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Enhanced Specificity of Multiplex Polymerase Chain Reaction via CdTe Quantum Dots

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Abstract.

Nanoparticles were recently reported to be able to improve both efficiency and specificity in polymerase chain reaction (PCR). Here, CdTe QDs were introduced into multi-PCR systems. It was found that an appropriate concentration of CdTe QDs could enhance the performance of multi-PCR by reducing the formation of nonspecific products in the complex system, but an excessive amount of CdTe QDs could suppress the PCR. The effects of QDs on PCR can be reversed by increasing the polymerase concentration or by adding bovine serum albumin (BSA). The mechanisms underlying these effects were also discussed. The results indicated that CdTe QDs could be used to optimize the amplification products of the PCR, especially in the multi-PCR system with different primers annealing temperatures, which is of great significance for molecular diagnosis.

Introduction

The PCR has become one of the most popular tool for molecular biology and pathogen detection as a high sensitive technique [1-3], but it still requires careful optimization on reaction conditions in order to eliminate nonspecific products in most PCR experiments, especially in multiplex PCR (multi-PCR). Optimized parameters could include the type and concentration of the enzyme, reaction buffer content, time and temperature of the annealing/extension process, primer design and use of a rapid heating-cooling response PCR machine [4,5]. It was reported that a variety of organic chemicals, including single-stranded DNA-binding proteins (SSBs) [6], amides and betaine [7], imidazole, tetramethylammonium chloride (TMAC) and TMAC derivatives [6-9], could act as accelerants in PCR mixture to increase yield and specificity.

As a novel material, nanoparticles (NPs) have many important applications in biology, for example in drug delivery, biomolecules separation, cancer thermal therapy, ultrasensitive biosensor and live cell imaging [10-15]. Recently, gold nanoparticles have been reported to be able to reduce the level of nonspecific products even at lower annealing temperature [9,16,17]. Li et al. [16] found that Au NPs can improve the efficiency of PCR and attributed the enhancement to the excellent heat conductivity of Au NPs. But some researchers reported some discriminating conclusions [17-19]. Yang et al. [18] evaluated the function of Au NPs in PCR systems and suggested that a complex interaction between Au NPs and native Taq DNA polymerase was accounted for the effects of Au NPs on PCR. Vuet et al. [19] demonstrated that AuNPs tended to favor smaller fragments of PCR products than larger ones owing to the different polymerase adsorptions. Possible reasons concerning these previous discrepant results might be referred to different nanoparticles used in terms of surface modification and some other different experimental conditions.

Quantum dots, as a new kind of fluorescent material, have many excellent features, such as size-tunable emission, broad excitation profiles and narrow/symmetric emission spectra, high photostability. The new generations of QDs have far-reaching potential for the study of intracellular processes at the single-molecule level, high-resolution cellular imaging, long-term in vivo observation of cell trafficking, tumor targeting and diagnostics [20]. CdTe QDs have the advantage of fluorescence at different wavelengths and could be used as potential fluorescent materials in real-time PCR. Here, CdTe quantum dots (QDs) were employed as a novel assisting agent to systematically investigate the effects of nanoparticles on PCR. First, two-round PCR was used to amplify a 310-bp fragment from Triticum aestivum

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Jicheng Xie

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Jun-Jie Zhu, Jing-Jing Li, Hai-Ping Huang, Fang-Fang Cheng, 2013-12-04 This book provides a broad introduction to all major aspects of quantum dot properties including fluorescence electrochemical photochemical and electroluminescence Such properties have been produced for applications in biosensing cell tracking in vivo animal imaging and so on It focuses on their special applications in DNA biosensing and provides readers with detailed information on the preparation and functionalization of quantum dots and the fabrication of DNA biosensors using examples to show how these properties can be used in DNA biosensor design and the advantages of quantum dots in DNA biosensing Further new emerging quantum dots such as metal nanoclusters and graphene dots and their applications in DNA biosensing have also been included **Dual Phase Multiplex Polymerase Chain Reaction**, 2008 Highly specific and sensitive methods were developed for multiplex amplification of nucleic acids on supports such as microarrays Based on a specific primer design methods include five types of amplification that proceed in a reaction chamber simultaneously These relate to four types of multiplex amplification of a target DNA on a solid support directed by forward and reverse complex primers immobilized to the support and a fifth type pseudo monoplex polymerase chain reaction PCR of multiple targets in solution directed by a single pair of unbound universal primers The addition of the universal primers in the reaction mixture increases the yield over the traditional bridge amplification on a solid support by approximately ten times Methods that provide multitarget amplification and detection of as little as 0 45 4 5 times 10 sup 12 g equivalent to 10 sup 2 10 sup 3 genomes of a bacterial genomic DNA are disclosed

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