

embodiment, the buffer comprises 22 U/ml *Thermococcus* species KOD thermostable polymerase complexed with anti-KOD antibodies, 66 mM Tris-SO<sub>4</sub> (pH 8.4), 30.8 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 11 mM KCl, 1.1 mM MgSO<sub>4</sub>, 330 μM dNTPs, as well as proteins and stabilizers (e.g., Invitrogen Life Technologies AccuPrime Pfx SuperMix manual, Cat. No. 12344-040). An alternatively exemplary embodiment can use 20 mM Tris-HCL (pH 8.8), 2 mM MgSO<sub>4</sub>, 10 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Triton-X-100, 0.1 mg/ml nuclease-free BSA (e.g., Stratagen Pfu DNA polymerase Instruction Manual Cat# 600135 Revision\$ 064003d), and/or the like. Primers is095, is096 and is101 can also be present in the reaction to approximately 7.5 pmol final.

[0113] After the amplification cycle, the pins 53, 57 are released and a pin over the air bladder is pushed to move the sample into the detection device 59. The analysis is performed in the same manner as described for Example 1. The amount of current generated (signal) is then measured as an indication of the number of amplicons bound at the biosensor. A signal at only the MBW biosensor is a mutant SNP sequence. A signal at the Sc biosensor is an indication of a wildtype SNP sequence, and a signal at both biosensors indicates that the patient is heterozygous for that SNP sequence. As mentioned above, when no signal is generated at both biosensors, it is an indication of an error occurring in either the amplification or detection process.

[0114] FIG. 11 illustrates the measured current profiles, termed chronoamperometric outputs, from the DNA cartridges, and specifically for the detection device 59. In the present example, PCR is performed in an Eppendorf Mastercycler egradient S, SN534502285. The PCR reaction was using primers described above specific for human C282Y SNP differentiation and used human DNA from a wild-type donor. The reactions were performed for 20, 22, 24, 26, 28, 30 and 35 cycles, prior to testing. An aliquot comprising 5% of the material from the amplification reaction was used in the detection device 59, generating the chronoamperometric data seen in FIG. 11.

[0115] The software for the instrument used for detection can be based on modified i-STAT 300 analyzer software (i-STAT Corporation, East Windsor, N.J.) that performs a series of steps in the detection process, although other suitable software processes or techniques can be used to implement the appropriate features and functionality of the instrument used for detection. The detection cartridge 59 is described in, for example, jointly-owned U.S. Application Publication No. 2003/0170881, the entire contents of which are incorporated by reference. Liquid containing the amplified target from the amplification cartridge is pneumatically pushed into the sensor chamber of the detection cartridge 59 to permit the capture steps. In a preferred exemplary embodiment, the temperature of a sensor chip in the detection cartridge 59 is set to approximately 47° C. as fluid containing amplicon is pushed back and forth over top of the capture oligonucleotide beads on the sensor to affect efficient capture of the amplicon. This step takes about 3 to about 10 minutes. Any liquid containing the uncaptured amplicon is then moved from the sensor area to a waste chamber, and a wash fluid containing an electroactive substrate is then applied to the sensor and set to collect data at a poise potential of, for example, +30 mV vs. Ag/AgCl electrode (at 2 pA/bit). The wash fluid is also forced into a waste chamber leaving a thin layer of analysis fluid containing p-aminophenol phosphate that can react with the

enzyme on the amplicon and be oxidized at the electrodes. Current generated as a function of time is recorded, as illustrated in FIG. 11.

[0116] In an alternative exemplary embodiment where the moiety is biotin and is bound to streptavidin-labeled alkaline phosphatase, the detection reagent can be p-aminophenol phosphate that is hydrolysed to form p-aminophenol by the enzyme. This is then electrochemically oxidized at the electrode surface of an amperometric sensor to generate a current proportional to the amount of moiety that is present. As mentioned above, this type of detection is illustrated in the current versus time plots of FIG. 11.

[0117] The instrument used for detection preferably includes a keypad for user entries and a suitable display. The instrument also includes a power source and suitable electrical and/or electronic circuitry and an embedded algorithm for controlling the temperature of the amplification chamber, as will be apparent to those skilled in the art. The instrument can also include an electrical connector of the type described in, for example, jointly-owned U.S. Pat. Nos. 4,954,087 and 5,096,669. The electrical connector can be used to make electrical connection to the sensors. Where it is desirable to perform the detection step at a controlled temperature, e.g., 37° C. or other suitable temperature, the connector can also incorporate suitable heating and thermistor elements that contact the back side of the silicon chip that provides the substrate for the sensor. These elements are of the same type as described for the amplification chamber 11. The instrument includes amperometric circuitry for controlling the potential of the sensor and measuring current. The instrument also includes a suitable embedded algorithm for controlling the entire analysis sequence performed by the instrument on the single-use device to make a nucleic acid determination and display a result on a display screen on the instrument. Where the electroactive species generated or consumed in proportion to the captured target is more appropriately detected by means of potentiometry or conductimetry, alternative circuitry (well known in the art) can be incorporated into the instrument.

[0118] While a preferred method of detection in the single-use cartridge is electrochemical, other sensing methods, including, but not limited to, fluorescence, luminescence, calorimetric, thermometric, fiber optics, optical wave guides, surface acoustic wave, evanescent wave, plasmon resonance and the like, can be used.

[0119] A preferred sensor comprises an amperometric electrode that is operated with a counter-reference electrode. The amperometric electrode comprises an approximately 100 μm diameter gold layer microfabricated onto a silicon chip. The silicon chip is treated in the first step of manufacture to produce an insulating layer of silicon dioxide on the surface, as is well known in the art. The electrode can be connected by means of a conducting line to a connector pad that makes contact with the electrical connector of the instrument. The conducting line is typically coated with an insulating layer of polyimide. Directly over the electrode or at an adjacent location on the chip are adhered polymer particles that have a ligand complimentary to and capable of capturing the amplified target. The counter-reference electrode can be microfabricated on the same silicon chip or one place adjacently in the second conduit. The counter-reference electrode can comprise a silver-silver chloride (Ag/AgCl) layer, of about 200 μm diameter, attached by a contact line to a contact pad that makes contact with the instrument