Electronic Supplementary Material (ESI) for Chemical Communications


# Supporting Information

# Aptamer－Based Polymerase Chain Reaction for Ultrasensitive Cell Detection

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## Materials and Measurement

KH1C12-FITC (FAM-ATCCAGAGTGACGCAGCATGCCCTAGTTACTACTACTCTTTTTAGCAAACGCCCTCGCTTTGGACACGGTGGCTTAGT), PCR primer (FW: ATCCAGAGTGACGCAGCA; RV: ACTAAGCCACCGTGTCCAA) were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. FAM-ACGCTCGGATGCCACTACAGTGGGAATAGTACCGTTATTCGGACTCCGTGACAATCTGCTCATGGACGTGCTGGTGAC, PCR primer (FW: ACGCTCGGATGCCACTACAG; RV: GTCACCAGCACGTCCATGAG) were synthesized by invitrogen technology Co., Ltd (Shanghai). HotMaster Taq DNA polymerase, ultrapure dNTP were obtained from Tiangen Biotech (Beijing) Co., Ltd. Shrimp alkaline phosphatase (SAP) was obtained from TaKaRa Biotechnology Co., Ltd. (Dalian, China). The dGTP-Fl and dUTP-Fl were purchased from Fermentas. PFP was synthesized according to the procedure in the literature [1]. 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's phosphate buffered saline with calcium chloride and magnesium chloride was prepared and used as wash buffer. The binding buffer used for selection processes of cells with aptamers or nonspecific DNA was prepared by adding BSA (1 mg/mL) into the wash buffer.

HL 60 (peripheral blood), Jurkat (T lymphocyte), A549 (pulmonary), SK-BR-3 (breast), fibroblast (normal), Hela (cervical), KB (breast), MCF-7 (Breast), A498 (renal), and HepG2 (Liver) cells were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). HL 60 cells were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) supplemented with 20% fatal bovine serum (FBS). Jurkat, SK-BR-3, KB cells were cultured in

RPMI 1640 supplemented with 10% FBS. Other cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS. They were incubated at 37 $^{o}$C in a 5% $CO_2$ atmosphere.

All the PCR reactions were carried out in Mycycler Thermocycler (Bio-Rad, cat. no. 170-9703). DYY-6C electrophoresis device (Beijing Liuyi Instrument Factory) was used for gel electrophoresis analysis. Fluorescence microscopy were snapped using fluorescence microscopy (Olympus 1×71with a filter D455/70 nm exciter, 500 nm beam splitter, and D525/30 nm emitter.) The magnification of object lens is 10×. Fluorescence spectra were obtained from a Hitachi F-4500 fluorometer equipped with a Xeron lamp excitation source.

**Cell-aptamers binding procedures:** KH1C12-FITC and nonspecific DNA sequence were denatured at 95 $^{o}$C for 5 min and quickly cooled on ice for 10 min. The FITC-labeled aptamer at a final concentration of 0.05 μM was incubated with target cells in 200 μL binding buffer containing 20% FBS. Approximately $5\times10^{6}$ cells were washed twice with wash buffer before incubation with aptamer or nonspecific DNA sequence, for adherent cells, they were trypsinized with 0.25% trypsin, washed with wash buffer twice, and suspended in binding buffer. KH1C12-FITC or nonspecific DNA sequence were subsequently added to cell suspensions and incubated for 2 h 4 $^{o}$C, respectively. And cells were washed with buffer through centrifugation at 1000 rpm for 5 min 3 times to remove unbound DNA sequences, and finally resuspended in 200 μL of $H_2O$ [2]. The cell density was determined using a hemocytometer, and this was performed prior to any experiments. For fluorescence microscopy imaging, a drop of cell suspension was placed on a glass slide and imaged immediately by fluorescence microscopy (Olympus 1×71) with 500 ms exposure time. Prior to PCR amplification, the cells were lysed by freezing and thawing three times.

