

[0197] The first step of the CPT method requires hybridizing a primary scissile primer (also called a primary scissile probe) to the target. This is preferably done at a temperature that allows both the binding of the longer primary probe and disassociation of the shorter cleaved portions of the primary probe, as will be appreciated by those in the art. As outlined herein, this may be done in solution, or either the target or one or more of the scissile probes may be attached to a solid support. For example, it is possible to utilize "anchor probes" on a solid support or the electrode which are substantially complementary to a portion of the target sequence, preferably a sequence that is not the same sequence to which a scissile probe will bind.

[0198] Similarly, as outlined herein, a preferred embodiment has one or more of the scissile probes attached to a solid support such as a bead. In this embodiment, the soluble target diffuses to allow the formation of the hybridization complex between the soluble target sequence and the support-bound scissile probe. In this embodiment, it may be desirable to include additional scissile linkages in the scissile probes to allow the release of two or more probe sequences, such that more than one probe sequence per scissile probe may be detected, as is outlined below, in the interests of maximizing the signal.

[0199] In this embodiment (and in other techniques herein), preferred methods utilize cutting or shearing techniques to cut the nucleic acid sample containing the target sequence into a size that will allow sufficient diffusion of the target sequence to the surface of a bead. This may be accomplished by shearing the nucleic acid through mechanical forces or by cleaving the nucleic acid using restriction endonucleases. Alternatively, a fragment containing the target may be generated using polymerase, primers and the sample as a template, as in polymerase chain reaction (PCR). In addition, amplification of the target using PCR or LCR or related methods may also be done; this may be particularly useful when the target sequence is present in the sample at extremely low copy numbers. Similarly, numerous techniques are known in the art to increase the rate of mixing and hybridization including agitation, heating, techniques that increase the overall concentration such as precipitation, drying, dialysis, centrifugation, electrophoresis, magnetic bead concentration, etc.

[0200] In general, the scissile probes are introduced in a molar excess to their targets (including both the target sequence or other scissile probes, for example when secondary or tertiary scissile probes are used), with ratios of scissile probe:target of at least about 100:1 being preferred, at least about 1000:1 being particularly preferred, and at least about 10,000:1 being especially preferred. In some embodiments the excess of probe:target will be much greater. In addition, ratios such as these may be used for all the amplification techniques outlined herein.

[0201] Once the hybridization complex between the primary scissile probe and the target has been formed, the complex is subjected to cleavage conditions. As will be appreciated, this depends on the composition of the scissile probe; if it is RNA, RNaseH is introduced. It should be noted that under certain circumstances, such as is generally outlined in WO 95/00666 and WO 95/00667, hereby incorporated by reference, the use of a double-stranded binding agent such as RNaseH may allow the reaction to proceed

even at temperatures above the T_m of the primary probe:target hybridization complex. Accordingly, the addition of scissile probe to the target can be done either first, and then the cleavage agent or cleavage conditions introduced, or the probes may be added in the presence of the cleavage agent or conditions.

[0202] The cleavage conditions result in the separation of the two (or more) probe sequences of the primary scissile probe. As a result, the shorter probe sequences will no longer remain hybridized to the target sequence, and thus the hybridization complex will disassociate, leaving the target sequence intact. The optimal temperature for carrying out the CPT reactions is generally from about 5° C. to about 25° C. below the melting temperatures of the probe:target hybridization complex. This provides for a rapid rate of hybridization and high degree of specificity for the target sequence. The T_m of any particular hybridization complex depends on salt concentration, G-C content, and length of the complex, as is known in the art.

[0203] During the reaction, as for the other amplification techniques herein, it may be necessary to suppress cleavage of the probe, as well as the target sequence, by nonspecific nucleases. Such nucleases are generally removed from the sample during the isolation of the DNA by heating or extraction procedures. A number of inhibitors of single-stranded nucleases such as vanadate, inhibitors of ACE and RNasin, a placental protein, do not affect the activity of RNaseH. This may not be necessary depending on the purity of the RNaseH and/or the target sample.

[0204] These steps are repeated by allowing the reaction to proceed for a period of time. The reaction is usually carried out for about 15 minutes to about 1 hour. Generally, each molecule of the target sequence will turnover between 100 and 1000 times in this period, depending on the length and sequence of the probe, the specific reaction conditions, and the cleavage method. For example, for each copy of the target sequence present in the test sample 100 to 1000 molecules will be cleaved by RNaseH. Higher levels of amplification can be obtained by allowing the reaction to proceed longer, or using secondary, tertiary, or quaternary probes, as is outlined herein.

[0205] Upon completion of the reaction, generally determined by time or amount of cleavage, the uncleaved scissile probes must be removed or neutralized prior to detection, such that the uncleaved probe does not bind to a detection probe, causing false positive signals. This may be done in a variety of ways, as is generally described below.

[0206] In a preferred embodiment, the separation is facilitated by the use of a solid support (either an internal surface of the device or beads trapped in the device) containing the primary probe. Thus, when the scissile probes are attached to the solid support, the flow of the sample past this solid support can result in the removal of the uncleaved probes.

[0207] In a preferred embodiment, the separation is based on gel electrophoresis of the reaction products to separate the longer uncleaved probe from the shorter cleaved probe sequences as is known in the art and described herein.

[0208] In a preferred embodiment, the separation is based on strong acid precipitation. This is useful to separate long (generally greater than 50 nucleotides) from smaller fragments (generally about 10 nucleotides). The introduction of