCCUGGAACCCUUGGtttaaaaaaaaaaaaaaaaaaaaaaaaaaaa
132	CAAACUUGACCCUGGAACCCUUGG
133	CUUAGGACCCGACUCACCCAGGGCAGACAtttaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
134	CUUAGGACCCGACUCACCCAGGGCAGACA

EXAMPLE 1: Assay Reagents and Protocols

The experiments described in the examples that follow may use one or more of the following reagents, although those skilled in the art will appreciate that other reagents may be substituted for those described here. Probe Matrix Lysis Reagent contained 0.1% (w/v) LLS, 20mM Lithium Succinate, and 1mM EDTA. Lysis Reagent contained 1% (w/v) LLS, 100mM Tris, 2.5 mM succinic acid, 10 mM EDTA, and 500 mM lithium chloride (LiCl) at pH 6.5-7.5. Target Capture Reagent contained 300 mM HEPES, 1.88 M lithium chloride, 100 mM EDTA, at pH 6.4, and 250 µg/ml of paramagnetic particles (0.7-1.05 µ particles, SERA-MAG™ MG-CM, Seradyn, Inc., Indianapolis, IN) with $(dT)_{14}$ oligomers covalently bound thereto. Wash Solution used in target capture contained 10 mM HEPES, 150 mM NaCl, 1 mM EDTA, and 0.1% (w/v) sodium dodecyl sulfate, at pH 7.5. Amplification Reagent was mixed with other reaction components produce a solution containing 50 mM HEPES, 33 mM KCl, 30 mM $MgCl_2$, 0.5 mM

of each dNTP (dATP, dCTP, dGTP, dTTP), 10 mM ATP, 2 mM CTP, 12.7 mM UTP, 2 mM GTP, at pH 7.7. Amplification oligonucleotides (primers, promoter primers, blocker oligonucleotides, promoter provider oligonucleotides), and optionally probes, may be added to the reaction mixture in the amplification reagent or separate from the amplification reagent. Enzyme Reagent contained 360 RTU/μl of Moloney murine leukemia virus (MMLV) reverse transcriptase (RT) and about 80 PU/μl of T7 RNA polymerase, 75 mM HEPES, 120 mM KCl, 10% TRITON® X-100, 160 mM N-acetyl-L-cysteine, and 1 mM EDTA at pH 7.0, where 1 RTU of RT incorporates 1 nmol of dTTP in 20 min at 37°C, and 1 PU of T7 RNA polymerase produces 5 nmol of RNA transcript in 20 min at 37°C. Hybridization Buffer contained 0.6M LiCl, 1% lithium lauryl sulfate, 10 mM of EDTA and 10 mM EGTA, 50 mM succinic acid, 115 mM lithium hydroxide, pH 4.7.

The transcription mediated amplification (TMA) reactions use substantially the procedures as disclosed in detail previously US Pats. 5,399,491 and 5,554,516 (Kacian et al.). Single primer transcription associated amplification use substantially the procedures disclosed in detail in US 2006-0046265 A1 and US Pat. 7,374,885 (Becker et al). The TAG amplification method used procedures substantially as disclosed in US 2007-0281317 A1 (Becker et al.). The use and detection of signal from AE-labeled probes to detect hybridization complexes with target sequences use the procedures substantially as disclosed in detail previously (US Pats. 5,283,174 and 5,656,744, Arnold et al., and US Pat. 5,658,737, Nelson et al.). The methods for using hairpin probes are well known, and include those already disclosed in detail in US Pats. 6,849,412, 6,835,542, 6,534,274, and 6,361,945, (Becker et al.).

By using various combinations of amplification oligonucleotides and labeled detection probes to provide a detectable signal, *P. acnes* 16S rRNA and 23S rRNA sequences were specifically detected in samples containing the appropriate target nucleic acids. The following examples illustrate some of the preferred embodiments for these methods and compositions for detection of *P. acnes* target sequences.

EXAMPLE 2: *P. acnes* Culture and Lysis

P. acnes were grown on reinforced clostridial agar (RCA) at 37°C under anaerobic conditions for three to four days. Colonies were selected and further grown in degassed reinforced clostridial broth at 37°C under anaerobic conditions for 24-48 hours. Bacteria (approximately 2 X 10^7 CFU) were pelleted and resuspended in 1mL of Probe Matrix Lysis

Reagent for 15-60 minutes at 80 or 100°C. The bacteria were detected using methods and reagents substantially as are described in detail previously (Arnold et al., 1989, *Clin. Chem.*, vol. 35, pp. 1588-94). Briefly, after lysing the bacteria, 1mL of 2X Hybridization Buffer was added to the Probe Matrix Lysis Reagent. Hybridization Buffer contained nucleic acid probes specific for rRNA sequences conserved among bacterial species (see US Pats. 4,851,330, 5,567,587, 5,601,984, 5,688,645, 5,714,324, and 5,723,579, Kohne, and 5,679,520, Hogan et al.) and the probes were labeled with acridinum ester (AE) (see US Pats. 4,946,958, Campbell et al.; and 5,185,439, 5,585,481 5,656,744, and 5,696,251, Arnold et al.). Non-hybridized probes were deactivated followed by activation of the AE and detection on a luminometer (see US Pats. 5,238,174 and 5,639,604, Arnold et al.). The amount of nucleic acid released was measured in relative light units (RLU). Table 7 shows the RLU signals over a course of time for the two different incubation temperatures. An alternative to using temperature controlled equipment, e.g. a heat block, bacteria can be lysed using a microwave oven. For example, microwaving at 30% power for 15 minutes may provide similar results as incubating at 95°C for 60 minutes. This reduces lysis time, however, due to microwave oven power differences, the power level and time may need to be adjusted.

Table 7: Lysis Comparison Between 80°C and 100°C

Temperature	15 minutes	30 minutes	45 minutes	60 minutes
80°C	30,834	65,154	124,729	156,817
100°C	69,578	186,024	244,604	361,665

In another embodiment, *P. acnes* bacteria may be filtered from a liquid using a filter system (e.g. MILLIPORE® Microfil S system). *P. acnes* were dispersed into 0.9% saline and passed through a Microfil S system. The filter was removed and placed in a container with 1mL of Probe Matrix Lysis Reagent. The container was incubated at 100°C for 30-60 minutes followed by the addition of 1mL of 2X Hybridization Buffer. Results in Table 8 show the RLU signals for pelleted bacteria and filtered bacteria.

Table 8: Lysis Comparison Between Pelleted and Filtered Bacteria

	0 minutes	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes
Pelleted	8,525 RLU	141,692	270,732	302,359	395,197	428,571
Filtered	16,302 RLU	159,674	201,704	260,828	338,774	333,503

In another embodiment, *P. acnes* can be lysed using known enzymes, (e.g. lysozyme,

mutanolysin, and protein K), alone or in combination. For example, *P. acnes* bacteria were incubated in 500ul TES buffer (30 mM Tris, pH 8.0; 5 mM EDTA; 50 mM NaCl) plus lysozyme for 30 minutes at 37°C. An additional 500ul of TES buffer plus 0.5% LDS, 1% Triton X-100 and mutanolysin or proteinase K was added and incubated an additional 30 minutes at 37°C followed by the addition of 1mL of 2X Hybridization Buffer. A comparison of Probe Matrix Lysis Reagent and protease digestion is shown in Table 9.

