

dependent on the direction of the electrical current, as described in, for example, U.S. Pat. Nos. 5,333,675 and 5,656,493. Many commercial temperature cycling devices are available, sold by, for example, Perkin Elmer (Wellesley, Mass.), Applied Biosystems (Foster City, Calif.), and Eppendorf (Hamburg, Germany). As these devices are generally large and heavy, they are not generally amenable to use in non-laboratory environments, such as, for example, at the point-of-care of a patient.

[0010] Microfabricated chamber structures for performing the polymerase chain reaction have been described in, for example, U.S. Pat. No. 5,639,423. A device for performing the polymerase chain reaction is described in, for example, U.S. Pat. No. 5,645,801 that has an amplification chamber that can be mated to a chamber for detection. For example, U.S. Pat. No. 5,939,312 describes a miniaturized multi-chamber polymerase chain reaction device. U.S. Pat. No. 6,054,277 describes a silicon-based miniaturized genetic testing platform for amplification and detection. A polymer-based heating component for amplification reactions is described in, for example, U.S. Pat. No. 6,436,355. For example, U.S. Pat. No. 6,303,288 describes an amplification and detection system with a rupturable pouch containing reagents for amplification. U.S. Pat. No. 6,372,484 describes an apparatus for performing the polymerase chain reaction and subsequent capillary electrophoretic separation and detection in an integrated device.

[0011] There are several nucleic acid amplification technologies that differ from the PCR reaction in that the reaction is run at a single temperature. These isothermal methods include, for example, the cycling probe reaction, strand displacement, INVADER™ (Third Wave Technologies Inc., Madison, Wis.), SNPase, rolling circle reaction, and NASBA. For example, U.S. Pat. No. 6,379,929 describes a device for performing an isothermal nucleic acid amplification reaction.

[0012] A microfluidic biochemical analysis system with flexible valve ports and with pneumatic actuation is described in, for example, Anderson et al., *Transducers '97*, pages 477-80; 1997 International Conference on Solid-State Sensors and Actuators, Chicago, Jun. 16-19, 1997. A fully integrated PCR-capillary electrophoresis microsystem for DNA analysis is described in, for example, Lagally et al., *Lab on a Chip*, 1, 102-7, 2001. A method of non-contact infrared-mediated thermocycling for efficient PCR amplification of DNA in nanoliter volumes is described in, for example, Huhmer and Landers, *Analytical Chemistry* 72, 5507-12, 2000. A single molecule DNA amplification and analysis microfluidic device with a thermocouple and valve manifold with pneumatic connections is described in Lagally et al., *Analytical Chemistry* 73, 565-70, 2001.

[0013] The polymerase chain reaction (PCR) is based on the ability of a DNA polymerase enzyme to exhibit several core features that include its ability to use a primer sequence with a 3'-hydroxyl group and a DNA template sequence and to extend a newly synthesized strand of DNA using the template strand, as is well known to those skilled in the art. In addition, DNA polymerases used in the PCR reaction must be able to withstand high temperatures (e.g., 90 to 99° C.) used to denature double stranded DNA templates, as well as be less active at lower temperatures (e.g., 40 to 60° C.) at which DNA primers hybridize to the DNA template. Fur-

thermore, it is necessary to have optimal DNA synthesis at a temperature at or above to the hybridization temperature (e.g., 60 to 80° C.).

[0014] Zhang et al. (2003, *Laboratory Investigation*, vol 83(8):1147) describe the use of a terminal phosphorothioate bond to overcome the limitations of DNA polymerases used for 3'-5' exonuclease activity. The phosphorothioate bond is not cleaved by 3'-5' exonucleases. This prevents DNA polymerases with 3'-5' exonuclease activities from removing the terminal mismatch and proceeding with DNA elongation, alleviating the lack of discrimination observed with normal DNA.

[0015] Another characteristic of DNA polymerases is their elongation rate. Takagi et al. (1997, *Applied and Environmental Microbiology*, vol 63(11): 4504) describes that *Pyrococcus* sp. Strain KOD1 (now *Thermococcus kodakaraensis* KOD1), *Pyrococcus furiosus*, Deep Vent (New England Biolabs, Beverly, Mass.), and *Thermus aquaticus* have elongation rates of 106 to 138, 25, 23 and 61 bases/second, respectively. The processivity rates of these enzymes are also described, and behave similarly to the elongation rates. Clearly, *Thermococcus kodakaraensis* KOD1 has much higher elongation and processivity rates compared to the other well known enzymes that would make this enzyme beneficial in applications where sensitivity and speed are an issue. Further, *Thermococcus kodakaraensis* KOD 1 possesses an exonuclease activity that would be detrimental for use in a 3'-allele specific primer extension assay used for SNP analysis.

[0016] Conventional detection methods for the final step in a nucleic acid analysis are well known in the art, and include sandwich-type capture methods based on radioactivity, colorimetry, fluorescence, fluorescence resonance energy transfer (FRET) and electrochemistry. For example, jointly-owned U.S. Pat. No. 5,063,081 (the '081 patent) covers a sensor for nucleic acid detection. The sensor has a permselective layer over an electrode and a proteinaceous patterned layer with an immobilized capture oligonucleotide. The oligonucleotide can be a polynucleotide, DNA, RNA, active fragments or subunits or single strands thereof. Coupling means for immobilizing nucleic acids are described along with methods where an immobilized nucleic acid probe binds to a complimentary 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 patent U.S. Pat. No. 5,096,669 that is directed to a single-use cartridge for performing assays in a sample using sensors. These sensors can be of the type described in the '081 patent.

[0017] Other divisional patents related to the '081 patent include, for example, U.S. Pat. No. 5,200,051 that is directed to a method of making a plurality of sensors with a permselective membrane coated with a ligand receptor that can be a nucleic component. For example, U.S. Pat. No. 5,554,339 is directed to 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 is directed to 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 is directed to methods for assaying a ligand using