Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary information

Cell-SELEX and Application Research of DNA Aptamer Against Esophageal Squamous Cell Carcinoma(ESCC) Cell Line TE-1

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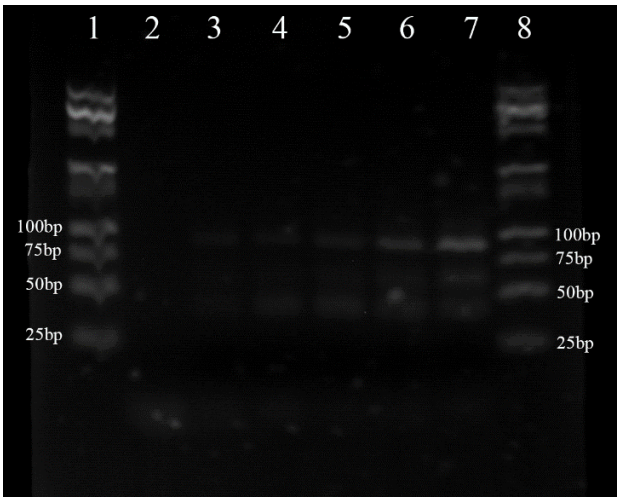

Figure S1. Optimization of asymmetric PCR template concentrations. Lane 2-7 correspond to 2.5ng/μL, 5ng/μL, 10ng/μL, 20ng/μL, and 40ng/μL of template respectively. Lane 1 and lane 8 are 500bp markers.

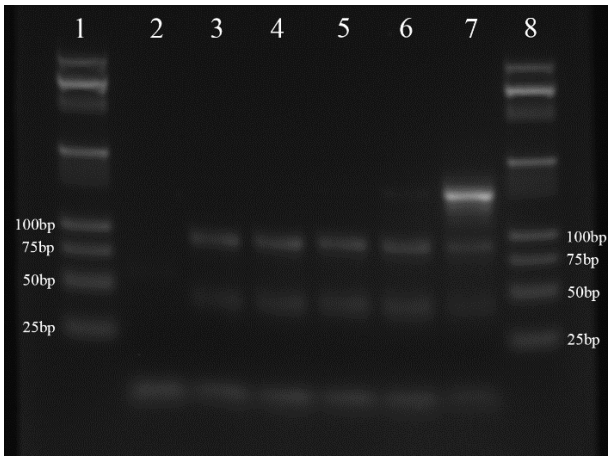

Figure S2. Optimization of asymmetric PCR cycle numbers. Lanes 2-7 correspond to 12, 14, 16, 18, and 20 cycles of reaction respectively. Lane 1 and lane 8 are 500bp markers.

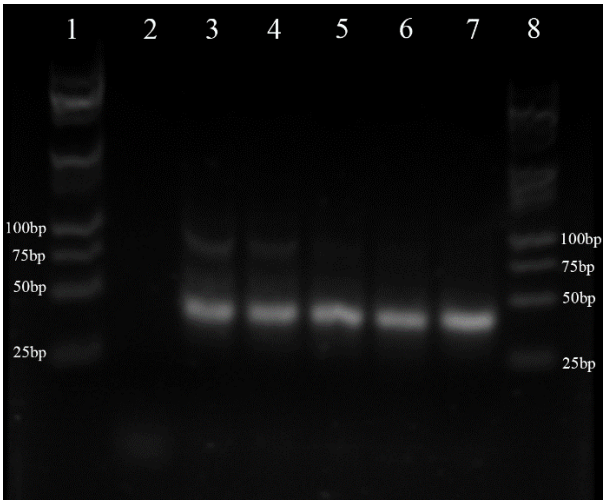

Figure S3. Optimization of asymmetric PCR primer ratios. Lanes 2-7 correspond to the ratio of forward and reverse primers equals 20:1, 40:1, 60:1, 80:1, and 100:1 respectively. Lane 1 and lane 8 are 500bp markers.

