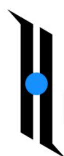
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HSAM

NOVEL NUCLEIC ACID AMPLIFICATION METHOD: HYBRIDIZATION SIGNAL AMPLIFICATION METHOD
(A NARRATIVE DESCRIPTION)

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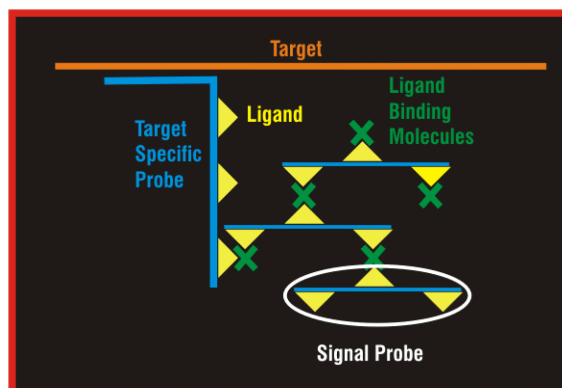
Many techniques have been developed to meet the growing demands of rapid, accurate detection of abnormal genes or infectious pathogens. Nucleic acid amplification systems, including polymerase chain reaction (PCR), ligase chain reaction (LCR), ligation-dependent PCR (LD-PCR), and strand displacement amplification (SDA), are powerful tools for the detection of minute amount of pathogenic sequences. However, these techniques have significant limitations. First, they require expensive equipment for amplification (PCR and LCR). Secondly, the characteristic run-to-run variation of these systems makes quantitation of target nucleic acid difficult. False amplification of non-target sequences and carry-over contamination are the other major problems associated with these systems. However, the branched DNA (bDNA) signal amplification method developed by Chiron Corp. is an example of an amplification system that provides a means to overcome problems such as carry-over contamination because it does not require amplification of target nucleic acid. Especially important is that the bDNA method provides a highly reliable quantitative measurement of target nucleic acid with equivalent sensitivity as other amplification (lower limit is about 10^2). However, due to special requirements and cost to synthesize the bDNA probe, the bDNA assay has been limited to a few applications.

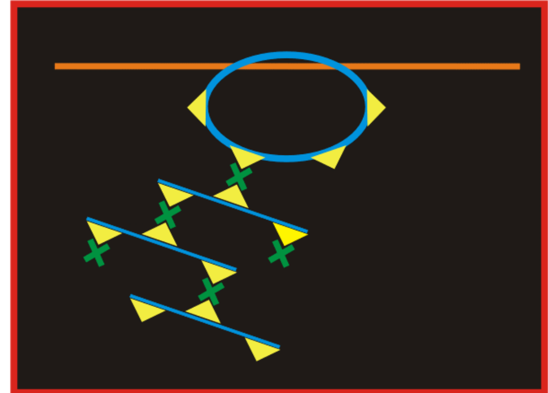
Hybridization Signal Amplification Method

1. Solid-support-based detection of nucleic acids: We have designed a novel and simple strategy, which we have dubbed HSAM (Hybridization Signal Amplification Method). The assay involves three components: a target-specific nucleic acid probe internally labeled with a ligand (e.g., biotin), a generic signal nucleic acid also internally labeled with a ligand similar to that in the target-specific probe, and a ligand binding molecule (e.g.streptavidin).

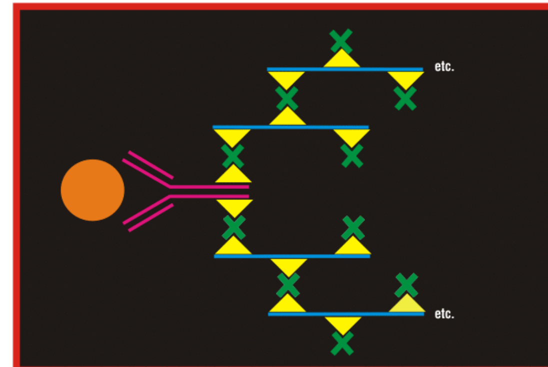


Typically the signal nucleic acid might be about 40 nucleotides long with three ligands, one incorporated at each end and one near the center of the sequence (Zecchini, 1995). The structure of the signal complex can be manipulated by the number type (valency) and positioning of the ligand molecules on the signal nucleic acid. A variety of detection moieties (e.g., a biotinylated fluorescent tag) also can be introduced into the system.

2. Detection of solid support-bound target by locked-on probe: An alternative embodiment of the HSAM principles is to use circular target probes to hybridize to target nucleic acid. This probe has a target-complementary region at each terminus, with a linker region between the complementary regions (Zhang et al 1998). The linker region will contain several biotin groups (depends on the length) that are incorporated during the probe synthesis. Upon hybridization to the target, the 5' end and the 3' end are brought in juxtaposition and a covalently linked circular probe is formed by incubation with DNA ligase. The covalently linked circular probe will be locked onto the target because of the helical nature of nucleic acid interactions (1 complete turn per 10 nucleotides of duplex structure) allowing extensive washing to remove unbound probes, thus reducing nonspecific background. The rest of the HSAM assay can then be performed by using biotinylated signal nucleic acids as described above. This design will be most useful for amplified *in situ* hybridization assays. This assay system can be used for the detection of loss of heterozygosity, dot blot assay for the detection of nucleic acids, and Southern or Northern blot.



3. Detection of proteins (antigen or antibody): Another embodiment of the HSAM principles is in the detection of either antibody or antigen. A biotinylated antibody/antigen or streptavidin-conjugated antibody/antigen (e.g., Dako) can be use to bind HSAM signal complexes to target antigen or antibody. After washing to remove unbound antibody or antigen probe, streptavidin and biotinylated signal nucleic acid and antiligand molecules are added to the reaction. A larger polymer will be generated as described above (# 2). This will significantly increase the sensitivity of an ELISA, Western blot or immunohistochemistry (IHC) assay. Other ligand/anti-ligand binding pairs, such as digoxigenin-anti-dig could also, in principle, be used to form the HSAM signal generating complexes.



Novelty of the Invention

This technique utilizes the property of multiple valences on the signal nucleic acids to generate large signal generating complexes, thus increasing assay sensitivity. The situation mimics the antigen and antibody complex formation.

Application of the Invention

HSAM is a novel nucleic acid amplification system with many unique features. It can be used for the detection of abnormal genes and infectious agents in liquid-based EIA format (antigen or antibody labeled with biotin), nitrocellulose membrane-base blot, dipstick format or DNA chip, and Latex agglutination assays where signal nucleic acids are coated on latex beads. It will be extremely useful in the *in situ* detection of nucleic acids in tissue sections and in gel matrix based single cell detection systems. In addition, combination of the method with previously described Ramification Amplification Method (RAM) will greatly enhance the sensitivity.