Electronic Supplementary Information

A Smart DNA-Gold Nanoparticle Probe for Detecting Single-base Changes on the Platform of Quartz Crystal Microbalance

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Methods

Materials Tris (2-carboxyethyl) phosphine hydrochlorides (TCEP) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The DNA oligonucleotides used in this study were synthesized in Sangon Biotechnology, Inc. (Shanghai, China). The oligonucleotides were purified through a high-performance liquid chromatography. Chloroauric acid (HAuCl4) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). (3-Aminopropyl)triethoxysilane (APTMS) was from Acros Organics (New Jersey). Individual DNA oligonucleotides were resuspended and stored in 0.1 M PBS buffer (10 mM PB pH balanced to 7.4, with 0.1 M NaCl) in the dark at -20 °C. The oligonucleotide concentrations were calculated based on the molar extinction coefficient of single-stranded DNA. The measured absorbance at 260 nm and the UV–Vis spectra were collected on a Shimadzu UV-2550 UV-Vis spectrophotometer. Nanopure water (18.2 MΩ) (Millipore Co., USA) was used throughout the study. All the other chemicals used were of analytical

grade. Tables S1 and S2 present the oligonucleotide sequences.

DNA-modified AuNP Accordingly, 13 nm AuNPs were prepared as previously described[1]. The resulting gold nanoparticles had a surface plasmon resonance maximum of $\lambda_{max} = 520\,nm$. The molar concentration of the gold nanoparticles was measured by using a UV–vis spectrophotometer. The molar extinction coefficient was $2.7\times10^{8}\,M^{-1}cm^{-1}$ at λ_{520nm} for a 13 nm gold nanoparticle. The gold nanoparticles were functionalized by using thiol-modified DNA, as previously described[1]. The final DNA-functionalized AuNP deposition was resuspended in 0.1 M PBS buffer and stored in 4 °C for further use.

Double-strand DNA complex preparation The linker-oligomer and the protector-oligomer were mixed at a 1:1.1 molar ratio, kept at 95 °C for 10 min, and slowly cooled to room temperature for more than 2 h.

Detection limit (DL) Two kinds of DL were listed in this study, as follows: $(DL)_{m13T}$ and $(DL)_{control}$. $(DL)_{m13T}$ denoted the lowest concentration of the perfect target that can be differentiated from the mutational sequence, and it was estimated using the following methodology: a series of concentrations for the correct and spurious target was measured. ΔF between the spurious and the correct target was derived with the same concentration. The linear relationship between [ln(ΔF)] and the target concentration was subsequently determined. The target concentration that results in [ln (ΔF)] ≈ 0 was calculated using the extrapolation method. This concentration was regarded as $(DL)_{m13T}$. $(DL)_{control}$ denoted the lowest perfect target concentration that can be effectively distinguished from the background baseline. A series of the QCM frequency shift at different target concentrations was measured. The linear relationship between the [ln($-F$)] and the target concentration was obtained. $(DL)_{control}$ was calculated using the triple signal-to-noise ratio method.

Native polyacrylamide gel electrophoresis (PAGE) analysis The proposed toehold exchange principle was also demonstrated by native PAGE. The experiments used 10% gel solution, which was prepared from 40% 19:1 acrylamide: bisacrylamide stock in 1 × tris-acetate-EDTA buffer (TAE) solution, and cast in 1.5-mm-thick glass gel cassettes. The loading dey containing TE/Mg^{2+}, 50% glycerol, and 0.2% each of Bromophenol Blue and Xylene Cyanol FF was added to all samples. Gel

was run at room temperature, 110V for 80min. After electrophoresis, the gel was stained with GelRed (Biotium) for 30 min and scanned. To distinguish DNA bands, a 13 T and 26 T segment was added to protect-oligomer and fuel-oligomer respectively to increase the molecular weight. The oligonucleotide sequences used in this section were listed in Table S1.

AFM characterization Accordingly, the samples were prepared as previously described[2]. Briefly, the amino chip surface was obtained by immersing freshly cleaved micas in ethanol containing 1% APTMS for ~5 min, and the micas were washed with ethanol and dried in air. Then, the mica surface was modified with 5% glutaraldehyde for 30 min. 0.5 μM amino-modified capture probe was immobilized on the micas through an amino-coupling reaction for 2h, followed by 2.6% sodium borohydride to block uncombined aldehyde group. 0.5 μM complex *S*, which is prepared by the hybridization of a linker-oligomer with a protect-oligomer, was dropped on the micas to incubate with capture probe for 1.5h. Subsequently, the mixture of 0.2 μM target (perfect or mutant) and 2 nM DNA-AuNP conjugates was introduced to trigger the strand displacement reaction. After 2h, the micas were rinsed with 6×SSC[3] at 37 °C followed by an ultrapure water wash at room temperature, and dried in air for AFM characterization. The surface morphology of the prepared micas was observed and recorded using atomic force microscope (AFM, VEECO Multimode V) operating at the tapping mode (Bruker AFM probe, OTESPA-R3). The oligonucleotide sequences for AFM analysis were listed in Table S2.

