Supplementary Information

Productive Screening of Single Aptamers with ddPCR

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1 Monitoring the progress of aptamer selection

We use the qPCR method to monitor the screening process.[1] At Round 1-3, four times washing was subsequently performed to continuously remove unbound and weakly bound DNAs. Add 200ul MES buffer to the MB-PDL1 beads for 10min at boiling water bath. Then, use the magnet fix MB-PDL1 beads to removing the supernatant (MB-PDL1 elution). Then was then incubated with MB-PD1 beads in binding buffer for 0.5 h at room temperature. The magnetic beads were washed three times with 100 μL of MES buffer. Then, use the magnet fix MB-PD1 beads to removing the supernatant (MB-PD1 elution). After that, we use qPCR method to detection retention rate Fig.S1(Round 1-3) .

After sorting the micro-beads, the samples were detected by qPCR and the control sample was the PCRmix. It can be seen from the FigS1 (Round 4 and 5)that the red and yellow lines (green and blue in Round 5) are positive samples, and the rest are control samples. It can be seen that the Cq value of the sample obtained by sorting is smaller than that of the control sample, indicating the effectiveness of this sorting process.

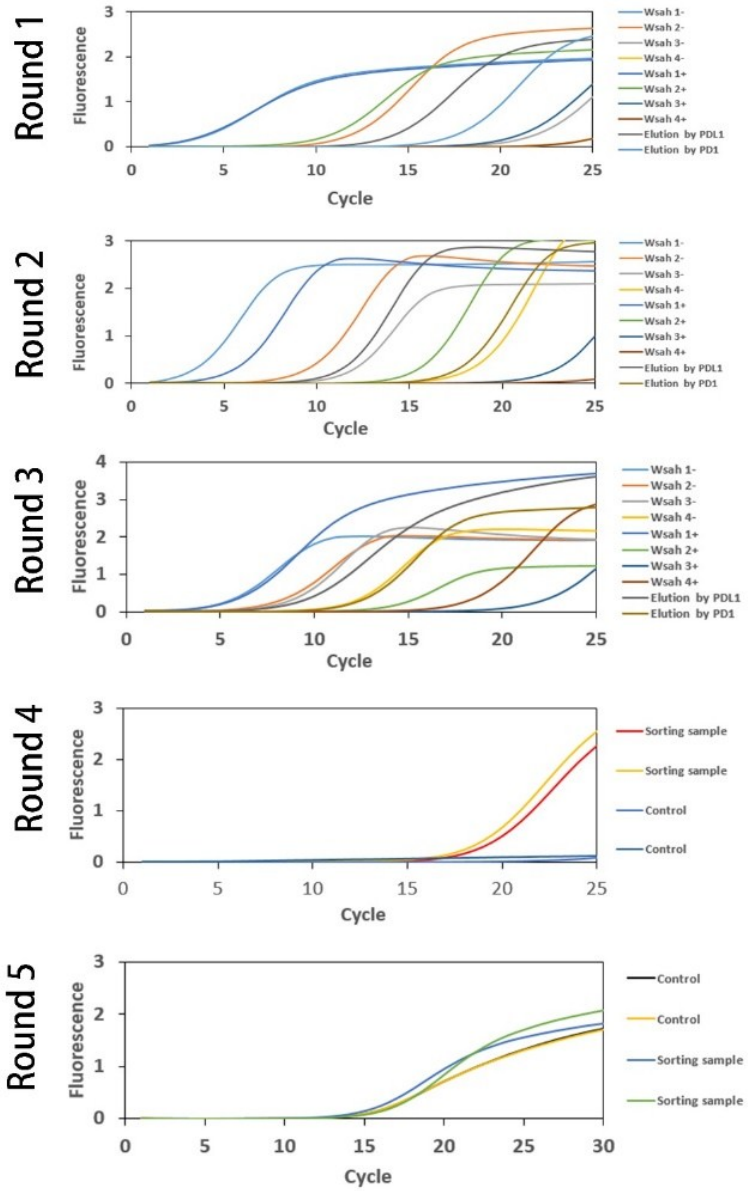

Fig.S1 qPCR to monitor the screening process. At Round 1-3, four times washing was subsequently performed to continuously remove unbound and weakly bound DNAs. We use this method to detection retention rate; Round 4 the red and yellow lines (green and blue in Round 5) are positive sorting samples, and the rest are control samples. It can be seen that the Cq value of the sample obtained by sorting is smaller than that of the control sample, indicating the effectiveness of this sorting process.

2 Retention rate of the screening (1-3rounds)

The traditional screening process needs to obtain the retention rate of each round as a basis for the reliability of the screening process. It can be seen from the FigS2 that the retention rate of the positive screening is significantly improved in the 1-3 rounds. So after third round, we combine the capillary based digital PCR system for single molecule amplification and flow sorting for screening.

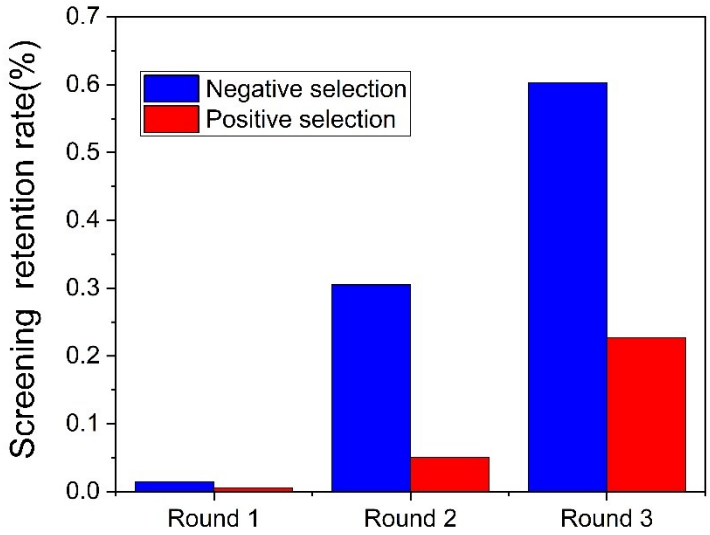

Fig.S2 Retention rate of the screening process with 1-3rounds

References

[1] Luo, Z. F.; He, L.; Wang, J. J.; Fang, X. N.; Zhang, L. Y., *Analyst* **2017,** *142*.