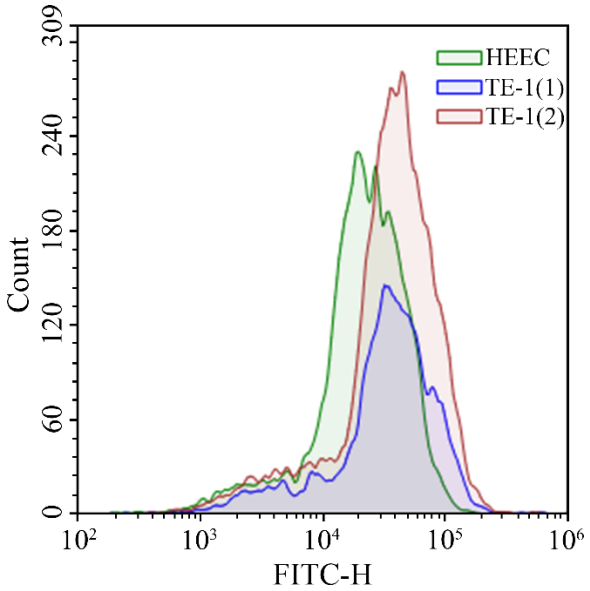

Figure S4. Monitoring of selection efficiency by flow cytometry. Products from 5th round of selection were amplified with FAM-labeled forward primer and incubated with TE-1 and HEEC cells, and flow cytometry analysis was conducted. The results show that products from the 5th round of selection already possess a certain degree of binding specificity.

Table S1. Selecting pressure applied on each round of selection [a]

Round number	Negative incubation time	Positive incubation time	Times of washing	Method of washing
1	0	60min	2	Rin
2	0	60min	2	Rin
3	0	55min	3	Rin
4	0	55min	3	Rin
5	0	50min	3	Rin
6	20min	50min	3	Con 1min
7	20min	45min	3	Con 1min
8	25min	40min	3	Con 2min
9	30min	35min	3	Con 2min
10	35min	30min	4	Con 2min
11	40min	30min	4	Con 3min
12	45min	20min	4	Con 4min
13	50min	20min	4	Con 5min
14	55min	15min	4	Con 5min
15	60min	15min	4	Con 5min

[a] "Rin" refers to rinse with PBS, and "Con" refers to elute on table concentrator for the corresponding time.

Table S2. Top ten sequences by frequency.

Sequence	Length	Frequency	Name
5’-TGCATGTCGTGTTCGCAACATTTAGATCTGCATGGTGAGG-3’	40bp	297	Te1
5’-GGTGGCATAGGTTCCCCATTGGAAAAGCGGGCTGTGGTGG-3’	40bp	234	Te2
5’-GGTGGCATAGGTTCCCCATTGGAAAAGCAGGCTGTGGTGG-3’	40bp	63	Te3
5’-TGGTAGCCTAAGCCTGTCCAGGAATCG-3’	27bp	27	——
5’-TGGTGGGGCATCTCGCGAAATTTGCTGAACTGGTCAGTGG-3’	40bp	17	Te4
5’-TGCATGCCGTGTTCGCAACATTTAGATCTGCATGGTGAGG-3’	40bp	15	Te5
5’-TGGTAGCCTAAGCCTGTCCAGGAATCGGC-3’	29bp	13	——
5’-TGCATGCCGTGTTCGCAACATTTAGATCTGCATGGTGAGG-3’	40bp	12	——
5’-TGGTAGCCTAAGCCTGTCCAGGAATCGGC-3’	29bp	12	——
5’-CCACCGGTCCGAATCCGTAGTACCGCCCGACGACAGCGGT-3’	40bp	10	——

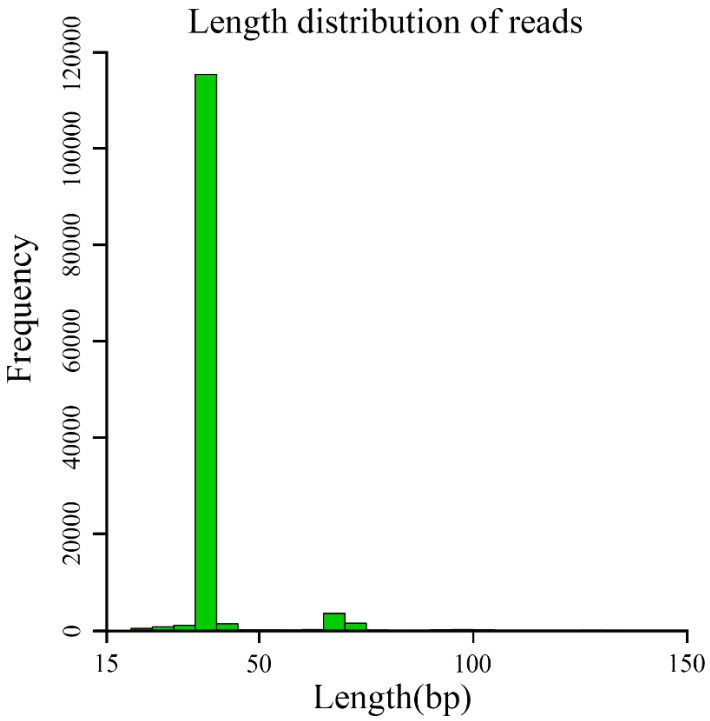

Figure S6. Statistics of the sequence length of SELEX products. After cutting off forward and reverse primers, the length of most sequences is located at 40bp, indicating that the SELEX was successful.

