Analyst

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Journal Name

View Article Online
DOI: 10.1039/C5AN01270H

ARTICLE

Single quantum dot-based biosensor for DNA point mutation assay

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Sensitive and selective detection of point mutation is essential to molecular biology research and early clinic diagnosis. Here, we demonstrate a single quantum dot (QD)-based biosensor for DNA point mutation assay. In this assay, the mutant target (G/C) remains unchanged after the endonuclease treatment, and the polymerase chain reaction (PCR) may be initiated with the assistance of primers and polymerase, generating a large number of mutant targets. The amplified mutant targets can be captured by the biotinylated probes during the process of denaturation and annealing, and the Cy5-dGTP may be assembled into the biotinylated probe with the catalysis of polymerase, leading to the formation of Cy5-labeled biotinylated probes. The Cy5-labeled biotinylated probes can be further assembled onto the QD surface to obtain Cy5-DNA-QD complex, resulting in the generation of fluorescence resonance energy transfer (FRET) between the QD donor and the Cy5 receptor. The mutant targets can be quantitatively evaluated through the measurement of Cy5 counts by total internal reflection fluorescence (TIRF) microscopy. While in the presence of wild-type targets (T/A), no Cy5-dGTP can be assembled into the biotinylated probe due to the presence of mismatch and consequently no FRET is observed. This single QD-based biosensor exhibits high sensitivity with a detection limit of 5.3 aM (or 32 copies) and can even discriminate as low as 0.01 % variant frequency from the mixture of mutant target and wild-type one. Importantly, this biosensor can be used for genomic analysis in human lung cancer cells, and may be further applied for early clinical diagnosis and personalized medicine.

1. Introduction

With the development of individualized medicine, there is an increasing need for the identification of genetic mutations in clinical samples. Genetic mutation consists of single-base substitution (point mutation), insertion, and deletion at specific position, and point mutation is one of the most abundant forms of genetic mutation in human genomes.[1] Point mutation usually results from the endogenous DNA replication/repair defects or the environmental mutagen exposures,[2,3] and it plays important roles through the regulation of functional encoded proteins in various biological events such as cellular growth, differentiation, and apoptosis.[4] More and more researches indicate that the point mutations in genomic DNA are closely associated with some severe diseases including cancers, Alzheimer's disease, sickle cell anemia and neuropsychiatric disorders.[5-8] Moreover, the individuals with different types of point mutations might have different responses to pathogens, drugs, vaccines and chemical agents.[9,10] Consequently, the accurate identification of point mutation in human genome is of great importance to early diagnosis of disease and prognosis in family genetic disorders as well as the pharmacological research.

So far, a variety of methods have been developed for point mutation assay including sequencing analysis,[11,12] single-strand conformation polymorphism (SSCP),[13,14] high resolution melting analysis (HRMA),[15-17] denaturing high performance liquid chromatography (DHPLC),[18] mutant-enriched PCR method,[19,20] microbead-based ligase reaction assay,[21] and label-free fluorescent molecular switch-based method.[22] Among them, the sequencing analysis is the most widely used method for point mutation assay, but it usually suffers from poor sensitivity and large sample consumption. The SSCP is another frequently used method with the advantage of high sensitivity, but it needs gel electrophoresis and is only suitable for qualitative analysis. The HRMA method relies on the differences of melting curve shape between the mutant target and the wild-type one, and has become an important tool for point mutation assay. However, it may only be used for the analysis of small and highly purified DNA fragments. The DHPLC seems to be a good alternative method due to its rapidness and reliability, but it is hard to distinguish the mutation types and it also involves expensive apparatus. The mutant-enriched PCR method is a restriction fragment length polymorphism (RFLP) analysis with high specificity and sensitivity. However, it involves two-step PCR and restriction

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digestion, and the RFLP analysis requires the electrophoresis. The microbead-based ligase reaction assay is a rapid point mutation detection method, but the involvement of molecular beacon increases the experimental cost. Therefore, the development of highly sensitive and specific methods for point mutation assay still remains a great challenge.

Recently, the semiconductor quantum dots (QDs) have gained increasing attention because of its excellent optical properties including high quantum yields, narrow and size-tunable fluorescence spectra, and less susceptible to photo-bleaching/quenching.[23-26] Moreover, the QDs are much brighter with longer lasting time than organic fluorophores and fluorescent proteins.[27] The QDs have been widely used in fluorescence imaging, biological labeling, biomedical research, and even in the field of single-molecule detection.[23] Especially, the QDs may function as the fluorescence resonance energy transfer (FRET) donors in the development of various biosensors for the detection of proteins, nucleic acids and small molecules.[28-30] Here, we develop a single QD-based biosensor for sensitive and selective detection of DNA point mutation. In this assay, the mutant targets are enriched by endonuclease digestion and PCR amplification, and then the single base extension reaction is initiated with the assembly of Cy5-dGTP into the biotinylated probe. The resultant Cy5-labeled biotinylated probes may be assembled onto the QD surface to form a Cy5-DNA-QD complex, leading to the occurrence of FRET between the QD and Cy5. While in the presence of wild-type targets, the single-base extension reaction cannot be initiated and no FRET occurs between the QD and Cy5. This method allows for highly sensitive detection of mutant targets with a detection limit of as low as 5.3 aM (or 32 copies), and it can even distinguish 0.01 % variant frequency from the mixture of mutant target and wild-type one. Importantly, this method can be used for genomic analysis in human lung cancer cells, and may be further applied for early clinical diagnosis and personalized medicine.

