

[0073] FIG. 26 shows a schematic of the PCR amplification method which differentiates between mutant and wild-type SNP sequences.

[0074] FIG. 27 provides a table of possible signal outcomes from a hemochromatosis test.

[0075] FIG. 28(a)-(b) show two views of a buccal sample device for direct application of a buccal sample to a PCR chamber. This extraction and amplification device attaches to the detection cartridge.

[0076] FIG. 29(a)-(b) show a comparison of signal which increases relative to the amount of control oligonucleotide.

[0077] FIG. 30(a)-(b) show the ability of the cartridge to discriminate between wild-type and mutant SNP sequences of hemochromatosis.

[0078] FIG. 31 shows an autoradiograph of ^{32}P radiolabelled synthetic oligonucleotides demonstrating that the ExoI enzyme is an active 3'→5' exonuclease, which has the ability to reduce the molecular weight down to about 6-7 nucleotides in length.

[0079] FIG. 32(a) shows PCR with phosphorothioate primers discriminating between wt/mut DNA templates using a 10% non-denaturing polyacrylamide gel; 6 μL sample+1.6 μL LD→6 μL loaded in each well (45 min SYBR Gold stain, photo-negative, experiment HFE 84-2, T_{hyb} 68° C. The seven columns were loaded as follows; 1 wildtype-selective PCR primer present with wildtype DNA template, generates anticipated ~150 bp band; 2 wildtype-selective PCR primer present with mutant DNA template, does not generate anticipated ~150 bp band; 3 a 10 base-pair ladder, prominent bands at 100,330 and 1660 bases; 4 mutant-selective PCR primer present with wildtype DNA template, does not generate anticipated ~150 bp band; 5 mutant-selective PCR primer present with mutant DNA template, generates anticipated ~150 bp band; 6 a 10 base-pair ladder, prominent bands at 100, 330 and 1660 25 bases; and 7 both selective PCR primers present with wildtype DNA template, does not generate any band. FIG. 32(b) shows the related chronoamperometry plot.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Nucleic Acid Separation Methods and Apparatuses Based on Magnetic Particles

[0080] The present disclosure demonstrates a rapid and simple protocol for isolating genomic DNA from whole blood for the primary purpose of performing an amplification reaction, e.g. polymerase chain reaction (PCR). The present method has the advantage of exhibiting a significant reduction in the common inhibitors of PCR, e.g. hemoglobin, found in prior art rapid DNA extraction protocols. In blood samples, added anticoagulation reagents such as chelating agents, heparin, EDTA and citrate can also act as inhibitors. The present method eliminates these inhibitors and other naturally occurring chelating agents as well as enzymes and proteins that can damage nucleic acid templates. It is important to note that this technique is also applicable to other sources of nucleic acid material, e.g. buccal swabs, urine, and other tissue samples, and can also be used in conjunction with other amplification methods.

[0081] By contrast with the prior art, for example that found in Dynabeads Genomic DNA Blood kit (Prod.No. 634.02, DYNAL BIOTECH CORP.), and also US patent 2003/0180754A1 where nucleic acid extraction takes 30-40 minutes, the present method reduces the time required for reproducible

DNA extraction to less than about 5 minutes and preferably and typically to about 2 minutes. This is a significant improvement when considering genetic analyses where the speed with which a result is obtained is crucial, e.g. the identification of highly infectious agents. It is also applicable to testing in the physician's office environment, or even at the bedside, where it is desirable to obtain a sample from a patient and deliver a result during a single physician visit.

[0082] The present method preferably uses coated beads, with an inner-core that is a paramagnetic material and a lysing and binding buffer. When a lysed cell solution containing genomic DNA is mixed with beads of the preferred embodiment, the surface chemistry on the beads weakly binds DNA with low specificity due to a strong negative surface charge, thus creating a bead-DNA complex. The preferred surface coating is a carboxylic acid coated surface and the paramagnetic beads typically have a 2.8 μm diameter, though beads in the diameter range of about 0.1 to 100 μm can be employed. Alternative anionic coatings for the beads include the following materials including very small diameter glass beads (e.g. Glass Milk), Whatman phosphocellulose and DEAE resin (e.g. DE52).

[0083] While non-magnetic beads may be used, it is certainly advantageous to use magnetic beads as these beads may be drawn to the side of a reaction vessel and held against the side by means of a magnet. This can occur within a short period of time, provides a means for concentrating the bead in one location and provides a means for moving and manipulating the beads. The magnetic field may be provided by a permanent magnet or by electromagnetic means, as is well known in the art.

[0084] In an example that uses a standard polypropylene PCR tube, a standard lysis buffer (DYNAL BIOTECH CORP.) containing; water 60-100% wt, sodium chloride (NaCl) 10-30%, lithium chloride (LiCl) 5-10%, tris-HCl 1-5%, lithium dodecylsulfate (LIDS) 0.1-1%, EDTA 0-1%, and dithiothreitol (DTT) 0-0.1%; was modified to include NaOH reagent at a final alkaline concentration of 0.65M. Other lysis buffers known in the art may also be used with the appropriate addition of base, e.g. NaOH. Whole blood (10 μL) was then added directly to the alkaline-modified lysis buffer with Dynabeads (23 μL). This induced the lysis of blood cells in about 15 seconds of manual pipette mixing, followed by about 15 seconds of dwell time for the adsorption of genomic DNA onto the beads. The bead-DNA complex was then captured against the side of a tube with a permanent magnet, which takes less than about 15 seconds. The entire supernatant of lysed cells was then removed by pipette. A wash buffer 50 μL , e.g. DYNAL WASH BUFFER (from a DYNAL KIT) was introduced by pipette and used to rinse the bead-DNA pellet that was captured against the tube wall. The wash solution was then entirely removed by pipette while the pellet remained captured against the tube wall. The remaining bead-DNA pellet (1-2 μL equiv. volume) was then removed and added to a new tube with a PCR cocktail (~25 μL) comprising polymerase enzyme, primers, dNTPs and buffer along with a mineral oil overlay (~10 μL) and placed into a conventional thermocycler. The total duration of this extraction process was found to be about two minutes. Note that it is demonstrated below that this novel purification protocol overcomes the problem associated with inhibitors of a PCR reaction remaining in the extract.

[0085] In a preferred embodiment, the extraction method employs alkaline lysis buffer, magnetic beads and also a wax