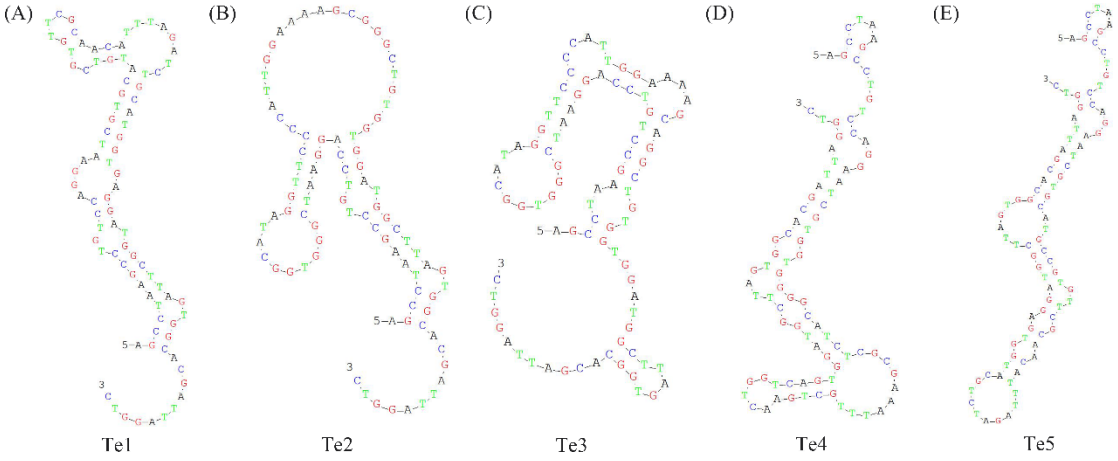

Figure S7. Secondary structures of five aptamer candidates simulated by DNAman software.

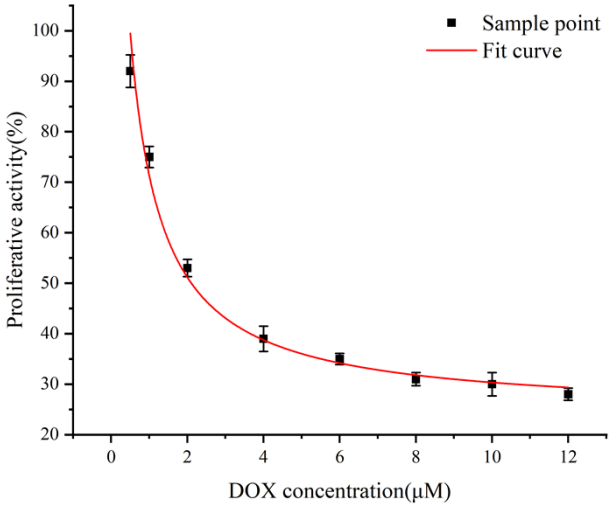

Figure S8. Optimization of DOX concentration used in the cytotoxic experiment. Different concentration of DOX was co-incubated with TE-1 cells to find out the concentration that controls cell viability at 50%. Data points were recorded and fitted with Origin software.