QCM-D experiments All detection processes were monitored online using a Q-Sense E4 QCM-D instrument (Q-Sense AB, Västra Frölunda, Sweden). Prior to modification, the crystal chips (5 MHz, AT - cut) (Hrbio Co., Ltd., Beijing, China) were immersed in a newly prepared piranha solution (a 3:1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) for 5 min followed by thorough rinsing with ultrapure water. Before loading onto the measuring cell, the chip was dried by using nitrogen gas.

The QCM-D measurements were performed at 25 °C with a flow rate of 15 μL min^{-1}. A 1 μM CP solution was pretreated with 10 mM TCEP for 1 h at room temperature. The CP solution was subsequently injected into the QCM measuring cell for on-line immobilization on a gilded chip

surface for ~2 h. Then, 1 mM MCH solution was injected for 30 min to remove the non-specific adsorption and to block the bare gold surface. The 250 nM double-strand complex solution was injected into the chamber to hybridize with the CP for 1.5 h. A mixture solution of the DNA–AuNP probe (3.6 nM) and target *X* (*m*, *n*) was injected for a minimum of 10 h. The QCM-D data simultaneously measured using the frequency and energy dissipation changes were obtained at seven overtones.

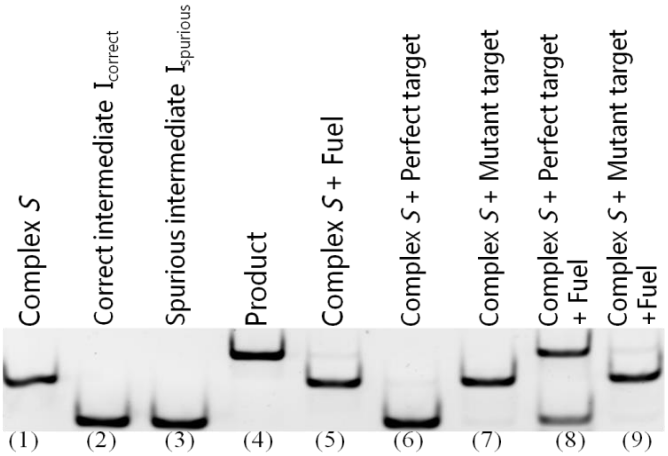

Fig. S1 Demonstration of toehold exchange reaction by native PAGE. (1) The complex *S* was prepared with a 1.1: 1.1: 1 ratio of capture probe to protect-oligomer to linker-oligomer, and then the solution was slowly annealed from 94°C to room temperature. (2), (3) and (4) were prepared with the same method except that the protect-oligomer was replaced by perfect target, mutant target, and fuel-oligomer respectively. (5) The fuel-oligomer was added into the complex *S* solution and the toehold exchange proceeded in a 30°C water bath for 1h. (6) The perfect target was added into the complex *S* solution and the toehold exchange proceeded in a 30°C water bath for 1h. (7) The mutant target was added into the complex *S* solution and the toehold exchange proceeded in a 30°C water bath for 1h. (8) The perfect target and fuel-oligomer were added into the complex *S* solution and the toehold exchange proceeded in a 30°C water bath for 1h. (9) The mutant target and fuel-oligomer were added into the complex *S* solution and the toehold exchange proceeded in a 30°C water bath for 1h. [Complex *S*] = [$I_{correct}$] = [$I_{spurious}$] = [Product] = [Perfect target] = [Mutant target] = [Fuel] = 200 nM.

