Materials and methods Supporting Information

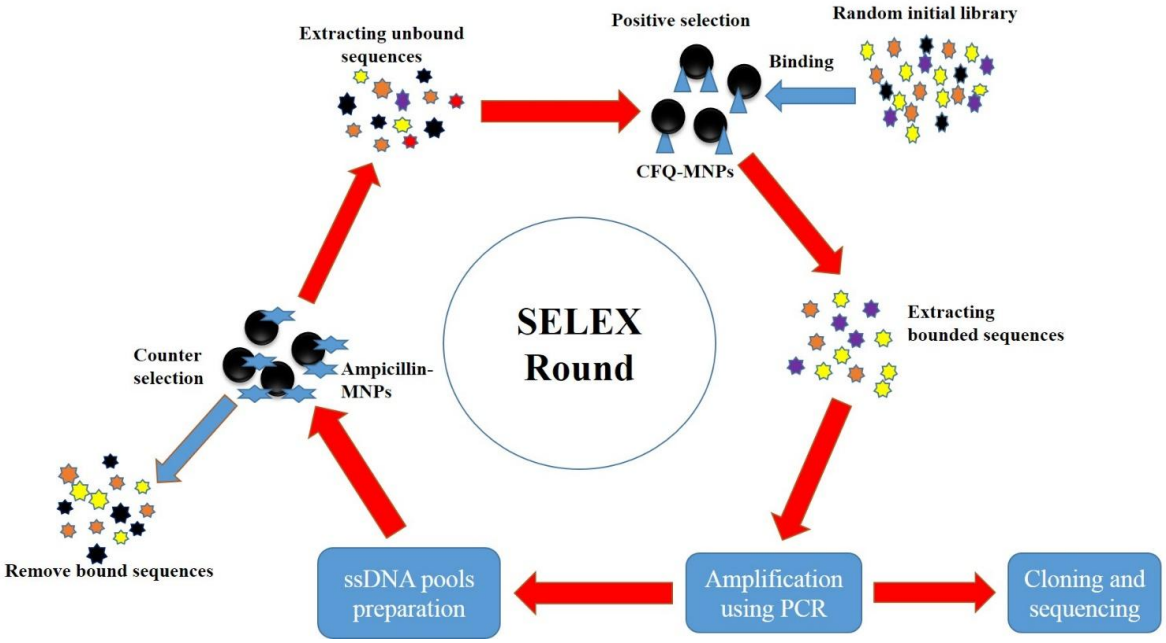
Selection of DNA aptamers against cefquinome.

Detailed counter selection procedure information: Before selection, 4 nmol starting library was denatured at 95 ℃ for 5 min followed by immediately cooled on ice for 10 min. Then, the CFQ-MNPs were incubated with the starting library for 1 hour at room temperature before removing the unbounded sequences and eluting the bounded sequences from the CFQ-MNPs by heating in boiling water for at least 10 min. The recovered DNA sequences were successively amplified via conventional PCR method using the former primer and biotin-labeled reverse primer, followed by preparation of secondary ssDNA library using SA-MNPs based magnetic separation. The prepared secondary library was used for the next round of selection.

Detailed counter selection procedure information: The prepared secondary library was first incubated with ampicillin linked MNPs to remove the bounded DNA sequences and the unbounded sequences were kept and incubated with CFQ-MNPs afterwards. The bounded sequences were then eluted and amplified by PCR method before ssDNA library preparation. In order to improve the binding affinity of the library, screening stress was always needed. From the fourth round of selection on, the incubation time of positive selection was gradually decreased, such as 60 min, 50 min, 40 min and 30 min; meanwhile, the incubation time of counter selection was gradually increased, such as 30 min, 40 min, 50 min and 60 min. Meanwhile, the washing time and the volume of washing buffer (40 mM of Tris–HCl, 10 mM of EDTA, 3.5 M urea, and 0.02 % aqueous solution of Tween 20, pH 8.0) were also gradually increased. Moreover, the amount of added ssDNA library was gradually decreased as the selection rounds increased, namely 4 nmols, 400 pmols, 200 pmols, 100 pmols and 50 pmols.

