

334 or 342, and (ii) a detection region. An example is an antibody specific for the FAM binding moiety, which has been modified with an alkaline phosphatase enzyme as the detection element.

Alternative Amplification Methods

[0133] An alternative embodiment of this method using the same detection cartridge can be used to perform a non-PCR nucleic acid amplification assay. A schematic for rolling circle amplification (RCA) is shown in FIG. 10 and one for strand displacement amplification (SDA) in FIG. 11. Note that the component elements correspond to those described for PCR as shown in FIG. 7(a). Both assays require a short ssDNA fragment with a 3'-OH moiety (308 and 310) made from the target, as shown by means of two different methods in FIG. 25. FIG. 25(a) shows a triggering event method, e.g. SNPase and cycling probe, and FIG. 25(b) shows the Invader™ method.

[0134] The same reagents are used as in the above section, however only one modified primer comprising a sequence of bases to a first region of said target nucleic acid is required. Again the mixture is cycled to provide multiple copies of an amplicon incorporating the 30 modified primer, followed by substantial elimination of any excess unincorporated modified primer from the mixture. Several methods can be used as discussed below. The mixture is then exposed to a capture oligonucleotide complementary to the single stranded hybridization region, followed by hybridization of the single stranded hybridization region of said amplicon incorporating said modified primer, with the capture oligonucleotide. Again the final step is detecting said moiety associated with said hybridization, e.g. electrochemical detection of an electroactive species generated by alkaline phosphatase. In the preferred embodiment, primers are attached to the polymerase-blocking region which, in turn is attached to a single stranded hybridization region.

[0135] For the rolling circle amplification strategy, the 3'-end of the primer has a blocking region, which could include a phosphate or a dideoxy nucleotide. A cleavage reaction similar to that found for the cycling probe reaction or the SNPase assay occurs, removing the blocking moiety, as shown in FIG. 25(a), comprising target DNA 300 and reagents 301, 302 and 309 participating in reaction 306. Pre-made circular molecules can be added to the reaction mixture. Extensions cannot occur with blocked primers, but do occur to cleaved primer molecules. The cleaved primer generate long single stranded molecules with duplications of specific regions complementary to the pre-made circular molecules. Synthetic oligonucleotides with detectable moieties are included in the mix, wherein the oligonucleotides are complementary to a region of the single stranded DNA, which can be found multiple times along the single stranded DNA. One region of the primer, which is single stranded and unique, binds to a capture oligonucleotide region. As this region is not complementary to the pre-made circular DNA, there is no competition of this region with the capture oligonucleotides. As shown in FIG. 10, in the rolling circle assay the ssDNA 3'-OH moiety (308,310) binds to the rolling circle reagent (311, 315) via reaction 312. Cycling incorporates a string of moieties 316 attached starting at the 3' end of 308 or 310, to produced 314. Detection of element 314 is achieved by binding its 5'-3'-OH region to complementary element 40 immobilized on bead 39 and labeled polynucleotide 317 comple-

mentary to 316. The label is then recognized by an antibody bound to alkaline phosphatase 318.

[0136] An alternative embodiment of this method using the same detection cartridge can be used to perform a non-PCR nucleic acid amplification assay. A schematic for strand displacement amplification is shown in FIG. 11. Note that component elements correspond to those in the PCR as used as in FIG. 7(a). Similar reagents are used as those described above, however the SDA primer must first be provided in a non-amplifiable format, which is converted to an amplifiable format. One approach to accomplishing this is to provide a primer with a blocked 3'-end block, for example using a 3'-terminal dideoxy sequence. A trigger event then occurs, which cleaves off the blocking 3'-end. One example of a trigger event could be an Invader reaction (Kwiatkowski R W, Lyamichev V, de Arruda M, Neri B. Clinical, genetic, and pharmacogenetic applications of the Invader assay. *Mol Diagn.* 1999; 4:353-364.), where the flapase activity cleaves at the hybridized junction of the blocked primer with the presence of genomic target nucleic acid, providing an available 3'-10 hydroxy group. This is shown in FIG. 25(b) with target DNA 300 and reagent comprising 304, 303, 305 and 309 participating in reaction 307. Alternatively, another example of a trigger event is a cycling probe reaction (Duck et al., 1990, *BioTechniques*, vol 9(2): 142), where the presence of the genomic target nucleic acid causes the cycling probe oligonucleotide to be cleaved at a four ribonucleotide sequence on the cycling probe oligonucleotide, in turn generating a free 3'-hydroxyl group. Another similar example is a mismatch to the genomic target nucleic acid and a repair enzyme, which as described for SNPase, generating a free 3'-hydroxyl group.

[0137] After the trigger event, which has generated a free 3'-hydroxyl group in the primer sequence, a complementary strand displacement primer is present. This SD primer is complementary at its 3' end for the primer described above, which generated a 3'-hydroxyl group. In addition, the SD primer has 3' to the 3'-hydroxyl group complementary oligonucleotide a region that when newly synthesized is cleaved by a Nickase restriction endonuclease, as described in U.S. Pat. No. 5,422,252. This allows the strand displacement reaction to generate many copies of newly synthesized sequence, which form the basis of a non-thio strand displacement amplification as described in U.S. Pat. No. 6,191,267. The next step in the process is to use these amplified newly synthesized fragments, complementary to the strand displacement primers as DNA bridges to generate a signal with the capture oligonucleotide, as described above. This is illustrated in FIG. 11, where in the strand displacement assay the ssDNA 3'-OH moiety (308, 310) binds to a region 320 at the 3' end of 319 composed of regions 320, 321 and 322. An extension reaction 323 then occurs which is then nicked in reaction 324 to produce a short portion of ssDNA 325 which accumulates by virtue of cycling reaction 326 of primer extensions and nicks. Detection of element 325 is achieved by binding a first portion of 325 to complementary 40 immobilized on bead 39 and a second portion of 325 to a labeled polynucleotide 317. The label is then recognized by an antibody bound to alkaline phosphatase 318.

Removal of Primers after Amplification

[0138] We describe several novel approaches to remove unused PCR primers from completed PCR reactions. It has been found that a consequence of seeking to develop systems incorporating rapid PCR reactions, i.e. completed amplifica-