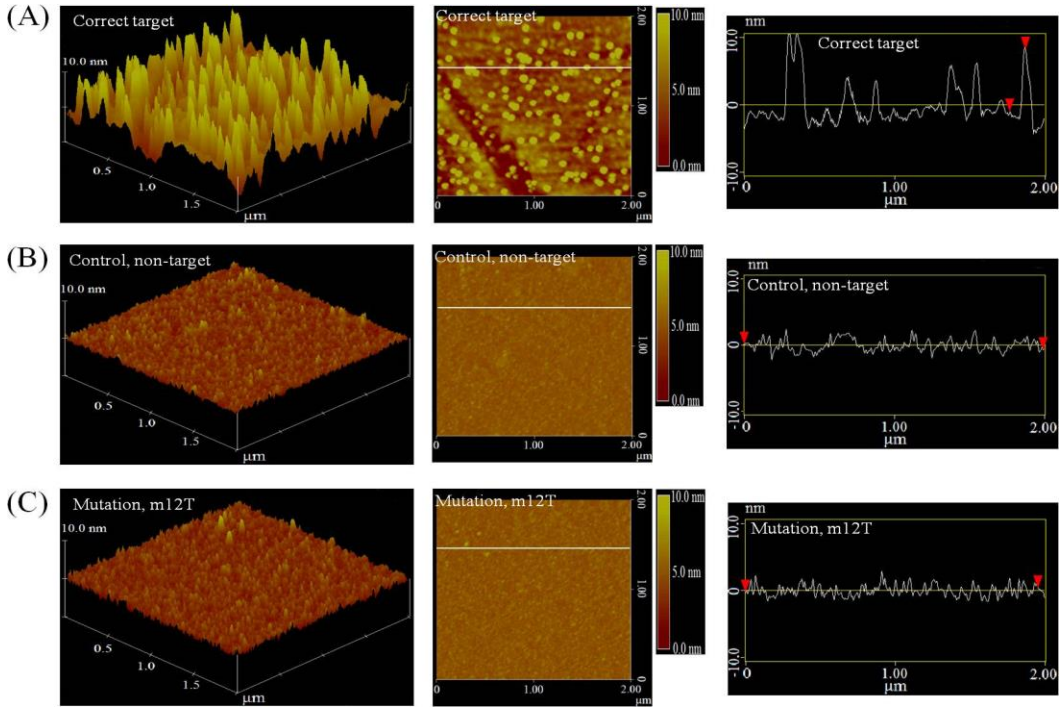

Fig. S2 AFM images of DNA-AuNP conjugates loaded micas chip surface. (A) The topographic images of micas surface incubated with the perfect target sample. The left is 3D topographic image. The middle is 2D image, and the right image is height profile by scanning the white line in 2D image. As shown in 3D and 2D images, many "islands" representing gold nanoparticles can be observed on the micas surface in perfect target sample. However, for the control and mutation sample (B and C), the micas surface is relatively smooth and the height profile is much lower than that of perfect target sample. The proposed assembly principle was also confirmed by the remarkable differences in surface morphology and height profile with AFM characterization.

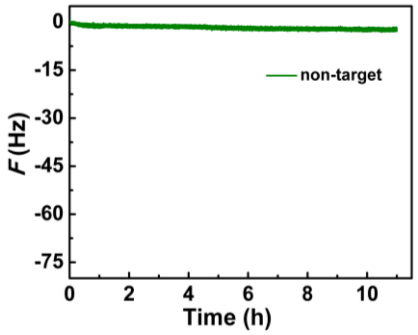

Fig. S3 The frequency response of QCM biosensor to the system without target *X* (*m*, *n*). [Complex] = 250 nM, [DNA-AuNP] = 3.6 nM.

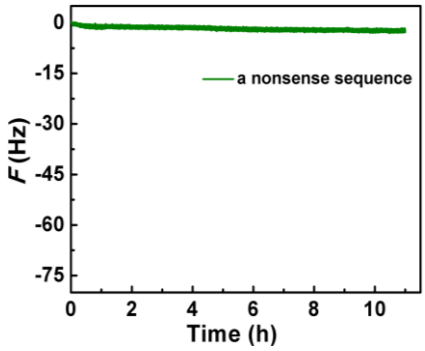

Fig. S4 The frequency response of QCM biosensor to the system with a nonsense sequence. [Complex] = 250 nM, [DNA-AuNP] = 3.6 nM.

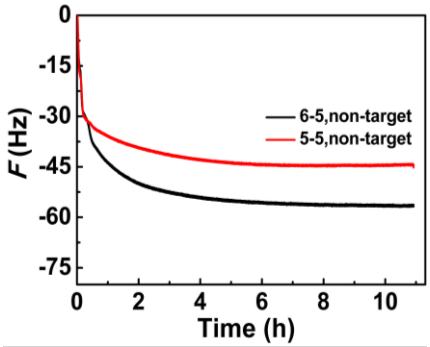

Fig. S5 The frequency responses of QCM biosensor to "6-5" and "5-5" systems without target. The label "6-5" means n=6, m=5. [Complex] = 250 nM, [DNA-AuNP] = 3.6 nM.

