

Scorpion probe) that hybridizes to B_x . Thus, using the UA universal probes described herein, a multiplicity of target sequences can be detected using a single probe. Most of the amplification steps involve amplification using a single primer (if U_1 and U_2 are the same) or primer pair. Methods for designing probes that recognize a specified sequence (e.g., B_x) are well known. For example, Molecular Beacons can be designed as described in Marras et al., 2003, Genotyping single nucleotide polymorphisms with molecular beacons. In Kwok, P. Y. (ed.), Single nucleotide polymorphisms: methods and protocols. The Humana Press Inc., Totowa, N.J., Vol. 212, pp. 111-128. Molecular Beacons can also be designed with the help of a dedicated software package called 'Beacon Designer,' which is available from Premier Biosoft International (www.premierbiosoft.com). However, it will be appreciated that the sequence of B_x can be, and generally is, an artificial sequence (i.e., not found in the initial sample nucleic acid) that can be recognized by the probe.

[0095] Primer concentration(s) will vary with the length, composition, and nature of the sample and targets. Those primer pairs with sequences specific to each of the targets (e.g., Type 1 and 2 primers) are required only in the first few rounds of amplification and can be provided in very small quantities (for example and not limitation, e.g., typically less than about 50 nM, more often less than about 30 nM and sometimes less than about 20 nM). Type 3 and 4 primers can be provided at somewhat higher concentration (for example and not limitation, e.g., typically from about 100 nM to 1 μ M, such as from about 200 nM to about 900 nM). The practitioner guided by this disclosure will be able to select appropriate concentration using routine methods.

[0096] The method can be modified in a variety of ways to achieve particular results. In one version of the method, a relatively small number of probe sequences can be used with a larger number of unique target sequences, with different classes of target specific sequences associated with differently labeled probes. For example, if target sequences 1-20 are characteristic of a viral pathogen, target sequences 21-40 are characteristic of a bacterial pathogen, and target sequences 41-45 are positive control sequences (human genes), amplification of a human patient sample could give zero, one or more than one of the following 45 amplification products:

[0097] Set 1: 20 products (from 5^{\prime} - U_F - B_V -target sequence1- $U_R^{1-3^{\prime}}$ to 5^{\prime} - U_F - B_V -target sequence20- $U_R^{1-3^{\prime}}$)

[0098] Set 2: 20 products (from 5^{\prime} - U_F - B_B -target sequence21- $U_R^{1-3^{\prime}}$ to 5^{\prime} - U_F - B_B -target sequence40- $U_R^{1-3^{\prime}}$)

[0099] Set 3: 5 products (from 5^{\prime} - U_F - B_C -target sequence41- $U_R^{1-3^{\prime}}$ to 5^{\prime} - U_F - B_C -target sequence45- $U_R^{1-3^{\prime}}$)

[0100] By using differently labeled probes B_V (hybridizes to Set 1 products), B_B (hybridizes to Set 2 products), and B_C (hybridizes to Set 3 products) the classes of amplification products can be detected and distinguished. Thus, if the human sample produced any amplicon to which Probe B_V hybridized and emitted signal it would indicate that the patient was infected with one of 20 viruses. If desired, the precise identity of the viral pathogen could be determined in a second assay step. Similarly, if the human sample produced any amplicon to which Probe B_B hybridized and emitted signal (different from the signal emitted by Probe B_V) it would indicate that the patient was infected with one of 20 bacteria. Other types of samples, such as food or agricultural sample can be screened for many different pathogens simultaneously and if any hits are detected the sample can be

selected for further analysis to determine which of the many pathogen(s) was responsible for the signal. If there is no signal, the sample can be concluded to be pathogen free.

[0101] Numerous primer pairs are known for detection and analysis of pathogens, and other primer combinations can be prepared using well established methods. For illustration and not limitation see, e.g., U.S. Pat. No. 6,503,722 "Detection of toxigenic strains of *Clostridium difficile* using a PCR-based assay" [e.g., 5^{\prime} CCCCAATAGAAGATTCAATATTAAG with 5^{\prime} ATGTAGAAGTAAACTTACT TGGATG to detect strains expressing toxin A; 5^{\prime} GGTGGAGCTTCAATTGGAGAG with 5^{\prime} GTGTAACCTACTTTTCATAACACCA to detect strains expressing toxin B; and 5^{\prime} AAGTGTCTGTAAACAGGTATAACC with 5^{\prime} GGTCCATTAGCAGCCTCACA to detect glutamate dehydrogenase (positive control for presence of bacteria)]; U.S. Pat. No. 6,723,505 "Method for identification of the indicators of contamination in liquid samples"; U.S. Pat. No. 6,632,642 "Genes for detecting bacteria and detection method by using the same"; U.S. Pat. No. 6,387,652 "Method of identifying and quantifying specific fungi and bacteria"; U.S. Pat. No. 6,013,435 "Drug resistance screening method using multiplex amplification"; U.S. Pat. No. 5,932,415 "Processes and agents for detecting listerias"; U.S. Pat. No. 6,225,094 "Method for the genus-specific or/and species-specific detection of bacteria in a sample liquid"; Di Pinto, 2005, A collagenase-targeted multiplex PCR assay for identification of *Vibrio alginolyticus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*, *J Food Prot.* 68(1):150-3; Maher et al., 2003, "Use of PCR to detect *Campylobacter* species in samples" *J. Clin. Micro.* 41(7):2980 [5^{\prime} AGTCGTAACA AGGTAGCCG with 5^{\prime} CYRYTGCCAAGGCATC-CACC]; Lim et al., "Use of PCR to detect *heliobacter pylori* in gastric mucosa of patients" *J. Clin. Micro.* 41(7):3387 [5^{\prime} ACTTTAAACGCATGAAGATAT with 5^{\prime} ATATTTGACCTTCTGGGGT]; and Wilson et al., 2003, "Use of PCR to detect *Legionella pneumophila*" *J. Clin. Micro.* 41(7):3327 [5^{\prime} GCAATGTCAACAGCAA with 5^{\prime} CATAGCGTCTTG-CATG].

[0102] Although the "Universal Amplification" method described above is suited for use in MPD-based analyses, it will be appreciated that this method can be used in a variety of formats (both microfluidic and nonmicrofluidic).

[0103] vi) Pooling of Amplicons

[0104] In some embodiments of the invention, following amplification and optional detection of amplicons, the contents of the sub-samples, including any amplicons in them, are pooled (i.e., allowed to combine or mix at least partially). Pooling combines the amplicons (if present) from multiple sub-samples. Pooling can be accomplished by, for example, opening the valves of an elastomeric microfluidic device in which partitioning and amplification occurred such that the contents of multiple sub-samples (e.g., at least about 10^3 , at least about 5×10^3 , at least about 10^4 , at least about 2×10^4 , at least about 3×10^4 , or at least about 10^5 sub-samples) are in fluidic communication with each other, constituting a "post-amplification sample" that consists of the contents of all of the chambers. Amplicons can mix by diffusion, which can be accelerated using thermal, mechanical, acoustic, or chemical energy. In one embodiment, pooling occurs primarily as a result of active mixing (e.g., by pumping the fluid through flow channels in the device using a rotary peristaltic pump or other mechanism). Alternatively or in combination with the methods above, a portion, all or substantially all of the post-amplification sample can be pumped out of or otherwise