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Supporting Information

Developing a Combined Strategy for Monitoring the Progress of Aptamers Selection

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Materials and Methods

Materials and Instruments

All chemicals were of the highest available quality and used as received without any further purification. Streptavidin and alternariol were purchased from Promega and Sigma. PD-1 Cell Line bought from CrownBio and Pseudomonas syringae was gifted by Qingdao entry-exit inspection and quarantine bureau. Dynabeads magnetic beads were purchased from Invitrogen. DPBS buffer (1X with calcium and magnesium, Corning) was used as received for SELEX no extra Ca^{2+} and Mg^{2+} addition.
DNA Library and primers were synthesized by a company (Sangon, Shanghai, China). All sequences are listed in the table S1.
Optical absorption spectra were acquired using a GE NanoVue™ Spectrophotometer spectrophotometer. Fluorescence was recorded on RF-5301PC Spectro-fluorophotometer (Shimadzu). Real-time PCR were performed on Light Cycler 96 Real-Time PCR System (Roche), or CFX Real-Time PCR System (Biorad).

Real-time PCR

Real-time PCR conditions experiments were conducted on Light Cycler 96 Real-Time PCR System (Roche), or CFX Real-Time PCR System (Biorad) according to the manufacturer's instructions. All reactions were performed in 20 μl reaction volumes in 96-well plates for PCR. A standard real-time PCR mixture contained 17 μl iTaq TM universal SYBR Green Supermix(500 nM primers, 200 uM dNTP), 1 μl DNA template (16 nM), 1 μl Taq DNA polymerase (concentration refer to the manual), 1 μl evagreen (4%). The alternative 50μl real-time PCR mixture contained 42.5 μl iTaq TM Universal SYBR Green Supermix (500nM primers, 200uM dNTP), 2.5 μl DNA template (16 nM), 2.5 μl Taq DNA polymerase (concentration refer to the manual), 2.5 μl evagreen (4%). Thermal cycling consisted of an initial denaturation at 98 ℃ for 30 s followed by 30 cycles of denaturation at 98 °C for 10 s or 30 s according to different DNA polymerase, annealing at 58 °C or 68 °C for 10 s or 30 s, and extension at 72 °C for 10 s or 30 s. After 30 cycles amplification, the melting curves analysis was performed from 50 °C to 95 °C. Threshold cycle (C_t) values were determined by automated threshold analysis.

SA aptamer selection

Carboxylic acid dynabeads (Invitrogen) were used to immobilize SA. The magnetic beads were activated with EDC and NHS and SA were immobilized after activation, following a manufacturer's procedure and a previously reported procedure with minor modifications[1]. The immobilized proteins were quantified using the NanoOrange® protein quantitation kit (Invitrogen). Each member of the ssDNA random library included 40 randomized nucleotides flanked by two 20-base primer binding sequences for qPCR. SA-coated magnetic beads were washed three times with 100 μL of DPBS

buffer (1X with calcium and magnesium, Corning) before each selection. 4.44×10^7 SA-coated beads were used in the first round of positive selection, and 2.22×10^7 SA-coated beads were used for the next two rounds to maintain highly stringent selection conditions. The ssDNA library ($\sim 10^{14}$ molecules) was denatured by heating at 95 °C for 10 min, and then quickly cooled on ice 10 min. This ssDNA library was incubated with SA-coated beads in binding buffer for 2 h at room temperature. After incubation, the beads were trapped by magnet.

The trapped beads were washed with binding buffer. Three times washing was subsequently performed to continuously remove unbound and weakly bound DNAs. The aptamer-bound beads were collected in a final volume of 1mL at the end of each round of selection. The eluted ssDNA aptamers were tested concentration by nanodrop absorption spectra, then diluted to 1.6 nM and amplified by qPCR to generate ssDNAs for next-round selection and high-throughput sequencing. To eliminate nonspecific aptamers, counter selections were also performed.