2. Experimental section

2.1 Materials and reagents

All oligonucleotides (Table 1) were synthesized and HPLC purified by Sangon Biotech (Shanghai) Co., Ltd. Taq DNA Polymerase, endonuclease MscI, shrimp alkaline phosphatase, E.coli exonuclease I, deoxynucleotide (dNTP) solution mixture were purchased from New England Biolabs (Beverly, MA, USA). Cyanine 5-dGTP (Cy5-dGTP) was obtained from PerkinElmer (Boston, MA, USA). 10000× SYBR Green I was purchased from Xiamen Bio-Vision Biotechnology (Xiamen, China). The streptavidin-coated 605 nm emission QD were obtained from Invitrogen Co. (Carlsbad, CA, USA). All other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 1 Sequences of targets, primers and probes [a]

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note	sequence (5'-3')
mutant target (double-stranded DNA)	AAC GTA CTG GTG AAA ACA CCG CAG CAT GTC AAG ATC ACA GAT TTT GGG CGG GCC AAA CTG CTG GGT GCG GAA GAG AAA GAA TAC CAT GCA GAA GGA GGC AAA
wild-type target (double-stranded DNA)	AAC GTA CTG GTG AAA ACA CCG CAG CAT GTC AAG ATC ACA GAT TTT GGG CTG GCC AAA CTG CTG GGT GCG GAA GAG AAA GAA TAC CAT GCA GAA GGA GGC AAA
forward primer	CTG GTG AAA ACA CCG CAG C
reverse primer	TGC CTC CTT CTG CAT GGT ATT
biotinylated probe	Biotin-GCA CCC AGC AGT TTG GCC C

[a] The underlined letter in mutant target and wild-type target indicates the point mutation.

2.2 Endonuclease digestion and PCR amplification

Because the recognition sequence of endonuclease MscI locates at the site of point mutation, the sequence of TGG↓CCA in wild-type target can be cleaved into two fragments, but the sequence of GGGCCA in mutant target cannot be cleaved. The endonuclease digestion reaction was performed in 10 μL of reaction mixture containing 5 U MscI, the targets at different concentrations, 1 × CutSmart buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9), and incubated at 37 °C for 4 h. Then the PCR amplification was carried out in 20 μL of reaction mixture containing 4 μL of MscI digestion products, 250 nM forward primer, 250 nM reverse primer, 0.2 mM dNTP, 1 U Taq DNA polymerase, and 1× LongAmp Taq reaction buffer (60 mM Tris-SO_4, 20 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 3 % Glycerol, 0.06 % IGEPAL CA-630, 0.05 % Tween 20, pH 9). The amplification involved the initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 20 s, 57 °C for 30 s, 65 °C for 30 s, and final extension at 65 °C for 5 min.

2.3 Single-base extension reaction

Before single-base extension reaction, the PCR products were treated with E.coli exonuclease I and shrimp alkaline phosphatase to degrade the excessive primers and the unincorporated dNTPs. 5 μL of PCR products were mixed with 5 μL of enzymatic mixture containing 1 U E.coli exonuclease I, 1 U shrimp alkaline phosphatase, and 1× CutSmart buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/ml BSA, pH 7.9), and incubated at 37 °C for 1 h, followed by enzyme inactivation at 80 °C for 20 min. The single-base extension reaction was performed in 20 μL of total volume containing 8 μL of enzymatic digestion products, 200 nM biotinylated probe, 2 μM Cy5-dGTP, 1 U Taq DNA Polymerase, and 0.5× LongAmp Taq reaction buffer (33 mM Tris-SO_4, 10 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, 1.5 % Glycerol, 0.03 % IGEPAL CA-630, 0.025 % Tween 20, pH 9.0). The

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extension reaction involved the initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 20 s, 60 °C for 40 s, and eventually at 4 °C. The extension products were further treated with 1 U shrimp alkaline phosphatase to digest the excessive Cy5-dGTP at 37 °C for 1 h, followed by enzyme inactivation at 65 °C for 10 min.

2.4 FRET detection

The 9 μL of reaction products was added to the incubation solution with a final volume of 100 μL containing 0.5 nM streptavidin-coated 605 nm emission QD and 1× incubation buffer (100 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, and 3 mM $MgCl_2$, pH 8.0). The mixture was incubated at room temperature for 10 min to make the streptavidin-coated QD conjugate with the biotinylated probes. At last, the fluorescence spectra of reaction solution were measured by a Hitachi F-4500 fluorometer (Tokyo, Japan) equipped with a xenon lamp as the excitation source. The spectra of QD and Cy5 were recorded at the excitation wavelength of 488 nm. The excitation and emission slits were set for 10 nm and 10 nm, respectively.

