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STRAND DISPLACEMENT AMPLIFICATION ON QUANTUM DOT ENCODED SILICA BEADS FOR MULTIPLEXED ASSAY OF MIRNAS

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MicroRNAs (miRNAs) are a group of small size (19–25 nucleotides in length), non-coding RNAs that play regulating roles in many critical biological processes. Recently, accumulating evidence indicates that the aberrant expression of miRNAs is associated with cancer initiation, tumor stage. Thus, miRNAs have been regarded as bio-markers in cancer diagnose. However, the unique characteristics of miRNA, such as their small size, sequence homology among family members, and low abundance, make miRNA more challenging to be analyzed. This article report a protocol of direct implementation of nicking enzyme assisted strand displacement amplification (SDA) on quantum dot-encoded core-shell silica beads (Qbead) and its application for multiplexed assay of human liver carcinoma miRNAs. Qbeads with remarkably improved chemical and photo-stability allows efficient bio-conjugation, hybridization and SDA on the surface. Multifunctional hairpin probes were used, which contain capture sequence specific target binding, nicking enzyme recognition site for the exponential amplification, the G-rich segment as the signal reporter with [Ru(bpy)2(DI)]2+ (Ru-DI), and 3'-end sequence triggering the strand-displacement amplification reaction. By the presence of target and enzyme, using molecular switch Ru-DI, the bead-SDA assay significantly improves the detection sensitivity. A dynamic range of 5 logs and a detection limit of 0.5 fM are achieved, owing to SDA-induced fluorescence signal amplification. More importantly, the SDA on Qbead results in color difference amplification allows color analysis be a conceivable quantification method for the Qbeads-based assay. With benefits of SDA-induced color difference amplification, quantify multiplex miRNAs in cancer cell lysates realized. The Qbead-SDA assay with color analysis could potentially enable the development of point-of-care diagnostics platforms.

Biography

Xiaojun Qu completed his Bachelor degree and Master degree from College of Veterinary Medicine-Northwest A&F University, China in 2012. In 2014, he started his Doctoral program at State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering-Southeast University, supervised by Professor Qingjiang Sun. As planned, he will complete his Doctoral degree in 2019. His dissertation mainly focuses on the isothermal amplification reaction on different kinds of quantum dot-encoded bead, which are applicable for ultrasensitive detection of biological molecules. Until now, he has joined and finished two projects and has co-authored three papers on the esteemed international journals.

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