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# A facile and pragmatic electrochemical biosensing strategy for ultrasensitive detection of DNA in real sample based on defective T junction induced transcription amplification

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ABSTRACT

A novel and pragmatic electrochemical sensing strategy was developed for ultrasensitive and specific detection of nucleic acids by combining with defective T junction induced transcription amplification (DTITA). The homogeneous recognition and specific binding of target DNA with a pair of designed probes formed a defective T junction, further triggered primer extension reaction and in vitro transcription amplification to produce numerous single-stranded RNA. These RNA products of DTITA could hybridized with the biotinylated detection probes and immobilized capture probes for enzyme-amplified electrochemical detection on the surface of the biosensor. The proposed isothermal DTITA strategy displayed remarkable signal amplification performance and reproducibility. The electrochemical DNA biosensor showed very high sensitivity for target DNA with a low detection limit of 0.4 fM (240 molecules of the synthetic DNA), and can directly detect target pathogenic gene of *Group B Streptococci* (GBS) from as low as 400 copies of genomic DNA. Moreover, the established biosensor was successfully verified for directly identifying GBS in clinical samples. This proposed strategy presented a simple and pragmatic platform toward ultrasensitive and handy nucleic acids detection, and would become a potential tool for general application in point-of-care setting.



## 1. Introduction

Highly sensitive detection of sequence-specific DNA plays essential roles in early clinical diagnosis (Debouck and Goodfellow, 1999), environmental monitoring (Palchetti and Mascini, 2008), forensic analysis (Divne and Allen, 2005) as well as counter-terrorist (Varma-Basil et al., 2004). Especially, point-of-care testing (POCT) for nucleic acid detection is attracting considerable interest owing to its general application in the setting where suitable facilities are unavailable and a rapid answer is required (Niemz et al., 2011). Over the past decades, PCR-based methods have facilitated rapid and accurate identification of DNA in central laboratories (Park et al., 2013; de-Paz et al., 2014). However, requirements of rigid control of temperature cycling, sophisticated and expensive equipments, and highly trained analysts limit its application in point-of-care (de-Paz et al., 2014; Tanaka et al., 2015). Thus, it is still urgent to develop highly sensitive, low-complexity, handy methods for DNA detection in point-of-care setting.

Electrochemical biosensor has attracted substantial attention as a potential POCT platform owing to its high sensitivity, easy to use, rapid response, low cost and inexpensive instrumentation (Wang, 2002; Dungchai et al., 2009; Lu et al., 2012). To explore the development of highly sensitive electrochemical DNA sensor, several isothermal amplification techniques have been used. These isothermal DNA amplification strategies, such as the strand displacement reaction (SDR) (Gao et al., 2014; Cheng et al., 2014), the hybridization chain reaction (HCR) (Gao et al., 2013; Li et al., 2014), rolling circle amplification (RCA) (Jiang et al., 2014; Deng et al., 2014), do not require special laboratory conditions for thermal cycling and is highly compatible to biosensor systems. But most of them trended to perform DNA target recycle or amplification directly on the electrode surface (Chen et al., 2011). The inherent features of heterogeneous formats, including the steric hindrance, restricted configurational freedom and the variant chemical microenvironment, lead to relatively low hybridization efficiency and enzyme kinetics (Vijayendran and Leckband, 2001). These disadvantages counteracted the sensitivity and reproducibility of the heterogeneous DNA amplification-based biosensor, especially in the setting of detection a low abundance target gene from matrix genomic DNA in real sample (Miranda-Castro et al., 2012).

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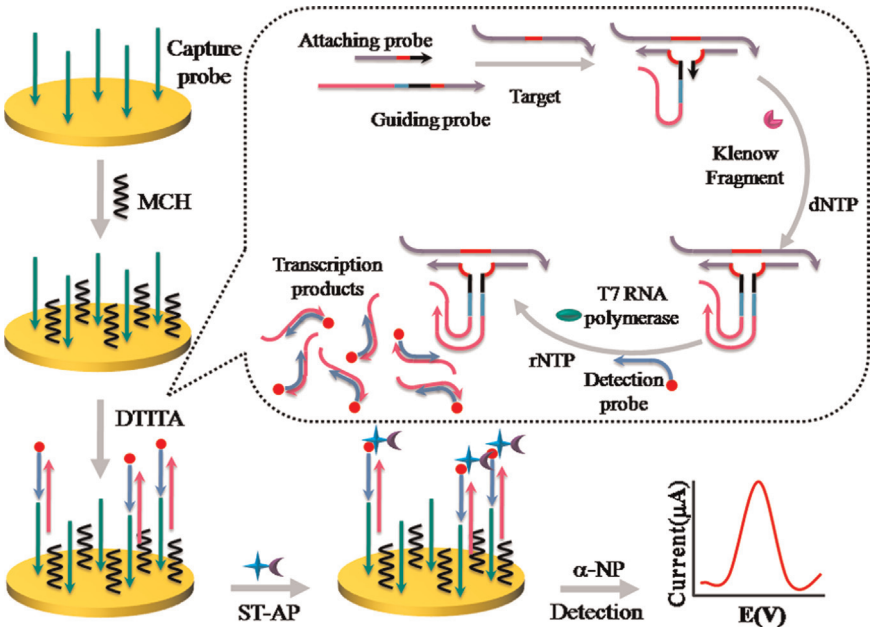


