

about 6-7 nucleotides in length. Further, it can process past the hexa-PEG region and it is inhibited in the double stranded region of the clam primers. Therefore, it demonstrates that ExoI is not prevented from being an exonuclease with the hexa-PEG region.

[0124] In another embodiment of the invention, gene copy mutations, e.g. ZNF217, are detected by using both the target gene and one or more housekeeping genes, e.g. actin or glyceraldehyde-3-phosphate dehydrogenase. This is accomplished with two sensors in the detection chamber **126**, with one for the target and the other for the housekeeper. Here, PCR primers are used to amplify both the housekeeping gene, as well as the gene of interest. If ZNF217 is present in the same copy number as the housekeeping gene, the level of signals is similar. However, when the ZNF217 gene is present in multiple copies, the level of signal at the ZNF217 sensor is greater than at the housekeeping gene sensor.

[0125] Another embodiment of the invention addresses genetic mutations which causes disease states includes gene expression mutations. Wildenhain et al., (1990, *Oncogene*, vol 5(6):879), describe the over-expression of the neu protein-tyrosine kinase, p185neu which is related to breast cancer. The c-Myc oncogene has been identified in many forms of cancer (Waikel et al., 1999, *Oncogene*, vol 18(34):4870). Other examples of oncogene overexpression were described by Ren (2004, *Curr. Opin. Hematol.* Vol 11(1):25). Over-expression mutations typically generate increased levels of mRNA, thus to detect mRNA in this invention, an initial step of cDNA synthesis is used prior to the PCR amplification. The synthesis of cDNA using reverse transcription is well known in the art, including amplification of this material by PCR. Using the PCR amplification previously described, the presence of a quantity of mRNA present in a cell can be determined by measuring the level of the signal. Comparing the signal for a particular oncogene, for example Her2/neu to 5 a housekeeping gene allows the discrimination of oncogene expression at normal levels, or at levels indicative of a disease state, and in particular with breast cancer in the case of Her2/neu.

[0126] FIG. 26 shows an alternative assay method schematic and experimental data for this method are shown in FIG. 32(a) by gel electrophoresis and FIG. 32(b) by chronoamperometry. Target nucleic acid (DNA or cDNA) **329** is shown flanked by two regions where PCR and/or Clam primers bind **330** and **333**, with an intervening sequence marked by **331**. During the PCR reaction, three primer sequences **31**, **341** and **37** are added to the reaction mixture, wherein **31** and **341** differ by a single nucleotide at their 3' end **340** as indicated by **337** and **338**.

[0127] Elements **31**, **341** and **37** act as PCR primers, wherein region **336** for primers **31** and **341** hybridize to region **330** on target molecule **329**. And region **35** of molecule **37** hybridizes to region **333** on target molecule **329**. Primer **37** can function as a complementary PCR primer for primers **31**, **341** or both **31** and **341**. Primer **37** also has the feature of a specific sequence of bases at region **35** wherein it hybridizes to target molecule **329** at location **333**. It has a DNA polymerase blocking group at **33**, another unique region at **36** which will form a single stranded region for later hybridization during detection and an optional binding moiety at **37**.

[0128] Clam primers **31** and **341** have many similar features, but also have some specific differences. Both clam primers **31** and **341** have an optional detectable moiety at **334**. This is for example a biotin molecule on **31** and a FAM tag on

341. However, these are different for **31** and **341** to allow later discrimination of the molecule. Both clam primers **31** and **341** have different designed single stranded binding regions **32** and **339** respectively. In addition, both clam primers **31** and **341** have DNA polymerase blocking groups **33** and both clam primers **31** and **341** have a point mutation designed into the fourth nucleotide base to assist in the discrimination of single nucleotide polymorphisms, as described by Lee et al., (2004, *Nucleic Acids Research*, vol 32(2):681), Newton et al., (1989, *Nucleic Acids Research*, vol 17(7):2503), and European Patent application No. 89302331.7. As already mentioned, region **336** of both clam primers **31** and **341** bind to region **330** on target molecule **329**, wherein a single nucleotide mutation at **337** or **338** discriminates between a single nucleotide difference. Both clam primers have a modified terminal phosphodiester bond at **340** that is resistant to 3' to 5' exonucleases present in certain thermostable DNA polymerases, which further assists in the discrimination of the two different molecules. This modified terminal phosphodiester bond can be a phosphorothioate or peptide nucleic acid (PNA). The Clam primers also have the feature of having intramolecular structure, which prevents the unincorporated single stranded primer molecules from binding to a capture oligonucleotide **40** or **30** at the detection stage, but permits them to hybridize, to the capture oligonucleotides **40** and **30** if incorporated into a newly synthesized PCR amplicons.

[0129] In the first round of PCR after denaturation of the double stranded target to single strands, primer **37** and either clam primers **31** or **341** or both **31** and **341** bind to target molecule **329**. When only either clam primer **31** or **341** binds to the target molecule **329** as is the case for a homozygote, the single nucleotide on both copies of two chromosomes are the same. When both **31** and **341** bind to two separate molecules of target **329** as is the case for a heterozygote, one chromosome has one single nucleotide base sequence, whereas the other chromosome has a different single nucleotide base sequence as is found in single nucleotide polymorphisms. This incorporates clam primers **31** or **341** or both **31** and **341**, as well as the PCR primer at the other end, **37** and the newly synthesized intervening region **331**.

[0130] PCR amplification is allowed to proceed for between 15 and 50 cycles to generate newly synthesized amplified molecules. In FIG. 26 we show an amplicon **344** with Clam primer **341** incorporated. This is done for illustrative purposes. If the other mutation were present, or if there was a different sequence on either of the chromosomes, an amplicon with **31** incorporated would be found. For simplicity, only the amplicon with **341** is shown in the figure.

[0131] During the detection step of the process, the newly synthesized PCR amplicon **344** with Clam primer **341** and PCR primer **37** incorporated binds to capture oligonucleotide **40** at region **339** based on the nature of complementary sequences binding to each other. Sequence **339** does not bind to a different physically separated capture oligonucleotide **30** which possesses a different sequence. Both capture oligonucleotides **30** and **40** are bound to a solid substrate or beads as indicated in **39**.

[0132] The detection of this hybridized complex can either be detected by a conjugate molecule which binds to binding moiety **334** in molecule **37**, or another single stranded oligonucleotide **318** binding at region **343** with region **36** of molecule **344** having its own detectable moiety **342** which can be detected by a conjugate molecule. The conjugate molecule has two features: (i) a region that binds to the binding moiety