

## SUPPORTING INFORMATION

### **Paper-based platform for detection by hybridization using intrinsically labeled fluorescent oligonucleotide probes on quantum dots**

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## 1. Detailed Experimental Procedures

### 1.1 Reagents and oligonucleotides

Oligonucleotides were from Integrated DNA Technologies (Coralville, IA) and were purified by the manufacturer using either standard desalting or HPLC. The oligonucleotide sequences were dissolved in autoclaved MilliQ water (purified water from a Milli-Q cartridge filtration system with a resistivity of 18.2 M $\Omega$ .cm) and stored at  $-20$  °C. Qdot® 565 ITK™ Streptavidin Conjugate Kits, Phire Hot Start II DNA polymerase, dNTPs, Phire reaction buffer (detergent free), 6x loading dye, generuler DNA ladder were from Life Technologies (ThermoFisher). Dynabeads M-280 (streptavidin conjugated magnetic beads) were from Invitrogen (ThermoFisher). 2-methylbenzothiazole ( $C_8H_7NS$ , 99%), 5-bromovaleric acid ( $Br(CH_2)_4COOH$ , 97%), 4-dimethylamino benzaldehyde ( $((CH_3)_2NC_6H_4CHO$ ,  $\geq 99.0\%$ , HPLC), acetic anhydride ( $((CH_3CO)_2O$ , ReagentPlus®,  $\geq 99\%$ ) N-hydroxysuccinimide (NHS,  $C_4H_5NO_3$ , 98%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC,  $C_8H_{17}N_3 \cdot HCl$ , purum  $\geq 98\%$ ), Goat Serum, lithium chloride (LiCl, ACS reagent,  $\geq 99\%$ ), sodium(meta)periodate ( $NaIO_4$   $\geq 99\%$ ), sodium cyanoborohydride ( $NaBH_3CN$ , reagent grade, 95%), Whatman chromatography paper (Grade 1), and water (molecular biology reagent, certified RNAase and DNAase free) were from Sigma-Aldrich (Oakville, ON). Diethyl ether ( $(C_2H_5)_2O$ , solvent grade) and methanol ( $CH_4O$ , solvent grade) were from Caledon Laboratory Chemicals. EZ-Link Amine-Peg<sub>3</sub>-biotin was from ThermoFisher. All buffer solutions were prepared using MilliQ water and were autoclaved prior to use. The buffer solutions included 100 mM tris-borate buffer (TB, pH 7.4), 100 mM bicarbonate buffer (BIC, pH 8.3), 10 mM Tris-HCl (pH 7.5), 100 mM HEPES (pH 8) and 50 mM borate buffer (BB, pH 9.25).

## 1.2 Instrumentation

Solution phase PL was determined using a HORIBA Instruments, Inc., (Piscataway, NJ, USA). The excitation source was a xenon arc lamp (Ushio, Cypress, CA) and the detector was a red-sensitive R928P photomultiplier tube (PMT, Hamamatsu, Bridgewater, NJ). UV-vis absorption spectra were acquired using a VWR® Double Beam UV-Vis Spectrophotometer. Epifluorescent images of the paper were taken using a Nikon Eclipse L150 epifluorescence microscope (Nikon, Mississauga, ON) which was custom modified with a motorized xyz translational stage. A 25 mW diode laser with an output of 402 nm (Radius 402, Coherent Inc, Santa Clara, CA) was used for the excitation source. The emission filters used were D570/20x and D630/30 for QD and dye emission, respectively (Chroma Technologies Corp., Bellows Falls, VT). Optical excitation on the surface of paper was done through a 4× Nikon WD Plan Fluor objective lens (NA = 0.13). Epifluorescence images were collected using a custom LabVIEW program (National Instruments, Austin, TX) that controlled the data acquisition and the operation of the xyz translational stage of the epifluorescence microscope. The images were collected by raster scanning the paper substrates using H5784-20 PMT (Hamamatsu, Bridgewater, NJ).