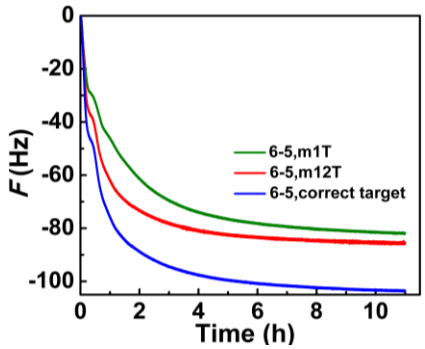

Fig. S6 Frequency responses of QCM biosensor to "6-5" systems with "m1T", "m12T" and correct target. The label "6-5" means n=6, m=5. "m1T" means the base at 1st position of target is mutated into T. [Complex] = 250 nM, [target *X* (*m*, *n*)] =62nM, [DNA-AuNP] = 3.6 nM.

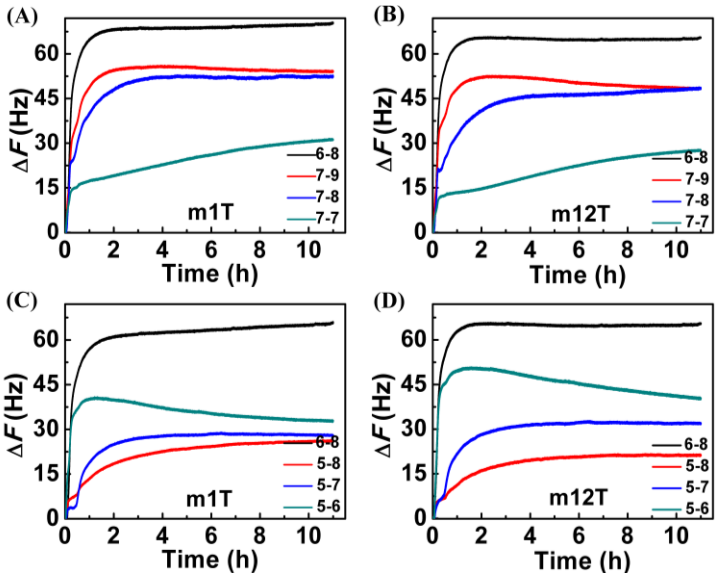

Fig. S7 Influence of the length of toehold domain on SNP detection. The label "6-8" means n=6 (a six-base domain $\overline{\gamma^n}$) and m=8 (an eight-base domain $\overline{\beta^m}$). The label "m1T" means the mutation occurs at the outermost (1st) position, and the base is changed into T. While the label "m12T" indicates the base mutation at the 12th position rather than the terminus; [Complex] = 250 nM, [Target *X* (*m*, *n*)] = 62 nM, [DNA-AuNPs] = 3.6 nM. [Complex] = 250 nM, [Target *X* (*m*, *n*)] =62nM, [DNA-AuNP] = 3.6 nM.

