Flexible multiplex system for detection, amplification and size-coded identification of nucleic acid targets

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Technology description

Summary

Problem or Unmet Need:
The advent of many pathogen-associated diseases like SARS and H1N1 demonstrate the urgency of establishing rapid, sensitive, specific and inexpensive tools for laboratory diagnosis of infectious diseases. To date, there is only a limited repertoire of diagnostic assays available that allow surveillance and clinical management of these pathogen-associated diseases. In addition, current tools have limited utility in detecting infection at early stages. Sensitive multiplex detection and characterization of genetic targets where precise target sequence may not be known are necessary for developing diagnostic assays for pathogen-associated diseases. Assays based on real-time PCR have the potential to meet this need. However, the utility in detecting related but not identical genetic targets is very low.
This invention is a system to detect and identify target nucleic acids (DNA or RNA) that is based on a strategy for enrichment of a reporter template for gene amplification methods. The enrichment is achieved by hybridization in solution of two different populations of polynucleotides complementary to two different regions on the target sequence. The first is a capture nucleic acid that is bound to a solid support and the second is a reporter nucleic acid containing a polymerase binding site for amplification. Binding of both capture nucleic acid and reporter polynucleotide allows for separation of specific reporter polynucleotides from all other non-reactive reporter polynucleotides. The target nucleic acid is then amplified using PCR or isothermal amplification. The template size is distinctive for each potential target, thus the specificity of the assay is dependent upon discrete changes in the length of an amplification product.

Application area

This technology can be used in molecular diagnostics, clinical microbiology, and genetic compatibility studies.
Determining the presence or absence of at least one target nucleic acid sequence in a sample where the precise target sequence may not be know.

Detecting microorganism and host transcripts and differentiating microorganism transcripts from host transcripts. Screening of blood products for infectious agents.
Transplant diagnostics: donor/recipient incompatibility

Advantages

This technology employs an optimal master reagent mix of primers, nucleotides, enzyme and other components increasing the sensitivity of the system and its compatibility with a large variety of sequence targets: broad based capture is enabled without sacrifice in sensitivity.
The assay is easily modified for use with various nucleic acid amplification platforms.
The assay is readily adapted to field conditions.
Correlation between numbers of targets and the templates carried forward into amplification facilitates standardization for target quantitation.

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