**Fig. 1.** Schematic representation of the DITIA strategy for electrochemical DNA biosensing.

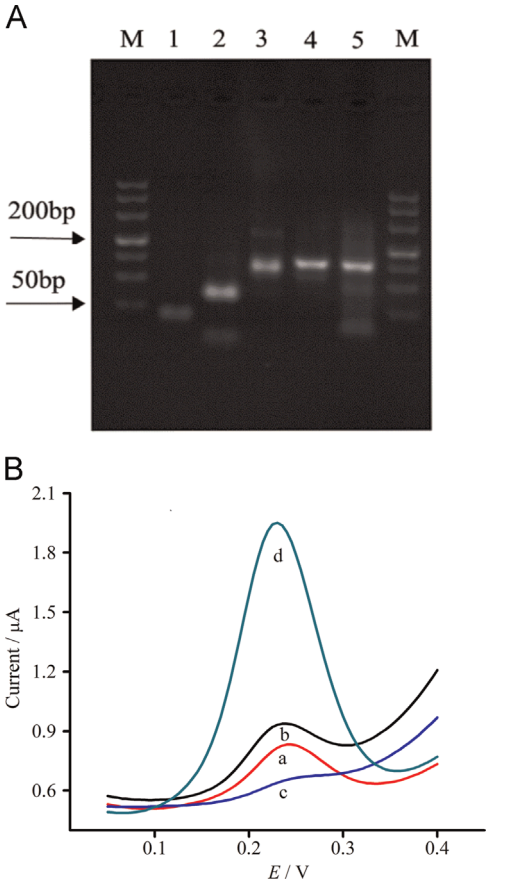


**Fig. 2.** (A) gel electrophoresis analysis of 2 μM target DNA (lane 1), 2 μM attaching probe and 2 μM guiding probe (lane 2), hybridization of 0.75 μM target DNA with 0.75 μM attaching probe and 0.75 μM guiding probe (lane 3), products of target DNA and two probes after primer extension reaction (lane 4) and transcription amplification (lane 5). (B) Typical DPV curves of designed biosensor responding to blank control (a), 1 nM target DNA without primer extension reaction (b), without transcription amplification (c), and with integrity DTITA (d), respectively.

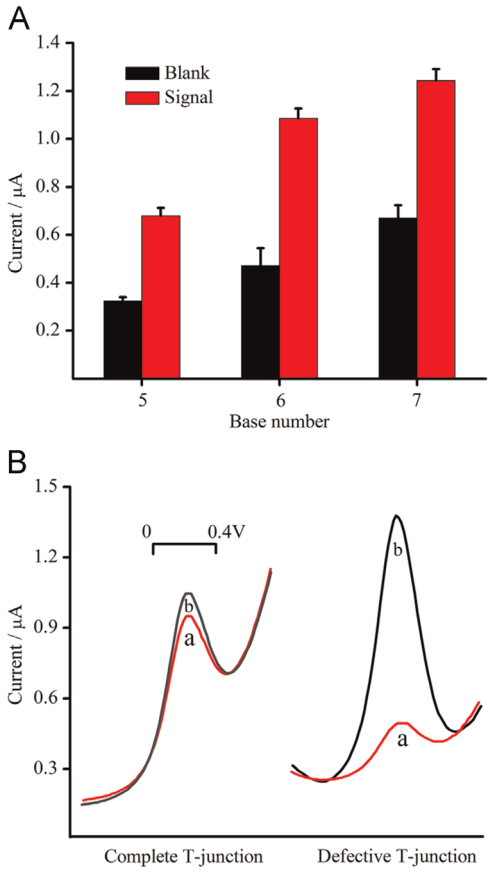


**Fig. 3.** (A) DPV signals of the designed biosensor by using complete T-junction in responding to blank and 1 nM target DNA with different base numbers for inter-hybridization of two probes, respectively. Error bars are standard derivation obtained from three independent experiments. (B) Typical DPV curves of the designed biosensor by using complete T-junction and defective T-junction in responding to blank (a) and 1 pM target DNA (b).

T7 RNA polymerase, a class of DNA-dependent RNA polymerase, only recognizes the specific promoter sequence and transcribes its downstream DNA sequence at approximate rate of