Electronic Supplementary Information (ESI)

Immobilisation of quantum dots by bio-orthogonal PCR amplification and labelling for direct gene detection and quantitation

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Experimental Section

Materials and Methods: Polymerase chain reaction (PCR) reagents, including Taq DNA polymerase with buffer, dNTP mixture and pCMV-GLuc plasmid, were obtained from New England Biolabs, Inc. (Ipswich, MA, USA). Synthetic oligonucleotides, labelled with biotin (Bt) or digoxigenin (Dig), were purchased from Integrated DNA Technologies (Coralville, IA, USA). Power SYBR Green PCR Master Mix was from Applied Biosystems (Foster City, CA, USA). Polymer-coated QDs containing carboxylic functional groups (QD-COOH) were prepared in accordance with literature reports.[1] Streptavidin (SA) was from Invitrogen (Carlsbad, CA, USA). 100 kDa molecular weight cut-off (MWCO) spin filters were purchased from Sartorius Stedim Biotech S.A. (Aubagne Cedex, France). Boric acid and *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anti-digoxigenin (antiDig) and blocking reagent were from Roche Diagnostics (Indianapolis, IN, USA). The water used was of Milli-Q grade purified by a Milli-Q UV Purification System of Sartorius Stedim Biotech S.A. (Aubagne Cedex, France). All other chemicals were of analytical grade obtained from commercial sources.

Preparation of streptavidin-coated QDs (QD-SA): Attachment of SA to QD-COOH was carried out using EDC as the coupling agent, which mediated the formation of covalent amide bonds between the carboxylic groups on the QD surface and the amine groups on SA. Briefly, QD-COOH (8 μM, 104 μL) were diluted in borate buffer (10 mM, pH 7.4, 729 μL),

SA solution (10 mg/mL, 200 µL), and EDC (10 mg/mL, 23.75 µL) and gently agitated at r.t. for 2 h. SA-coated QDs (QD-SA) formed were washed five times with borate buffer (50 mM, pH 8.3, 5 mL) on the 100 kDa MWCO spin filter to remove any excess unbound protein. The purified QD-SA was dissolved in borate buffer (50 mM, pH 8.3, 2 µM) and stored at 4 °C in the dark until use.

Preparation of 384-microtiter plate coated with Dig antibody (antiDig-plate): NUNC Maxisorp F384 black plate was used for the surface immobilization of antiDig, which exhibits high affinity to polar groups/hydrophilic molecules through its hydrophilic surface. The procedure for coating of the microtiter plate with antiDig was a slight deviation from the manufacturer's instructions (Roche). Wells of microtitre plate were coated with antiDig in PBS (10 µg/mL, 20 µL) at 4°C for 12 h. One well was only incubated with PBS without antiDig as a negative control (blank). Thereafter, the wells are treated with blocking buffer (1% blocking reagent in PBS, 100 µL) to prevent non-specific binding.

PCR amplification on pCMV-GLuc template (287 bp arbitrary target): Each PCR reaction was carried out in standard Taq reaction buffer (50 µL), Taq DNA polymerase (1.25 U), dNTPs (200 µM), primers (0.3 µM) and template (1.0 ng). The PCR was performed with a Bio-Rad DNA Engine Tetrad 2 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA), using the following conditions: initial denaturation at 95°C for 30 s, followed by 25× three-step cycles (95°C for 30 s, 60°C for 30 s and 68°C for 45 s) and a final extension at 68°C for 5 min. The unlabeled primers used were 5'-AGATAACTACGATACGGGAGGG (forward) and 5'-GAATGAAGCCATACCAAACGAC (reverse). In addition, the labelled forward primer contains 5'-Bt (tetraethyleneglycol spacer) and the reverse primer 5'-Dig (with C9-spacer). The following four PCR experiments were carried out using different combinations of labelled and unlabelled primers: labelled forward and reverse primers (entry C), labelled forward and unlabelled reverse primers (entry D), unlabelled forward and labelled reverse primers (entry E), unlabelled forward and reverse primers (entry F). The corresponding no-template control (NTC) of the four PCR amplifications was also conducted in the absence of the template (entries G-J correspond to NTC of entries C-F respectively, Fig. S1). Analysis of the PCR and NTC products (entries C-F and G-J) was carried out using 2% w/v agarose gel electrophoresis with 1×SYBR Safe DNA gel stain (Invitrogen) and visualization carried out using Synene G:Box Gel Documentation system (Synoptics Ltd., Cambridge, UK) (Fig. S1).

QD-based immunodetection assay: The PCR amplicon solution (30 μL) was treated with QD-SA (2 μM, 4 μL) and PBS (10×, 5 μL) and topped up with water to 50 μL. The conjugation reaction was gently agitated for 30 min at 30°C to yield QD-SA/amplicon solution. Thereafter, 7 μL of the QD-SA/amplicon solution was diluted with blocking buffer (8 μL) and was incubated with antiDig-plate at 37°C for 4 h. Two negative controls were carried out: blank containing water in lieu of PCR reaction mixture (entry A), and conjugation reaction on uncoated microtitre wells (entry B). The wells were washed with PBS containing 0.1% Tween 20 (100 μL) and was added borate buffer (50 mM, pH 8.3, 50 μL). Fluorescence readings were measured at 365 nm excitation and 590 nm emission by Tecan Infinite M200 microplate reader (Tecan Group Ltd., Männedorf, Switzerland). The experiments were carried out in triplicates (Fig. 1). To determine the sensitivity of the assay, dual-labelled amplicons (entry C) were purified from PCR reaction mixture using Qiagen QIAquick PCR purification kit in accordance with manufacturer's instruction and quantitated by UV (BioSpec-Nano, Shimadzu). The amplicon was diluted to a starting concentration of 25 ng/μL. The experimental protocol was carried out as previously described using measured quantities of DNA amplicon from 0-80 ng. The experiments were carried out in triplicates (Fig. 2).