Before total internal reflection fluorescence (TIRF) microscopy imaging, 100 μL of reaction solution was diluted 25-fold with the dilution buffer containing 67 mM glycine-KOH (pH 9.4), 50 μg/mL BSA, 2.5 mM $MgCl_2$, 1 mg/mL Trolox. In order to minimize the photobleaching, an oxygen-scavenging buffer (1 mg/mL glucose oxidase, 0.4 % (w/v) D-glucose, and 0.04 mg/mL catalase) was added into the imaging buffer. The glass slides were pretreated according to the method proposed by Chan et al.[31] The QDs were excited by a Jive 488-nm DPSS laser (Cobolt) via TIRF. Both the QD and the Cy5 fluorescence signals were collected by an oil immersion objective (NA 1.45, 100×, Olympus), and imaged onto the two halves of an Andor Ixon DU897 EMCCD with a time resolution of 50 ms. A series of sequential frames on different locations were acquired from a single slide. The images were obtained with Image J (version 1.48e19, Broken Symmetry Software, U.S.A.). An imaging region of 200×200 pixels was selected for data analysis. The Cy5 counts were obtained by summing up five frames with the 'analyze particles' data of Image J.

2.5 Electrophoresis

The products were analyzed by 12% PAGE gel electrophoresis at room temperature in 0.5× TBE buffer（45 mM Tris-borate, 45 mM boric acid, 1 mM EDTA, pH 8.3) with 1× loading buffer and 1× SYBR Green I as the fluorescent indicator. The images of gel electrophoresis were acquired with a Kodak Image Station 4000MM (Woodbridge, CT, USA).

2.6 Extraction of genomic DNA

The H460 cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10 % fetal bovine serum, 100 μg/mL penicillin and 100 μg/mL streptomycin. The cells were incubated at 37 °C in humidified air with 5 % CO_2. The genomic DNA of H460 cells was extracted from 2.5× 10^6 cells using the Takara MiniBEST universal genomic DNA extraction kit according to the manufacturer's procedures, and the extracted genomic DNA was eluted with 50 μL of elution buffer.

3. Results and discussion

3.1 Principle of point mutation assay

As shown in Scheme 1, the point mutation assay involves three steps including PCR amplification, single base extension reaction, and FRET detection. We chose the L858R point mutation in human epidermal growth factor receptor (EGFR) gene as a model.[32] In the first step, the mutant targets remain uncleaved after treatment with endonuclease MscI, but most wild-type targets are cleaved into two fragments. With the assistance of primers and Taq polymerase, the mutant targets and the uncleaved wild-type targets are amplified by PCR. In the second step, the amplified mutant target are captured by the biotinylated probes during the process of denaturation and annealing, and meanwhile the Cy5-dGTP is assembled into the biotinylated probe with the catalysis of Taq polymerase at 60 °C. When the temperature is increased to 94 °C, the duplexes are denatured, releasing both the Cy5-labeled biotinylated probes and the mutant targets. The released mutant targets can hybridize with new biotinylated probes to initiate the single-base extension cycle, generating large numbers of Cy5-labeled biotinylated probes. Although the wild-type targets may be captured by the biotinylated probes, the Cy5-dGTP cannot be assembled into the biotinylated probe due to the presence of mismatched base. In the third step, the Cy5-labeled biotinylated probes are attached onto the QD surface through biotin-streptavidin interaction to form a Cy5-DNA-QD complex, resulting in the generation of FRET between the QD donor and the Cy5 receptor. When the Cy5-DNA-QD complex is excited by a 488 nm laser, the fluorescence signals of both QD and Cy5 can be observed simultaneously. The mutant target can be quantitatively evaluated by the measurement of Cy5 counts. Even though some free Cy5-dGTP molecules may exist in the solution, they are far away from the QD surface and no FRET between the QD and Cy5 can be observed in the single-particle/molecule imaging.

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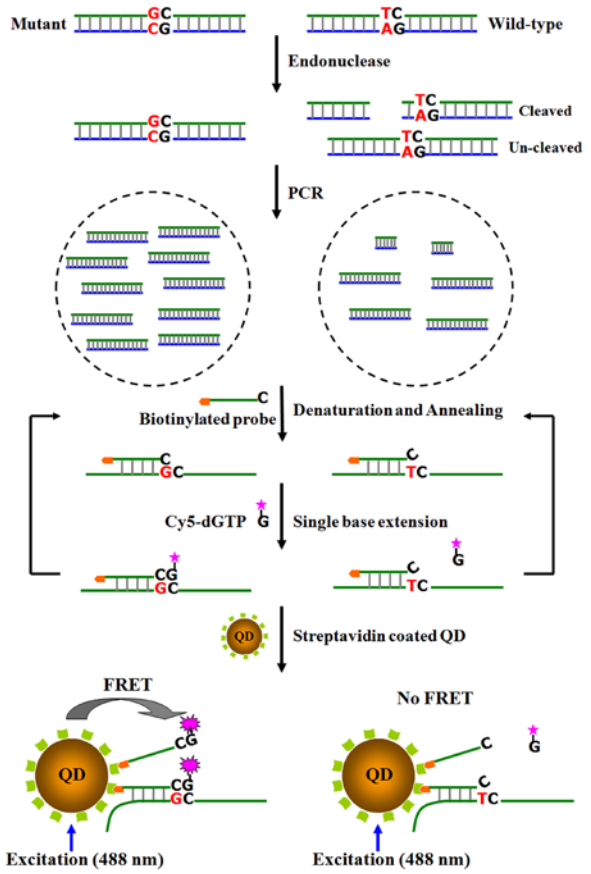

Scheme 1 Schematic illustration of single QD-based biosensor for DNA point mutation assay.

