

Ultrasensitive detection of circulating miRNA by branched-rolling circle amplification coupled with graphene fluorescence-based sensor

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Abstract

In this study, we developed an isothermal microRNAs (miRNAs) detection platform based on high efficient branched-rolling circle amplification (BRCA) system coupled with graphene oxide (GO)-based fluorescence assay. In the strategy, cancer-associated miR-21 was selected as a model target. Target miRNA complementary binds a linear single-stranded DNA (ssDNA) probe and turns to circularized conformation under reaction containing T4 RNAligase2. The BRCA products were amplified after adding secondary primers in the present of Phi29 DNA polymerase, then quantified by measuring fluorescent level after adding GO-sensing complexes. The limit of detection of our developed platform was 0.87 fM, which is lower than that of the original BRCA method. Our platform also can discriminate a single mismatch miRNA mutant and can be applied to determine amounts of target miRNA in total RNA extracts from cancer cell lines. These indicate that our platform has a potential for miRNA detection in clinical diagnosis.

Keywords: MicroRNA; graphene oxide; branched-rolling circle amplification; isothermal amplification

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