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Energy driven cascade recognition for selective detection of nucleic acids with high discrimination factor at room temperature

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ABSTRACT

In this article, we demonstrated a cascade recognition strategy for the detection of single strand nucleic acid with high discrimination factor at room temperature. The cascade recognition strategy contains a toehold mediated strand displacement and a double-toehold mediated double strand displacement reaction, thus enable the high ability to discern point mutation of target. The discrimination factor of the model target is between 45 and 109, with the medium of 70. This strategy is homogeneous, easy operation, enzyme-free, isothermal, and can be easily adapted to high-throughput devices without the need of designing complicated instruments.

1. Introduction

Nucleic acids including DNA and RNA are essential for all known life forms, because these acids encode and regulate the expression of hereditary information within living organisms. The biologically meaningful detection of nucleic acids has yielded the development of polymerase chain reaction (PCR) (Mestdagh et al., 2009; Schena et al., 1995), microarray (Gunderson et al., 2005; Schena et al., 1995; Sendroiu et al., 2011) and fluorescent in situ hybridization (Bartlett, 2004; Ge et al., 2014).

The detection of nucleic acids in real samples generally involves two steps: amplification of nucleic acids (usually based PCR, such as asymmetric PCR, biotin–streptavidin separation, lambda exonuclease digestion and size separation on denaturing urea polyacrylamide gel electrophoresis) and detecting of the amplification product based DNA hybridization (Meric et al., 2002; Tosar et al., 2010). DNA hybridization experiments require optimization because this process does not strictly follow the Watson–Crick pairing rules (Zuker, 2003). An oligonucleotide presents potential to pair with numerous sites on the probe, including one or several mismatches. This phenomenon can leads to false-positive results. On the other hand, the desired target sites of single-stranded DNA or RNA are often folded into stable secondary structures that must be unfolded to allow for probe binding. A highly stable fold of the target will provide limited probe for binding, leading to a false-negative test result. Moreover, other artifacts encountered with DNA, such as probe folding and probe dimerization, can lead to false results. To solve this controversy, researchers have developed various methods, including use of molecular beacons (Tyagi and Kramer, 1996; Zheng et al., 2015), strand displacement reaction (Li et al., 2015; Zhang and Seelig, 2011; Zhang et al., 2013), and elevation of the reaction temperature near the melting temperature (T_m) (Jin et al., 2015; Yang et al., 2014). Molecular beacons, which were proposed by Tyagi et al., are single-stranded, hairpin-shaped oligonucleotide probes comprising a stem and a loop structure. The loop region contains a complementary sequence to a target sequence. The stem is formed by annealing complement arm sequences that act as a regulated region. The thermodynamic properties of molecular beacons are related to the relatively free energy changes between target/molecular beacons (or point mutation target/molecular beacons) and molecular beacons, thus enabling the differentiation between the target and point mutation target. However, Molecular beacons are difficult to use in designing and detecting long oligonucleotides because of abnormal kinetics and limit of length. Elevation of the reaction temperature near T_m is another efficient method used to enhance the specificity of nucleic acid hybridization. For the simple linear capture probe and target hybridized at T_m, half of the double-

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stranded capture probe/target has dissociated and the hybridization reaction reaches the equilibrium. At T_m, the minimum change in target sequence could yield an evident change in free energy change and hybridization. Thus, marginally different sequences maybe distinguished. For strand displacement reaction, displacement activity occurs readily in the target, whereas a weaker affinity in SNP results in high specificity.

