Electronic Supplementary Material (ESI) for Analytical Methods.

Whole-cell Based Aptamer Selection for Selective Capture of Microorganisms in the Microfluidic Device

Congxiao Zhang[1], Xuefei Lv[1], Dalei Li, Zhiguang Zhang, Yan Man, Xu Han, Hong Qing*, and Yulin Deng*

School of Life Science, Beijing Institute of Technology, Beijing 100081, China

[1] These authors contributed equally to this work.

*Corresponding Authors

Tel: +86-10-68915996. Fax: +86-10-68914907. E-mail address: deng@bit.edu.cn ;

Tel: +86-10-68918409. Fax: +86-10-68914907. E-mail address: hqing@bit.edu.cn

The highly enriched aptamer pools were cloned and sequenced and a total of 40 sequences were obtained. Screened aptamer sequences of eight families without primers are listed in Table S1. The sequence alignment was analyzed using sequence alignment programs ClustalX1.83. The sequences were grouped into eight families based on the homology of the oligonucleotides of individual clones with each group containing similar sequence.

Information on composition and distribution of putative Quadruplex forming G-Rich sequences (QGRS) in nucleotide sequences using QGRS Mapper is listed in Table S2, The result showed that most of aptamer sequence got higher G-scores, which indicated the sequences would be preferable to form a G-quadruplex.

The flow cytometry results of binding assays of aptamers with *E. coli* cells are shown in Figure S1-S5 for the binding affinity and selectivity. A FC-500 flow cytometer (Beckman coulter Inc., U.S.) was used to assess the binding of the evolved aptamer pools and individual aptamer sequences toward *E. coli* 11775. The aptamer

pools after the selection were fluorescently tagged via PCR amplification with 5'-FAM modified primers (Invitrogen) and the individual aptamers sequences were obtained with the fluorescent label 5'-FAM from Invitrogen (Shanghai). The DNA aptamers were heated to create folded ssDNA at 95 ℃ and subsequent fast cooling on ice prior to incubation with the bacteria. The binding affinity of aptamers was determined by incubating 10^8 bacterial cells with 100 pmol FAM-labeled aptamers for 45 min in binding buffer. Cells were then washed once with washing buffer (1× binding buffer with 0.05% BSA), suspended in 0.5 mL of binding buffer, and subjected to flow-cytometric analysis within 30 min. Forward scatter, side scatter, and fluorescence intensity were measured, and gated fluorescence intensity above background (cells with no aptamer added) was quantified as well.

The detection of bacterial cells in real sample (drinking water) was carried out in the microfluidic device accordingly, and the results are shown in Fig. S6.

Table S1.
Screened aptamer sequences of eight families without primers

Family	name	Sequence of Screened aptamer for *E. coli*
Family 1	EA1	GATAGAGGGGGGGGGAGGTGGTTAGTGTAGGGTAGGTGG
	EA8	GGCACGGGGGGGGGGGAAGTGGGGGTGCGAGGGTATGGTGG
	EA17	GAGGGGGGGGTGACGGGTCAGGGTACGGGGGGCACTAGTG
	EA25	GCGGCATTGGGGCGGGACAGGAGGTCGGGATGGATTAGGT
	EA26	AGGGTGGTGAGGTGAGGGAGGGTAGGTGCGGTGGGGTGG
Family 2	EA11	GAGTGAGGGAGGGGGAAGTAGGGCATTAGGGGGGTGTGGT
	EA19	GGGGCAGTAAAGGGTCGGGGTGAGTGGGTGTGAGGAGGAG
	EA21	GGGGGAGGGACAGGAGCTGGGTGGAGAGTGGTGTGCGT
	EA24	GGGGGTAATGGGGTGGGGGTGTGTCAGATGGGTGGAGGCG
	EA27	GGGGGTTGGAAGGGTGGCTGGGACAGCGGGGTGTTGACGG
	EA29	GATGTCAGCCAGGGAGCGGGATAGGGCAGGGGTCTTGTGG
	EA33	GGGGGGAGGAAGGGTTGGGGCTTGTTGGGTGGTCTGGG
Family 3	EA6	GGGGATGGGGTAGGTTGTCAGGAGGGGAAGGGGGTGTGG
	EA9	GGACATGGCGGGTGATAGGGTGAAGGGGGGGGTAGTGGTTG
	EA13	GGCAGGGAACAGGGGGAGTCACGGGCAGTGGTGGGGATTG
	EA14	GTGGGAGGGAGGGTATGGGGTAGGCAGGGGGGGGGGGTGTA
	EA23	GGCGGACTGGGGTCGTGGGGTAAGGGGGGTCAGGGAAT
	EA35	GCAGGGTAGGAGGTAGGGGGGTTGGGCTGGGGAGTGGAG
Family 4	EA4	GAGAGCTGAGCCGCGTGGGGGAGACTCGTGGGGTGTAATG
	EA7	GGGGGTGAGGTGCGGGGAGTGGGGGGGGACAAGTTGGGCG
	EA31	GAGGAACGATGCGATCGTGTGGGGATTGAAAGGTTAGGGG
	EA36	GTCAGGGCCGGGGCGGAAGGATGCGATACGTGGTGGGGG
Family 5	EA10	GGAAAGCGTGGAGAGACCCAGACGTGGATAGAGGGACGCC
	EA28	GCTGGGGGCAGCGAGTAAAAATAACCGTGCGCGGGGGGTC
	EA30	GGTGTGCCACGGCAGTTGCGCGGGGGGTGTCTCTGAACGG
	EA32	CGGGGAGCAAGGACAGGCCAAGGGACCTCGCGCGGGGAGG
Family 6	EA18	CGGGGCAGTGGGGAGGTTAGAGGTTGGAGTTGGTGGGAT
	EA20	GCGAGAGGTGGGTTGGCGGTCTGGGGGTGGTCGGATGGGG
	EA38	GGGCGGTGGGGGGCCTGGTAGCGGGAGGATGGGCTGGAGT
	EA39	CGGAGAAGCGGGGAGGCTCGTGGCACGGGCGGGTAGGGG
	EA40	GGGTCCGGGGCCGAGGGTTGGGAGGGGGGGTTGGTGGGAT
Family 7	EA2	CGTGAGCACTAGGGAAAGGGGAGCACGCAGGGTGAGGGGT
	EA3	GTCAGGGGGGACGGGGACGGACGGAGGGAAGGGGAGGGTG
	EA34	GGCAGAAGGGCAGGGAAACGGGGGGAAAAGGGTAGGAGGG
Family 8	EA5	GGCCACCCGACGGATTCCGGGAGGGAAGGTGGAACCATGG
	EA12	GGGGACGGTCGGGGTGTGGAGGGTGGAGGGTTAATGGTGG
	EA15	GGGAGGGTACGGGATGGTGTGGCAGGTGCAATTGATGAGG
	EA16	GGGACGGCTTCGGGGGGGGGGTGGGGAAGTGGCACGGT
	EA22	CATTGCCAAGGGGAAGGGGCGGTGGGGGCACGGAGGCTCG
	EA37	GAGGGACACGGGGGAGGCGGGGGTTTGGGGCAGGGTGGGT