## 1.3 Data Analysis: Modified FRET ratio (MFR)

The epifluorescent microscope was used to take two images of the paper. One of the images was taken using the D570/20x filter that corresponded to the wavelength band of significant QD PL, and the other was taken using the D630/30 filter which corresponded to the wavelength band of significant dye PL. The images were processed using ImageJ software (version 1.46r, National Institutes of Health, USA). The modified FRET ratio was calculated using the following formula

$$MFR = \left(\frac{EM_{dye}}{EM_{QD}}\right)_{target} - \left(\frac{EM_{dye}}{EM_{Qd}}\right)_{probe} \quad \text{Equation S1}$$

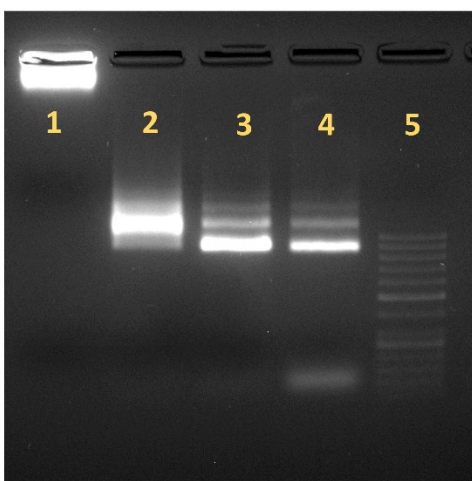
where  $EM_{\text{dye}}$  is the integrated PL intensity for an image taken using the dye emission filter and  $EM_{\text{QD}}$  is the integrated PL intensity for an image taken using the QD emission filter. The subscript *target* represents the image of the spot taken in the presence of the target DNA and the subscript *probe* represents the images of the spot taken in the absence of the target DNA.

## **2. Additional Results and Discussion**

### **2.1 Loading capacity of the QDs**

The loading capacity of Qdot™ 565 ITK™ Streptavidin Conjugates has been calculated using a method published elsewhere<sup>1</sup>. Briefly, QD-probe conjugates were prepared by mixing DNA probes labeled with 3' biotin and 5' Cy-5 dye with streptavidin coated QDs at 70x equivalents in 100 mM Tris Borate (TB) buffer with 20 mM NaCl at pH 7.4. After an hour, the QD-probe conjugates were purified from any excess DNA by centrifugation using Amicon Ultra-0.5, 50 kDa centrifugal filter (Millipore Corporation, Billerica, MA). The absorption spectrum of the purified QD-probe conjugates was measured in addition to two reference samples. One of the reference samples was a 5  $\mu\text{M}$  solution of the labeled DNA probe and the other, 200 nM solution of streptavidin conjugated QDs. The ratio of the absorbances at 400 nm of the reference QD sample and the QD of the purified sample, was used to calculate the concentration of the QDs in the purified sample. The ratio of the absorbances at 640 nm of the reference probe sample and the probe in the purified sample were used to calculate the concentration of the probe. The ratio of the concentration of the probe to the QD concentration was used to calculate the loading capacity of the QDs.

## 2.2 Gel electrophoresis

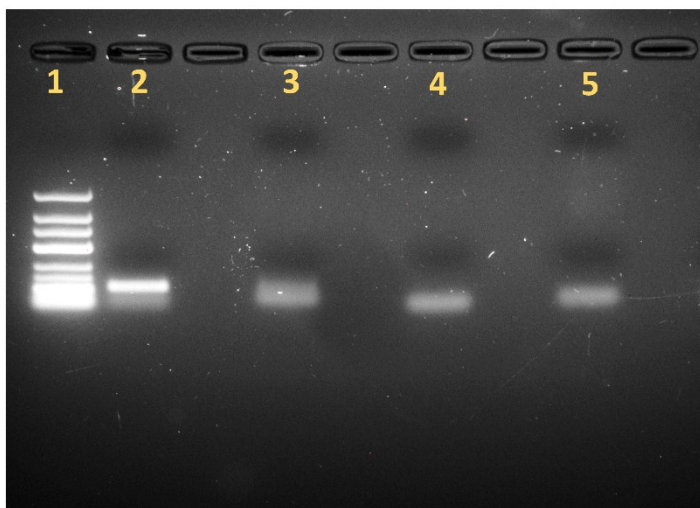


### 2.2.1 QD-Probe conjugates

**Figure S1.** Image of gel electrophoresis slab. 1 – streptavidin coated QDs; 2 – streptavidin coated QDs with 35x DNA probe; 3 - streptavidin coated QDs with 50x DNA probe; 4 - streptavidin coated QDs with 70x DNA probe; 5 – DNA ladder

