

between the ETM and the electrode is then detected to indicate the presence or absence of the original target sequence, as described below. Alternatively, detection modules utilizing fluorescence are made, as described below.

[0153] In a preferred embodiment, the amplification is target amplification. Target amplification involves the amplification (replication) of the target sequence to be detected, such that the number of copies of the target sequence is increased. Suitable target amplification techniques include, but are not limited to, the polymerase chain reaction (PCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA).

[0154] In a preferred embodiment, the target amplification technique is PCR. The polymerase chain reaction (PCR) is widely used and described, and involve the use of primer extension combined with thermal cycling to amplify a target sequence; see U.S. Pat. Nos. 4,683,195 and 4,683,202, and PCR Essential Data, J. W. Wiley & sons, Ed. C. R. Newton, 1995, all of which are incorporated by reference. In addition, there are a number of variations of PCR which also find use in the invention, including "quantitative competitive PCR" or "QC-PCR", "arbitrarily primed PCR" or "AP-PCR", "immuno-PCR", "Alu-PCR", "PCR single strand conformational polymorphism" or "PCR-SSCP", "reverse transcriptase PCR" or "RT-PCR", "biotin capture PCR", "vector PCR", "panhandle PCR", and "PCR select cDNA subtration", among others.

[0155] In general, PCR may be briefly described as follows. A double stranded target nucleic acid is denatured, generally by raising the temperature, and then cooled in the presence of an excess of a PCR primer, which then hybridizes to the first target strand. A DNA polymerase then acts to extend the primer, resulting in the synthesis of a new strand forming a hybridization complex. The sample is then heated again, to disassociate the hybridization complex, and the process is repeated. By using a second PCR primer for the complementary target strand, rapid and exponential amplification occurs. Thus PCR steps are denaturation, annealing and extension. The particulars of PCR are well known, and include the use of a thermostable polymerase such as Taq I polymerase and thermal cycling.

[0156] Accordingly, the PCR reaction requires at least one PCR primer and a polymerase. Mesoscale PCR devices are described in U.S. Pat. Nos. 5,498,392 and 5,587,128, and WO 97/16561, incorporated by reference.

[0157] In a preferred embodiment, the target amplification technique is SDA. Strand displacement amplification (SDA) is generally described in Walker et al., in *Molecular Methods for Virus Detection*, Academic Press, Inc., 1995, and U.S. Pat. Nos. 5,455,166 and 5,130,238, all of which are hereby expressly incorporated by reference in their entirety.

[0158] In general, SDA may be described as follows. A single stranded target nucleic acid, usually a DNA target sequence, is contacted with an SDA primer. An "SDA primer" generally has a length of 25-100 nucleotides, with SDA primers of approximately 35 nucleotides being preferred. An SDA primer is substantially complementary to a region at the 3' end of the target sequence, and the primer has a sequence at its 5' end (outside of the region that is complementary to the target) that is a recognition sequence for a restriction endonuclease, sometimes referred to herein

as a "nicking enzyme" or a "nicking endonuclease", as outlined below. The SDA primer then hybridizes to the target sequence. The SDA reaction mixture also contains a polymerase (an "SDA polymerase", as outlined below) and a mixture of all four deoxynucleoside-triphosphates (also called deoxynucleotides or dNTPs, i.e. dATP, dTTP, dCTP and dGTP), at least one species of which is a substituted or modified dNTP; thus, the SDA primer is modified, i.e. extended, to form a modified primer, sometimes referred to herein as a "newly synthesized strand". The substituted dNTP is modified such that it will inhibit cleavage in the strand containing the substituted dNTP but will not inhibit cleavage on the other strand. Examples of suitable substituted dNTPs include, but are not limited, 2'-deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate. In addition, the substitution of the dNTP may occur after incorporation into a newly synthesized strand; for example, a methylase may be used to add methyl groups to the synthesized strand. In addition, if all the nucleotides are substituted, the polymerase may have 5'→3' exonuclease activity. However, if less than all the nucleotides are substituted, the polymerase preferably lacks 5'→3' exonuclease activity.

[0159] As will be appreciated by those in the art, the recognition site/endonuclease pair can be any of a wide variety of known combinations. The endonuclease is chosen to cleave a strand either at the recognition site, or either 3' or 5' to it, without cleaving the complementary sequence, either because the enzyme only cleaves one strand or because of the incorporation of the substituted nucleotides. Suitable recognition site/endonuclease pairs are well known in the art; suitable endonucleases include, but are not limited to, HincII, HindII, AvaI, Fnu4HI, TthIII, NciI, BstXI, BamI, etc. A chart depicting suitable enzymes, and their corresponding recognition sites and the modified dNTP to use is found in U.S. Pat. No. 5,455,166, hereby expressly incorporated by reference.

[0160] Once nicked, a polymerase (an "SDA polymerase") is used to extend the newly nicked strand, 5'→3', thereby creating another newly synthesized strand. The polymerase chosen should be able to initiate 5'→3' polymerization at a nick site, should also displace the polymerized strand downstream from the nick, and should lack 5'→3' exonuclease activity (this may be additionally accomplished by the addition of a blocking agent). Thus, suitable polymerases in SDA include, but are not limited to, the Klenow fragment of DNA polymerase I, SEQUENASE 1.0 and SEQUENASE 2.0 (U.S. Biochemical), T5 DNA polymerase and Phi29 DNA polymerase.

[0161] Accordingly, the SDA reaction requires, in no particular order, an SDA primer, an SDA polymerase, a nicking endonuclease, and dNTPs, at least one species of which is modified.

[0162] In general, SDA does not require thermocycling. The temperature of the reaction is generally set to be high enough to prevent non-specific hybridization but low enough to allow specific hybridization; this is generally from about 37° C. to about 42° C., depending on the enzymes.

[0163] In a preferred embodiment, as for most of the amplification techniques described herein, a second amplification reaction can be done using the complementary target