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

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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. With the in-plane mode cantilever for high-Q resonating in liquid, the stx2 gene of *E.coli* O157:H7 is directly identified by accurate digestion of the *EcoRV* enzyme. 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-stand 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 samples and some controls. Streptavidin-modified mesoporous-silica is used as the gravimetric sensing material to enhance the mass-adsorption and sensitivity. Specific binding between the biotinylated-DNA and the sensing-material is confirmed by electrophoresis analysis [see Fig.2(d)]. The streptavidin-functionalized mesoporous silica is immobilized on our lab-made cantilever. Shown in Fig.2(b) the cantilever vibrates in an in-plane swing resonance-mode for high-Q operation in liquid [3]. A microfludic chamber is fabricated, with the cantilever embedded inside to identify the DNA.

The real-time double-strand gene detection is with the experimental results shown in Fig.2(a). After the biotinylated-DNA products injected into the microfluidic-chamber, resonance-frequency of the cantilever continually decreases along with the process of the DNA binding onto the cantilever. The frequency reaches stable within 10min while the binding is completed. The frequency-shift of $\Delta f_1=15.9kHz$ is obtained. Then, with the enzyme site-specific digestion, the frequency reversely increases by $\Delta f_2=10.2kHz$ within 6min. The ratio of $\Delta f_2/\Delta f_1=64.2\%$ should equals to the ratio between the changes in strand-length (i.e. the mass changes) during DNA-binding and enzyme-cutting, respectively, i.e. $\Delta l_{cut}/l_{DNA}=64.2\%$. According to the known cutting-site, theoretically the chain-length ratio between the removed DNA segment and the whole DNA should be $\Delta l_{cut}/l_{DNA}=2635/3776=69.8\%$. 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.

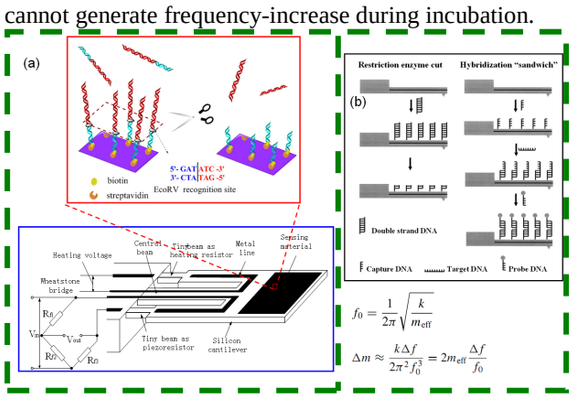

Fig.1: (a) Schematic of DNA detection. Biotinylated double-strand DNA is specific-bound on cantilever via streptavidin. The DNA is site-specific digested and recognized by EcoRV-enzyme. The cut-out mass is measured by cantilever frequency-signal. (b) Traditional sandwich-hybridization detection to single-strand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

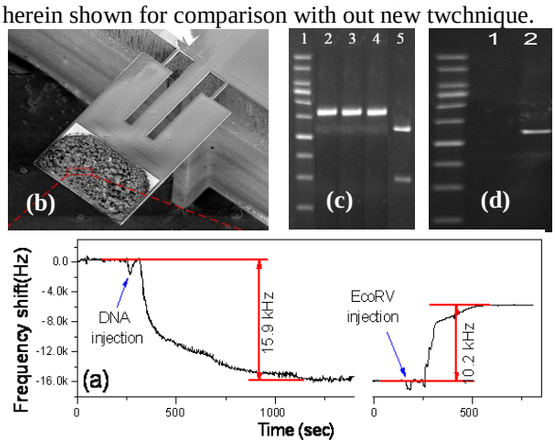

Fig.2: (a) Detection results for double-strand DNA (stx2 gene of *E.coli* O157:H7). (b) SEM of the cantilever sensor. (c) and (d) Electrophoresis analyses which confirm the biotinylated-DNA and the specific binding.

References:

[1] B. Cha, et al, *Biosens. Bioelectronics*, 25(2009): 130.
[2] R.J. Roberts, *CRC Crit. Rev. Biochem.* 4(1976): 123.
[3] Y. Tao, et al, *Sens. Actuators B*, 157(2011) : 606.