**PCR amplification conditions:** The PCR mixture contained 2 μL of 10×HotMaster Taq DNA polymerase PCR buffer, 1 μL of 10 μM FW primer, 1 μL of 10 μM RV primer, 2 μL of dNTP and dUTP-Fl, dGTP-Fl mixture (10 μM of dATP, dCTP, 5 μM of dUTP-Fl, dGTP-Fl, dUTP and dGTP), 0.2 μL of 2.5 unit/ μL HotMaster Taq DNA polymerase, and 2 μL template DNA in a total volume of 20 μL solution. The templates are different concentrations of aptamers and cells-aptamers conjugates, respectively. After 95 $^{o}$C for 3 min, the thermocycling parameters were as follows: 30 cycles at 94 $^{o}$C for

30 s and 60 °C for 1min. After the cycle reaction, the extension reaction was carried out for 5 min at 65 °C, and then the reaction products were held at 4 °C.

**Fluorescence measurement:** Before fluorescent measurement, 4 μL shrimp alkaline phosphatase (0.5 unit/μL) was added into PCR products and was incubated at 37 °C for 30 min to degrade excess dNTP-Fl. After that, 586 μL HEPES, 8 μL 15 μM PFP, and 6 μL the product were added into a cuvette. Then the emission spectra were measured an excitation wavelength of 380 nm. The slit width and PMT voltage of the measurements were 5 nm and 700 V, respectively.

**LOD determination:** The limit of detection (LOD) of cell-specific aptamers PCR approach was obtained from Equation 1 in which $S_0$ is the standard deviation of the background and S is the sensitivity [3]. LOD of this method was determined to be 0.13 cells.

$$LOD = 3 \times S_0 / S \qquad (1)$$

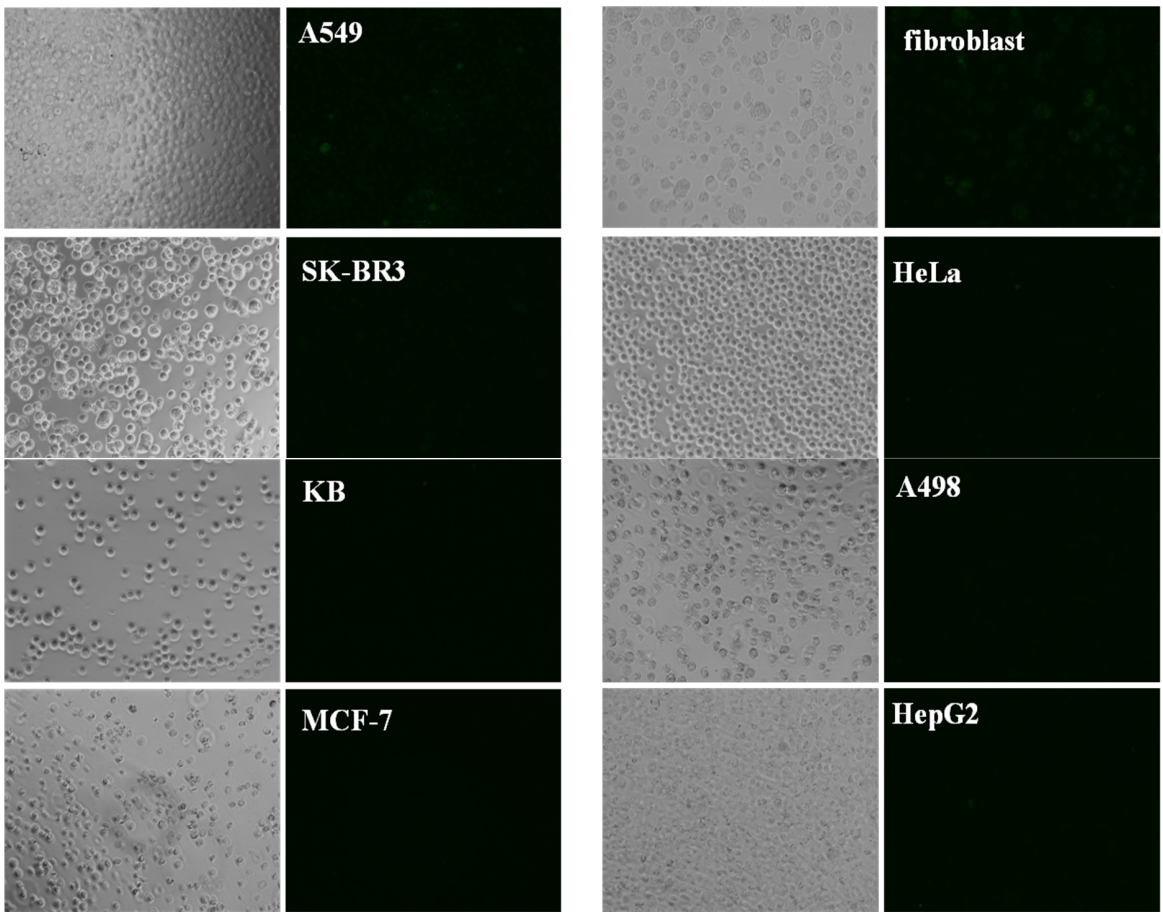


**Figure S1**. Phase contrast bright-field images (left) and fluorescence images (right) of A549, fibroblast, SK-BR-3, Hela, KB, A498, MCF-7, HepG2 cells after incubated with FITC-KH1C12. Images were

snapped using fluorescence microscopy (Olympus 1×71) with 500 ms exposure time. The false color of fluorescein is green and the type of light filter is D455/70 nm exciter, 500 nm beam splitter, and D525/30 nm emitter. The magnification of object lens is 10×.

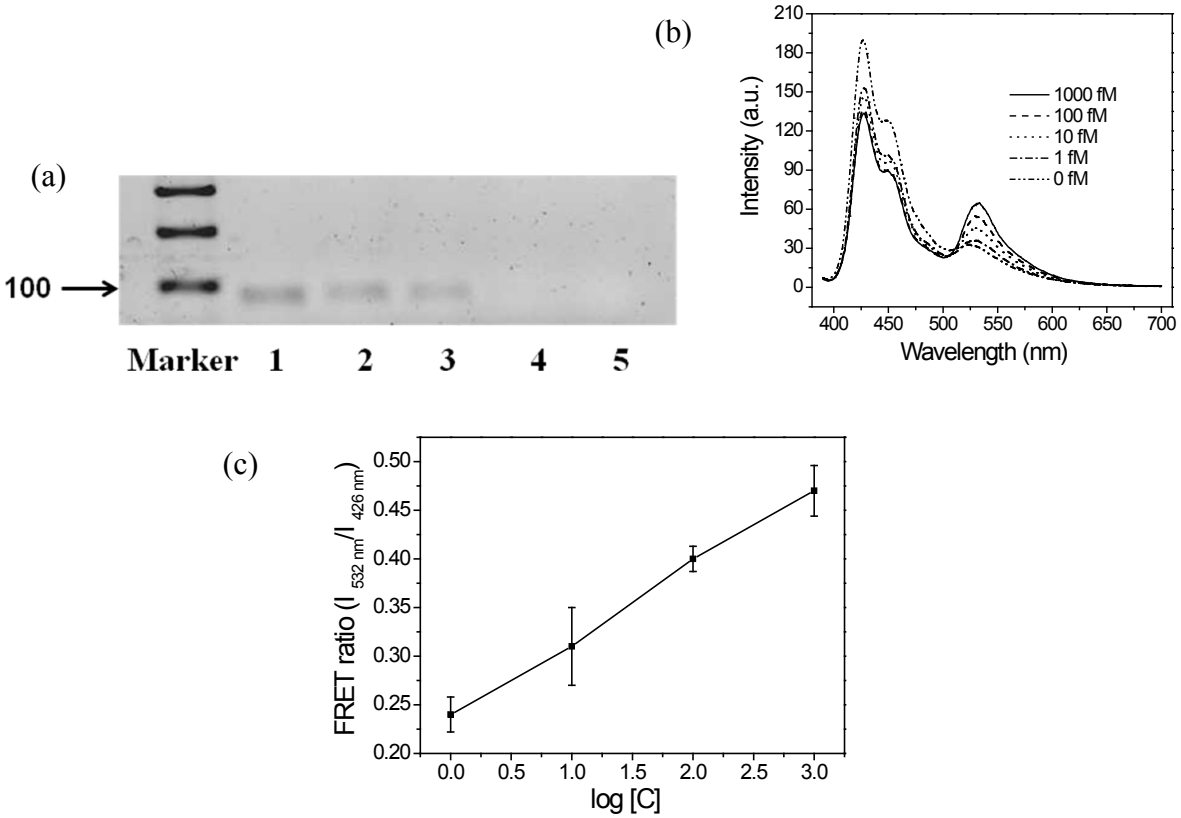


**Figure S2**. (a) Gel electrophoresis analysis of PCR-amplified products of which different concentrations of aptamers (1-5 stand for 1000 fM, 100 fM, 10 fM, 1 fM, 0 fM) were used as template. (b) Emission spectra from solutions containing PFP and five PCR-amplified products. The final concentration of PFP is $2\times10^{-7}$ M in RUs, and the excitation wavelength is 380 nm. (c) FRET ratios ($I_{532\ nm}/I_{426\ nm}$) of four samples vs. log [C]. Error bars represent the standard deviation of three experiments.

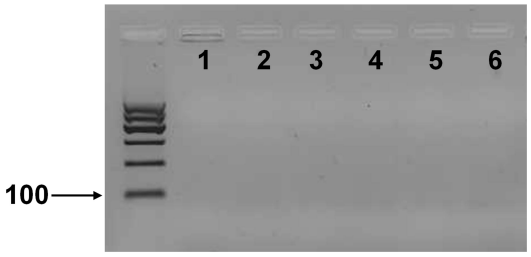

**Figure S3**. Gel electrophoresis analysis of PCR-amplified products. 1, 2, 3, 4, 5, 6 stand for PCR systems containing 2 μL of $10^6$ cell/mL, $10^5$ cell/mL, $10^4$ cell/mL, $10^3$ cell/mL, $10^2$ cell/mL lysed HL 60 cell suspensions, and pure water as negative control, respectively.

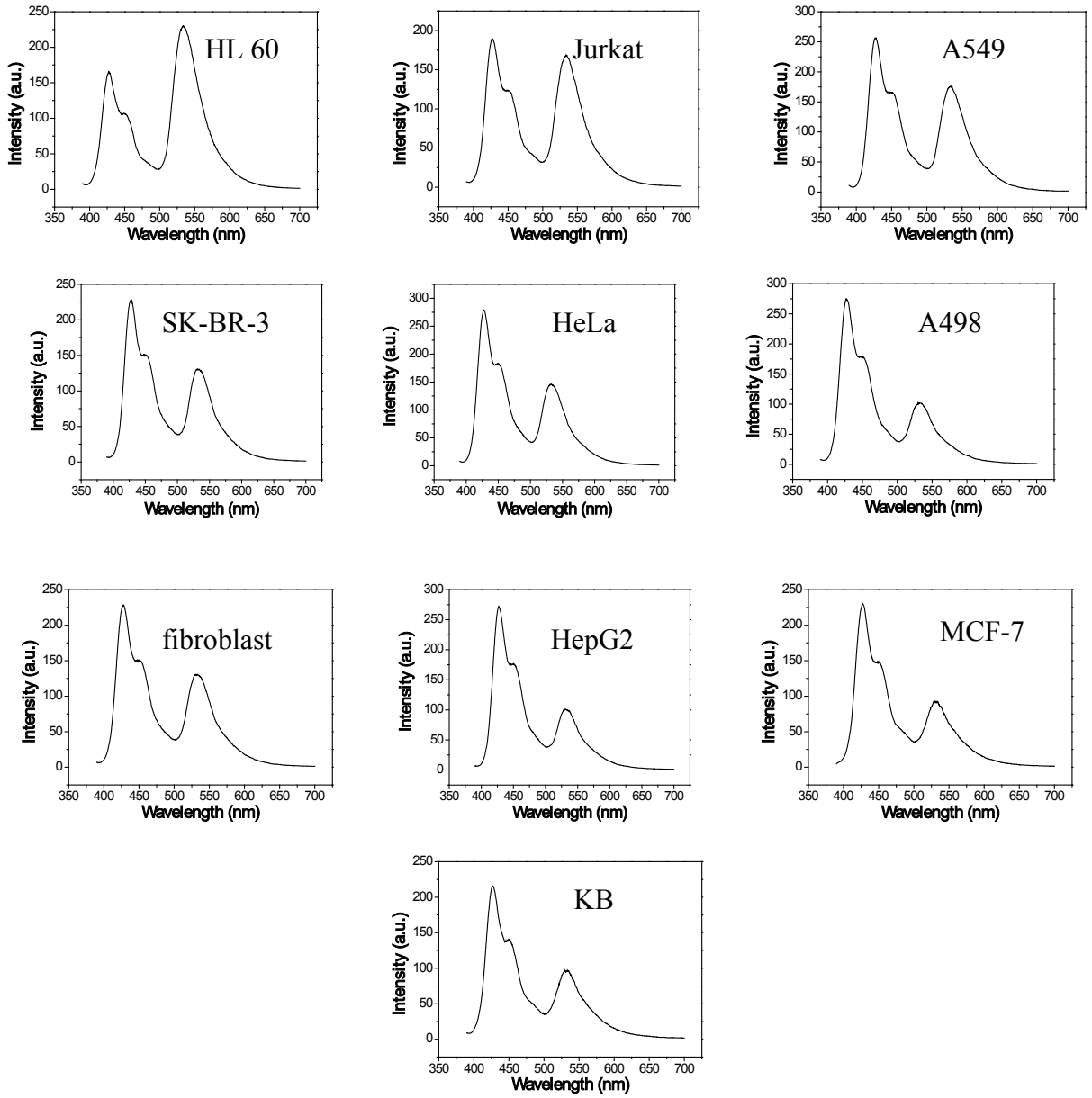


**Figure S4**. Fluorescence emission spectra of PFP with PCR products mixture of different cells-aptamer conjugate as template. The final concentration of PFP is $2\times10^{-7}$ M in RUs, and the excitation wavelength is 380 nm.

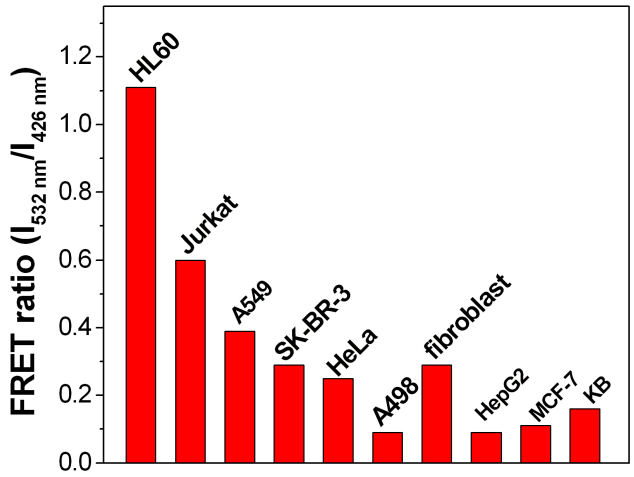


**Figure S5.** FRET ratio ($I_{532nm}/I_{426nm}$) as a function of cell types. The values were calculated from emission spectra, subtracting the signal of the negative control. For each cell type, 2000 cells were used as template for FRET response measurement.

