

gate is a final concentration of 350 ug/ml. Alternatively a control oligonucleotide sequence was used in place of the amplified DNA. The control oligonucleotide sequence was manufactured as a positive control for chronoamperometric detection. This single-stranded sequence is analogous to **36** as shown in FIG. **7(a)** and is complementary to region **40** and contains a 36-FAM fluorescent species. Note that FIG. **7(b)** shows the undesired competition of a standard primer in the detection step, whereas with the clam-like primer, as in FIG. **7(c)** this is obviated. The results from both of these samples are shown in FIGS. **8(a)** and **8(b)**. FIG. **8(a)** shows the chronoamperometric reading for anti-FITC ALP conjugate alone versus the conjugate with amplicon hybridized to the sensor. FIG. **8(b)** shows the chronoamperometric reading for anti-FITC ALP conjugate alone versus the conjugate with a positive control oligonucleotide sequence.

[0119] The detection cartridge operated as follows, a 20 μ L portion of the 100 μ L aliquots was loaded into an enzyme-linked DNA hybrid sensor cartridge, as described in jointly owned US 20030170881 and placed into an i-STAT model 300 electrochemical analyzer (i-STAT Corporation). The sensor cartridge contained multiple (2 or 4) amperometric sensors coated with specific DNA oligomers. In this example, the oligomers were 5'-biotinylated oligonucleotides and were bound to streptavidin-coated beads which were adsorbed onto the sensor surface. One of the sensors was coated with the complementary single-stranded DNA oligomer to one of the single-stranded portions of the PCR primers, as a control. Also present within this cartridge was a separate anti-FAM-alkaline phosphatase conjugate.

[0120] In the preferred embodiment, the PCR amplified product and anti-FAM ALP conjugate dissolved into a single solution were brought into contact with the DNA capture sensors. Note that alternatively the PCR product may be contacted with the sensor first, followed by the conjugate. In the preferred embodiment, the double-stranded PCR products, containing both single-stranded hybridization regions, binds to the capture region on the amperometric sensor. Binding of the alkaline phosphatase label can occur either in solution before capture of the PCR product or after it has bound to the bead. After a controlled period of time, typically 5 to 15 minutes and at a controlled temperature preferably 37° C., the solution is moved out of the sensor region and delivered to a waste chamber within the cartridge. A wash solution, containing substrate for ALP, is brought over the sensor washing excess a FAM ALP conjugate away from the sensor region. A trailing portion of the wash solution remains on the sensor and provides an electrogenic substrate for the ALP label. Note that in an alternative embodiment a wash solution may be used first, followed by a second solution containing the substrate. Note also that where an optical sensor or other type of sensor is used, other appropriate substrates are used. In the preferred embodiment, the measured current at the capture sensor is essentially directly proportional to the number of ALP labels present on the sensor. An adjacent amperometric sensor which is not coated with the complementary DNA binding sequence can be used as a control sensor to offset any nonspecific binding of the ALP reagent on the sensors, thus improving the detection limit. Alternatively a capture oligonucleotide with a sequence different from the complimentary DNA binding sequence can be used as a negative control.

[0121] Referring to FIG. **8(a)** and FIG. **8(b)**, these show the measured current profiles, or chronoamperometric output,

from DNA cartridges. PCR product with conjugate shows an increase in measured current, over conjugate alone, in FIG. **8(a)**. Here, competing unbound primers may be reducing signal. A similar increase in signal is observed with the positive-control oligonucleotide sequence that is labeled with 36-FAM species, as shown in FIG. **8(b)**. It has also been found that the net current is proportional to the number of PCR amplicons in the sample, see FIG. **9(a)**, where the steady-state current is shown to increase with increasing amplicon concentration. These data are plotted in FIG. **9(b)**.

[0122] The software used for the instrument **200** and **650** (see FIGS. **6** and **21**) in this example is a modified i-STAT 300 analyzer (i-STAT Corporation) which performs a series of steps in the detection process. In the first step, the instrument makes contact with and identifies the cartridge, and then conducts a battery check and other internal instrument checks. It then initiates and completes a thermal cycle to heat the sensor chip to 37° C. The liquid containing the amplified target is then pneumatically pushed from conduit **125** into the sensor chamber **126** to permit the capture steps. A push pin **213** in the instrument then makes contact with element **135** during the second motor motion of the instrument causing the analysis fluid **134** to be dispensed from the analysis pack into the analysis into conduit **125** which acts a temporary holding chamber. The temperature set-point for the sensor chip is then increased to 47° C. and a conductivity sensor on the chip is initialized. The target liquid is then pushed back and forth over top of the capture oligonucleotide beads to effect efficient capture of the amplicon. This step takes about 3 to 9 minutes. Note that the conductivity sensor is used to monitor the position of the fluid during this capture process. Before the last two oscillations, the software in the instrument causes the heating of the chip to be turned off and the remaining cycles are conducted at ambient temperature. The liquid containing the uncaptured amplicon is then moved slowly to the sample inlet side of the waste chamber **137**, and the sensors are set to collect data at a poise potential of +30 mV vs. Ag/AgCl electrode (at 2 pA/bit). As this liquid is pushed into the waste chamber a locking wick mechanism closes a vent when it becomes saturated. This mechanism is of the type described in jointly owned US 20030170881 which is incorporated here by reference. The software then causes the instrument to actuate the cartridge such that analysis fluid is drawn across the sensors to wash the remaining unbound material from the capture oligonucleotide, leaving a thin layer of analysis fluid containing p-aminophenol phosphate which can react with the enzyme and be oxidized at the electrodes. Current generated as a function of time is recorded, as shown in FIG. **9(a)**, and can be used by the software algorithm to display a result.

[0123] It is known in the art that ExoI can be used to degrade un-incorporated single stranded oligonucleotides in DNA sequencing reactions, however it was not known if unnatural DNA, like the hexa-PEG region would be degraded by the ExoI enzyme. To demonstrate that ExoI works on this unnatural base, the experiment shown in FIG. **31** was performed. This figure shows an autoradiograph of ³²P radiolabelled synthetic oligonucleotides after ExoI treatment. In FIG. **31**, the is015 oligonucleotide in lane 1 is the same as oligo 1 above. The oligonucleotides labeled is026 and is027, like is015 contained an HPEG spacer, while the is020 oligonucleotide did not contain an HPEG spacer. FIG. **31** demonstrates that the ExoI enzyme is an active 3'->5' exonuclease, which has the ability to reduce the molecular weight down to