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(54) Title: ANALYSIS OF TARGET MOLECULES WITHIN A SAMPLE VIA HYBRIDIZATION CHAIN REACTION

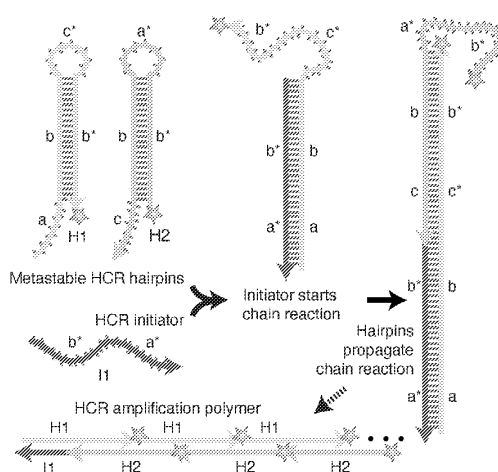


FIG. 1A

(57) Abstract: Methods of analysis of a sample using hybridization chain reaction (HCR) are provided herein. Some embodiments involve one, two, or all three of the following aspects: 1) repeated signal detection, 2) overlapping binding sites, and 3) catalytic reporter deposition (CARD). Compositions and kits relating to these are also provided. Some embodiments encompass a method for repeated signal detection with reporter-labeled HCR hairpins involving providing a sample possibly containing one or more targets as well as possibly other molecules that are not targets, providing one or more probe sets each comprising either: a) one or more HCR initiator-labeled probes, or b) one or more probe units each comprising two or more HCR fractional initiator probes, providing one or more HCR amplifiers (each labeled with one or more reporters), detecting one or more signals from one or more reporters. In some embodiments, a probe unit comprises two or more HCR fractional initiator probes, wherein an HCR fractional initiator probe comprises a target-binding region and a fractional initiator, wherein the target-binding regions within a probe unit are configured to bind to overlapping or non-overlapping binding sites on the target, and wherein the fractional initiators on the probes within each probe unit are configured to bind to overlapping or non-overlapping binding sites on an HCR hairpin. Some embodiments encompass a method for HCR-mediated catalytic reporter deposition (CARD) for signal detection with hapten-labeled HCR hairpins involving providing a sample possibly containing one or more targets as well as possibly other molecules that are not targets, providing one or more probe sets each comprising either: a) one or more HCR initiator-labeled probes, or b) one or more probe units each comprising two or more HCR fractional initiator probes, providing one or more HCR amplifiers (each labeled with one or more haptens), providing

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NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW,
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one or more anti-haptens labeled with one or more reporter entities, wherein the reporter entity is an enzyme that mediates CARD, providing one or more CARD-substrates leading to deposition of one or more reporters, and detecting one or more signals from one or more reporters.