Table 9: Lysis Comparison Between Heat and Enzyme

	RLU
Negative control (Hybridization Reagent at room temperature)	1,644
Probe Matrix Lysis buffer 100°C for 60 minutes	399,713
Lysozyme and mutanolysin in TES buffers	865,296
Lysozyme and proteinase K in TES buffers	170,466

EXAMPLE 3: Design and Initial Testing of *P. acnes* 16S Amplification Oligomer Sets

Amplification and detection oligonucleotides were designed to amplify and detect *P. acnes* 16S rRNA. The designs were based on a *P. acnes* 16S rRNA gene sequence (GenBank accession number AY642048.1 and GI number 50082571). Two sets of amplification and detection oligomers were designed to be specific for target sequences within the first 500 bases of GI number 50082571 (SEQ ID NO. 199), in which the first set of oligomers are specific for target sequences contained in bases 1-200 (SEQ ID NO. 200) and the second set of oligomers are specific for target sequences contained in bases 325-500 (SEQ ID NO. 201). The *P. acnes* 16S rRNA amplification and detection oligomers are SEQ ID Nos. 1-8, 16-26, 35-54, 75-98, 135-160, 181-182, 184, 189-193, 210, and 211-216.

Known amounts of *P. acnes* 16S rRNA sequences were amplified using real time single primer transcription associated amplification, (substantially as described in detail in US20060046265 A1, Becker et al.), using combinations of the oligonucleotides in Table 1 and Table 2. Oligomer combinations were tested using 0 and 10^5 copies of *P. acnes* 16S rRNA. The reaction mixtures containing the amplification oligonucleotides, target and amplification reagents (but not enzymes) were covered to prevent evaporation, incubated 5 min at 95°C, then 2 min at 42°C, then enzymes were added, the reactions were mixed and incubated for 30 min at 42°C, measuring fluorescence at regular time intervals (i.e., every 30 sec) during the amplification reaction after enzyme addition. The reaction mixture was monitored using a real

time fluorescent detection system (e.g. Bio-Rad Laboratory's Opticon™ or Chromo4™, or a FluoDia® T70 instrument). Real time algorithms are well known in the art (e.g., see *Biochem. Biophys. Res. Commun.* 2002 Jun 7;294(2):347-53 and *Nucleic Acids Res.* 2004 32(22):e178). Negative controls, (reactions performed in the same manner using zero copies of *P. acnes* 16S rRNA), were tested to determine background fluorescent signal levels. Oligomer combinations were evaluated on signal emergence time, maximum relative fluorescent units (RFU) signal, slope of the sigmoid curve, and background signal. Assay oligomer combinations that produced a fluorescent curve at 10^5 copies of *P. acnes* 16S rRNA and had less than 0.1 relative fluorescent units (RFU) in the negative controls were selected for further testing.

The following assay oligomer combinations were further tested for sensitivity: (1) SEQ ID Nos. 8, 20, 35 and 75; (2) SEQ ID Nos. 7, 20, 35, and 79; (3) SEQ ID Nos. 7, 20, 35 and 77; (4) SEQ ID Nos. 1, 16, 37, and 75; (5) SEQ ID Nos. 1, 18, 37, and 75; (6) SEQ ID Nos. 3, 20, 41, and 75; (7) SEQ ID Nos. 8, 19, 37, and 79; (8) SEQ ID Nos. 1, 16, 37, and 79; (9) SEQ ID Nos. 3, 19, 37, and 95; (10) SEQ ID Nos. 4, 22, 47, and 81; and (11) SEQ ID Nos. 4, 23, 51, and 81. Each combination was tested at 0, 10^3, 10^4, and 10^5 copies of *P. acnes* 16S rRNA. These results were used to identify assay oligomer combinations that displayed emergence times around 20 minutes for reactions that contained 10^5 copies of *P. acnes* 16S rRNA, with maximum signals from 0.98 to 1.5 RFU, and background signal levels at less than 0.2 RFU. The results of these tests showed that selected assay oligomer combinations amplified as few as 10^4 copies of *P. acnes* 16S rRNA. Results obtained by using the 16S amplification and detection oligomer combinations of combination 1 (SEQ ID Nos. 8, 20, 35 and 75), combination 2 (SEQ ID Nos. 7, 20, 35, and 79), and combination 3 (SEQ ID Nos. 7, 20, 35 and 77) are shown in Table 10, reported as emergence of the signal (TTime) and average RFU ("AveRange" or mean of detected RFU for replicate samples in the same condition, averaged following subtraction of the minimum detected RFU for a test result from the maximum detected RFU from the same test result).

Table 10: 16S rRNA Amplification and Detection Oligomer Combinations for *P. acnes*

Amplification Oligonucleotides	Copies rRNA	AveRange (RFU)	TTime (minutes)
Combination 1	0	0.208	35.1
	10^3	0.527	33.0
	10^4	1.176	28.2
	10^5	1.452	23.1
Combination 2	0	0.116	28.0
	10^3	0.215	27.9
	10^4	0.771	26.6
	10^5	0.984	22.2
Combination 3	0	0.085	42.6
	10^3	0.362	31.9
	10^4	0.962	27.7
	10^5	1.248	22.3

Amplification and detection oligomer combinations 1 (SEQ ID Nos. 8, 20, 35 and 75), 2 (SEQ ID Nos. 7, 20, 35, and 79) and 3 (SEQ ID Nos. 7, 20, 35, and 77) were tested for cross reactivity against the following challenge organisms: *P. granulosum, P. cyclohexanicum, P. freudenreichii, actinomyces israelii, P. avidum* and *P. theonii.* Each oligomer combination was tested for each challenge organism using single primer transcription associated amplification. The oligomer combinations 1, 2 and 3 were tested using 10^4 CFU for *P. granulosum, P. cyclohexanicum, P. freudenreichii* and *A. israelii,* and 10^2 CFU for *P. avidum* and *P. theonii.* Amplification and detection oligomer combinations did not amplify or detect any of the challenge organisms. Data from the cross reactivity test is shown in Table 11 reported as described above for each of the oligomer combinations.

Table 11: Cross Reactivity for the 16S rRNA Assay Oligomer Combinations

Amplification Oligonucleotides	Challenge Organism	AveRange (RFU)	TTime (minutes)
Combination 1	none	0.294	33.8
	P. avidum	0.068	No signal
	P. cyclohexanicum	0.177	36.5
	P. freudenreichii	0.063	No signal
	P. granulosum	0.064	No signal
	P. israellii	0.062	No signal
	P. theonii	0.055	No signal
Combination 2	none	0.134	26.5
	P. avidum	0.035	No signal
	P. cyclohexanicum	0.093	14.7
	P. freudenreichii	0.041	No signal

	P. granulosum	0.037	No signal
	P. israellii	0.036	No signal
	P. theonii	0.049	No signal
Combination 3	none	0.134	26.5
	P. avidum	0.035	No signal
	P. cyclohexanicum	0.093	14.7
	P. freudenreichii	0.041	No signal
	P. granulosum	0.037	No signal
	P. israellii	0.036	No signal
	P. theonii	0.049	No signal

The amplification and detection oligomer combinations were further tested ensure that challenge organisms (i.e., related non-*P.acnes* species), did not inhibit amplification of *P. acnes* 16S rRNA. Three challenge organisms, *P. cyclohexanicum, P. avidum and P. theonii*, were chosen because *P. avidum* exhibits the highest degree of homology to *P. acnes*, whereas *P. theonii* and *P. cyclohexanicum* exhibited the strongest affects on the fluorescent curves in the initial cross reactivity studies. Zero or 10^6 copies of *P. acnes* 16S rRNA were tested with 10^4 copies of *P. Cyclohexanicum* or 10^2 copies of *P avidum* or *P theonii*. The tests were repeated using 0 or 10^4 CFU *P. acnes* lysate and 10^4 copies of *P. cyclohexanicum*, *P. avidum* or *P. theonii*. Samples without *P. acnes* showed no amplification where as samples with *P. acnes* showed amplification. The presence of other bacteria reduced the background signal and the maximum RFU's, however, the emergence time was not affected. Data from the tests is show in Table 12.