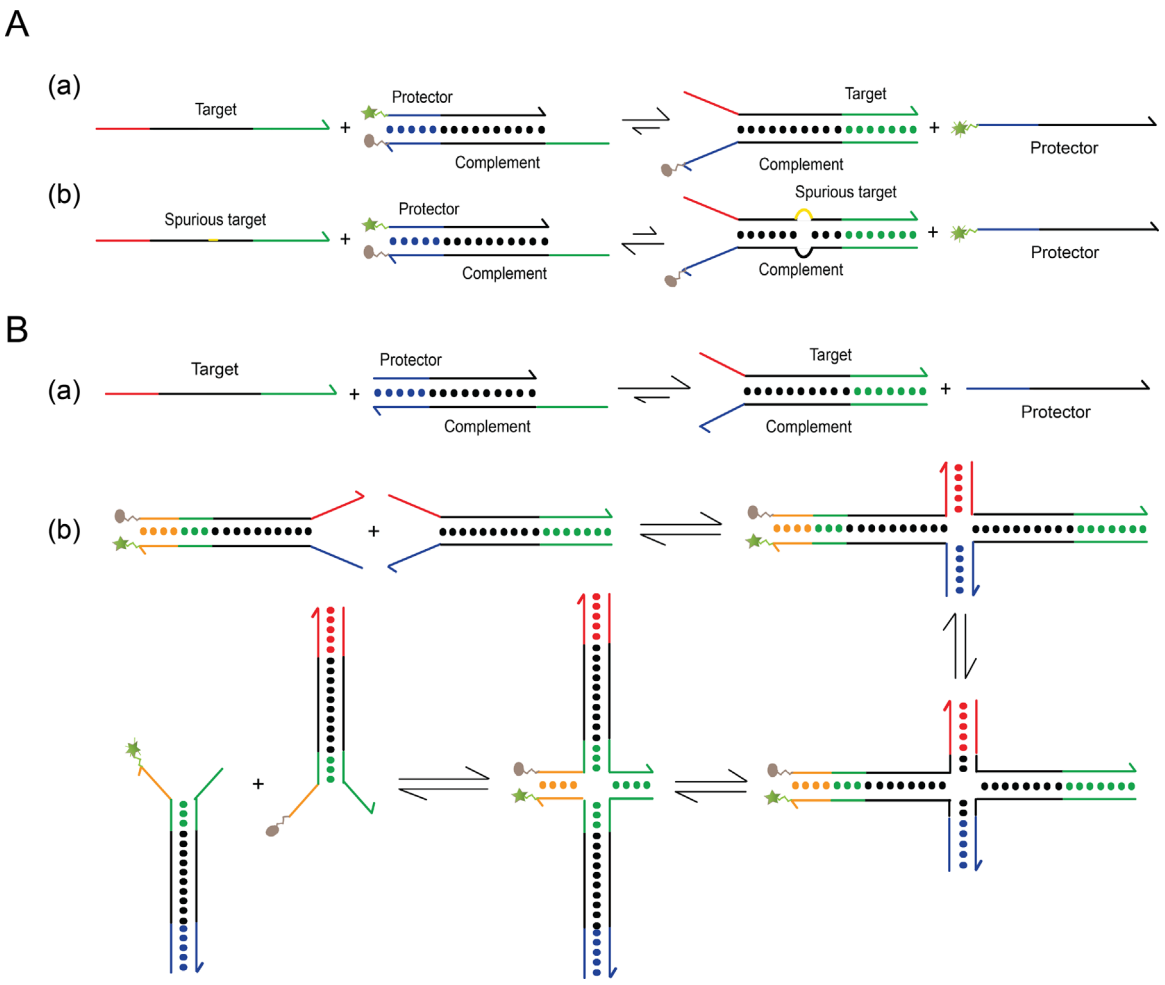
In this article, we report a new class of probes based on a mode of reaction that we call energy driven cascade toehold-mediated strand displacement. This cascade recognition was achieved as follows: in the first reaction, we used a double-stranded probe which consists of two complementary oligonucleotides of different lengths, denoted by protector and complement strands. The complement strand is partially complementary to the target and forms overhangs at one end. An ideal protector fulfills the following requirements: it must not be so competitive to hinder the formation of perfectly matched probe-target hybrids but it must be suffciently competitive to block non-specific hybridization. An ideal protector would be a single-stranded oligonucleotide that possesses low vigour compared to target. Thus, the protector forms a stable duplex in the absence of the target and can be displaced in the presence of the target. The newly formed target/complement duplex possess 5 nucleotide (nt) single-stranded overhangs at the 5′ and 3′ end of each strand and acts as initial toeholds. The initial toeholds should only be sufficiently long because an excessively long toehold may induce stable hybridized overhangs, whereas excessively short toehold may not form hybridized overhangs. As shown in Scheme 1B, the formed target/complement double-stranded nucleic acids undergo a double-stranded toehold exchange reaction with the quencher/fluorophore-labelled double-strand probe; step-to-step branch migration then leads to the separation of the quencher and fluorophore.

2. Experimental section

2.1. Materials

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA) and used as received unless otherwise mentioned. All oligonucleotides were synthesized and further purified using HPLC by Sangon (Shanghai, China). 6-carboxylfluorescein (FAM) and 4-((4-(dimethylamino) phenyl)azo) benzoic acid (Dabcyl) were used as fluorescence and quencher according to fluorescence resonance energy transfer (FRET). The sequences are as follows:

Protector: 5′- GTAGTTTG TAGTCAA -3′
FAM labeled protector (5nt): 5′ FAM -TAGTAGTTTGTAGTCAA-3′
FAM labeled protector (6nt): 5′ FAM -AGTAGTTTGTAGTCAA-3′
FAM labeled protector (7nt): 5′ FAM -GTAGTTTGTAGTCAA-3′
FAM labeled protector (8nt): 5′ FAM -TAGTTTGTAGTCAA-3′
Complement: 5′-TTGACTACAA ACTACTACCTCA -3′
Dabcyl labeled Complement: 5′-TTGACTACAAACTACTACCTCA -3′Dabcyl
Target: 5′-TGA GGT AGT AGT TTG TAC AGT T-3′
11Mismatch: 5′-TGA GGT AGT ACT TTG TAC AGT T -3′
8Mismatch: 5′-TGA GGT ACT AGT TTG TAC AGT T-3′



Scheme 1. Scheme illustration of the proposed (A) strand displacement reaction and (B) cascade recognition strategy.