The affinity and selectivity of the selected aptamers for SA was determined by Biacore assay as used before.[2]

PD1-Cell SELEX:

To select aptamers targeting PD1, we used a PD1-overexpressing 293 cancer cell line. SELEX method was used as previously described with some modification.[3, 4] In brief, in the first round of selection, DNA library (1μM) was dissolved in binding buffer (DPBS buffer from Corning), denatured by heating at 95 °C for 10 min, and cooled on ice for 10 min. The library was then incubated with target control cell confluent monolayer in a flask at room temperature. Then, the supernatant of the control cell was added into the target cells and incubated 1 hour at room temperature. Cells were then washed three times using binding buffer. Bound ssDNA aptamers were digested by trypsin. The cell solution was directly used as the template of qPCR. The counter selection was used to reduce background binding and to exclude DNA sequences that could bind to common biomarkers present on the surface of both cell lines. The recovered single-stranded DNAs (ssDNAs) were then dissolved in 1 mL of binding buffer and quantified by ultraviolet measurement. The affinity was determined by fluorescent spectrometry. The binding assays were carried out by incubating 200 pmol of fluorescently labeled aptamer/aptamer pool with 10^7 cells for 30 min in binding buffer and then washing the cells once prior to resuspension in binding buffer for immediate flow cytometric analysis. Forward scatter, side scatter, and fluorescence intensity were measured, and gated fluorescence intensity above background (cells with no aptamers added) was quantified.

Live Bacteria SELEX

The SELEX procedure used in this work was based on a method developed by Hamula et al. with some modifications.[5] Pseudomonas syringae were grown 6 hours in liquid culture and harvested upon reaching logarithmic phase (minimum OD600 of 0.6). The cells were centrifuged at 8000 rpm at 4 °C to remove media and

subsequently washed twice in 3ml binding buffer (DPBS buffer from Corning) at room temperature. SELEX was initiated with the ssDNA library (1 μM), which was denatured by heating at 94 °C for 10 min and subsequently cooling at 0 °C for 10 min. The denatured ssDNA library was incubated with 10^9 cfu/mL pseudomonas syringae cell in binding buffer at room temperature for 2 hours. Following incubation, unbound ssDNA were washed away with 3 mL of binding buffer by centrifugation at 8000 rpm and 4 °C for 10 min. Bound ssDNA aptamers were eluted by heating the bacteria bound aptamer complexes at 94 °C for 10 min. The mixture was centrifuged as described above, and the supernatant containing aptamers that had affinity to pseudomonas syringae was isolated. The collected fractions were amplified by qPCR. All qPCR products were purified using a Qiagen MinElute PCR Purification Kit (Qiagen Inc., Valencia, CA). Negative controls consisting of cells incubated with all medium components but without the oligonucleotide libraries were prepared for each round of selection. The counter-selection against a mixture of other bacteria (*E. coli*) were introduced in the 3rd and the 5th rounds, respectively, to ensure that the selected aptamers maintain high species specificity for the target Pseudomonas syringae. The affinity was determined by fluorescent spectrometry.

Alternariol aptamer selection

A single-stranded DNA (ssDNA) pool containing 40 randomized nucleotides was designed to have 5□ terminal complemented to the capture oligonucleotide (5□-biotin-3□). The biotin-capture oligonucleotides could bind to the streptavidin-immobilized Dynabeads as a library sample. SA immobilized dynabeads (Invitrogen) were prepared as in SA selection.

For a given round of selection, ssDNA pool and 3'-biotin-labeled capture oligonuecleotide were denatured in elution buffer at 95 °C for 5 min and then cooled down slowly to room temperature. The hybridized DNA sample was subsequently incubated and bound with streptavidin-agarose beads for 15 min at room temperature to bind onto the beads based on the biotin-streptavidin interaction. Unbounded DNA was washed away. Then the ssDNA library-capture oligonucleotide complex bound magnetic beads were subjected to a positive selection process. The magnetic bead was treated with alternariol solution and incubated at room temperature for 2 hours. Then the wash solution was collected for 3 times to obtain the eluted nucleicacid sequences that are supposed to have good binding with alternariol. All the collected fractions were amplified by qPCR. The new enriched DNA pool was used for the next round of selection. The affinity of enriched DNA library was determined by fluorescent spectrometry. Fluorescence of alternariol (10 μM) was detected at 430 nm with excitation at 330 nm under different concentration of ssDNA (0-20 μM).