To confirm the PCR amplification, single base extension reaction, and FRET detection involved in this assay, we performed nondenaturating polyacrylamide gel electrophoresis (PAGE) analysis (Fig. 1A and 1B) and fluorescence measurement (Fig. 1C). As shown in Fig. 1A, a distinct band is observed in the presence of mutant target after PCR amplification, but no band is observed in the blank group. Notably, a weak band is observed in the presence of wild-type target after PCR amplification, because endonuclease MscI is unable to digest the wild-type targets completely. We used PAGE to analyze the products of single-base extension reaction (Fig. 1B). The left image of Fig. 1B is obtained via the sliver staining and the right image is acquired by a Kodak Image Station 4000MM at an excitation wavelength of 635 nm (for Cy5 molecule). In the left image, a distinct 19 bp band from the biotinylated probe is observed in the lanes of blank and wild-type target (WT). While in the presence of mutant target, a new band appears in the lane of mutant target (Mut), which results from the assembly of Cy5-dGTP into the biotinylated probe in single-base extension reaction (left image of Fig. 1B). This is confirmed by the appearance of a new band upon the excitation of Cy5 fluorescence in the presence of mutant target (right image of Fig. 1B). After the addition of QDs into the products of single-base extension reaction, we measured the fluorescence of both the QDs and Cy5 (Fig. 1C). The addition of mutant target induces the decrease of QD fluorescence intensity and the increase of Cy5 fluorescence intensity, suggesting the occurrence of FRET between the QD and Cy5 due to the formation of a Cy5-DNA-QD complex. In contrast, both the QD and Cy5 fluorescence intensities remain unchanged in the presence of wild-type target as compared with that in the blank group, suggesting no occurrence of FRET between the QD and Cy5. This result also demonstrates the high specificity of the proposed method for DNA point mutation assay.

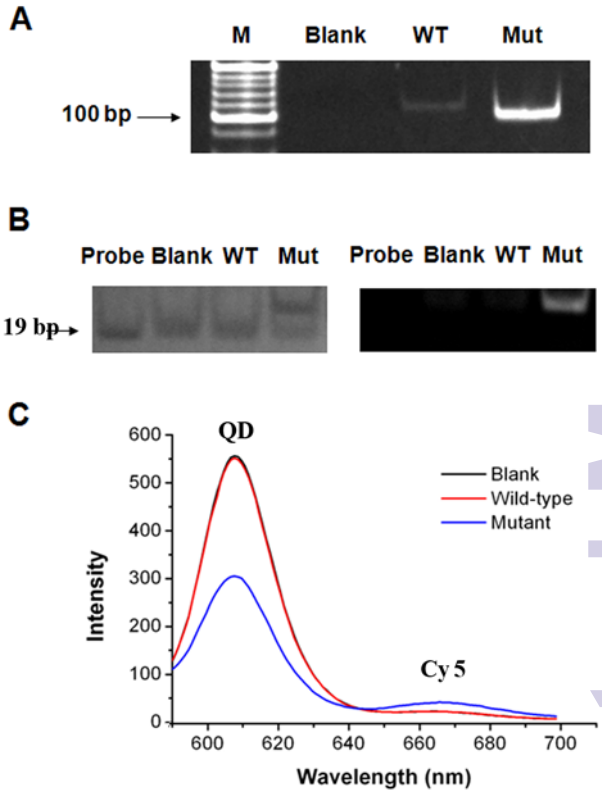

Fig. 1 Verification of single QD-based biosensor for DNA point mutation assay. (A) PAGE analysis of the PCR-amplified products. (B) PAGE analysis of the single-base extension products. The left image is obtained via the sliver staining and the right image is acquired by a Kodak Image Station 4000MM at an excitation wavelength of 635 nm (for Cy5 molecule). (C) Fluorescence spectra of the single-base extension products with the addition of QDs. The concentration of mutant target is 1 fM, and the concentration of wild-type target is 1 fM. The M, WT and Mut represent the DNA maker, wild-type target, and mutant target, respectively.

3.2 Detection of point mutation with TIRF microscope

To further verify the feasibility of single QD-based biosensor for DNA point mutation assay, we performed the fluorescence images with TIRF microscopy in the presence of mutant target and wild-type one, respectively. As shown in Fig. 2, in the presence of mutant target (Fig. 2A and 2B), the fluorescence signals of QD (Fig. 2A, green color) and Cy5 (Fig. 2B, red color) are observed simultaneously. The presence of mutant target enables the assembly of Cy5-dGTP into the biotinylated probe and the formation of Cy5-DNA-QD complex, making the Cy5 molecule in close proximity to the QD and consequently the occurrence of FRET

between the QD and Cy5. However, in the presence of wild-type target (Fig. 2C and 2D), only the fluorescence signal of QD can be detected (Fig. 2C, green color), but no Cy5 fluorescence signal is observed (Fig. 2D), suggesting no occurrence of FRET between the QD and Cy5 because no Cy5-dGTP can be assembled into the biotinylated probe. Notably, the QD fluorescence signals in the presence of mutant target (Fig. 2A) are much weaker than those in the presence of wild-type target (Fig. 2C) due to the quenching of QD fluorescence as a result of energy transfer from the QD donor to the Cy5 acceptor in the Cy5-DNA-QD complex.