Table 12: Specific Amplification of *P. acnes* 16S rRNA in the Presence of Challenge Organisms

Amplification Oligomer	***P. acnes* rRNA**	**Challenge Organism**	**AveRange (RFU)**	**TTime (minutes)**
Combination 1	0	none	0.657	35.9
	10^6	none	1.894	22.4
	0	*P. avidum*	0.067	No signal
	10^6	*P. avidum*	1.393	23.2
	0	*P. cyclohexanicum*	0.345	38.1
	10^6	*P. cyclohexanicum*	1.815	23.0
	0	*P. theonii*	0.060	No signal
	10^6	*P. theonii*	0.920	22.3
Combination 2	0	none	0.249	27.8
	10^6	none	1.316	19.0
	0	*P. avidum*	0.033	No signal
	10^6	*P. avidum*	0.909	19.4

	0	*P. cyclohexanicum*	0.103	23.7
	10^6	*P. cyclohexanicum*	1.182	19.7
	0	*P. theonii*	0.049	0.9
	10^6	*P. theonii*	1.040	19.4
Combination 3	0	none	0.371	35.7
	10^6	none	1.752	21.0
	0	*P. avidum*	0.048	No signal
	10^6	*P. avidum*	1.407	21.5
	0	*P. cyclohexanicum*	0.203	38.2
	10^6	*P. cyclohexanicum*	1.633	21.5
	0	*P. theonii*	0.047	No signal
	10^6	*P. theonii*	1.380	21.1

EXAMPLE 4: Design and Testing of *P. acnes* 16S rRNA Target Capture Oligomers

Target capture is used to purify and prepare nucleic acid samples for subsequent amplification (see US Pats. 6,110,678, 6,280,952, and 6,534,273, Weisburg et al.). Briefly, samples were prepared containing known amounts of target nucleic acid in Lysis Reagent. Samples were mixed with a target capture oligomer that contained the target-specific sequence with a covalently attached dT_3A_{30} tail, and polydT-magnetic particles. The mixtures were incubated for 30 min at 60°C, and then for 30 min at room temperature to form hybridization complexes that captured the target RNA onto the magnetic particles. Magnetic particles were separated from the solution by applying a magnetic field to the outside of the container and transferring the magnetic particles to another solution. The magnetic particles with the target sequence were washed once with wash solution. The magnetic particles with the target nucleic acid were resuspended in amplification reagent, ready for subsequent amplification. Target capture can be performed manually or with an automated device (e.g. Target Capture System (Gen-Probe Incorporated) or ' KingFisher™system (Thermo Labsystems))

Three target capture oligonucleotides were designed for the *P. acnes* 16S rRNA, two specific oligonucleotides that correspond to bases 221 and 250 of *E. coli* accession number V00331 and GI number 42756 (SEQ ID Nos. 127-130) and one non-specific capture oligonucleotide. Use of non-specific capture oligonucleotides has been described previously (see US 2008-0286775 A1, Becker et al.). Briefly, the non-specific capture probes used in the following tests are oligonucleotides of random sequences of G's and U's, 18 bases in length that non-specifically bind to nucleic acid. Target capture oligonucleotides were tested to confirm they did not interfere with the amplification reaction. Specifically, 2.5 pmol of target capture

oligomers (SEQ ID Nos. 127 or 129) or 2.5 pmol of non-specific capture probe were spiked in samples containing 10^5 copies of target RNA. Samples were amplified using real-time single primer transcription associated amplification with one of the assay oligomer combinations described earlier herein. Target capture oligonucleotides exhibited minimal affects on the amplification reaction, data shown in Table 13.

Table 13: Affect of Target Capture Oligonucleotides on Amplification

	AveRange (RFU)	TTime (minutes)
No Target Capture Oligomer	1.270	15.0
SEQ ID NO. 127	1.035	14.3
SEQ ID NO. 129	1.030	14.0
Non-Specific Capture Probe	0.940	14.6

Target capture oligomer sensitivity was determined by: (1) keeping the amount of *P. acnes* 16S rRNA constant and varying the amount of target capture oligomers, and (2) keeping the amount of target capture oligomers constant and varying the amount of *P. acnes* 16S rRNA. The first test, 0 or 10^6 copies of *P. acnes* 16S rRNA were amplified using 0, 2.5, 5, 10, or 20 picomoles of capture probe per mL of lysis reagent. In the second test, 0, 10^3, 10^4, 10^5, and 10^6 copies of *P. acnes* 16S rRNA were amplified using 5 picomoles of capture probe per mL of lysis reagent. In both tests, nucleic acids samples were amplified using real time single primer transcription associated amplification, using assay oligomer combination 1. Data from the sensitivity experiments is shown in Tables 14 and 15.

Table 14: Sensitivity of 16S Target Capture Oligonucleotides Test 1

Target Capture Probe	Amt. Target Capture	AveRange (RFU)	TTime (minutes)
SEQ ID NO. 127	0	0.178	17.3
	2.5	0.330	15.0
	5	0.355	15.3
	10	0.354	15.5
	20	0.351	16.4
SEQ ID NO. 129	0	0.196	19.2
	2.5	0.321	15.1
	5	0.315	15.0
	10	0.335	16.0
	20	0.334	16.1
Non-specific Capture Probe	0	0.177	17.6
	2.5	0.332	17.0
	5	0.338	16.9
	10	0.306	15.3

	20	0.310	17.0

Table 15: Sensitivity of 16S Target Capture Oligonucleotides Test 2

Target Capture Probe	Amt. *P. acnes* rRNA	AveRange (RFU)	TTime (minutes)
SEQ ID NO. 127	0	0.173	17.3
	10^3	0.194	16.7
	10^4	0.200	17.9
	10^5	0.309	16.7
	10^6	0.356	14.3
SEQ ID NO. 129	0	0.146	16.0
	10^3	0.169	16.2
	10^4	0.186	16.2
	10^5	0.293	16.5
	10^6	0.333	14.2
Non-Specific Capture Probe	0	0.158	17.4
	10^3	0.140	16.0
	10^4	0.168	18.3
	10^5	0.229	17.9
	10^6	0.301	16.3

Target capture oligonucleotides were also tested for cross reactivity against the following challenge organisms: *P. cyclohexanicum, P. avidum and P. theonii.* Briefly, target capture probes were added to samples containing the 10^4 CFU of challenge organism. Controls were run containing 0 or 10^6 copies of *P. acnes* 16S rRNA. All samples were amplified using real time single primer transcription associated amplification, using assay oligomer combination 1. Data is show in Table 16.

Table 16: Cross Reactivity of Target Capture Oligomers

Target Capture Probe	Amt. *P. acnes* 16S rRNA	Challenge Organism	AveRange (RFU)	TTime (Minutes)
SEQ ID NO. 127	0	none	0.123	20.8
	10^6	none	0.461	16.1
	0	*P. avidum*	0.059	7.8
	0	*P. cyclohexanicum*	0.125	19.3
	0	*P. theonii*	0.062	7.8
SEQ ID NO. 129	0	none	0.136	19.8
	10^6	none	0.525	16.7
	0	*P. avidum*	0.049	5.7
	0	*P. cyclohexanicum*	0.137	20.8
	0	*P. theonii*	0.067	5.9

Target capture oligomers were further tested for cross reactivity to ensure the challenge organisms, *P. cyclohexanicum, P. avidum and P. theonii*, did not inhibit amplification. Briefly, target capture probes were added to samples containing 0, 10^3, 10^4, 10^5, or 10^6 copies of *P. acnes* 16S rRNA with and without the 10^4 CFU of the challenge organism. Following target capture, all samples were amplified using real time single primer transcription associated amplification, using assay oligomer combination 1Results of the tests in which target capture was performed using SEQ ID NO. 129 are shown in Table 17.

