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Methods and Compositions for Amplification and Detection of MicroRNAs

FEATURES

Gene amplification is a viable means to amplify genetic signals when the quantities of starting materials are limited. The present invention provides methods to amplify, detect, and quantify minute amounts of microRNAs (miRNAs) and noncoding RNAs (ncRNAs) in biological samples. One major obstacle when profiling miRNAs is their low expression level. miRNAs are estimated to constitute only 0.01% of total RNA. As a result, large quantities of starting materials are required for miRNA profiling. This requirement makes it difficult to study tissue- and cell-type specific expression of miRNAs. Another difficulty of profiling miRNAs is their small size, as they are predominantly smaller than 22-25 nt. The small size of miRNAs makes direct amplification difficult, if not impossible, with conventional RNA or DNA amplification strategies. Moreover, miRNAs lack an appropriate sequence to anchor a primer for the first strand DNA synthesis, which is usually the first step of the majority of RNA amplification procedures. The invention provides methods, compositions, and kits for the amplification of small polynucleotide, especially miRNA from nanogram quantities of total RNA or enriched small RNAs, as well as applications of the amplification methods. The compositions and methods herein employ signature sequence generation to the population of target miRNAs and/or small polynucleotide sequences in biological samples.

BENEFITS

The invention provides methods to generate a signature sequence for detection and quantification of target miRNAs and/or small polynucleotide sequences in an accurate, reproducible, and cost-effective approach.

A novel methodology for amplification of individual and/or multiple miRNAs developed by the investigators termed miRNA signature sequence amplification (SSAM) is employed. The SSAM technology enables downstream genetic manipulations including miRNA microarray and real-time quantitative PCR (qPCR) based methods. The generation of a signature sequence to the desired target miRNAs and/or small polynucleotide sequences is fundamentally different from other methods which attach a variety of sequences of interest, such as a bacteriophage RNA synthesis promoter, to a cDNA copy of mRNA through reverse transcription. Signature sequences using the SSAM technology are generated in the presence of target miRNA. However, the ‘first strand DNA’ is not a cDNA copy of a RNA molecule. Rather, it is a DNA copy of a DNA molecule. Instead of being a template, miRNA serves as a primer, and a sequence-specific first oligonucleotide is a template for a DNA dependent DNA synthesis. Although a signature sequence includes target miRNA sequences, a SSAM oligonucleotide primer is designed to be significantly longer than the target miRNA sequence itself, and is suitable for use in a variety of paradigms.

Signature sequences have been generated in the presence of target miRNAs. The ‘first strand DNA’ in the SSAM method is not a cDNA copy of a RNA molecule. Rather, it is a DNA copy of a DNA molecule. Instead of being a template, miRNA serves as a primer, and a sequence-specific first oligonucleotide (FON) is a template for a DNA dependent DNA synthesis. A key step of the SSAM method is to generate a signature sequence library representing the specific miRNA population and/or overlapping miRNA ensembles within a defined sample preparation. A signature sequence is generated only in the presence of a specific target miRNA, and does not bind to different miRNAs. When the intended target RNA is absent, no hybrid molecule is generated.

The SSAM procedure is a novel technology for amplification of individual miRNAs and/or multiple miRNA ensembles. A two primer (FON and SON) system enables reproducible presence detection and quantitation.

INTELLECTUAL PROPERTY STATUS

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