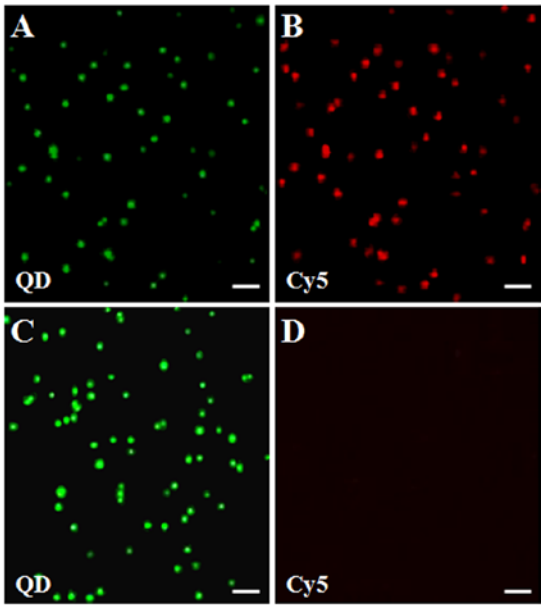

Fig. 2 Fluorescence images of the single QD-based biosensor in the presence of mutant target (A and B) and wild-type target (C and D), respectively. A and C are the images of QDs. B and D are the images of Cy5. The mutant target concentration is 1 fM, and the wild-type target concentration is 1 fM. The scale bar is 2 μm.

3.3 Optimization of experimental condition

To ensure the good performance of the single QD-based biosensor, we optimized the probe-to-QD ratio to obtain high FRET efficiency. We synthesized a double-stranded DNA probe with a length of 20 bp. One strand is labeled with biotin at the 5' end (5'-biotin-CAA TTC CCG TCG CTA TCA AA-3'), and its complementary strand is labeled with Cy5 at the 5' end (5'-Cy5-TTT GAT AGC GAC GGG AAT TG-3'). We calculated the FRET efficiency according to the equation of $E = 1 - (F / F_0)$, where F is the fluorescence intensity of QD donor in the presence of Cy5 acceptor, and F_0 is the fluorescence intensity of QD donor in the absence of Cy5 acceptor. As shown in Fig. 3A, the FRET efficiency improves with the increase of probe-to-QD ratio from 1 to 36, and levels off beyond the ratio of 36. Therefore, the probe-to-QD ratio of 36 is used in the following research.

The concentration of Cy5-dGTP is a key factor in the single base extension reaction and influence the subsequent FRET efficiency. Theoretically, the high-concentration Cy5-dGTP may lead to high single-base extension efficiency and the assembly of more Cy5 molecules into the biotinylated probes and consequently the high FRET efficiency. To optimize the Cy5-dGTP concentration, we measured the FRET efficiency at various concentrations of Cy5-dGTP. As shown in Fig. 3B, the FRET efficiency improves with the increase of Cy5-dGTP concentration from 0.5 μM to 2 μM, but remains unchanged beyond the concentration of 2 μM. Therefore, we chose 2 μM as the optimal Cy5-dGTP concentration in the subsequent research.

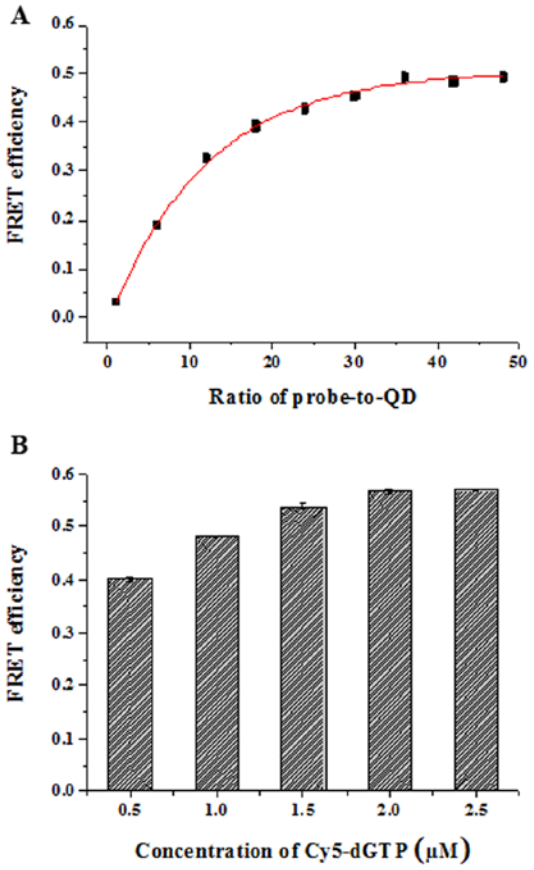

Fig. 3 (A) Variance of FRET efficiency with the ratio of probe-to-QD. (B) Variance of FRET efficiency with the concentration of Cy5-dGTP. Error bars show the standard deviation of three experiments.