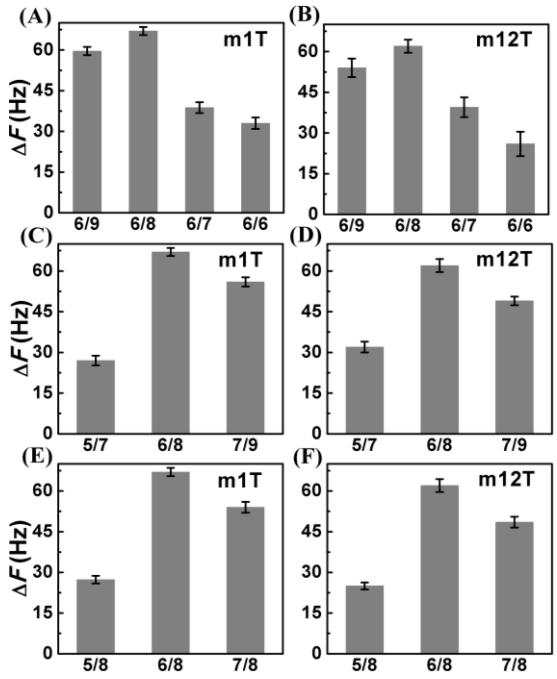

Fig. S8 Effect of the toehold domain's length on discrimination capability. The differences in QCM frequency shifts between the systems of spurious and perfect target *X* (*m*, *n*). For example, in Fig. 2(A), the label "6-8" means *n*=6 (a six-base domain $\overline{\gamma^n}$) and *m*=8 (an eight-base domain $\overline{\beta^m}$). The label "m1T" means the mutation occurs at the outermost (1st) position, and the base is changed into T. While the label "m12T" indicates the base mutation at the 12th position rather than the terminus. The error bars are standard deviations of three repetitive measurements. As demonstrated in Fig. S8, when the toehold domain's length is fixed (e.g. the system of *n*/*m*=6/8), the value of Δ*F* between the system of "m1T" and "m12T" is similar, and this phenomenon can be contributed by the following statements. As previously described[4], the discrimination capability depends crucially on the length of toehold domain. When the length is fixed, the difference in signal intensity (Δ*F*) between "m1T" and "m12T" is small. Additionally, for the toehold exchange reaction on the chip surface, due to the high density of the immobilized double-stranded DNA and the steric hindrance, the oligonucleotides adopt the extended conformation. This conformation results in the enhanced toehold exchange efficiency but weakens the discrimination of base mutation's signal intensity at different positions[5]. The sensitivity of instrument also may be responsible for the small differences in signal intensities of mutations at various positions.

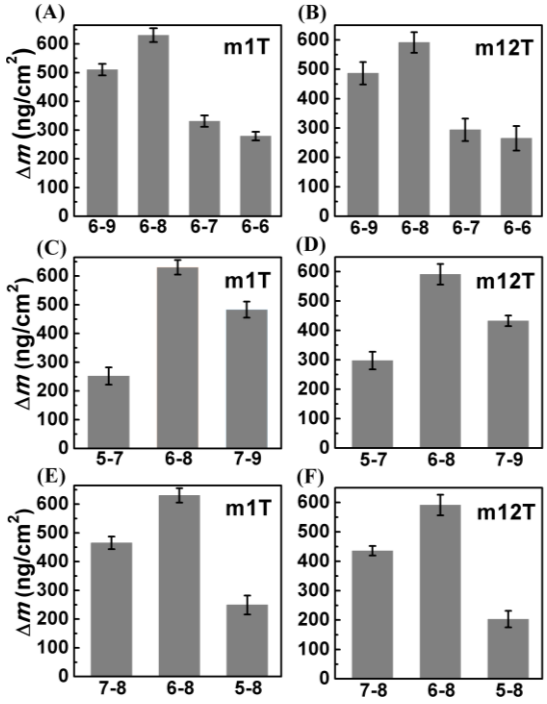

Fig. S9 The differences in mass accumulation between the systems of spurious and perfect target. The mass accumulation on the chip surface of QCM biosensor was calculated by Sauerbrey equation. The error bars are standard deviations of three repetitive measurements.

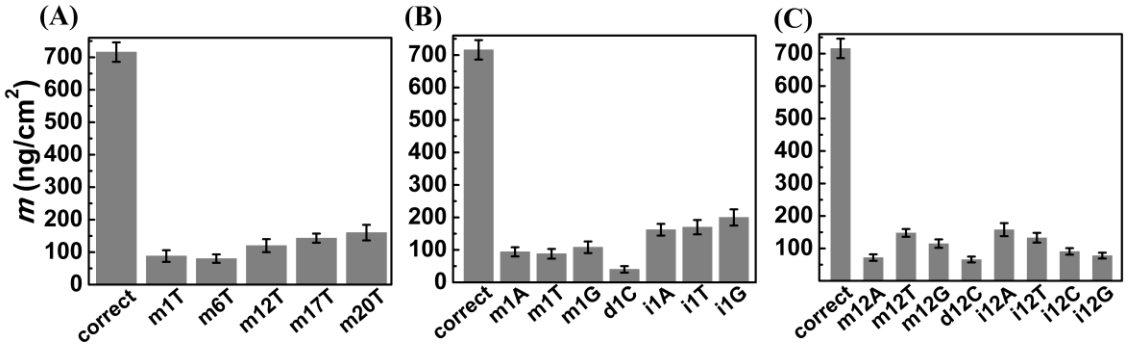

Fig. S10 The mass accumulation for the systems with perfect and spurious target on gold electrode surface of QCM biosensor. The results were obtained by Sauerbrey equation. The error bars are standard deviations of three repetitive measurements.

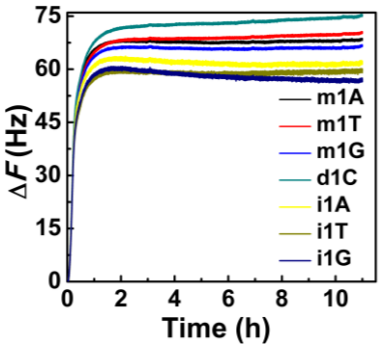

Fig. S11 Discrimination of all mutation schemes at 1st position. Differences in frequency shifts of QCM biosensor between the correct and the spurious target *X* (*m*, *n*) with all types of single-base changes; the labels of "m", "d" and "i" represent SNP, deletion and insertion, respectively. [Complex] = 250 nM, [Target *X* (*m*, *n*)] =62 nM, [DNA-AuNP] = 3.6 nM.