The immobilization of the dye-labeled probes on the surface of the QDs was confirmed using gel electrophoresis (Figure S1). The first well was loaded with QDs with no probe; the second, third and fourth wells were loaded with QD-probe conjugates at 35, 50 and 70 equivalents respectively. The slab was made from 2% agarose gel in 1× TAE running buffer at pH 8.0. The stock for the TAE running buffer was made using 242g TRIZMA base, 57.1mL glacial acetic acid, and 100 mL of 500 mM EDTA. The concentration of the QDs in the 25  $\mu$ L samples was in the range of 20-25 nM. The gel electrophoresis image shows evidence of QD-probe conjugation as the samples that were incubated with the DNA probes moved further down the gel than the sample of QDs that was not incubated with DNA probes. These results were expected as the electrophoretic mobility of the QD-probe conjugates is higher than the unconjugated QDs due to the negative charge associated with the phosphate groups of the DNA probe backbones. The 35x QD-probe conjugates did not travel the same distance as the 50x and 70x which suggests lower probe density on the surface of

the QDs. The lane containing the 70x QD-probe conjugates has an additional band containing the excess DNA probes. The lane containing 50x QD-probe conjugates do not have an additional band suggesting surface saturation which supports the experimentally determined loading capacity of 46 probes for these streptavidin coated QDs.



### 2.2.2 Analysis of PCR sample

**Figure S2.** Image of gel electrophoresis slab. 1 – GeneRuler Low Range DNA ladder; 2 – PCR mix with template and both primers; 3 – PCR mix with template and Forward primer; 4 – PCR mix with template and Reverse primer; 5 – PCR mix with both primers and no template

Amplification of the target samples was verified using gel electrophoresis (Figure S2). The gel was prepared using 2.5 g of agarose in 125 mL of running 1x TAE buffer prepared from a stock TAE. The amplification of the target was evident from the presence of a band characteristic of a 100mer oligonucleotide in Lane 2. Lanes 4, 5 and 6 did not contain the template, the forward primer, or both primers respectively and do not show any amplification. The 3<sup>rd</sup> also lane had a band that is characteristic of a 100mer oligonucleotide. This was due to asymmetric amplification of the template caused by the presence of the forward primer and the absence of the reverse primer.

Since asymmetric amplification is linear in nature, the band was much fainter than the one produced by standard PCR.

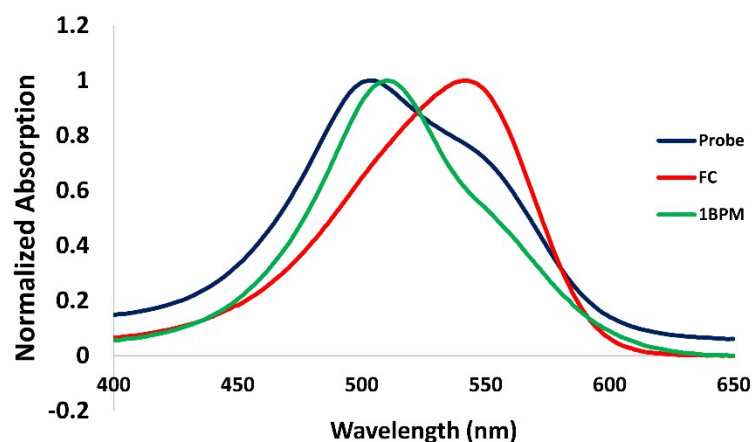
### 2.3 Quantum yield calculations

The quantum yield of the dye-labeled probe in presence and the absence of the target in 100 mM TB buffer (pH 7.4) was estimated to be 15.6 % and 8.1 % respectively using a single point approximation technique. The reference dye was Alexa 546 with a reported quantum yield of 79 % in 50 mM potassium phosphate, 150 mM NaCl, pH 7.2<sup>2</sup>.

$$Q = Q_R \left( \frac{I}{I_R} \right) \left( \frac{OD_R}{OD} \right) \left( \frac{n^2}{n_R^2} \right)$$

Equation S2

where Q represents quantum yield; OD and OD<sub>R</sub> stands for absorption of dye and reference respectively; I and I<sub>R</sub> are the integrated fluorescence intensity of dye and references respectively; and n is the refractive index of the solution<sup>2</sup>. The integrated fluorescence intensity was calculated using trapezoidal approximation. The excitation wavelength used was 545 nm (Figure S3). The refractive indices were assumed to be equal to 1.33.