3.4 Sensitivity of point mutation assay

To investigate the sensitivity of the proposed method for DNA point mutation assay, we measured different-concentration targets under the optimally experimental conditions. Fig. 4A shows the variance of Cy5 counts with the target concentration in the range from 0 to 10 fM. Despite of the increasing concentration of wild-type target, the Cy5 counts show no significant change (Fig. 4A). In contrast, the Cy5 counts increase gradually with the increasing concentration of mutant target, and a linear correlation is obtained between the Cy5 counts and the mutant target concentration in the range from 0 aM to 3 fM (Fig. 4B). The regression equation is $N = -116.42 + 194.92 \log_{10} C$ with a correlation coefficient of 0.9858, where N is the Cy5 counts and C is the concentration of mutant target (aM), respectively. The limit of detection is calculated to be 5.3 aM (or 32 copies) based on the evaluation of the average signal of control plus three times standard deviation. Notably, the sensitivity of single

QD-based biosensor has improved by as much as 6 orders of magnitude as compared with that of surface enhanced Raman scattering-based assay (1.4 pM),[33] by more than 5 orders of magnitude as compared with that of the rolling circle amplification-based electrochemiluminescence assay (0.1 pM),[34] and by more than 2 order of magnitude as compared with that of ligation reaction-based electrochemical assay (0.1 fM).[35] The improved sensitivity may be attributed to both the high amplification efficiency of PCR and the high signal-to-noise ratio of single QD-based biosensor.

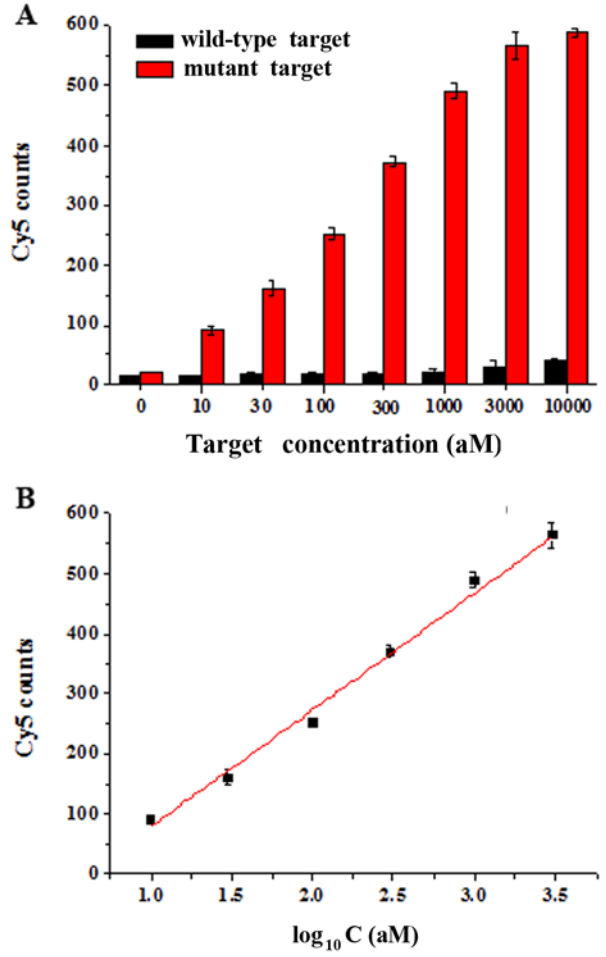

Fig. 4 (A) Variance of the Cy5 counts with the concentration of mutant target (red column) and wild-type one (black column). (B) The Cy5 counts show a log-linear correlation with the concentration of mutant target in the range from 10 aM to 3 fM. Error bars show the standard deviation of three experiments.

3.5 Measurement of variant frequency

In general, there is only a trace amount of mutant gene at the early stage of diseases, and their information is usually hided by the presence of abundant wild-type gene.[36] It is particularly imperative to accurately identify the minimal amount of mutant target among plentiful wild-type ones for early clinical diagnosis. To evaluate the feasibility of single QD-based biosensor for variant frequency assay, we measured the artificial mixtures of mutant target and wild-type one at various percentages. The total concentration of mutant target and wild-type one is 10 pM, and the mixture includes 0, 0.01 %, 0.05 %, 0.1 %, 0.5 %, 1 %, 5 %, 10 %, 50 %, and 100 % mutant target, respectively. As shown in Fig. 5, the Cy5 counts increase with the increasing percentage of mutant target in the mixture. It is worth noting that the proposed method can even distinguish as low as 0.01 % variant frequency, in which the concentration of wild-type target is 10000 times higher than that of the mutant target. The proposed method with the discrimination capability of 0.01 % variant frequency is superior to most currently used approaches for point mutation assay including the cationic conjugated polymer-based FRET method (2-8%),[37,38] the microbead based ligase detection reaction assay (0.25 %),[21] and the mutant-enriched PCR method (0.05 %).[19]

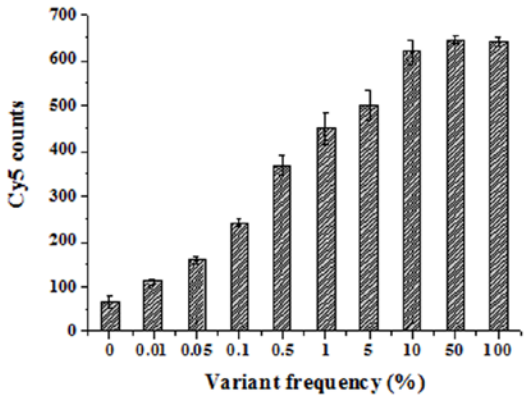

Fig. 5 Variance of the Cy5 counts as a function of variant frequency. The total concentration of mutant target and wild-type one is 10 pM. Error bars show the standard deviation of three experiments.