Supplementary Scheme Captions

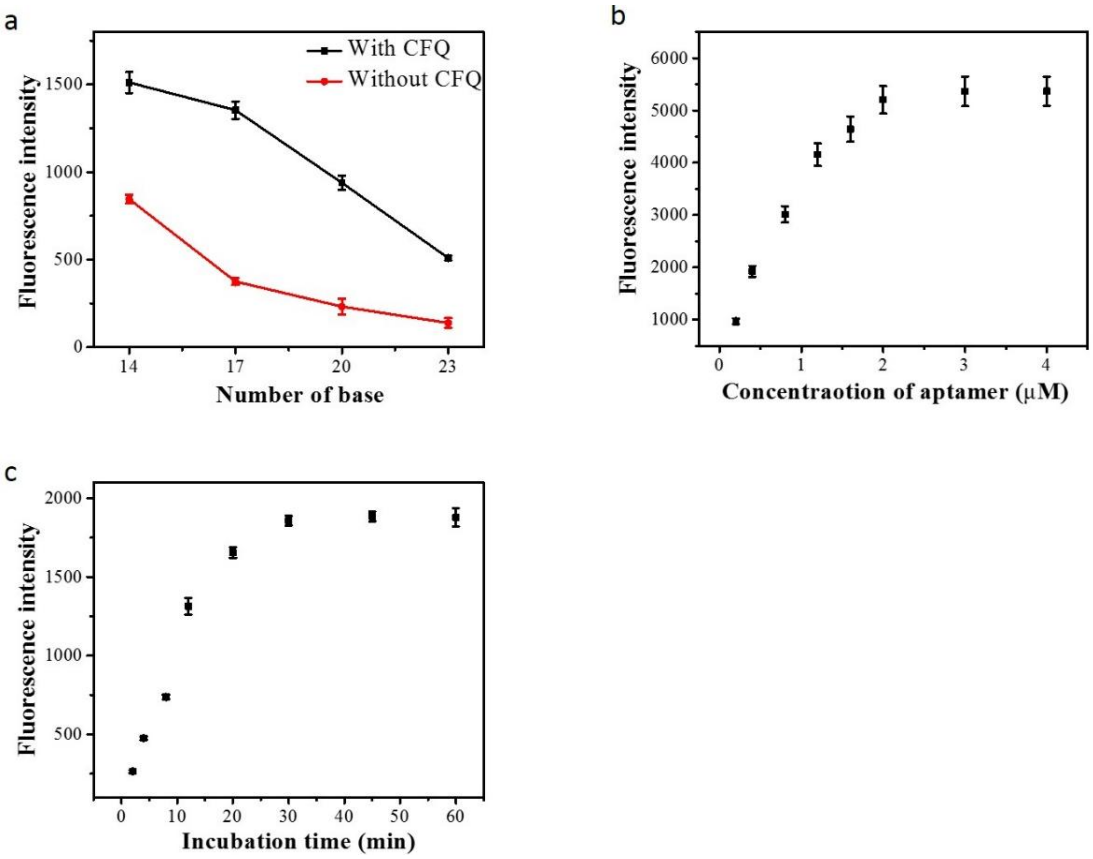
Supplementary Scheme S1. The illustration of SELEX processes of selecting CFQ specific ssDNA aptamers using magnetic nanoparticle-based method *in vitro*.



Supplementary Scheme. S1

Supplementary Figure Captions

Supplementary Fig. S1. The optimization of the established aptasensor. (a) The optimization of probe length in the detection of CFQ. (b) The optimization of aptamer concentration in the detection of CFQ. (c) The optimization of incubation time in the detection of CFQ.



Supplementary Fig. S1