Electronic supplementary information

Evaluation of Aptamer Specificity with or without Primers Using Clinical Samples for C-reactive Protein by Magnetic-Assisted Rapid Aptamer Selection

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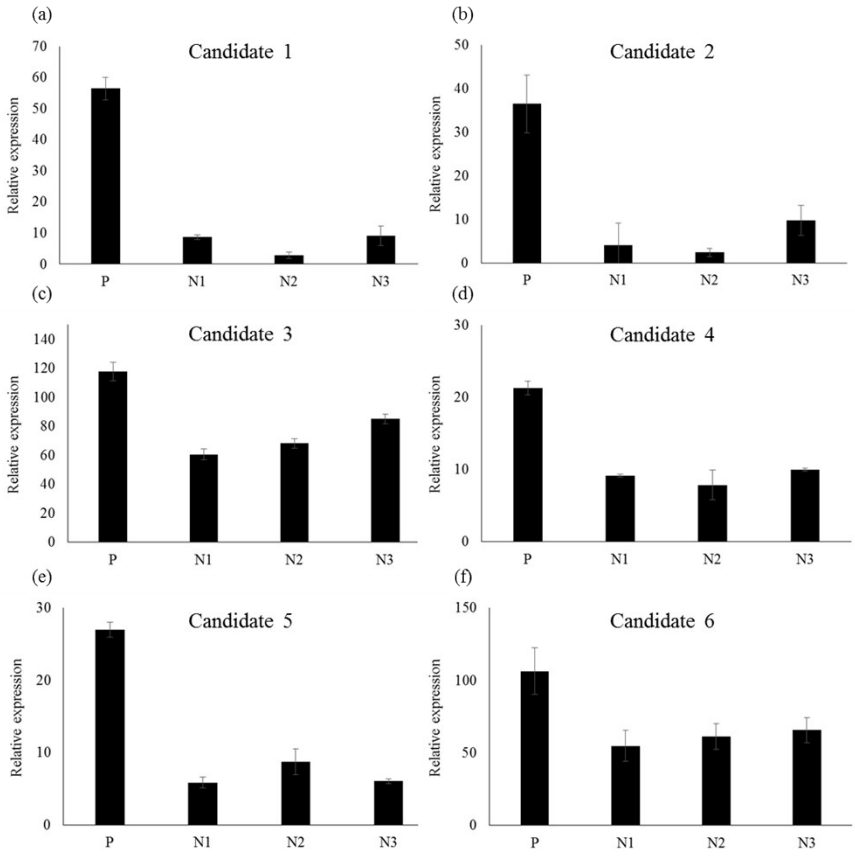
Procedure for Self-synthesizing Forward Single Strand Aptamers

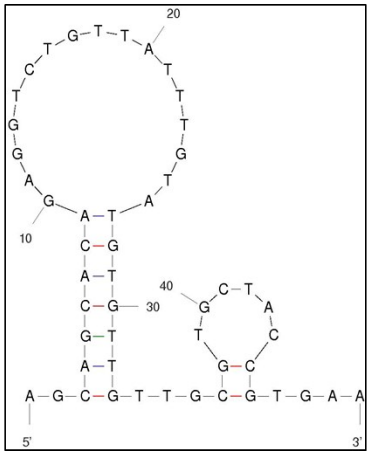
For each plasmid picked from the cloning experiments, 10 ng of aptamer clone plasmid was used as a PCR template to generate double strand DNA (dsDNA) with 50N-LAB-S and Biotin-50N-LAB-AS primers. The PCR condition and procedure were as described in the main text. After the completion of PCR amplification, the PCR product was incubated with SA-MNPs, obtained by magnetic separation from 5 μl of SA reagent. Forward single strand aptamers (non-biotinylated strand) were separated from the immobilized complementary strand, by being incubated with 0.15 N of fresh NaOH for 5 minutes. The bound SA-MNPs were removed with a magnetic stand. An equal amount of 0.15 N of HCl was added to the collected supernatant to adjust the final pH to 7.0, after which the forward ssDNA was precipitated with 1 ml of 100% ice-cold alcohol. The concentration of the single strand aptamers was determined with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The self-synthesized aptamers were then used for the preliminary reverse validation.

Figure S1. Results of the preliminary reverse validation of self-synthesized aptamers, including candidate aptamers #1-6 (a) to (f), with positive control (CRP: P1) and negative controls (N1, N2, and N3). From the result, Candidate 1 and 2 were selected for further experiments and named as CRP-aptamer-1 and CRP-aptamer-2, respectively.

Figure S2. The secondary structure of selected aptamers. (a) CRP-aptamer-1 and (b) CRP-aptamer-2 predicted by MFold analysis


(a)
Candidate 1
Relative expression
70
60
50
40
30
20
10
0
P
N1
N2
N3
(b)
Candidate 2
Relative expression
50
40
30
20
10
0
P
N1
N2
N3
(c)
Candidate 3
Relative expression
140
120
100
80
60
40
20
0
P
N1
N2
N3
(d)
Candidate 4
Relative expression
30
20
10
0
P
N1
N2
N3
(e)
Candidate 5
Relative expression
30
20
10
0
P
N1
N2
N3
(f)
Candidate 6
Relative expression
150
100
50
0
P
N1
N2
N3

(a)



(b)