Gene Direct Identification by Site-specific Enzyme Cutting Double-strand DNA on a Resonant-Cantilever

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Abstract:

We develop a novel gene identification method for direct detection of double-strand DNA. After bound on a gravimetric resonant cantilever, the double-strand DNA chain is with its certain-length segment cut out by its site-specific restriction enzyme digestion (see Fig.1a). The cut-down ratio in the strand-length is proportional to the ratio of mass change on the cantilever and, thus, can be online measured by the cantilever frequency-shift signal.

The double-strand DNA detection method is expected in applications of fast and accurate gene identification.

Traditional DNA identification used hybridization mechanism, thus, it only can detect single-strand DNA. Such gene identification suffers nonspecific adsorption induced false-positives and the time-consuming and laborious preparation of single-strand sample (including the asymmetric PCR) and hybridization process. Such sensors often used a complicated 'sandwiched structure' (see Fig.1b) where the targeted DNA should be linked to a probe-DNA and a capture-DNA from double-sides [1]. Direct detection to double-strand DNA samples will largely simplify the process and facilitate on-site identification. Since a double-strand DNA fragment at least features one unique cutting-site for a certain restriction enzyme [2], i.e. enzyme cutting position is accurate, the cut-out proportion of the double-strand length is the same as the ratio of its mass change that can be directly measured by a gravimetric sensor. Herein we use resonant micro-cantilever sensor to directly detect double-strand DNA samples by online recording the frequency-shift signal.

A 3776-bp directed-PCR product (from E. coli O157:H7) is amplified with forward primer that was pre-modified with biotin. The cutting-site of EcoRV enzyme is known as 1141-bp away from the biotinylated terminal of the double-strand. Fig.2(c) shows the electrophoresis analysis results that confirm the biotinylated-DNA and the specific binding. With a small part of inevitably uncompleted enzyme-digestion taken into account, the measured ratio of 64.2% is really consistent to the theoretical 69.8%.

When the DNA without biotin-modification is introduced into the chamber, no detectable sensing-signal is observed that indicates negligible nonspecific adsorption of DNA. Besides, the directed-PCR product of negative control (E. coli DH5α) does not induce detectable sensing-signal that validates our detection method. Biotinylated-DNA without EcoRV site produces similar frequency-decrease of 14.2kHz, however, it cannot generate frequency-increase during incubation.

![Fig.1: (a) Schematic of DNA detection. Biotinylated double-strand DNA is specific-bound on cantilever via streptavidin. The DNA is site-specific digested and recognized by EcoRV enzyme. The cut-out mass is measured by cantilever frequency-signal. (b) Traditional sandwich-hybridization detection to single-strand DNA is herein shown for comparison with out new technique.](image1)

![Fig.2: (a) Detection results for double-strand DNA (stx2 gene of E. coli O157:H7). (b) SEM of the cantilever sensor. (c) and (d) Electrophoresis analyses which confirm the biotinylated-DNA and the specific binding.](image2)

References: