



# Ultrasensitive FRET-based DNA sensor using PNA/DNA hybridization

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## ABSTRACT

In the diagnosis of genetic diseases, rapid and highly sensitive DNA detection is crucial. Therefore, many strategies for detecting target DNA have been developed, including electrical, optical, and mechanical methods. Herein, a highly sensitive FRET based sensor was developed by using PNA (Peptide Nucleic Acid) probe and QD, in which red color QDs are hybridized with capture probes, reporter probes and target DNAs by EDC-NHS coupling. The hybridized probe with target DNA gives off fluorescent signal due to the energy transfer from QD to Cy5 dye in the reporter probe. Compared to the conventional DNA sensor using DNA probes, the DNA sensor using PNA probes shows higher FRET factor and efficiency due to the higher reactivity between PNA and target DNA. In addition, to elicit the effect of the distance between the donor and the acceptor, we have investigated two types of the reporter probes having Cy5 dyes attached at the different positions of the reporter probes. Results show that the shorter the distance between QDs and Cy5s, the stronger the signal intensity. Furthermore, based on the fluorescence microscopy images using microcapillary chips, the FRET signal is enhanced to be up to 276% times stronger than the signal obtained using the cuvette by the fluorescence spectrometer. These results suggest that the PNA probe system conjugated with QDs can be used as ultrasensitive DNA nanosensors.

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## 1. Introduction

As the average human life expectancy increases, DNA analysis technology for the early diagnosis and monitoring of diseases is becoming increasingly important. However, most DNA analysis systems include a target amplification step, a washing step to separate the unhybridized DNA, and the immobilization of target DNAs on glass or a chip using the binding kinetics [1–6]. In order to reduce such troublesome and time consuming steps, we chose the single molecule Förster resonance energy transfer (FRET) detection technique using quantum dots (QDs). First, because the brightness of QDs is 10–100 times higher than that of organic dyes [7], QDs can be utilized as donors without the target amplification step. Typically, it is difficult to transfer the energy from the donors to acceptors, in which the distance is further than 10 nm in the single-molecule FRET system. Thus, the washing step is not essential in the single-molecule FRET system for removing the unhybridized DNA. However, the recent studies [8–9] reported that the 10-nm is not the limitation for energy transfer. Many groups reported QD-FRET systems in antibody-antigen reactions, ligand-receptor interactions, and DNA-DNA hybridizations [10–16] with molecular probes such as molecular beacon [3] and TaqMan [2]. Typically, they have sub-nM detection limit with DNA-DNA hybridization. In spite of providing a cascaded FRET system in conjugated polymer/quantum

dot/dye-labeled DNA complexes for DNA hybridization detection, the detection limit was 5nM [17]. Especially, Zhang et al. reported DNA nanosensors using QDs and ultrasensitive optical systems in capillary chips to enhance the detection limit [18]. Also, they reported the fluorescent bursts for the comparison between nanosensors with sandwiched hybrids and molecular beacons. The nanosensor shows much better sensitivity than the molecular beacons, which is 100 fold better than the molecular beacon at 0.96 nM target concentration. In addition, they controlled the velocity of the microflows in the microfluidic channel to break the FRET limit and enhance the FRET signal [19].

In this study, in order to enhance the detection sensitivity, we have investigated a DNA sensor using PNA (Peptide Nucleic Acid) probes. PNA is an artificially created nucleic acid with a neutral polyamide backbone of n-(2-amino ethyl)glycine, whereas the backbone of DNA is composed of phosphodiester linkers with negative charges. Therefore, PNA is highly reactive because of the absence of electrostatic repulsion with the target DNA [20]. Furthermore, PNA binds strongly and selectively with a specific DNA; even though a single mismatch prevents the hybridization reaction in a wide range of temperature and salt concentration [21,22,23]. In the system, 620 nm QDs are conjugated with either sandwiched hybrid DNA/DNA or PNA/DNA composed of reporter probes/capture probes/target DNAs. The conjugation is carried out by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)-N-hydroxysulfosuccinimide (NHS) coupling protocol. Additionally, we have examined two types of reporter probes to elicit the effect of the distance between the donor and the acceptor. The first is a fluorophore

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