

or oil-filtering medium. Again, the method can be performed as a manual procedure, as described here, or as the basis of an automated analysis in a disposable device. The use of wax or oil as a filtering medium overlaying the lysed-cell bead-DNA complex mixture eliminated the need for further fluid movement and assisted in purifying the bead-DNA complex. For instance, blood was combined with the lytic buffer and beads and the resulting DNA-bead complex was pelleted and drawn through an upper filtering layer with a permanent magnet, thus selectively separating the complex from the bulk of solution. This is illustrated in detail in FIG. 1.

[0086] FIG. 1 shows a tube 1 contains a wax filtering medium 2 above a lytic buffer 3 and magnetic beads 4. Typically the tube is stored at ambient temperature, so the tube is first heated to melt the wax. Generally, this is a temperature change to above about 35° C. Blood 5 is introduced with a pipette 6 and the blood is well mixed so that cells lyse in the buffer. Nucleic acid 7 then binds to the beads via non-specific surface bonds. A magnet 8 is then used to draw the beads and some extra lysed material and buffer to side of the tube to form a pellet. The magnet is then moved along side the tube to draw the pellet upwards through the wax layer. It has surprisingly been found that this effectively filters the pellet, as excess aqueous fluid is excluded by the greater surface tension of the wax. Optionally, after this step, the wax may be re-hardened by removing the heat. The resulting bead-nucleic acid pellet remains trapped in a thin layer of wax easily accessible at the side of the tube, while the lytic buffer and blood remains trapped below the wax. The bead-nucleic acid pellet can then be removed from the side of the tube and introduced to a new tube with the PCR cocktail present. The nucleic acid elutes off the bead during the first heating cycle of PCR, as it has been found that water at a temperature of above 800 C is sufficient for elution. It has also been found that neither the beads nor the wax interfere with PCR.

[0087] Ideal characteristics of waxes for this application include waxes which melt from a solid to a liquid at between 25 to 450 C. Further, these preferred waxes do not significantly evaporate at temperatures in the range 60 to 900 C. When these waxes are solid they prevent movement of bead and other solutions that are trapped by their presence, however, when these waxes are in a liquid state their viscosity is sufficiently low to permit passage of magnetic beads under a magnetic field. The waxes also have the property of being compatible with reagents for DNA amplification. Four examples of waxes that can be used in the present invention are heneicosane (98%, m.p. 40-42° C., Sigma), docosane (99%, m.p. 43-45° C., Sigma), tricosane (99%, m.p. 48-50° C., Sigma) and tricosaheneicosane. The preferred wax is heneicosane. Other organic liquids that can be used to form the barrier layer through which the beads pass include silicone oil and mesitylene.

[0088] FIG. 2 demonstrates the successful removal of a purified DNA sample from blood using the beads transiting through wax process, with the presence of the anticipated bands (gel lanes 5 and 6 matching lane 2). This figure shows a polyacrylamide gel of PCR products with and without beads and with and without blood and also purified DNA controls. Note that the band labeled “*” represents the anticipated base-pair length for symmetrical PCR with a modified wild-type Hemachromatosis oligonucleotide primer set prepared on a known wild-type alleles ACD blood tube sample. The positive control (lane 2) also represents genomic DNA purified using a Qiagen commercial kit for sample preparation

(wild-type 15 alleles) and the negative control (lane 1) features with no DNA added to the PCR cocktail. In this example, PCR was performed in a conventional thermocycler, with a mineral oil overlay, using 30 cycles. A volume of 10 uL of sample plus 2 uL of loading dye was added into each well of a 10% non-denaturing polyacrylamide gel, 1xTBE buffer, as shown in FIG. 2.

[0089] FIG. 3 contrasts the successful removal of purified DNA from blood using the beads transiting through wax protocol (gel lanes 1-4) to the protocol without using the wax as a filter medium (gel lane 5). The band labeled “*” represents the anticipated base-pair length for symmetrical PCR with a modified wild-type Hemachromatosis oligonucleotide primer set prepared on a known wild-type alleles ACD blood tube sample. PCR was performed in a conventional thermocycler, with mineral oil overlay, using 30 cycles. A volume of 10 uL of sample plus 2 uL of loading dye was added into each well of a 10% non-denaturing polyacrylamide gel, 1xTBE buffer, as shown in FIG. 3.

[0090] The principles demonstrated by the above description can be incorporated into an individual nucleic acid extraction device based on manual manipulations of the type shown in FIG. 1, or into an automatic device as described below, where the user only needs to add the sample to the device and all the other steps are performed automatically.

Nucleic Acid Separation Methods and Apparatuses Based on Absorbent Filters

[0091] An alternative approach to quickly extract and isolate nucleic acids found in bodily fluids is provided. It is based on the use of filter materials. The disclosed devices and processes significantly improve upon the existing art by marrying chemically impregnated solid-substrate technologies to a miniaturized filtering apparatus. It also conveniently minimizes the time for extraction of an amplifiable quantity of genomic DNA from a low volume of bodily fluid. While the device may be used as an individual separation device, it is particularly amenable to integration into a disposable cartridge device for DNA isolation, amplification and optionally detection.

[0092] The individual device can be used, for example, in clinical and research environments as a rapid means for taking a small volume of fluid, such as blood or buccal cells, and quickly isolating DNA amenable to amplification. Alternatively, when incorporated into a disposable cartridge, microfluidic elements are used to automatically move the sample within the cartridge and to affect the extraction process. Both applications are described.

[0093] The primary features of the device and method combine; (i) rapid nucleic acid isolation, typically in less than two minutes, (ii) elements amenable to incorporation in a disposable cartridge, (iii) generation of either bound or unbound nucleic acid in a form compatible with amplification, (iv) utilization of small sample volumes, e.g. blood, buccal cells and tissue, and (v) utilization of small volumes of other liquid reagents to perform the operation.

[0094] Regarding the device, the supporting structure of a low-volume filter holding apparatus was used for the placement of a chemically-impregnated solid-substrate matrix. It functions as a filtering layer that extracts and isolates DNA from an applied sample by retaining these nucleic acids within its matrix. The filtering matrix was impregnated with lytic salts and optionally detergent, which after the binding step is then flushed or washed with a solvent, preferably