

tion in less than about minutes, that it is necessary to increase the primer concentrations. However, this typically can generate an increased primer background in the detection step, which can reduce signal generation on the capture oligonucleotide. Experiments using purified amplicons and increased unlabelled target oligonucleotides, amongst labeled control oligonucleotides, demonstrated that these background oligonucleotides were able to remove or reduce the signal. One approach or a combination of the approaches described below can be used to reduce the background signal.

[0139] One way for providing for easy removal of primers from the reaction amplification mixture is to use a clam-like oligonucleotide primer. This oligonucleotide predominantly exhibits a certain desired secondary structure in solution, when in a first temperature range, but not in a second higher temperature range. In this example, the oligonucleotide is capable of priming the target nucleic acid in the second temperature range, but not in the first temperature range. This is achieved by designing the oligonucleotide such that the primary structure results in a secondary structure with one or more regions that hybridize, preferably predominantly in an intra-molecular manner, but also in an inter-molecular manner. This can occur in the first temperature range but not in said second temperature range, thus changing the temperature will enable switching the primer between a priming and non-priming form. As a result, lowering the temperature at the end of the amplification reaction effectively removes excess primer from the mixture. It has been found that clam-like primers of this type may be prepared incorporating a polymerase blocking region, a single stranded hybridization region and optionally a detectable moiety. Alternative methods for removing primer at the end of the amplification reaction have also been devised. These are by electrophoresis, post-PCR hybridization and enzymatic conversion.

Electrophoretic Separation

[0140] The first approach described is electrophoretic separation. It is well known that nucleic acids can be separated based on their molecular weight. By exploiting the size differences between the PCR amplicon and the oligonucleotide primers it is possible to rapidly purify the amplicon. In the preferred embodiment, an electrophoresis module is incorporated into a single-use device. For example, the electrophoretic purification module can be situated at a point along a channel in the device at a position convenient to effect purification, as shown in FIG. 12. The device is comprised of an electrode **50** in a channel of the device and a second electrode **51** in an adjacent cavity **52**. Each electrode is connected to an electrical contact pad **53**. A channel **54** in the device provides a means through which fluid moves from an earlier stage e.g. a PCR amplification step, to a later stage e.g. a detection step.

[0141] The purification module shown in FIG. 12 can be situated on either side of the channel and above or below. It can have two or more electrodes. For example, an additional third electrode can be situated in a position between the two electrodes that are shown. For the two-electrode embodiment shown in FIG. 12, a capture membrane for the primer sequences is used which effectively irreversibly absorbs the primer. Suitable materials include nitrocellulose, Whatman DE52 membrane, and other DNA binding membranes, well known in the art.

[0142] In one embodiment, solidified gel matrix, e.g. agarose, with an electrophoresis buffer is positioned in the cavity.

A sample segment of PCR amplified material is then moved through the channel and positioned over the cavity. Optionally a second pair of conductivity electrodes can be used to sense the position of the material as it moves through the channel, as described in jointly owned U.S. Pat. No. 5,096,669 incorporated herein by reference. Once the sample is positioned appropriately, an electrophoretic charge is applied across the two electrodes, with **50** being negative, and **51** being positive. This causes electrophoretic movement of the molecules in the gel matrix, with the smaller synthetic oligonucleotide primers moving the fastest and the larger per amplicons moving slower. Once the fragments have moved an appropriate distance, i.e. out of the channel and into the cavity, the electrophoretic charge is reversed, causing the fragments to move in the opposite direction. After a certain amount of time and with a particular charge and voltage the larger molecule will have transferred back into the channel, leaving the smaller primer molecules in the gel material. This is thus a way of effecting purification of the amplicons.

[0143] In another embodiment, a third electrode is positioned between the two electrodes shown in FIG. 12. Here electrodes **50** and **51** are set as negative and positive respectively. After a time when the primer molecules have passed the third middle electrode, but the amplicon has not, electrode **50** is reversed to positive charge, leaving electrode **51** as positive. At this point, the third middle electrode is made negative. This causes the primer to continue moving away from the channel, and reverses the direction of the amplicon back towards the channel.

[0144] FIG. 13 (a)-(g) illustrates the steps involved using charged dyes in a device. FIG. 13(a) shows a modified i-STAT cartridge base of the type described in jointly owned U.S. Pat. No. 5,096,669. It has an entry port **71**, a channel **72**, a cavity **73** adjacent to the channel and three electrodes **74**, **75** and **76**, two of which are in the cavity and one in the channel. The cavity contains 1% agarose with buffer as a transparent gel. A sample comprising 5 μ L of common electrophoresis loading dyes, bromophenol blue and xylene cyanol, both negatively charged, is added through the entry port and enters the channel as a fluid segment **77**, as shown in FIG. 13(b). Note that these dyes migrate at roughly 25 to 50 by sizes, where as with actual DNA separation will be of 50 by and 300 by fragment.

[0145] In FIG. 13(c) a negative potential is applied to **74** and a positive one to **76**, in this case 50V. The charged dyes quickly move into the agarose gel, towards **76**. The two dyes migrate at different rates according to their charge-to-mass ratio through gel. As shown in FIG. 13(d) the dyes are resolved into two bands **78** and **79** either side of **75**. This takes about three minutes. At this point **74** and **76** were made positive and **75** made negative, thus driving the two migrating dyes in opposite directions as shown in FIG. 13(e) until the xylene cyanol dye re-enters the channel, FIG. 13(f). Finally, the xylene cyanol is pneumatically moved down the channel for further downstream applications, as shown in FIG. 13(g) while the other dye remains in the cavity.

[0146] Clearly, the behavior of the two dyes is representative of different length nucleotide sequences or any other chemical species with different charge-to-mass ratios that could be separated from one another quickly using electrophoresis. Furthermore, the electrophoretic properties and capabilities of this device can be tailored according to gel density, buffer-salt selection, applied potential and duration, physical dimensions and the like, to achieve any desired separation.