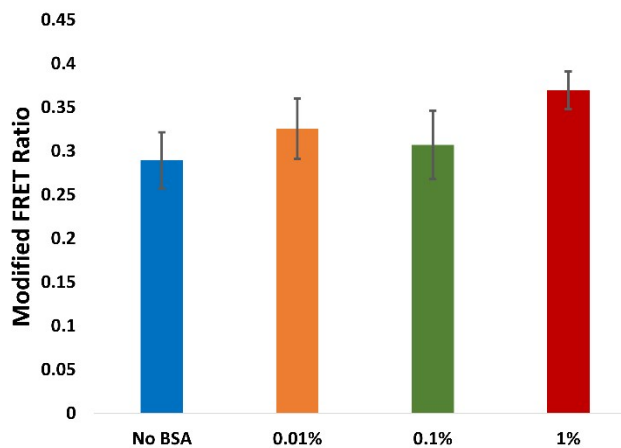


## 2.4 Absorption spectrum

**Figure S3.** Absorption spectrum of dye-labeled probe in the presence and absence of a complementary target as well as in the presence of a target with a single base pair mismatch.

## 2.5 Non-specific adsorption

The surface of the paper was blocked using BSA to determine whether some DNA mass transfer may have been impeded by non-specific adsorption on the paper. No significant difference between the signals in the presence and the absence of BSA were observed which suggests that



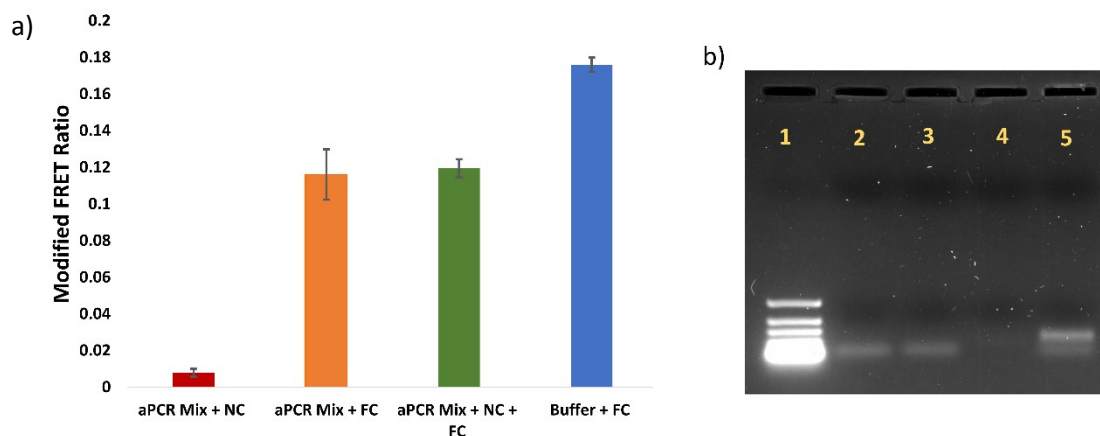
there is no significant loss of DNA (Figure S4).



**Figure S4.** Response of the assay to FC target in the presence of varying concentrations (%w/v) of BSA used to block the surface of the paper.

## 2.6 Assay Selectivity in aPCR samples

To examine whether the assay will be useful to analyze samples produced by asymmetric PCR



(aPCR), the signal obtained from pure single-stranded DNA target dissolved in buffer was compared to the signal from a aPCR mix spiked with the pure target. The aPCR was conducted using a 30:1 ratio of reverse to forward primer. The results showed that the signal obtained from a spiked aPCR mix was similar to the signal obtained from the clean buffer. Any difference between clean buffer and the aPCR mix could be attributed to the presence of excess primers.

**Figure S5. a)** Response of the assay to FC target in comparison to the response obtained from NC target in the presence of aPCR mix. **b)** Image of gel electrophoresis slab. 1 – GeneRuler Low Range DNA ladder; 2 – PCR mix with reverse primer and no template; 3 – PCR mix with reverse primer and NC template; 4 – PCR mix with template and no primer; 5 – PCR mix with template and reverse primer.

## References

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3. J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: New York, 2006