Table 17: Specific Amplification of *P. acnes* 16S rRNA in the Presence of Challenge Organisms

Challenge Organism	Amt. *P. acnes* rRNA	AveRange (RFU)	TTime (minutes)
None	0	0.105	13.3
	10^3	0.109	15.7
	10^4	0.153	19.8
	10^5	0.306	23.3
	10^6	0.430	20.4
P. avidum	0	0.067	6.5
	10^3	0.066	9.3
	10^4	0.074	4.2
	10^5	0.068	6.5
	10^6	0.098	9.6
P. cyclohexanicum	0	0.097	10.9
	10^3	0.105	12.1
	10^4	0.193	18.2
	10^5	0.348	24.0
	10^6	0.580	21.2
P. theonii	0	0.073	8.4
	10^3	0.077	6.8
	10^4	0.080	7.6
	10^5	0.094	7.9
	10^6	0.324	19.8

EXAMPLE 5: TAG Amplification and Real Time Detection of *P. acnes* 16S rRNA

Methods for reducing background fluorescence are known and one such method, called TAG amplification, was used to reduce the background signal for real time detection of *P. acnes* 16S rRNA. TAG amplification has been described in detail previously (see US. 2007-0281317 A1 Becker et al.). Briefly, TAG amplification modifies one of the amplification oligmers (referred to as the TAG oligomer) to include a non-target binding sequence at the 5' end. TAG oligmers may be included with the target capture probe during the target capture step. Thus, the

sample nucleic acid simultaneously hybridizes to both the target capture probe and the TAG oligmer. All non-bound nucleic acid is washed away, including excess TAG oligmers. Samples are amplified, the first round of amplification uses the TAG oligomer whereas subsequent rounds of amplification use oligmers specific for the TAG sequence. Embodiments of TAG oligomers for amplifying *P. acnes* 16S rRNA are shown in Table 18, in which the lower case letters represent the TAG sequences.

Table 18: *P. acnes* 16S rRNA TAG Oligonucleotides

Seq ID	Sequence
135	GaacctagttgggcgagttacggaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
136	CCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
137	AACCTGCCCTgactggtacatgcaagctacCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
138	AACCTGCCCTTGgactggtacatgcaagctacCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
139	AACCTGCCCTTGACgactggtacatgcaagctacCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
140	AACCTGCCCTTGACTTgactggtacatgcaagctacCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
141	AACCTGCCCTgttgacgtaccgtattgaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
142	AACCTGCCCTTGgttgacgtaccgtattgaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
143	AACCTGCCCTTGACgttgacgtaccgtattgaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
144	AACCTGCCCTTGACTTgttgacgtaccgtattgaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
145	AACCTGCCCTcattggcatagctagaccCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
146	AACCTGCCCTTGcattggcatagctagaccCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
147	AACCTGCCCTTGACcattggcatagctagaccCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
148	AACCTGCCCTTGACTTcattggcatagctagaccCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
149	AACCTGCCCTTGACTTTgtgacccaatgatctaacaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
150	AACCTGCCCTTGACgtgacccaatgatctaacaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
151	AACCTGCCCTTGgtgacccaatgatctaacaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
152	AACCTGCCCTgtgacccaatgatctaacaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT

153	AACCTGCCgtgacccaatgatctaacaCCTGAAGTTATCCCAAAGTCAAGGG CAGGTT
154	gtgacccaatgatctaacaCCTGAAGTTATCCCAAAGTCAAGGGCAGGTT
155	gtgacccaatgatctaacaGAGCACCCCACAAAAGCAG
156	CTGCTTTTGTGGGGTGCTCTGTgtgacccaatgatctaacaGAGCACCCCAC AAAAGCAG
157	CTGCTTTTGTGGGGTGCTCgtgacccaatgatctaacaGAGCACCCCACAAA AGCAG
158	CTGCTTTTGTgtgacccaatgatctaacaGAGCACCCCACAAAAGCAG
159	CTGCTTTTgtgacccaatgatctaacaGAGCACCCCACAAAAGCAG
160	CTGCTTgtgacccaatgatctaacaGAGCACCCCACAAAAGCAG
189	Gaacctagttgggcgagttacgga
190	Gactggtacatgcaagctac
191	Gttgacgtaccgtattga
192	Cattggcatagctagacc
193	Gtgacccaatgatctaaca

Initial testing using TAG amplification was conducted on samples containing 0 or 10^5 copies of *P. acnes* 16S rRNA. Target capture was performed using a target specific capture probe and the TAG oligomer. Real time single primer transcription associated amplification was performed, using a variation of assay oligomer combination 1. The variation included: (1) substituting SEQ ID NO. 135 for SEQ ID NO. 20 and (2) adding an additional amplification oligomer SEQ ID NO. 189. TAG amplification greatly reduced the background fluorescence, however, the emergence time was delayed and there were several false positives. Several experiments were conducted to eliminate the false positives by varying the amplification conditions. Zero or 10^5 copies of *P. acnes* were amplified with and without blocker oligomers added during target capture, with and without extra blocker oligomers added during amplification, and with or without an additional incubation at 60 or 65°C for five minutes, after target capture. Due to the ubiquitous nature of *P. acnes*, the false positives may be due to contamination, rather than problems with the amplification system. Data is shown in Table 19.

Table 19: Initial Testing Using the TAG System to Amplify *P. acnes* 16S rRNA

Amplification System	Amt. *P. acnes* rRNA	AveRange (RFU)	TTime (minutes)	False Positives	False Negatives
Single Primer Transcription Associated Amplification	0	0.258	20.7	0	0
	10^5	0.554	18.0		
TAG	0	0.433	34.1	3/5	0
	10^5	0.607	29.5		
TAG more replicates	0	0.114	33.1	2/12	0
	10^5	0.493	28.7		

TAG plus extra incubation	0	0.173	35.5	1/5	1/5
	10^5	0.725	33.0		
TAG plus extra incubation and extra blocker	0	0.044	5.7	0/5	0/5
	10^5	0.721	32.0		

Modified TAG oligomers were designed (SEQ ID Nos. 137-160) to reduce the false positives and false negatives. The modified TAG oligomers form a hairpin structure using a closing sequence that hybridizes to all or part of the target binding sequence and or the TAG sequence. The closing sequence ranges from 6-22 bases in length. The target binding region of a hairpin TAG oligomer will preferentially bind to the target, thus unfolding the hairpin, rather than stay in the hairpin conformation. The hairpin reduces non-target hybridization of the TAG oligomer. Samples were tested using assay oligomer combination 2 with two changes. SEQ ID NO. 20 was substituted with a sequence selected from SEQ ID Nos. 137-160. If SEQ ID Nos. 137-140 were used, then SEQ ID NO. 190 was added to the amplification oligomers. If SEQ ID Nos. 141-144 were used, then SEQ ID NO. 191 was added to the amplification oligomers. If SEQ ID Nos. 145-148 were used, then SEQ ID NO. 192 was added to the amplification oligomers. If SEQ ID Nos. 149-160 were used, then SEQ ID NO. 193 was added to the amplification oligomers. Samples were amplified using real time single primer transcription associated amplification. Data is shown in Table 20 for SEQ ID Nos. 137-148.