3.6 Real sample analysis

To further validate the feasibility of the proposed method for real sample analysis, we measured the spiked wild-type target / mutant target in the genomic DNA of H460 cells. The H460 is a wild-type lung cancer cell without L858R point mutation.[39,40] We used the pure buffer solution and the genomic DNA without any targets as the control group. As shown in Fig. 6, no significant difference is observed between the control group and the wild-type group with the wild-type target spiked in the genomic DNA and buffer solution. However, high Cy5 counts are observed in both the mutant target-spiked genomic DNA sample and the mutant target-spiked buffer solution (Fig. 6). These results demonstrate that the proposed method can be applied for DNA point mutation assay in complex genomic DNA sample with good accuracy and reliability.

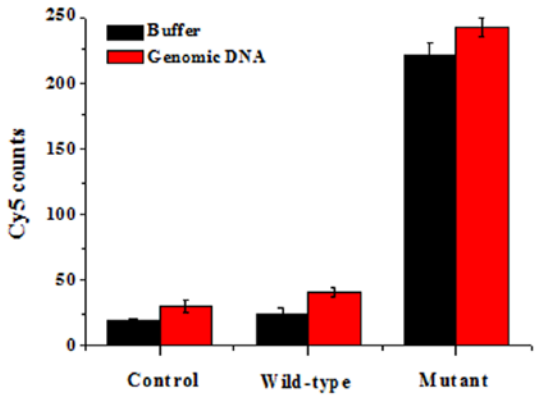

Fig. 6 The Cy5 counts obtained under different conditions. From left to right: a buffer solution, a 10 % extracted genomic DNA sample, 100 aM wild-type target in the buffer solution, 100 aM wild-type target in the genomic DNA sample, 100 aM mutant target in the buffer solution, and 100 aM mutant target in the genomic DNA sample. Error bars show the standard deviation of three experiments.

4. Conclusions

In summary, we have developed a single QD-based biosensor for sensitive and selective detection of DNA point mutation. This biosensor takes advantage of the excellent specificity of endonuclease MscI and single base extension reaction, and it can distinctly differentiate the mutant target from the wild-type one. Due to the high amplification efficiency of PCR and the high signal-to-noise ratio of single-molecule detection, this biosensor can sensitively detect the mutant target with a detection limit of 5.3 aM (or 32 copies), and it can even discriminate as low as 0.01 % variation frequency from the mixture of mutant target and wild-type one. More importantly, this biosensor can be used for genomic analysis in human lung cancer cells, and may be further applied for early clinical diagnosis and personalized medicine.

Acknowledgements

This work was supported by the National Basic Research Program 973 (Grant. 2011CB933600), the National Natural Science Foundation of China (Grant No. 21325523), the Award for the Hundred Talent Program of Nanyue of Guangdong Province, and the Award for Pengcheng Scholar of Shenzhen City.

