World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:11, No:08, 2017

Ultrasensitive Detection and Discrimination of Cancer-Related Single Nucleotide Polymorphisms Using Poly-Enzyme Polymer Bead Amplification

Authors : Lorico D. S. Lapitan Jr., Yihan Xu, Yuan Guo, Dejian Zhou

Abstract : The ability of ultrasensitive detection of specific genes and discrimination of single nucleotide polymorphisms is important for clinical diagnosis and biomedical research. Herein, we report the development of a new ultrasensitive approach for label-free DNA detection using magnetic nanoparticle (MNP) assisted rapid target capture/separation in combination with signal amplification using poly-enzyme tagged polymer nanobead. The sensor uses an MNP linked capture DNA and a biotin modified signal DNA to sandwich bind the target followed by ligation to provide high single-nucleotide polymorphism discrimination. Only the presence of a perfect match target DNA yields a covalent linkage between the capture and signal DNAs for subsequent conjugation of a neutravidin-modified horseradish peroxidase (HRP) enzyme through the strong biotin-nuetravidin interaction. This converts each captured DNA target into an HRP which can convert millions of copies of a non-fluorescent substrate (amplex red) to a highly fluorescent product (resorufin), for great signal amplification. The use of polymer nanobead each tagged with thousands of copies of HRPs as the signal amplifier greatly improves the signal amplification power, leading to greatly improved sensitivity. We show our biosensing approach can specifically detect an unlabeled DNA target down to 10 aM with a wide dynamic range of 5 orders of magnitude (from 0.001 fM to 100.0 fM). Furthermore, our approach has a high discrimination between a perfectly matched gene and its cancer-related single-base mismatch targets (SNPs): It can positively detect the perfect match DNA target even in the presence of 100 fold excess of co-existing SNPs. This sensing approach also works robustly in clinical relevant media (e.g. 10% human serum) and gives almost the same SNP discrimination ratio as that in clean buffers. Therefore, this ultrasensitive SNP biosensor appears to be well-suited for potential diagnostic applications of genetic diseases.

Keywords : DNA detection, polymer beads, signal amplification, single nucleotide polymorphisms
Conference Title : ICNB 2017 : International Conference on Nanotechnology and Biosensors
Conference Location : Venice, Italy
Conference Dates : August 14-15, 2017