Table 20: Initial Testing of the TAG oligomer redesign for *P. acnes* 16S rRNA

SEQ ID NO. 20 Substitution	**Copies *P. acnes* rRNA**	**AveRange (RFU)**	**TTime (Minutes)**
SEQ ID NO. 137	0	0.055	No signal
	10^5	0.913	24.1
SEQ ID NO. 138	0	0.058	No signal
	10^5	0.711	25.2
SEQ ID NO. 139	0	0.052	No signal
	10^5	0.745	26.5
SEQ ID NO. 140	0	0.062	No signal
	10^5	0.745	25.5
SEQ ID NO. 141	0	0.503	36.2
	10^5	0.424	25.6
SEQ ID NO. 142	0	0.075	No signal
	10^5	0.350	26.5
SEQ ID NO. 143	0	0.217	35.4
	10^5	0.415	26.4
SEQ ID NO. 144	0	0.366	35.5
	10^5	0.383	26.7

SEQ ID NO. 145	0	0.964	25.6
	10^5	0.912	17.2
SEQ ID NO. 146	0	0.716	25.9
	10^5	0.760	17.7
SEQ ID NO. 147	0	0.679	25.7
	10^5	0.982	19.0
SEQ ID NO. 148	0	0.336	25.5
	10^5	0.800	21.1

SEQ ID Nos. 137-140 provided the best background signal reduction and were further tested for sensitivity. The oligomer combinations were tested at 0, 10^4 and 10^5 copies of *P. acnes* 16S rRNA, the data is shown in Table 21. Assay oligomer combination with SEQ ID NO. 137 was further tested for sensitivity. The SEQ ID NO. 137 oligomer combination resulted in 1/15 false positives and no false negatives at 10^4 and 10^5 copies of 16S rRNA, and 1/15 false negatives at 10^3 copies.

Table 21: Sensitivity Testing for the TAG Oligomer Redesigned for *P. acnes* 16s rRNA

SEQ ID NO. 20 Substitution	**Copies *P. acnes* rRNA**	**AveRange (RFU)**	**TTime (Minutes)**	**False negatives**
SEQ ID NO. 137	0	0.063	31.4	1/6
	10^4	0.790	30.1	
	10^5	1.013	26.3	
SEQ ID NO. 138	0	0.104	33.0	3/6
	10^4	0.526	29.6	
	10^5	0.993	27.4	
SEQ ID NO. 139	0	0.039	No signal	5/6
	10^4	0.204	33.9	
	10^5	1.062	27.8	
SEQ ID NO. 140	0	0.043	No signal	3/6
	10^4	0.300	30.6	
	10^5	0.887	29.8	

EXAMPLE 6: Increasing Sensitivity in *P. acnes* 16S rRNA Detection

Experiments were conducted to determine if sensitivity may be improved by adding one or more helper probes, selected from SEQ ID Nos. 195-198 (see Table 22), to the target capture step. Helper probes have been described previously (see US Pat. 5,030,557, Hogan). Briefly, helper probes are short probes that help reduce hybridization inhibition due to secondary structure. Their small size allows them to bind areas near secondary structures and then the secondary structure changes which allows a longer oligomer to hybridize to the nucleic acid.

Table 22: Target Capture Helper Oligomers for *P. acnes* 16S rRNA

SEQ ID NO.	Sequence
195	CATGCAGCAGGAGCTCCTATC
196	GCTCCTATCCGGTATTAGC
197	GCAGGAGCTCCTATCCGGTA
198	GCAGGAGCTCCTATCCGGTATTAGC

Samples were tested using assay oligomer combination 2 with two changes: SEQ ID NO. 141 replaced SEQ ID NO. 20, and SEQ ID NO. 191 was added to the amplification oligonucleotides. The first test compared samples with and without helper probes, the second test compared the different helper probes. Both tests used real time single primer transcription associated amplification. In the first test, 0, 10^3 or 10^5 copies of *P. acnes* 16S rRNA underwent target capture using a specific capture probe (SEQ ID NO. 129) with and without helper probe (SEQ ID NO. 195). The results of the first test did not improve sensitivity, but did improve the slope of the curve. In the second test, 0 or 10^4 copies of *P. acnes* 16S rRNA underwent target capture using a specific capture probe (SEQ ID NO. 129) and one helper probe chosen from SEQ ID Nos. 196-198. The results of the second test showed amplification inhibition or problems with the experimental set up because data for SEQ ID NO. 195 is lower than previous tests. Data for the two tests is show in Tables 23 and 24, "AveSlope" is the average slope of the real time amplification curve; "AveRange" stands for average (mean) of detected RFU for replicate samples tested in the same condition.

Table 23: *P. acnes* Amplified With and Without Target Capture Helper Probes

	Copies *P. acnes* rRNA	AveSlope	AveRange (RFU)	TTime (minutes)
No helper	0	0.018	0.040	No signal
	10^3	0.026	0.099	29.0
	10^5	0.050	0.603	26.0
With helper SEQ ID NO. 195	0	0.024	0.092	28.3
	10^3	0.025	0.098	27.1
	10^5	0.063	0.716	25.2

Table 24: *P. acnes* Comparison of Target Capture Helper Probes

Helper Probe	Copies *P. acnes* rRNA	AveSlope	AveRange (RFU)	TTime (minutes)
SEQ ID NO. 195	0	0.019	0.083	20.7
	10^4	0.018	0.100	14.1
SEQ ID NO. 196	0	0.018	0.063	No signal

	10^4	0.019	0.075	No signal
SEQ ID NO. 197	0	0.020	0.062	No signal
	10^4	0.018	0.073	9.1
SEQ ID NO. 198	0	0.019	0.064	No signal
	10^4	0.018	0.086	20.5

EXAMPLE 7: Reducing False Positives With Base Treatment

Due to the prevalence of *P. acnes* in the environment and on human skin, buffers and oligomers that are made in the presence of people may contain trace amounts of *P. acnes* thus causing false positives. Pre-treating amplification oligomers and buffers with base may reduce false positives by degrading any *P. acnes* RNA that is present in the reagents. One method for base treating the oligomers uses lysis reagent (see Example 1) that is not brought to pH 7.5; this will be referred to as base or basic lysis reagent. Target capture and some of the amplification oligomers are added to the base lysis reagent, heated at 60°C for 90 minutes and cooled to room temperature. After cooling, the base lysis reagent is neutralized by injecting hydrochloric acid into the tube using a sterile syringe. Once neutralized, the sample may be added to the lysis buffer and oligomers.

EXAMPLE 8: Reducing False Positives With Modified Target Capture Probes

One method to reduce false positives uses target capture probes that have been modified to include a hairpin formation. Hairpin target capture probes have been described previously (see US 2006-0068417 A1 Becker et al). Embodiments of hairpin target capture probes for capturing *P. acnes* nucleic acid are listed in Table 25, in which lower case letters represent the hairpin closing sequence.

