

target sequence in a sample. Detection is preferably electrochemical and is based on a labeled probe that also binds to a different region of the target. Alternatively, an immobilized antibody to the hybrid formed by a probe and polynucleotide sequence can be used along with DNA binding proteins. The '081 patent incorporates by reference the jointly owned U.S. Pat. No. 5,096,669 which covers a single-use cartridge for performing assays in a sample using sensors. These sensors can be of the type described in '081.

[0025] Other divisional patents related to '081 include U.S. Pat. No. 5,200,051 which covers a method of making a plurality of sensors with a permselective membrane coated with a ligand receptor that can be a nucleic component. U.S. Pat. No. 5,554,339 covers microdispensing, where a nucleic acid component is incorporated into a film-forming latex or a proteinaceous photoformable matrix for dispensing. U.S. Pat. No. 5,466,575 covers methods for making sensors with the nucleic component incorporated into a film-forming latex or a proteinaceous photoformable matrix. U.S. Pat. No. 5,837,466 covers methods for assaying a ligand using the sensor components including nucleic components. For example, a quantitative oligonucleotide assay is described where the target binds to a receptor on the sensor and is also bound by a labeled probe. The label is capable of generating a signal that is detected by the sensor, e.g. an electrochemical sensor. U.S. Pat. No. 5,837,454 covers a method of making a plurality of sensors with a permselective membrane coated with a ligand receptor that can be a nucleic component. Finally, jointly owned U.S. Pat. No. 5,447,440 covers a coagulation affinity-based assay applicable to nucleotides, oligonucleotides or polynucleotides. These jointly owned patents are incorporated herein by reference.

[0026] It is noteworthy that jointly owned U.S. Pat. No. 5,609,824 discloses a thermostated chip for use within a disposable cartridge applicable to thermostating a sample, e.g. blood, to 37° C. Jointly owned U.S. Pat. No. 6,750,053 and pending US 20030170881 address functional fluidic elements of a disposable cartridge relevant to various tests including DNA analyses. These additional jointly owned patents and applications are incorporated herein by reference. Several other patents address electrochemical detection of nucleic acids, for example U.S. Pat. No. 4,840,893 discloses detection with an enzyme label that uses a mediator, e.g. ferrocene. U.S. Pat. No. 6,391,558 discloses single stranded DNA on the electrode that binds to a target, where a reporter group is detected by the electrode towards the end of a voltage pulse and uses gold particles on the electrode and biotin immobilization. U.S. Pat. No. 6,346,387 discloses another mediator approach, but with a membrane layer over the electrode through which a transition metal mediator can pass. U.S. Pat. No. 5,945,286 is based on electrochemistry with intercalating molecules. U.S. Pat. No. 6,197,508 discloses annealing single strands of nucleic acid to form double strands using a negative voltage followed by a positive voltage. Similar patents include U.S. Pat. No. 5,814,450, U.S. Pat. No. 5,824,477, U.S. Pat. No. 5,607,832 and U.S. Pat. No. 5,527,670 which disclose electrochemical denaturation of double stranded DNA. U.S. Pat. No. 5,952,172 and U.S. Pat. No. 6,277,576 disclose DNA directly labeled with a redox group.

[0027] Several patents address devising cartridge-based features or devices for performing nucleic acid analyses, these include for example a denaturing device U.S. Pat. No. 6,485,915, an integrated fluid manipulation cartridge U.S.

Pat. No. 6,440,725, a microfluidic system U.S. Pat. No. 5,976,336 15 and a microchip for separation and amplification U.S. Pat. No. 6,589,742.

[0028] Based on the forgoing description there is a need for a convenient and portable analysis system capable of performing nucleic acid testing.

OBJECTS OF THE INVENTION

[0029] An object of the invention is to provide an integrated nucleic acid test cartridge capable of performing extraction, amplification and detection.

[0030] A further object of the invention is to provide an integrated nucleic acid test cartridge with optical and electrochemical detection.

[0031] A further object of the invention is to provide an integrated nucleic acid test cartridge with an extraction step based on filter extraction or on particle transit through a layer that is immiscible with an aqueous fluid.

[0032] A further object of the invention is to provide an integrated nucleic acid test cartridge capable of performing extraction and amplification.

[0033] A further object of the invention is to provide an integrated nucleic acid test cartridge capable of performing amplification and detection.

[0034] An object of the invention is to provide an integrated cartridge for nucleic acid testing that operates in conjunction with a reader instrument.

[0035] An object of the invention is to provide an integrated nucleic acid testing system and method suitable for analyses performed at the bedside, in the physician's office and other locations remote from a laboratory environment where testing is traditionally performed.

[0036] An object of the invention is to provide a device and method of nucleic acid extraction from a sample with a purification step involving particle transit through a layer that is immiscible with an aqueous fluid.

[0037] An object of the invention is to provide a device and method of filter-based nucleic acid extraction from a sample with an elution step prior to amplification.

[0038] An object of the invention is to provide a simple method and component for separating nucleic acid from a sample suitable for integration into a device for performing genetic analyses.

[0039] An object of the invention is to provide electrophoretic separation of primers from amplicons after amplification capable of integration with a nucleic acid testing cartridge.

[0040] An object of the invention is to provide a DNA polymerase enzyme that generates the most synthesis in the shortest time period, therefore a DNA polymerase with an elongation rate of over 100 bases per second or a processivity rate of over 300 bases.

[0041] It is another object of the invention to provide a DNA polymerase enzyme that functions in a miniaturized thermocycler device in a short time period.

SUMMARY OF THE INVENTION

[0042] In one embodiment, the invention is directed to a nucleic acid separation method, comprising: exposing a sample comprising cells containing nucleic acid to an aqueous mixture comprising a lytic reagent and one or more beads capable of binding the nucleic acid released from said cells to form a nucleic acid-bead complex, and passing the nucleic