High-throughput sequencing and bioinformatics analysis

High-throughput sequencing was done by the Novogene Bioinformation Technology Co., Ltd (Tianjin, China). Barcodes were attached by PCR to the pools of all selection rounds for SA. All the selection rounds pools for one target mixed together as one

sample. Then samples were sent to Tianjin Novogene Bioinformatics Technology Co., Ltd for sequencing. Reads processing and data analysis were conducted for each selection enriched library were mapped to each read using RazerS.[6]

Supported Table and Figures

Table S1 the sequences of initial library and the primers

Target	DNA species	Sequences
SA	DNA library	**Lib1** AGCAGCACAGAGGTCAGATG(N40)CCTATGCGTGCTACCGTGAA
	Primers	Lib1F1: AGCAGCACAGAGGTCAGATG Lib1F2: FAM-AGCAGCACAGAGGTCAGATG Lib1R1: TTCACGGTAGCACGCATAGG Lib1R2: polyA20(C18)-TTCACGGTAGCACGCATAGG
Pseudomonas syringae	DNA library	**Lib1** AGCAGCACAGAGGTCAGATG(N40)CCTATGCGTGCTACCGTGAA
	Primers	Lib1F1: AGCAGCACAGAGGTCAGATG Lib1F2: FAM-AGCAGCACAGAGGTCAGATG Lib1R1: TTCACGGTAGCACGCATAGG Lib1R2: polyA20(C18)-TTCACGGTAGCACGCATAGG
PD1-Cell	DNA library	**Lib1** AGCAGCACAGAGGTCAGATG(N40)CCTATGCGTGCTACCGTGAA
	Primers	Lib1F1: AGCAGCACAGAGGTCAGATG Lib1F2: FAM-AGCAGCACAGAGGTCAGATG Lib1R1: TTCACGGTAGCACGCATAGG Lib1R2: polyA20(C18)-TTCACGGTAGCACGCATAGG
Alternariol	DNA library	**Lib2** ATTGGCACTCCACGCATAGG(N40)CCTATGCGTGCTACCGTGAA
	Primers	Lib2F1: ATTGGCACTCCACGCATAGG Lib2F2: FAM-ATTGGCACTCCACGCATAGG Lib2F3: CCTATGCGTGGAGTGCCAAT-biotin Lib1R1: TTCACGGTAGCACGCATAGG Lib1R2: polyA20(C18)-TTCACGGTAGCACGCATAGG

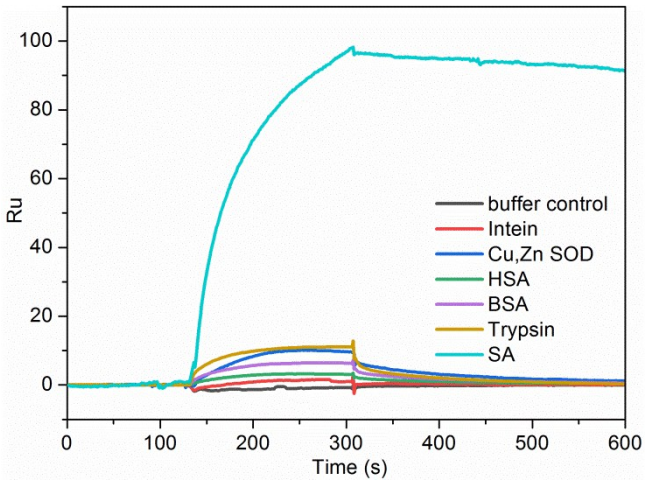

Figure S1 Selectivity analysis of pool 4 interaction with SA and other proteins by SPR. Intein is RecA inteins from *Mycobacterium tuberculosis*. Cu,Zn SOD is superoxide dismutase with Cu^{2+} and Zn^{2+} ions. HAS is human serum albumin. BSA is bull serum albumin. The concentration of the selected aptamers was 0.1 μM. Data are presented as real-time graphs of response units (RU) against time and evaluated using BIA evaluation 4.0 software (Biacore).

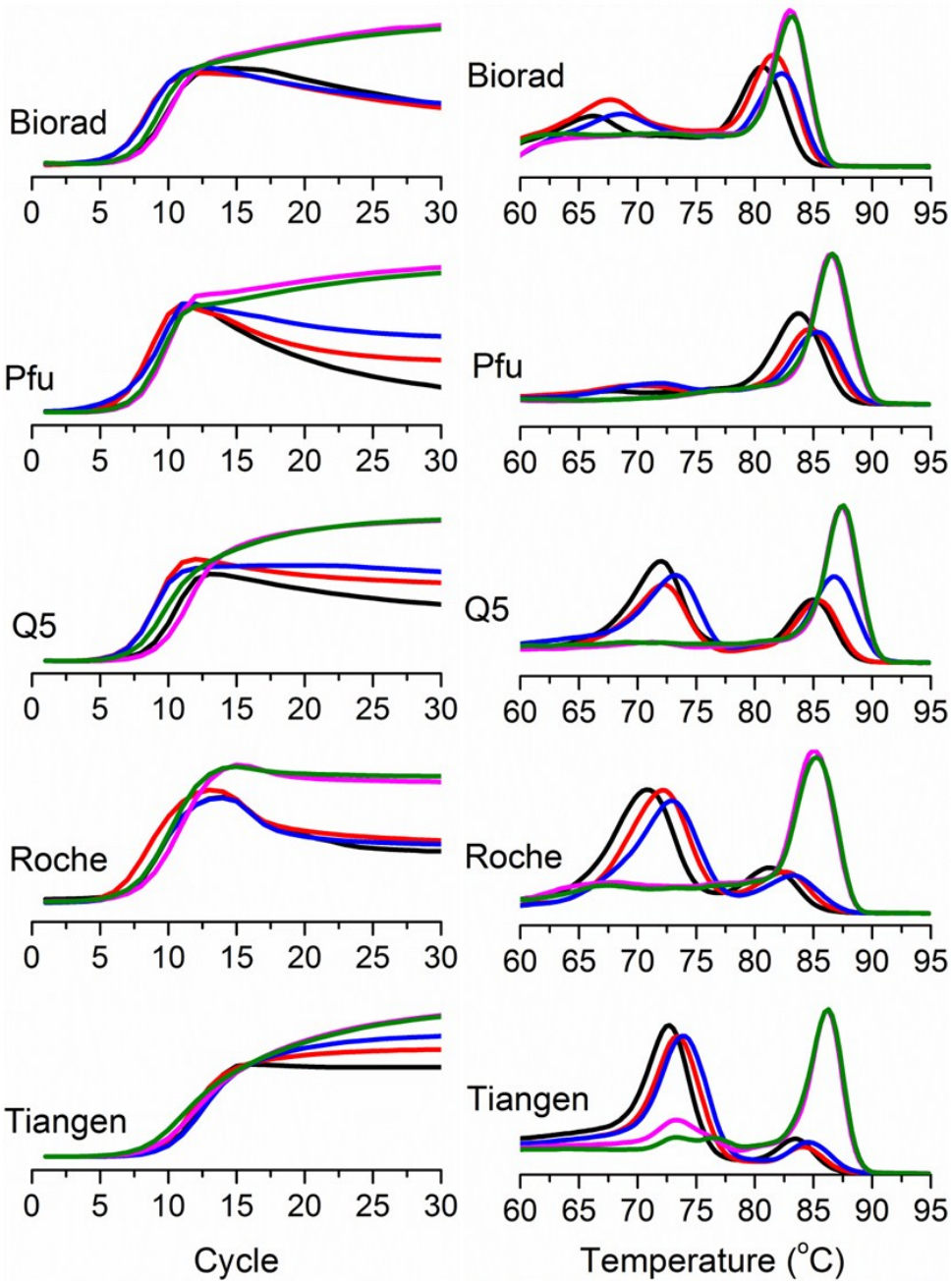

Figure S2. Different DNA polymerases were used in qPCR according to the manufacturer's instructions. Amplification curve (right) and melting curve (left) of every round ssDNA library during SA aptamer selection. Black, red, blue, magenta and olive are for the first, second, third, fourth and fifth round selection. 20 μL reaction volumes containing 500 nM primers, 200 μM dNTP, 16 nM DNA template, 4% evagreen and DNA Polymerase (according to the manufacturer's instructions, different name was purchased from different company) on Light Cycler 96 Real-Time PCR System (Roche).

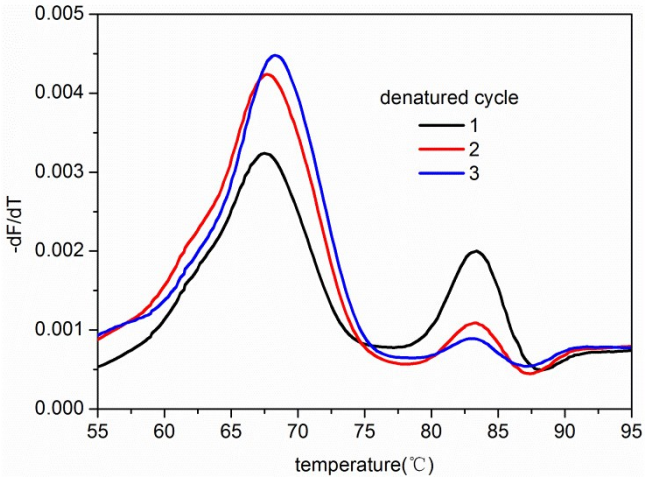

Figure S3 melting curve of pool 3 to SA aptamer selection under three times repetitive annealing.

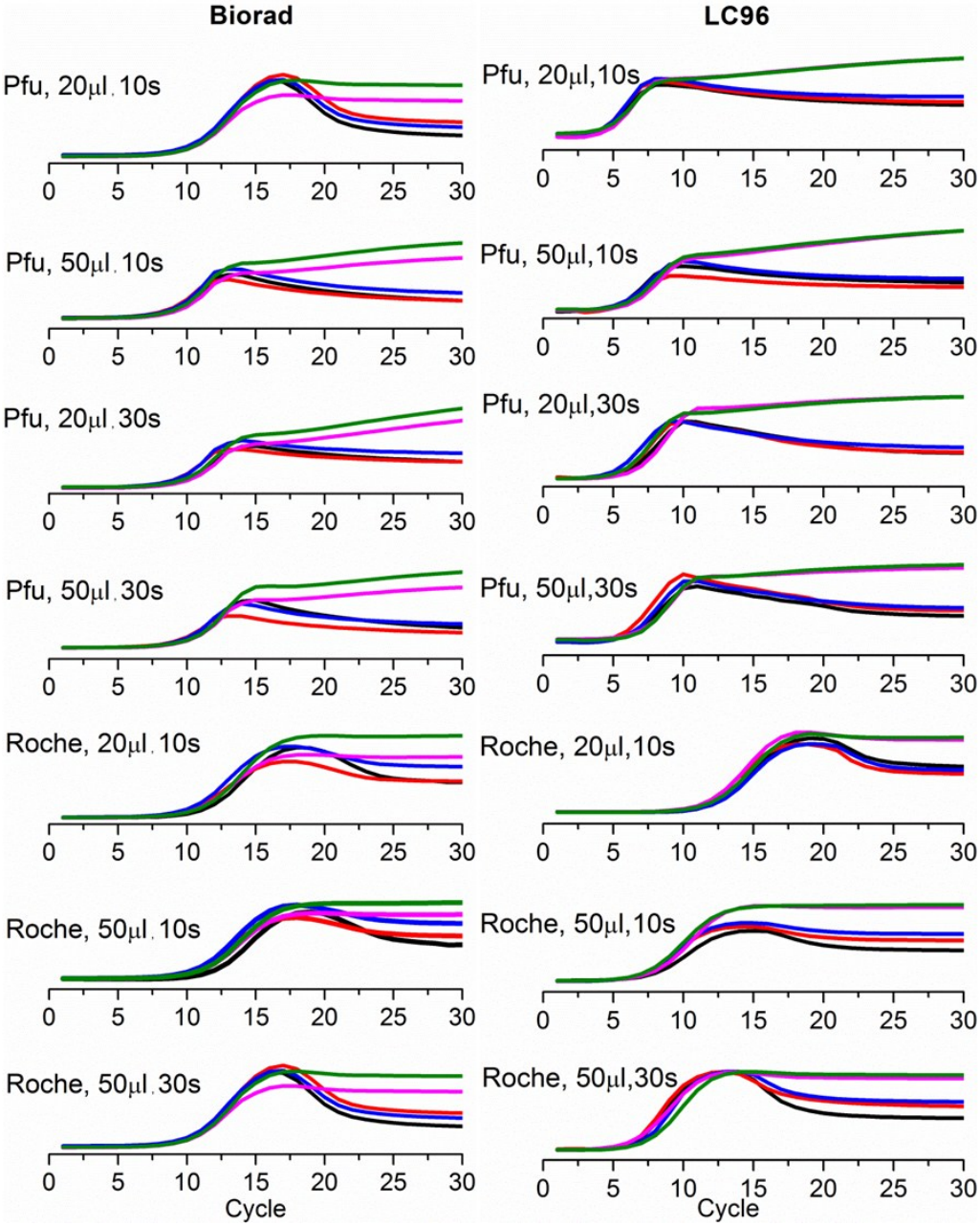

Figure S4. Amplification curves changes under different volume(50 or 20 μL), extension time(at 72 °C 30 s or 10 s extension), DNA polymerase(Pfu DNA polymerase from Sangon or Roche DNA polymerase from Roche) and qPCR devices (Cycler 96 Real-Time PCR System, Roche, or CFX Real-Time PCR System, Biorad). Black, red, blue, magenta and olive are for the first, second, third, fourth and fifth round selection of SA aptamer.

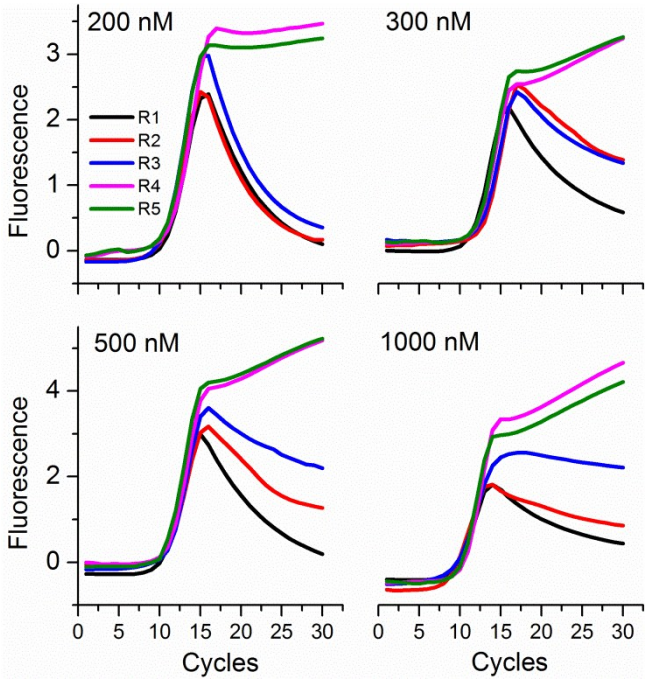