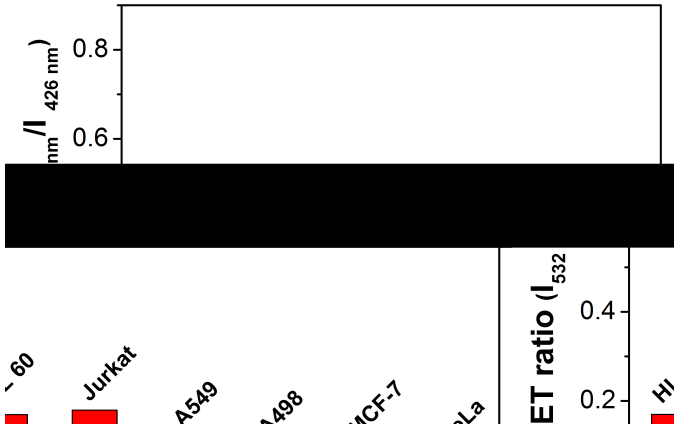


**Figure S6.** FRET ratio ($I_{532nm}/I_{426nm}$) as a function of cell types for nonspecific DNA sequence. The values were calculated from emission spectra, subtracting the signal of the negative control. For each cell type, 2000 cells were used as template for FRET response measurement.

## References

1. M. Stork, B.S. Gaylord, A. J. Heeger, G. C. Bazan, *Adv. Mater.* **2002**, *14*, 361-366.
2. Sefah, K.; Tang, Z.W.; Shangguan, D.H.; Chen, H.; Lopez-Colon, D.; Li, Y.; Parekh, P.; Martin, J.; Meng, L.; Phillips, J.A.; Kim, Y.M.; Tan, W.H. *Leukemia* **2009**, *23*, 235-244.
3. Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Anal. Chem.* **1980**, *52*, 2242-2249.