Table 25: Hairpin Target Capture Oligomers

SEQ ID NO	Sequence
219	GCUGAUAAGCCGCGAGUTTTAAAAAAAAAAAAAAAAAAAAAAAAAAA
220	GCUGAUAAGCCGCGAGUaucagcTTTAAAAAAAAAAAAAAAAAAAAAAAAAAA
221	GCUGAUAAGCCGCGAGUuaucagcTTTAAAAAAAAAAAAAAAAAAAAAAAAAA
222	GCUGAUAAGCCGCGAGUuuaucagcTTTAAAAAAAAAAAAAAAAAAAAAAAAA
223	GCUGAUAAGCCGCGAGUcuuaucagcTTTAAAAAAAAAAAAAAAAAAAAAAAA
224	GCUGAUAAGCCGCGAGUgcuuaucagcTTTAAAAAAAAAAAAAAAAAAAAAAA
225	GCUGAUAAGCCGCGAGUcggcuuaucagcTTTAAAAAAAAAAAAAAAAAAAA

	AAAAAAAAAA
226	GCUGAUAAGCCGCGAGUcgcggcuuaucagcTTTAAAAAAAAAAAAAAAAAA AAAAAAAAAAAA

Linear target capture probes (SEQ ID NO. 219) were compared to hairpin target capture probes with hairpin closing sequences that were 10, 12, or 14 bases long (SEQ ID Nos. 224, 225, and 226 respectively). Target capture was preformed with the target capture probes and amplification oligomers (SEQ ID Nos. 210 and 8). After separating the target capture complexes, the samples underwent real-time single primer transcription associated amplification using SEQ ID Nos. 35, 193 and 181. Results are shown in Table 26. For the linear target capture probes, 6 out of 12 negative samples were positive. For the hairpin target capture probes with 10 or 12 base closing sequences, 1 out of 12 negative samples were positive. For the hairpin target capture probes with 14 base closing sequence there were no false positives. Although the hairpin target capture probes reduced the number of false positives, they also reduced the sensitivity of the assay.

Table 26: Linear and Hairpin Target Capture Probes

Target Capture Probe	Copies rRNA	AveRange (RFU)	TTime (minutes)
SEQ ID NO. 219	0	0.202	28.6
	10^4	0.471	23.3
	10^5	0.505	22.5
SEQ ID NO. 224	0	0.023	28.0
	10^4	0.024	27.5
	10^5	0.353	28.2
SEQ ID NO. 225	0	0.026	27.2
	10^4	0.209	29.5
	10^5	0.405	28.9
SEQ ID NO. 226	0	0.026	0
	10^4	0.207	28.0
	10^5	0.143	29.9

To improve sensitivity while reducing false positives, hairpin target capture was combined with base treating the target capture probes and some of the amplification oligomers as described in Example 7. The closing sequences for the hairpin target capture oligomer were reduced to 7, 8, or 9 bases in length (SEQ ID Nos. 221, 222, or 223 respectively). SEQ ID Nos. 210 and 8 were added to a tube containing base lysis buffer with either SEQ ID Nos. 219 or 221

or 222 or 223 and heated at 60°C for 90 minutes. The tubes were cooled to room temperature and were sterilely injected with hydrochloric acid to neutralize the buffer. After separating the target capture complexes, the samples underwent real-time single primer transcription associated amplification using SEQ ID Nos. 35, 193 and 181. The results are shown in Table 27. The base treatment produced no false positives, even when using the linear target capture oligomer. Additionally, the assay sensitivity was equivalent between the linear target capture probe and the hairpin target capture probe.

Table 27: Linear and Hairpin Target Capture Probes with Base Treatment

Target Capture Probe	Copies rRNA	AveRange (RFU)	TTime (minutes)
SEQ ID NO. 219	0	0.041	0
	10^4	0.415	27.1
	10^5	0.413	24.0
SEQ ID NO. 221	0	0.031	0
	10^4	0.415	27.4
	10^5	0.408	24.0
SEQ ID NO. 222	0	0.044	23.0
	10^4	0.604	26.6
	10^5	0.613	23.8
SEQ ID NO. 223	0	0.047	12.8
	10^4	0.578	24.5
	10^5	0.596	22.5

EXAMPLE 9: Reducing False Positives With Scavenger Oligomers

Scavenger oligomers are short oligomers (generally less than 20 bases) that are designed to hybridize to non-target bound oligomers, e.g. excess amplification oligomers or capture probes. Hybridizing the excess oligomers with scavengers helps prevent the excess oligomers from non-specifically hybridizing to non-target material. Scavenger oligomers are generally designed to hybridize to a region on either the target capture oligomer or the amplification oligomer that may potentially hybridize to a non-specific target. For example, target capture probes have a poly A tail, therefore any poly-T sites in a non-specific target may non-specifically hybridize to the poly-A tail. Alternatively, scavengers may hybridize to a structural region such as a hairpin. Examples of scavenger oligomers are listed in Table 28 below. SEQ ID Nos. 211, 212, 213, 214, 215, and 216 may be used with a TAG oligomer (SEQ ID NO. 210). SEQ ID Nos. 217 and 218 may be used with a target capture oligomer of SEQ ID Nos. 219, 220, 221, 222, 223, 224, 225, or 226 (see Table 25 above).

Table 28: Scavenger Oligonucleotides

SEQ ID NO	Sequence
210	CTGCTTTTGTGGGGgtgacccaatgatctaacaGAGCACCCCACAAAAGCAG
211	CTGCTTTTGTGGGGTG
212	CTGCTTTTGTGGGG
213	CTGCTTTTGTGG
214	CTGCTTTTGT
215	GTGGGGTGCTCTGT
216	GGGGTGCTCTGTTAG
217	UUUUUUUAAAACUCGCGGCU
218	TTTTTTTAAAACTCGCGGCT

Scavengers oligomers were added to either the target capture probes or the amplification oligomers. For assays where scavenger oligomers were added to the target probes, one scavenger oligomer was selected from SEQ ID Nos. 211-218 and added to SEQ ID NO. 219, 210, and 8. Zero or 10^4 copies of *P. acnes* rRNA was added and target capture was performed as described in Example 4. After target capture the samples were amplified by real-time single primer transcription associated amplification, as described in Example 3 using SEQ ID Nos. 8, 35, 181, and 193. For assays where scavenger oligomers were added to the amplification oligomers, target capture was performed using SEQ ID Nos. 219, 210, and 8 on zero or 10^4 copies of *P. acnes* rRNA. After target capture the samples were amplified by real-time single primer transcription associated amplification using SEQ ID Nos. 8, 35, 181, and 193, plus one sequence selected from SEQ ID Nos. 211-218. Results are shown in Tables 28 and30. Adding scavenger oligomers to the target capture probes did not eliminate false positives (results reported below are number of false positives per 15 total assays performed for each condition reported). Adding scavenger oligomers to the amplification oligomers did not inhibit amplification and SEQ ID Nos. 215 and 217 did not have any false positives.