Figure S5 Different concentrations of primer (200-1000 nM) affect the amplification curve during qPCR. 20 μL reaction volumes containing 1.6 nM DNA template, 200 μM dNTP, 4% evagreen, DNA template(50-0.01 nM) and Q5® high-fidelity DNA Polymerase(20 U mL-1) on Light Cycler 96 Real-Time PCR System (Roche).

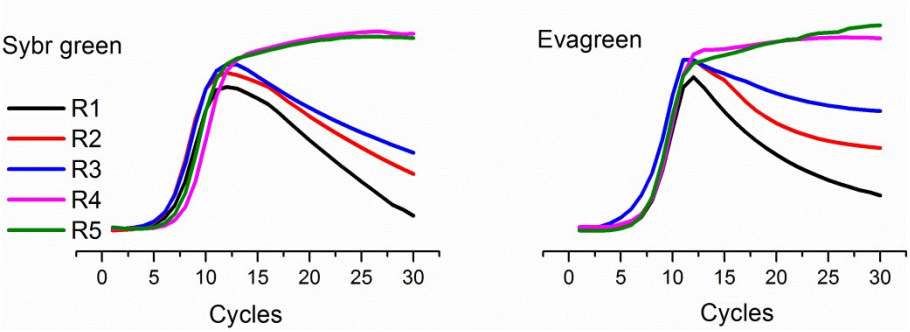

Figure S6. Different dyes affect the amplification curve during qPCR. 20 μL reaction volumes containing 1.6 nM DNA template, 200 μM dNTP, 200nM primer, DNA template(50-0.01 nM) and Q5® high-fidelity DNA Polymerase(20 U mL-1) on Light Cycler 96 Real-Time PCR System (Roche).

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