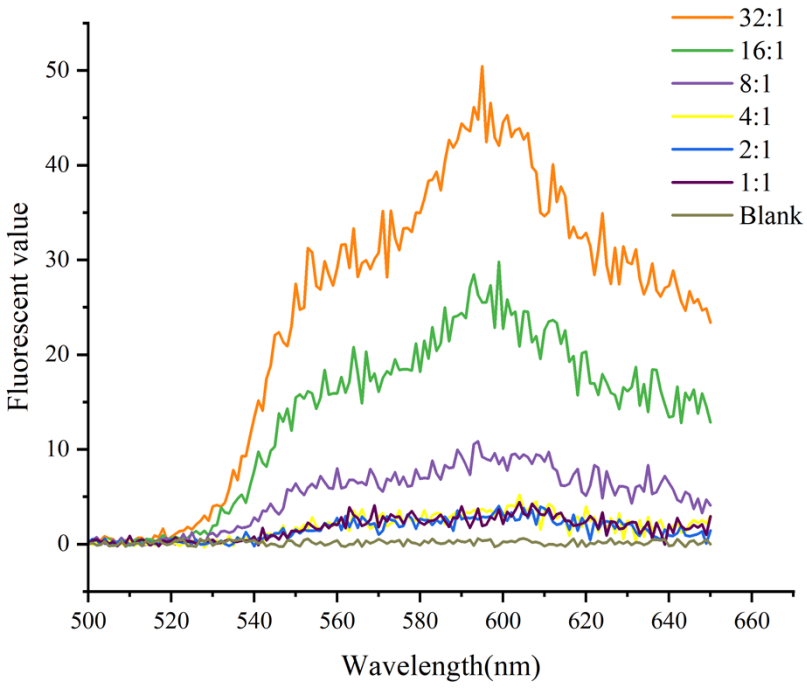

Figure S9. Results of the pre-experiment to determine the drug-carrying ability of Te4 aptamers. Different ratios of DOX and Te4 were mixed and self-assembled to form Te4-DOX. Since the fluorescence of DOX quenches when binding with DNA, the best-matching DOX:Te4 ratio can be determined by the point that the fluorescence signal of the whole system stops decreasing, which was proved to be 4:1.

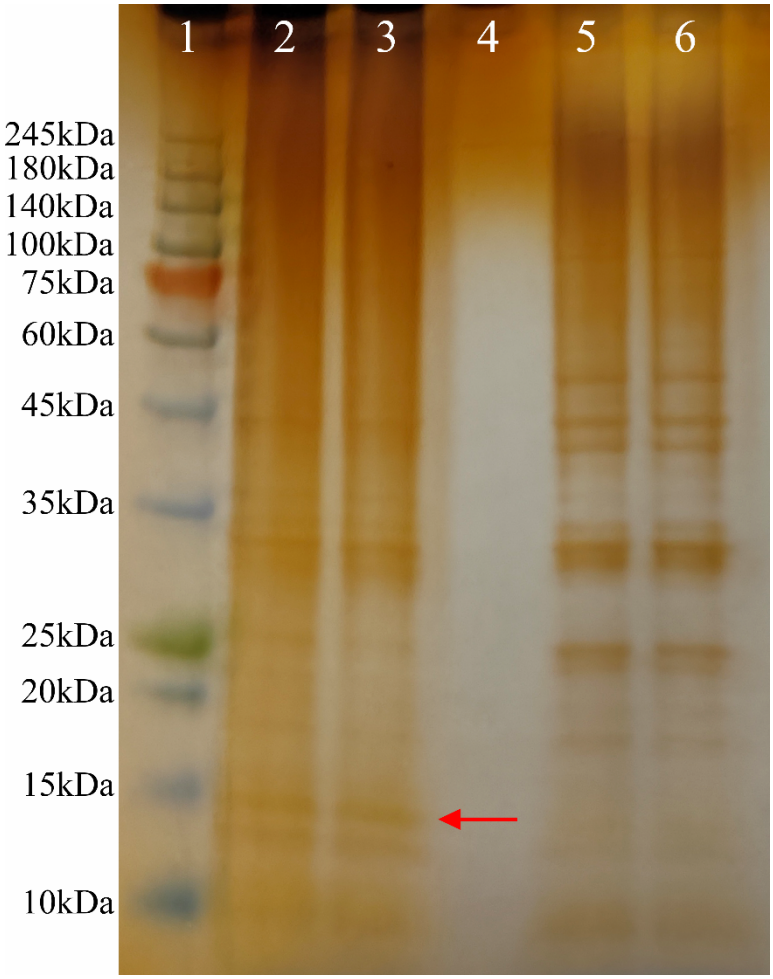

Figure S10. Membrane protein captured by Te4 aptamer. SDS-PAGE analysis was performed and the gel was treated with silver staining. Lanes 2 and 3 are proteins captured by Te4 aptamer from TE-1 cells with magnetic separation, and lanes 5 and 6 are total proteins from TE-1 cells. Specific bands can be observed(the red arrow) in lane 2 and lane 3, indicating that the molecular target of Te4 is a kind of protein.