

[0147] In another embodiment, the original liquid sample is moved out of the region of the channel abutting the cavity and is replaced with a smaller amount of a different liquid prior to reversing the polarity of the electrodes. This can effect a concentration of the amplicon, which in turn can increase hybridization rates at a later stage in the assay process. In another embodiment, the primers are brought in contact with a capture membrane or particle within the cavity, which effects irreversible binding, thus preventing the primer from moving back towards the channel. In another embodiment, the agarose may be replaced with a different matrix including acrylamide, a mixture of agarose and locust bean gum, hydrocolloids, or other appropriate separation media. In another embodiment, the device is manufactured as a subcomponent on silicon and inserted into a micro-device, as shown in FIG. 12. In another embodiment, to address constraints associated with integration of this separation component into a genetic testing device, the electrophoretic channel may be L-shaped with electrode 75 located at or near the elbow of the 'L.' For example FIG. 24 shows the L-shaped channel feature 655 incorporated into an integrated testing device 651 abutting conduit 409, with electrodes 652, 653 and 654 with entry port 657 and matrix 656. Other elements are as for FIG. 19.

[0148] FIG. 14 demonstrates the operation of the electrophoresis device with an amplicon and primer from a PCR reaction. Lane (A) shows a portion of the PCR reaction product after electrophoresis into gel cavity and back out again and into a fresh second recovery aliquot and applied to a 6% non-denaturing acrylamide gel. Lane (B) shows a portion of sample that remained in original aliquot removed after one direction migration. Lane (C) is a control of equivalent concentration to the sample and lane (D) is a 10 base-pair ladder at a three times greater concentration than in the sample and control. The ladder major species base-pair lengths are 330, 100 and 10.

Clam-Like Oligonucleotides

[0149] Normally, for PCR applications reducing the amount of secondary structure is a desirable approach when designing synthetic oligonucleotide sequences, as this helps in reducing non-specific and poor priming of the target. The predicted folding structure of an oligonucleotide that is complementary to the HfeI gene, that has a five base pair adenosine spacer sequence and that has a free single stranded region is shown in FIG. 15. The FIG. 15 sequence is 5'-ACTTCATACACAACCTCCCGCGTTGCATAACTAAA-AACTGGCAAGGGTAAACAGATCCCC-3' (SEQUENCE ID No. 5). As a theoretical prediction of potential molecular folding an RNA folding program (Vienna RNA) predicts an oligonucleotide with single stranded nature at any temperature above 10° C. By designing synthetic oligonucleotides with secondary structure at low temperatures, but which lose their secondary structure during the denaturation step of PCR and PCR hybridization, we can effect hybridization of amplicons but not the primer molecules at the later stage of hybridization and detection. Using the isO15 sequence as a starting point, oligonucleotides with a hairpin loop structure were designed and modeled as shown in FIG. 16(a) and (b). The base pair sequence in FIG. 16(a) is 5'-TTGCCAGACTTCATACACAACCTCCCGCGTTGCATAACTAAAAGTATGAAG TCTGGCAAGGGTAAACAGATCCCC-3' (SEQUENCE ID No. 6), and that 30 of FIG. 16(b) is 5'-ACCCTTGCCAGACTTCATACCCGCGTTGCATAACTAAAAA-GTATGAAGTCTGGCAAGGG-

TAAACAGATCCCC-3' (SEQUENCE ID No. 7). In the models of FIG. 16, a five base pair sequence shown in the box is used to model the effect of an HPEG spacer. Based on the models in FIG. 16, two oligonucleotides designated CLAM1 and CLAM2 were. The two sequences differ by four nucleotides.

CLAM1 :
5' -TTGCCAGACTTCATACACAACCTCCCGCGTTGCATAACT -HPEG-

GTATGAAGTCTGGCAAGGGTAAACAGATCCCC -3'

CLAM2 :
5' -ACCCTTGCCAGACTTCATACCCGCGTTGCATAACT -HPEG-

GTATGAAGTCTGGCAAGGGTAAACAGATCCCC -3'

[0150] In the CLAM1 sequence, the sequence located 5' to the HPEG spacer, 5'-TTGCCAGACTTCATACACAACCTCCCGCGTTGCATAACT-3' is designated as SEQUENCE ID No. 8 and the sequence located 3' to the HPEG spacer, 5'-GTATGAAGTCTGGCAAGGGTAAACAGATCCCC-3' is designated as SEQUENCE ID No. 9.

[0151] In the CLAM2 sequence, the sequence located 5' to the HPEG spacer, 5'-ACCCTTGCCAGACTTCATACCCGCGTTGCATAACT-3' is designated as SEQUENCE ID NO. 10 and the sequence located 3' to the HPEG spacer, 5'-GTATGAAGTCTGGCAAGGGTAAACAGATCCCC-3' is designated as SEQUENCE ID NO. 11.

[0152] These oligonucleotide sequences maintain the key primary sequence features for HfeI priming in PCR reactions and for binding to the capture oligonucleotide, but additional sequences have been added to generate intramolecular binding, generating these "clam-like" structures. Note that the HPEG spacer region sequence is indicated with the five 'A's and it was anticipated that these sequences will have no secondary structure above about 40° to 45° C.

[0153] FIGS. 7(b) and 7(c) compare and contrast the differences between using PCR primer sequences with little or no secondary structure and the CLAM PCR primers. At temperatures during PCR, particularly at temperatures at or above hybridization, the CLAM primers do not form secondary structures and once it is incorporated into a PCR amplicon it loses its ability to form a clam structure. At temperatures below PCR hybridization and at temperatures used for hybridization of the capture oligonucleotides, the CLAM PCR primers do form secondary structure. Therefore, unincorporated CLAM PCR primers do not bind to the capture oligonucleotides and do not interfere with signal generation.

[0154] FIG. 7(b) shows a PCR reaction using a non-CLAM oligonucleotide sequence and hybridizing to a target nucleotide sequence. A sequence like isO15 with no secondary structure is used as one of two PCR primers 81. The PEG spacer generates single stranded regions in the PCR amplicon and excess primer sequences are generated in the reaction 82. In step 83, both the PCR amplicon and the unreacted primer sequences can bind to the capture oligonucleotide bound to a solid substrate like a bead. Typically, the unreacted primer is in significant molar excess compared to the PCR amplicon and reduces the signal detection.

[0155] FIG. 7(c) shows a PCR reaction using a CLAM oligonucleotide sequence hybridizing only the PCR amplicon to a target nucleotide sequence. Using a modification to the isO15 sequence to generate either CLAM1 or CLAM2 sequences, a PCR reaction is performed 81. At temperatures