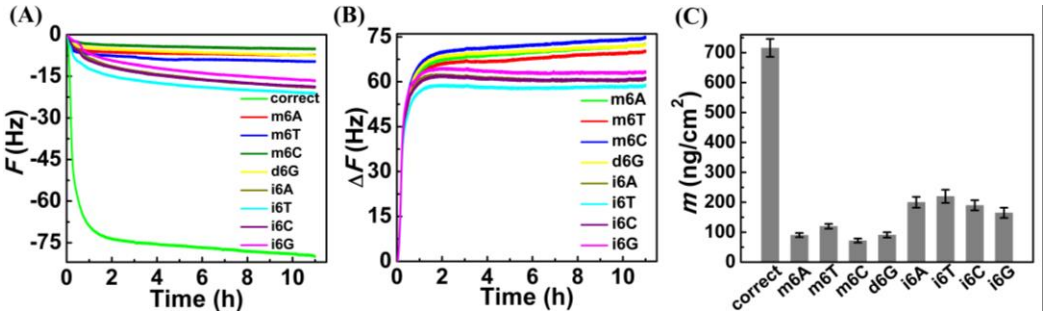

Fig. S12 Discrimination of all mutation schemes at 6th position. (A) Frequency responses of QCM biosensor to the correct and the spurious target *X* (*m*, *n*) with all types of single-base changes. (B) The corresponding difference in frequency shifts between the spurious target *X* and the correct one. The labels of "m", "d" and "i" represent SNP, deletion and insertion, respectively. [Complex] = 250 nM, [Target *X* (*m*, *n*)] =62 nM, [DNA-AuNP] = 3.6 nM. (C) The mass accumulation on the chip surface of QCM biosensor is calculated by Sauerbrey equation. The error bars are the standard deviations of three repetitive measurements.

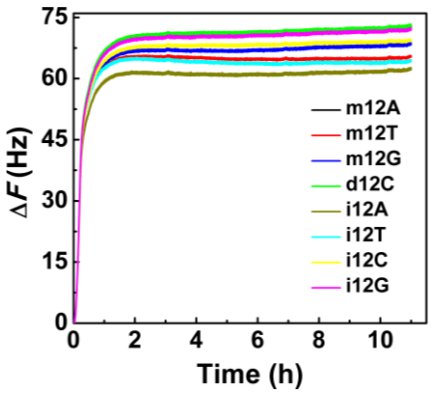

Fig. S13 Discrimination of all mutation schemes at 12th position. Differences in frequency shifts of QCM biosensor between the correct and the spurious target *X* (*m*, *n*) with all types of single-base changes; the labels of "m", "d" and "i" represent SNP, deletion and insertion, respectively. [Complex] = 250 nM, [Target *X* (*m*, *n*)] =62 nM, [DNA-AuNP] = 3.6 nM.

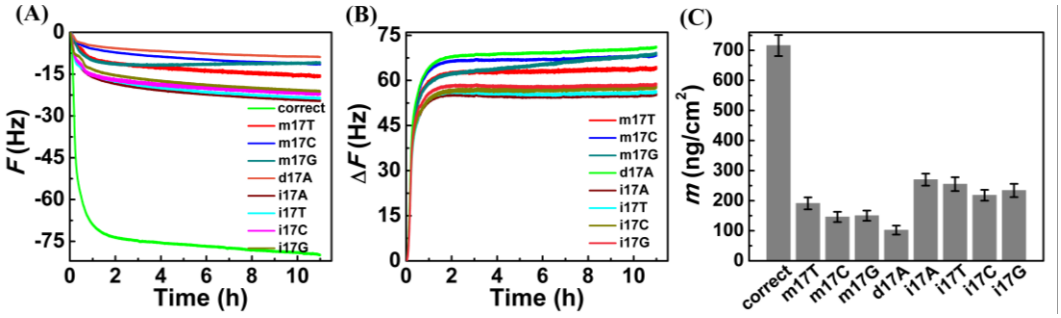

Fig. S14 Discrimination of all mutation schemes at 17th position. (A) Frequency responses of QCM biosensor to the correct and the spurious target *X* (*m*, *n*) with single-base changes with all of styles. (B) The corresponding difference in frequency shifts between the spurious target *X* and the correct one. The labels of "m", "d" and "i" represent SNP, deletion and insertion, respectively. [Complex] = 250 nM, [Target *X* (*m*, *n*)] =62 nM, [DNA-AuNP] = 3.6 nM. (C) The mass accumulation on the chip surface of QCM biosensor calculated by Sauerbrey equation. The error bars are standard deviations of three repetitive measurements.