Table 29: Scavenger Oligomers Added to Target Capture

Scavenger Oligomer	**Copies rRNA**	**AveRange (RFU)**	**TTime (minutes)**	**False Positives per 15 assays**
No scavenger oligomer	0	1852.47	20.77	3
	10^4	10314.00	15.43	
SEQ ID NO. 211	0	1606.53	24.33	3
	10^4	10588.78	15.73	
SEQ ID NO. 212	0	1377.40	21.69	2
	10^4	10816.67	15.59	
SEQ ID NO. 213	0	1486.00	28.72	4
	10^4	10827.00	15.35	

SEQ ID NO. 214	0	3650.87	20.03	5
	10^4	11179.89	16.17	
SEQ ID NO. 215	0	768.93	16.84	2
	10^4	10537.89	16.38	
SEQ ID NO. 216	0	1573.60	24.35	2
	10^4	10911.11	16.69	
SEQ ID NO. 217	0	4026.00	20.25	8
	10^4	11943.33	14.12	
SEQ ID NO. 218	0	3997.93	16.10	6
	10^4	11163.33	14.45	

Table 30: Scavenger Oligomers Added to Amplification Oligomers

Scavenger Oligomer	Copies rRNA	AveRange (RFU)	TTime (minutes)	False Positives per 15 assays
No scavenger oligomer	0	2114.67	24.52	4
	10^4	11133.89	15.94	
SEQ ID NO. 211	0	1183.33	20.41	1
	10^4	9467.11	15.75	
SEQ ID NO. 212	0	2268.33	25.67	3
	10^4	10056.00	15.64	
SEQ ID NO. 213	0	1276.93	20.33	3
	10^4	10000.56	15.80	
SEQ ID NO. 214	0	1937.13	21.51	3
	10^4	10274.67	15.74	
SEQ ID NO. 215	0	657.47	n/a	
	10^4	10306.44	15.41	
SEQ ID NO. 216	0	1854.33	22.69	5
	10^4	10293.33	16.24	
SEQ ID NO. 217	0	683.47	n/a	
	10^4	10018.44	15.11	
SEQ ID NO. 218	0	613.47	25.59	1
	10^4	9820.78	15.99	

EXAMPLE 10: Design and Initial Testing of *P. acnes* 23S Amplification Oligomer Sets

Amplification and detection oligonucleotides were designed to amplify and detect *P. acnes* 23S rRNA. In general, amplification and detection were targeted to bases 608,745-609,295 of accession number AE017283 and GI number 50839098 (SEQ ID NO. 202). The first set of amplification and detection oligomers corresponds to bases 608,775-609,045 (SEQ ID NO. 203) and the second set of amplification and detection oligomers corresponds to bases 608,995-609,275 (SEQ ID NO. 204) of accession number AE017283 and GI number 50839098 (SEQ ID Nos. 9-15, 27-34, 55-72, 99-126, 183, and 185-188). Known amounts of *P. acnes* 23S

rRNA sequences were amplified using real time transcription associated amplification using combinations of the oligomers in Tables 4 and 5.

Amplification oligonucleotide combinations were tested at 0 and 10^5 copies of *P. acnes* 23S rRNA, under conditions similar to Example 3. Oligomer combinations were evaluated on signal emergence time, maximum RFU signal, slope of the sigmoid curve, and background signal levels. Assay oligomer combinations targeting the first region of *P. acnes* 23S rRNA were: (1) SEQ ID Nos. 11, 27, 61 and 107; (2) SEQ ID Nos.11, 32, 61, and 105; (3) SEQ ID Nos.11, 27, 61, and 99; (4) SEQ ID Nos. 11, 32, 61, and 103; (5) SEQ ID Nos.11, 31, 61, and 103; and (6) SEQ ID Nos.11, 32, 59, and 101. Assay oligomer combinations, targeting the second region of *P. acnes* 23S rRNA, were: (7) SEQ ID Nos. 14, 34, 69, and 117; (8) SEQ ID Nos. 14, 34, 67, and 117; (9) SEQ ID Nos. 14, 33, 67, and 117; (10) SEQ ID Nos. 14, 33, 67, and 119; and (11) SEQ ID Nos. 183, 185, 186, and 188. Data for assay oligomer combinations 2 and 11 are shown in Table 31.

Table 31: Data for oligomer combinations 2 and 11 amplifying 23S rRNA

Amplification Oligonucleotides	Copies rRNA	AveRange (RFU)	TTime (minutes)
Combination 2	0	0.107	25.3
	10^3	0.110	27.8
	10^4	0.921	20.8
	10^5	2.145	16.4
Combination 11	0	3.191	44.4
	10^3	2.333	34.7
	10^4	2.556	30.5
	10^5	2.618	26.6

EXAMPLE 11: TAG Amplification Oligomers for *P. acnes* 23S rRNA

Oligomers that may be used to amplify *P. acnes* 23S rRNA using the TAG amplification system are listed in Table 32, lowercase letters represent the TAG sequence. As described in Example 5, this method is used to reduce background fluorescence in a real time detection assay. Using selected combinations of assay oligomers from the group shown in Table 32, amplification is performed substantially as described in Example 5, but using 23S RNA transcripts for the target for amplification. The amplified product is detected during amplification (i.e., in real time) by using a probe, such as a molecular beacon or molecular torch, that hybridizes specifically to the amplified product made from the amplified tag sequence or specifically hybridizes to the amplified 23S sequence located between the selected amplification

oligomers in Table 32, which include those selected from the 23S rRNA sequence specific probes are shown in Table 6.

Table 32: 23S rRNA TAG Amplification Oligomers

SEQ ID NO	Sequence
161	GTGTGACGGGTGGGAAgttgacgtaccgtattgaATTCCCACCCGTCAC AC
162	GTGTGACGGGTGGGgttgacgtaccgtattgaATTCCCACCCGTCACAC
163	GTGTGACGGGTGgttgacgtaccgtattgaATTCCCACCCGTCACAC
164	GTGTGACGGGgttgacgtaccgtattgaATTCCCACCCGTCACAC
165	GTGTGACGGGTGGGAAcattggcatagctagaccATTCCCACCCGTCAC AC
166	GTGTGACGGGTGGGcattggcatagctagaccATTCCCACCCGTCACAC
167	GTGTGACGGGTGcattggcatagctagaccATTCCCACCCGTCACAC
168	GTGTGACGGGcattggcatagctagaccATTCCCACCCGTCACAC
169	GTGTGACGGGTGGGAAgcatgcaggttaacgtagacATTCCCACCCGTC ACAC
170	GTGTGACGGGTGGGgcatgcaggttaacgtagacATTCCCACCCGTCACA C
171	GTGTGACGGGTGgcatgcaggttaacgtagacATTCCCACCCGTCACAC
172	GTGTGACGGGgcatgcaggttaacgtagacATTCCCACCCGTCACAC
173	GTGTGACGGGTGGGAAgtgacccaatgatctaacaATTCCCACCCGTCA CAC
174	GTGTGACGGGTGGGgtgacccaatgatctaacaATTCCCACCCGTCACA C
175	GTGTGACGGGTGgtgacccaatgatctaacaATTCCCACCCGTCACAC
176	GTGTGACGGGgtgacccaatgatctaacaATTCCCACCCGTCACAC
177	TCGTGTCAGTGGTGAAgtgacccaatgatctaacaGCTTCACCACTGACA CGA
178	TCGTGTCAGTGGTGgtgacccaatgatctaacaGCTTCACCACTGACACG A
179	TCGTGTCAGTGGgtgacccaatgatctaacaGCTTCACCACTGACACGA
180	gtgacccaatgatctaacaGCTTCACCACTGACACGA
191	gttgacgtaccgtattga
192	cattggcatagctagacc
193	gtgacccaatgatctaaca
194	gcatgcaggttaacgtagac

CLAIMS:

1. A composition comprising at least two oligomers specific for a *P. acnes* 16S rRNA sequence or DNA encoding 16S rRNA contained within SEQ ID NO:199 selected from the group consisting of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, the completely complementary sequences of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, and the RNA equivalent of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, wherein when a first oligomer of the at least two oligomers is selected from the group consisting of SEQ ID Nos. 16 to 26, 135 to 160, 184 and 210, then a second oligomer is selected from the group consisting of SEQ ID Nos. 35 to 54.

