

## **Supplementary material**

### **Development of DNA aptamer selection method based on Heterogeneous sandwich form, and its application to Colorimetric assay for detection of Influenza A virus**

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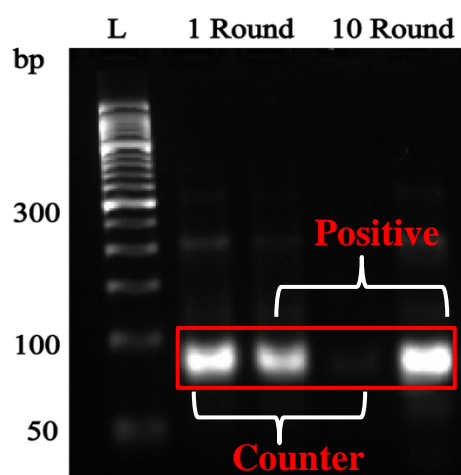
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**Table S1.** Primers and aptamer candidates obtained by H-sandwich SELEX.

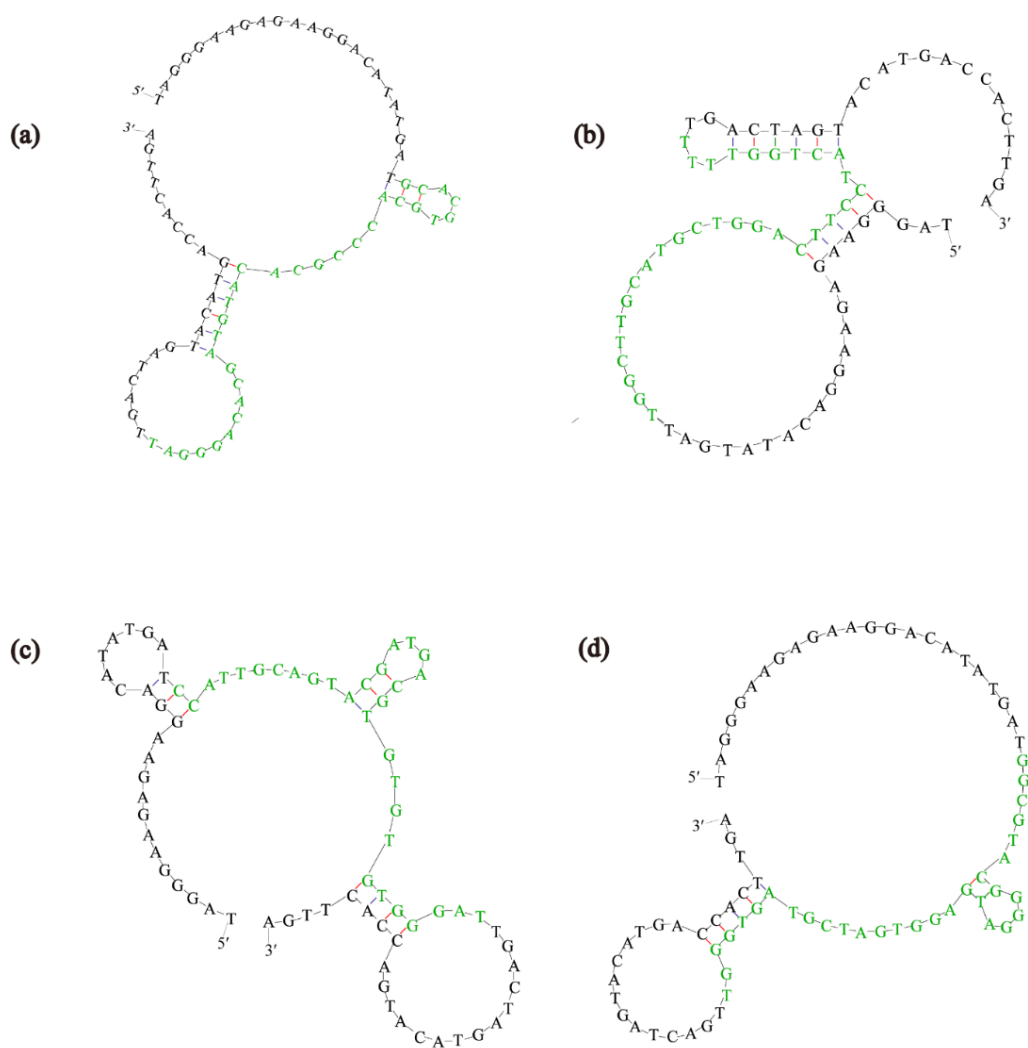
<b>5'→3'</b>	<b>Forward primer</b>	<b>Sequence</b>	<b>Reverse primer</b>
INFA-apt1	TAGGGAAGAGAAGGACAT ATGAT	GCACGTGCACCCGCACATG TAGCACAGGGA	TCAAGTGGTCATGTACT AGTCAA
INFA-apt2	TAGGGAAGAGAAGGACAT ATGAT	TGGCTTGCATGCTGGACTTC CTACTGGTTT	TCAAGTGGTCATGTACT AGTCAA
INFA-apt3	TAGGGAAGAGAAGGACAT ATGAT	CCATTGCAGTACGATGACGT GTGTGTGGGA	TCAAGTGGTCATGTACT AGTCAA
INFA-apt4	TAGGGAAGAGAAGGACAT ATGAT	GGCGTACGGGGATGAGGTG ATCGTAGTGGG	TCAAGTGGTCATGTACT AGTCAA