Notes and references

1 A.C. Syvanen, *Nat. Rev. Genet.*, 2001, **2**, 930-942.
2 E.R. Fearon and B. Vogelstein, *Cell,* 1990, **61**, 759-767.
3 E.R. Fearon , *Science,* 1997, **278**, 1043-1050.
4 M. Cargill, D. Altshuler, J. Ireland, P. Sklar, K. Ardlie, N. Patil, C.R. Lane, E.P. Lim, N. Kalyanaraman, J. Nemesh, L. Ziaugra, L. Friedland, A. Rolfe, J. Warrington, R. Lipshutz, G.Q. Daley and E.S. Lander, *Nat. Genet.,* 1999, **22**, 231-238.
5 H.C. Erichsen and S.J. Chanock, *Br. J. Cancer,* 2004, **90**, 747-751.
6 G.D. Schellenberg, I. D' Souza and P. Poorkaj, *Curr. Psychiatry Rep.,* 2000, **2**, 158-164.
7 A. Cole-Strauss, K. Yoon, Y. Xiang, B.C. Byrne, M.C. Rice, J. Crynn, W.K. Holloman and E.B. Kmiec, *Science,* 1996, **27** , 1386-1389.
8 J. McClellan and M.C. King, *Cell,* 2010, **141**, 210-217.
9 W.E. Evans and M.V. Relling, *Science,* 1999, **286**, 487-491.
10 S. Fang, Z. Wang, J. Guo, J. Liu, C. Li, L. Liu, H. Shi, L. Liu, H. [illegible] C. Xie, X. Zhang, W. Sun and M. Li, *OncoTargets Ther.,* 2014, **7**, 1185-1193.
11 P.Y. Kwok, C. Carlson, T.D. Yager, W. Ankener and D. Nickerson, *Genomics,* 1994, **23**, 138-144.
12 H. Zakeri, G. Amparo, S.M. Chen, S. Spurgeon and P.Y. Kwok, *BioTechniques,* 1998, **25**, 406-414.
13 K. Hayashi, *PCR Methods Appl.,* 1991, **1**, 34-38.
14 M. Inazuka, H.M. Wenz, M. Sakabe, T. Tahira and K. Hayashi, *Genome Res.,* 1997, **7**, 1094-1103.
15 H. Do, M. Krypuy, P.L. Mitchell, S.B. Fox and A. Dobrovic, *BMC Cancer ,* 2008, **8**, 142-154.
16 J. Gonzalez-Bosquet, J. Calcei, J.S. Wei, M. Garcia-Closas, M.E. Sherman, S. Hewitt, J. Vockley, J. Lissowska, H.P. Yang, J. Khan and S. Chanock, *Plos One. ,* 2011, **6**, e14522.
17 H. Sun, Y. Yang, L. Yang, B. Su, G. Jiang, K. Fei and D. [illegible] *Biomed Res. Int.,* 2014, **2014**, 407537.
18 V. Cohen, J.S. Agulnik, J. Jarry, G. Batist, D. Small, M. Kreisman, N.A. Tejada, W.H. Miller Jr and G. Chong, *Cancer,* 2006, **107**, 2858-2865.
19 H. Asano, S. Toyooka, M. Tokumo, K. Ichimura, K. Aoe, S. [illegible] K. Tsukuda, M. Ouchida, M. Aoe, H. Katayama, A. Hiraki, K. Sugi, K. Kiura, H. Date and N. Shimizu, *Clin. Cancer Res.,* 2006, **12**, 43-48.
20 C. He, L. Zheng, Y. Xu, M. Liu, Y. Li and J. Xu, *Clin. Chim. Acta* 2013, **425**, 119-124.
21 S. Watanabe, K. Hagihara, K. Tsukagoshi and M. Hashimoto, *Anal. Chem.,* 2014, **86**, 900-906.
22 H. Xu, F. Geng, Y. Wang, M. Xu, X. Lai, P. Qu, Y. Zhang and S. Liu, *Chem. Commun.,* 2015, **51**, 8622-8625.
23 I.L. Medintz, H.T. Uyeda, E. Goldman and H. Mattoussi, *Nat. Mater.,* 2005, **4**, 435-446.
24 R.C. Somers, M.G. Bawendi and D.G. Nocera, *Chem. Soc. Rev.,* 2007, **36**, 579-591.
25 Q. Wang, Y. Liu, Y. Ke, H. Yan, *Angew. Chem., Int. Ed.* 2008, **47**, 316.
26 A. Samanta, Y. Zhou, S. Zou, H. Yan, Y. Liu. *Nano Lett.* 2014, **14**, 5052-5057.
27 X. Michalet, F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, J. Li, G. Sundaresan, A.M. Wu, S.S. Gambhir, S. Weiss, *Science* 2005, **307**, 538-544.
28 K. Boeneman, B.C. Mei, A.M. Dennis, G. Bao, J.R. Deschamps, H. Mattoussi and I.L. Medintz, *J. Am. Chem. Soc.,* 2009, **13**, 3828-3829.
29 C.Y. Zhang, H.C. Yeh, M.T. Kuroki and T.H. Wang, *Nat. Mater.* 2005, **4**, 826-831.
30 C.Y. Zhang and L.W. Johnson, *Anal. Chem. ,* 2009, **81**, 3051-3055.
31 H.M. Chan, L.S. Chan, R.N.S. Wong and H.W. Li, *Anal. Chem. ,* 2010, **82**, 6911-6918.
32 S.V. Sharma, D.W. Bell, J. Settleman and D.A. Haber, *Nat. Rev. Cancer ,* 2007, **7**, 169-181.
33 S.Q. Huang, J. Hu, G. Zhu and C.Y. Zhang, *Biosens. Bioelectron. ,* 2014, **65**, 191-197.
34 Q. Su, D. Xing and X. Zhou, *Biosens. Bioelectron. ,* 2010, **2**[illegible] 1615-1621.
35 Y. Huang, Y.L. Zhang, X. Xu, J.H. Jiang, G.L. Shen and R.Q. [illegible] *J. Am. Chem. Soc.,* 2009, **131**, 2478-2480.

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DOI: 10.1039/C5AN01270H

36 M. Hashimoto, M.L. Hupert, M.C. Murphy and S.A. Soper, *Anal. Chem.* , 2005, **77**, 3243-3255.
37 J. Song, Q. Yang, F. Lv, L. Liu and S. Wang, *ACS Appl. Mater. Interfaces* , 2012, **4**, 2885-2890.
38 Q. Yang, T. Qiu, W. Wu, C. Zhu, L. Liu, J. Ying and S. Wang, *ACS Appl. Mater. Interfaces* , 2011, **3**, 4539-4545.
39 T.T. Chao, C.Y. Wang, C.C. Lai, Y.L. Chen, Y.T. Tsai, P.T. Chen, H.I. Lin, Y.C.T. Huang, C.W. Shiau, C.J. Yu and K.F. Chen, *J. Pharmacol. Exp. Ther.,* 2014, **351**, 352-358.
40 B.S. Oh, E.A. Shin, J.H. Jung, D.B. Jung, B. Kim, B.S. Shim, M.C. Yazdi, M. Iranshahi and S.H. Kim, *Phytother. Res.,* 2015, **29**, 844-849.