2. The composition of claim 1, further comprising at least one third oligomer selected from the group consisting of SEQ ID Nos. 1 to 8, 75 to 98, 127 to 130, 181, 182, 195 to 198, and 211 to 226, the completely complementary sequences of SEQ ID Nos. 1 to 8, 75 to 98, 127 to 130, 181, 182, 195 to 198, and 211 to 226, and the RNA equivalents of SEQ ID Nos. 1 to 8, 75 to 98, 127 to 130, 181, 182, 195 to 198, and 211 to 226.

3. The composition of claim 1, wherein the at least two oligomers include a promoter provider oligomer selected from the group consisting of SEQ ID Nos. 35 to 54 and 135 to 160.

4. The composition of claim 2, wherein the at least one third oligomer is selected from the group consisting of SEQ ID Nos. 1 to 8, the completely complementary sequences of SEQ ID Nos. 1 to 8, and the RNA equivalent of SEQ ID Nos. 1 to 8, and wherein the third oligomer selected from this group includes a 3' blocked end.

5. The composition of claim 2 wherein the at least one third oligomer is selected from the group consisting of SEQ ID Nos. 75 to 98, 181, and 182, the completely complementary sequences of SEQ ID Nos. 75 to 98, 181, and 182, and the RNA equivalent of SEQ ID Nos. 75 to 98, 181, and 182, and wherein the third oligomer selected from this group includes a label attached to the third oligomer.

6. A composition comprising at least two oligomers specific for a *P. acnes* 23S rRNA sequence or DNA encoding 23S rRNA contained within SEQ ID NO:202 selected from the group consisting of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, the completely complementary sequences of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, and the RNA equivalent of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, wherein when a first oligomer of the at least two oligomers is selected from the group consisting of SEQ

ID Nos. 27 to 34, 161 to 180, and 185, then a second oligomer is selected from the group consisting of SEQ ID Nos. 55 to 72, 186, and 187.

7. The composition of claim 6, further comprising at least one third oligomer selected from the group consisting of SEQ ID Nos. 9 to 15, 99 to 126, 131 to 134, 183, and 188, the completely complementary sequences of SEQ ID Nos. 9 to 15, 99 to 126, 131 to 134, 183, and 188, and the RNA equivalent of SEQ ID Nos. 9 to 15, 99 to 126, 131 to 134, 183, and 188.

8. The composition of claim 6, wherein the at least two oligomers include a promoter provider oligomer selected from the group consisting of SEQ ID Nos. 55 to 72, 186, and 187, the completely complementary sequences of SEQ ID Nos. 55 to 72, 186, and 187, and the RNA equivalent of SEQ ID Nos. 55 to 72, 186, and 187.

9. The composition of claim 7, wherein the at least one third oligomer is selected from the group consisting of SEQ ID Nos. 9 to 15 and 188, the completely complementary sequences of SEQ ID Nos. 9 to 15 and 188, and the RNA equivalent of SEQ ID Nos. 9 to 15 and 188, and wherein the third oligomer selected from this group includes a 3' blocked end.

10. The composition of claim 7 wherein the at least one third oligomer is selected from the group consisting of SEQ ID Nos. 99 to 126 and 183, the completely complementary sequences of SEQ ID Nos. 99 to 126 and 183, and the RNA equivalent of SEQ ID Nos. 99 to 126 and 183, and wherein the third oligomer selected from this group includes a label attached to the third oligomer.

11. A method for specifically detecting a *P. acnes* in a sample by detecting a target nucleic acid sequence that is a *P. acnes* rRNA or DNA encoding a *P. acnes* rRNA comprising the steps of:

(a) providing a sample that contains a *P. acnes* target region in a 16S rRNA or DNA encoding 16S rRNA contained in SEQ ID NO:199, and/or a target region in a 23S rRNA or gene encoding 23S RNA contained in SEQ ID NO:202;

(b) amplifying a sequence in the *P. acnes* target region to make an amplified product by using at least two amplification oligomers in an *in vitro* amplification reaction that includes enzymatic elongation of at least one amplification oligomer,

wherein when the target sequence is in the 16S rRNA or DNA encoding the 16S rRNA contained in SEQ ID NO:199, the at least two amplification oligomers are selected from the group consisting of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, the completely complementary sequences of SEQ ID Nos. 16 to 26, 35 to 54, 135 to 160, 184, and 210, and the RNA equivalent of SEQ ID Nos. 16 to 26,

35 to 54, 135 to 160, 184, and 210, and

wherein when the target sequence is in the 23S rRNA or DNA encoding 23S rRNA contained in SEQ ID NO:202, the at least two amplification oligomers are selected from the group consisting of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, the completely complementary sequences of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187, and the RNA equivalent of SEQ ID Nos. 27 to 34, 55 to 72, 161 to 180, and 185 to 187; and

(c) detecting the amplified product.

12. The method of claim 11, wherein the target sequence is in the 16S rRNA or DNA encoding the 16S rRNA, the at least two amplification oligomers use a first amplification oligomer selected from the group consisting of SEQ ID Nos. 16 to 26, 135 to 160, 184 and 210, with a second amplification oligomer selected from the group consisting of SEQ ID Nos. 35 to 54.

13. The method of claim 11, wherein the amplifying step for the target sequence in the 16S rRNA or DNA encoding the 16S rRNA further includes a third oligomer selected from the group consisting of SEQ ID Nos. 1 to 8, the completely complementary sequences of SEQ ID Nos. 1 to 8, and the RNA equivalent of SEQ ID Nos. 1 to 8, and wherein the third oligomer selected from this group includes a 3' blocked end.

14. The method of claim 11, wherein the detecting step for the amplified product made from the target sequence in the 16S rRNA or DNA encoding the 16S rRNA uses a detection probe selected from the group consisting of SEQ ID Nos. 75 to 98, 181, and 182, the completely complementary sequences of SEQ ID Nos. 75 to 98, 181, and 182, and the RNA equivalent of SEQ ID Nos. 75 to 98, 181, and 182.

15. The method of claim 11 further comprising a target capture step to purify the 16S rRNA or DNA encoding the 16S rRNA from the sample by hybridizing to the 16S rRNA or DNA encoding the 16S rRNA a target capture probe selected from the group consisting of SEQ ID Nos. 127 to 130.

16. The method of claim 11, wherein the target sequence is in the 23S rRNA or DNA encoding the 23S rRNA, the at least two amplification oligomers use a first amplification oligomer selected from the group consisting of SEQ ID Nos. 27 to 34, 161 to 180, and 185, with a second amplification oligomer selected from the group consisting of SEQ ID Nos. 55 to 72, 186, and 187.

17. The method of claim 11, wherein the amplifying step for the target sequence in the 23S rRNA or DNA encoding the 23S rRNA further includes a third oligomer selected from the group consisting of SEQ ID Nos. 9 to 15 and 188, the completely complementary sequences of SEQ ID Nos. 9 to 15 and 188, and the RNA equivalent of SEQ ID Nos. 9 to 15 and 188, and wherein the third oligomer selected from this group includes a 3' blocked end.

18. The method of claim 11, wherein the detecting step for the amplified product made from the target sequence in the 23S rRNA or DNA encoding the 23S rRNA uses a detection probe selected from the group consisting of SEQ ID Nos. 99 to 126 and 183, the completely complementary sequences of SEQ ID Nos. 99 to 126 and 183, and the RNA equivalent of SEQ ID Nos. 99 to 126 and 183.

19. The method of claim 11 further comprising a target capture step to purify the 23S rRNA or DNA encoding the 23S rRNA from the sample by hybridizing to the 23S rRNA or DNA encoding the 23S rRNA a target capture probe selected from the group consisting of SEQ ID Nos. 131 to 134.

20. The method of claim 11, wherein the amplified product is detected by using a detection probe that includes a label.