**Table S2.** The conditions for aptamers selection. Reactions were performed in 10 rounds with decreasing ssDNA concentration (nmol) and incubation time (min).

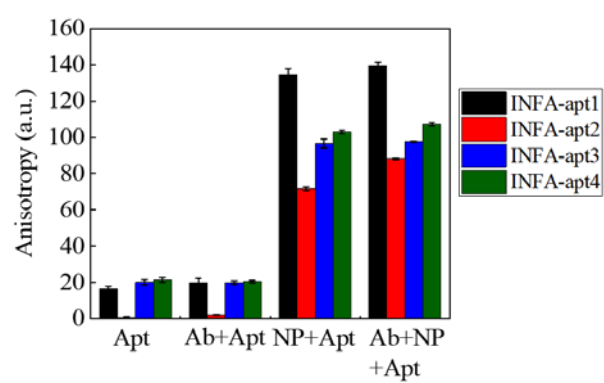
Selection round	ssDNA (pmol)	Incubation time (min)
1	1000	60
2-4	20	40
5-7	10	30
8-10	4	30



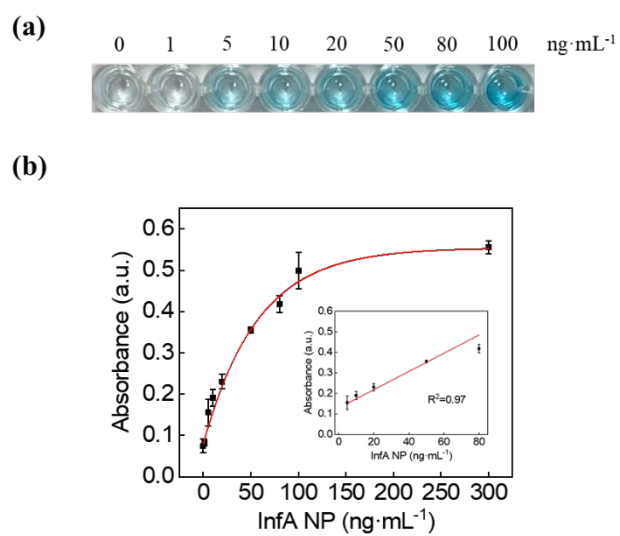
**Figure S1.** Comparison between the PCR products of ssDNA bound to targets (lane 2 and 4) and ssDNA bound to antibody-BSA (lane 1 and 3) in 1<sup>st</sup> and 10<sup>th</sup> selection round.



**Figure S2.** Predicted secondary structure of aptamer candidates obtained by H-sandwich SELEX (a-d). Portion predicted to bind to the antibody-antigen complex (green) and that to the linear structure (black).



**Figure S3.** Binding affinity of aptamer candidates in heterogeneous sandwich form with fluorescence polarization.



**Figure S4.** Antibody-based colorimetric assay with the sandwich form. Detection limit of the InfA NP, as determined by the naked eye (a) and titration curve (b). Inset: the absorption is a linear correlation with the concentration of InfA NP in the range from 5 to  $80 \text{ ng} \cdot \text{mL}^{-1}$  ( $y=0.00444x+0.12918$ ,  $R^2=0.97$ ). Error bars show the standard deviation of three experiments.