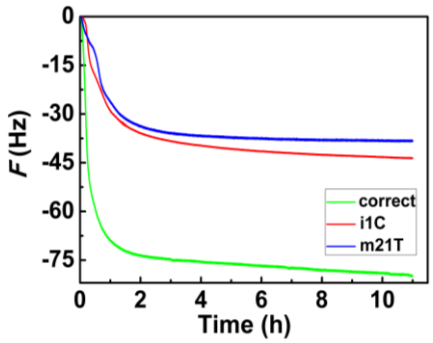

Fig. S15 Discrimination of base C insertion at 1st position and mismatch at 21th position. The labels of "m" and "i" represent SNP and insertion, respectively. [Complex] = 250 nM, [Target *X* (*m*, *n*)] =62 nM, [DNA-AuNP] = 3.6 nM.

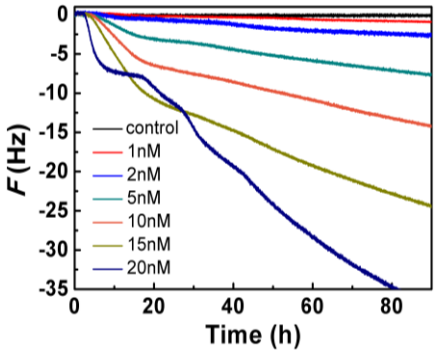

Fig. S16 The response of QCM biosensor to a traditional sandwich-type assay (composed of the capture probe, the target, and the DNA-AuNP probe). The detection limit of this assay is ~1 nM.

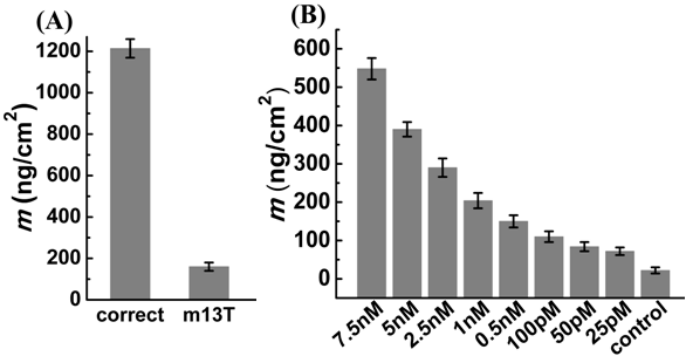

Fig. S17 (A) The mass accumulation on the chip surface of QCM biosensor for system of the correct and spurious target p53. (B) The mass accumulation on the chip surface of QCM biosensor at different target concentrations. The error bars are standard deviations of three repetitive measurements.

Table S1. The ssDNA sequences used in the native PAGE analysis. Sequences start from the 5’ end.

Linker-oligomer	CTACCTTCACAAACACGCACCTTAAAGCTCACTCTCACC CTAC
Protector-oligomer	AGGTGCGTGTTTGTGAAGGTAGTTTTTTTTTTTTTT
Perfect target	GCTTTAAGGTGCGTGTTTGTG
Mutant Target	GCTTTAAGGTGTGTGTTTGTG
Capture probe	TTTTTTGTAGGGTGAGAGTGA
Fuel-oligomer	AAGGTGCGTGTTTGTGAAGGTATTTTTTTTTTTTTTTTTT TTTTTTTT

Table S2: The ssDNA sequences used in AFM characterization. Sequences start from the 5’ end.

Linker-oligomer	CTACCTTCACAAACACGCACCTTAAAGCTCACTCTCACC CTAC
Protector-oligomer	AGGTGCGTGTTTGTGAAGGTAG
Perfect target	GCTTTAAGGTGCGTGTTTGTGA
Mutant Target	GCTTTAAGGTGTGTGTTTGTGA
Capture probe	H_2N-TTTTTTGTAGGGTGAGAGTGA
DNA-AuNP Fuel	AAGGTGCGTGTTTGTGAAGGTAGTTTTTTTTTT-SH

Table S3: The random sequences used for optimization and verification of the detection system on QCM biosensor platform. The sequences started from the 5’end.