PCR amplification on cDNA library (HDC as target): Each PCR reaction was carried out in standard Taq reaction buffer (25 μL), Taq DNA polymerase (1.25 U), dNTPs (200 μM), primers (0.3 μM) and template (total DNA, 1 μL, 25 ng). The PCR was performed with a Bio-Rad DNA Engine Tetrad 2 thermal cycler using the conditions as before. The forward primer used was 5'-Bt-CTCCACATCGATGCTGCTTA and the reverse 5'-Dig-CCTTGACCCAGAACCCAGTA. The PCR cycle number was varied from 20-34 cycles. Conjugation to QD-SA and antiDig-plate, and fluorescence determination was carried out as previously described, which corresponded to 4.2 ng of total DNA template of HDC. The experiments were carried out in triplicates (Fig. 3). In addition, to determine the sensitivity of the assay, PCR of cycle number 30 was also carried out using varying amounts of total DNA template from 1.25-100 ng. As before, successful conjugation was achieved using the established protocols and the experiments were carried out in triplicates, equalling 0.21-16.8 ng of total DNA template of HDC (Fig. S2).

Real-time PCR amplification on cDNA library (HDC as target): The PCR reaction was carried out in Power SYBR Green PCR Master Mix (20 μL), primers (0.5 μM) and template

(total DNA, 1 μL, 25 ng). The PCR was performed with CFX96 real-time PCR detection system (Bio-Rad Laboratories, Foster City, CA, USA) using the following conditions: initial AmpliTaq Gold polymerase activation at 95°C for 10 min, followed by 40×two-step cycles (95°C for 15 s, 60°C for 1 min). The forward primer used was 5'-CTCCACATCGATGCTGCTTA and the reverse 5'-CCTTGACCCAGAA-CCCAGTA (Fig. S3).

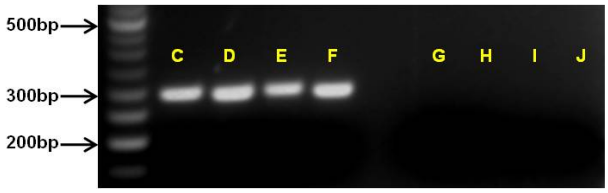

Fig S1. Gel electrophoresis analysis of PCR and NTC using combinations of labelled and unlabelled primers.

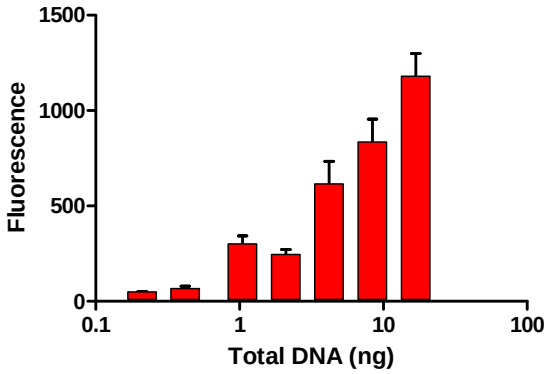

Fig S2. Fluorescence response of immunoassay on cDNA library against HDC using varying amounts of template DNA.

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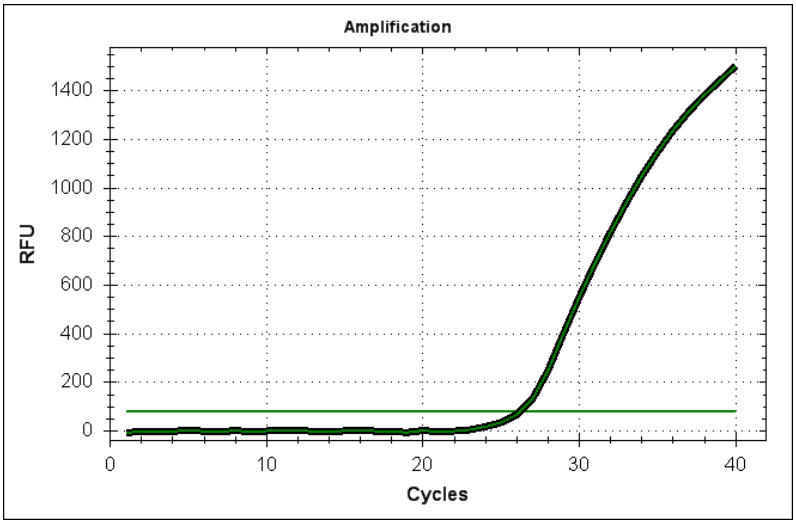

Fig S3. Real-time PCR on cDNA library against HDC.

Reference

1. (a) Y. C. Chen, R. Thakar and P. T. Snee, *J. Am. Chem. Soc.*, 2008, **130**, 3744, (b) C. B. Murray, D. J. Noms and M. G. Bawendi, *J. Am. Chem. Soc.*, 1993, **115**, 8706.