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Abstract:

MicroRNAs (miRNA) are short, 18-24 nucleotide long noncoding RNAs. These small RNAs, which are initially transcribed in the nucleus, are transported into the cell cytoplasm where they regulate protein translation either through direct cleavage of mRNA, or indirect inhibition through binding to mRNA and disrupting the protein translation machinery. Recently, miRNAs have gained much attention due to their implication in numerous diseases and cancers. It has been found that heightened or lowered levels of miRNA in diseased cells vs. healthy cells are linked to disease progression. It is therefore immensely important to be able to detect these small molecules. Current detection methods of Northern blotting, microarrays, and qRT-PCR suffer from drawbacks including low sensitivity, a lack of simplicity, being semi-quantitative in nature, time-consuming, and requiring expensive instruments. This work aims to develop novel miRNA technologies which will address these above problems. Bioluminescent labels are promising alternatives to current methods of miRNA detection. Bioluminescent labels are relatively small, similar in size to fluorescent proteins, and they emit very intense signals upon binding to their substrate. Bioluminescent labels are advantageous to fluorescent labels in that they do not require an external excitation source, rather, the excitation energy is supplied through a biochemical reaction. Therefore, background signal due to excitation is eliminated. They also have the advantage of being produced in large amounts through bacterial expression. Four miRNA detection methods are presented which utilize luminescence-based methods. Three employ Renilla luciferase, a bioluminescent protein, and one is based on fluorescence. The presented methods are capable of detecting miRNA from the picomole (nanomolar) level down to the femtomole (picomolar) level. These methods are rapid, sensitive, simple, and quantitative, can be employed in complex matrices, and do not require expensive instruments. All methods are hybridization-based and do not require amplification steps.

Description:

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