linker-oligomer	GGTGAGGTAGGTGAGTAAGTGTATGGCTGGAGTGAGT GTGAGGGTAG
Protector-oligomer:7-9	CATACACTTACTCACCTACCTCA
Protector-oligomer:7-8	CATACACTTACTCACCTACCTC
Protector-oligomer:7-7	CATACACTTACTCACCTACCT
Protector-oligomer:6-9	CCATACACTTACTCACCTACCTCA
Protector-oligomer:6-8	CCATACACTTACTCACCTACCTC
Protector-oligomer:6-7	CCATACACTTACTCACCTACCT
Protector-oligomer:6-6	CCATACACTTACTCACCTACC
Protector-oligomer:5-8	GCCATACACTTACTCACCTACCTC
Protector-oligomer:5-7	GCCATACACTTACTCACCTACCT
Protector-oligomer:5-6	GCCATACACTTACTCACCTACC
Protector-oligomer:5-5	GCCATACACTTACTCACCTAC
Target:correct	CTCCAGCCATACACTTACTCA
Target:m1A	ATCCAGCCATACACTTACTCA
Target:m1T	TTCCAGCCATACACTTACTCA
Target:m1G	GTCCAGCCATACACTTACTCA
Target:d1C	TCCAGCCATACACTTACTCA

Target:i1A	ACTCCAGCCATACACTTACTCA
Target:11T	TCTCCAGCCATACACTTACTCA
Target:i1C	CCTCCAGCCATACACTTACTCA
Target:i1G	GCTCCAGCCATACACTTACTCA
Target:m6A	CTCCAACCATACACTTACTCA
Target:m6T	CTCCATCCATACACTTACTCA
Target:m6C	CTCCACCCATACACTTACTCA
Target:d6G	CTCCACCATACACTTACTCA
Target:i6A	CTCCAAGCCATACACTTACTCA
Target:i6T	CTCCATGCCATACACTTACTCA
Target:i6C	CTCCACGCCATACACTTACTCA
Target:i6G	CTCCAGGCCATACACTTACTCA
Target:m12A	CTCCAGCCATAAACTTACTCA
Target:m12T	CTCCAGCCATATACTTACTCA
Target:d12C	CTCCAGCCATAGACTTACTCA
Target:m12	CTCCAGCCATAACTTACTCA
Target:i12A	CTCCAGCCATAACACTTACTCA
Target:i12T	CTCCAGCCATATCACTTACTCA
Target:i12C	CTCCAGCCATACCACTTACTCA
Target:i12G	CTCCAGCCATAGCACTTACTCA
Target:m17T	CTCCAGCCATACACTTTCTCA
Target:m17C	CTCCAGCCATACACTTCCTCA
Target:m17G	CTCCAGCCATACACTTGCTCA
Target:d17A	CTCCAGCCATACACTTCTCA
Target:i17A	CTCCAGCCATACACTTAACTCA
Target:i17T	CTCCAGCCATACACTTTACTCA
Target:i17C	CTCCAGCCATACACTTCACTCA
Target:i17G	CTCCAGCCATACACTTGACTCA
Target:m20T	CTCCAGCCATACACTTACTTA
Target:m21T	CTCCAGCCATACACTTACTCT
Capture Probe	SH-TTTTTTTTTTTTTTTCTACCCTCACACTCA
DNA-AuNP Probe	GCCATACACTTACTCACCTACCTCACCTTTTTTTTTTTTTT-SH

Table S4: The ssDNA sequences used in the detection experiments associated with the mutation hotspot R273H in p53 gene. Sequences start from the 5’ end.

Linker-oligomer	CCCACCTCAATCACAAACACGCACCTCAAAGCTCACTCTCACCCTAC
Protector-oligomer	AGGTGCGTGTTTGTGATTGAGGT
Target :correct	GCTTTGAGGTGCGTGTTTGTG

Target :m13T	GCTTTGAGGTGCTTGTTTGTG
Capture probe	SH-TTTTTTTTTTGTAGGGTGAGAGTGA
DNA-AuNP	GAGGTGCGTGTTTGTGATTGAGGTGGGTTTTTTTTTTTT-SH

1 (a) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *Journal of the American Chemical Society*, 1998, **120**, 1959; (b) T. Song and H. Liang, *Journal of the American Chemical Society*, 2012, **134**, 10803.

2 (a)D. Wang, G. Chen, H. Wang, W. Tang, W. Pan, N. Li and F. Liu, *Biosensors and Bioelectronics*, 2013, **48**, 276.

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4 T. Song, S. Xiao, D. Yao, F. Huang, M. Hu and H. Liang, *Advanced Materials*, 2014, **26**, 6181.

5 H. Xu, W. Deng, F. Huang, S. Xiao, G. Liu and H. Liang, *Chemical Communications*, 2014, **50**, 14171.