Table S2.
Information on composition and distribution of putative Quadruplex forming G-Rich Sequences (QGRS) in nucleotide sequences using QGRS Mapper

Name	Position	Length	QGRS	G-Score
EA1	11	20	GGTATGAGAGGATAGAGGGG	14
	31	11	GGGGGAGGTGG	21
	50	23	GGGTAGGTGGGAAGTTATCGCGG	13
EA2	32	28	GGGAAAGGGGAGCACGCAGGGTGAGGGG	38
EA3	25	30	GGGGGGACGGGGACGGACGGAGGGAAGGGG	36
EA4	37	25	GGGGGAGACTCGTGGGGTGTAATGG	14
EA5	40	13	GGAGGGAAGGTGG	20
EA6	20	11	GGGGGATGGGG	19
	41	19	GGAGGGGAAGGGGGTGTGG	20
EA7	22	27	GGGGTGAGGTGCGGGGAGTGGGGGGGG	55
EA8	10	23	GGGTATGAGAGGGCACGGGGGGG	36
	33	30	GGGGAAGTGGGGGTGCGAGGGTATGGTGGG	42
EA9	20	29	GGGACATGGCGGGTGATAGGGTGAAGGGG	40
	50	24	GGTAGTGGTTGGAAGTTATCGCGG	13
EA10	\	\	\	\
EA11	27	25	GGGAGGGGGAAGTAGGGCATTAGGG	40
EA12	21	30	GGGGACGGTCGGGGTGTGGAGGGTGGAGGG	39
EA13	20	26	GGGCAGGGAACAGGGGGAGTCACGGG	38
	50	12	GGTGGGGATTGG	18
EA14	23	17	GGGAGGGAGGGTATGGG	40
	43	14	GGCAGGGGGGGGGG	21
EA15	10	24	GGGTATGAGAGGGGAGGGTACGGG	37
	36	25	GGTGTGGCAGGTGCAATTGATGAGG	11
EA16	20	27	GGGGACGGCTTCGGGGGGGGGGTGGGG	56
EA17	11	17	GGTATGAGAGGAGGGGG	15
	28	23	GGGTGACGGGTCAGGGTACGGGG	41
EA18	24	20	GGCAGTGGGGAGGTTAGAGG	19
	46	27	GGAGTTGGTGGGATGAAGTTATCGCGG	10
EA19	22	27	GGGCAGTAAAGGGTCGGGGTGAGTGGG	38
	54	20	GGAGGAGGAAGTTATCGCGG	12
EA20	20	25	GGCGAGAGGTGGGTTGGCGGTCTGG	20
	45	14	GGGTGGTCGGATGG	21
EA21	10	20	GGGTATGAGAGGGGGGAGGG	35
	33	19	GGAGCTGGGTGGAGAGTGG	18
EA22	31	23	GGGAAGGGGCGGTGGGGGCACGG	21
EA23	20	30	GGGCGGACTGGGGTCGTGGGGTAAGGGGGG	42
EA24	10	29	GGGTATGAGAGGGGGTAATGGGGTGGGGG	39
	50	11	GGTGGAGGCGG	21
EA25	23	27	GGCATTGGGGCGGGACAGGAGGTCGGG	20

Table S2. Information on composition and distribution of putative Quadruplex forming G-Rich Sequences (QGRS) in nucleotide sequences using QGRS Mapper (continued)

Name	Position	Length	QGRS	G-Score
EA26	11	22	GGTATGAGAGAGGGTGGTGAGG	14
	36	25	GGGAGGGTAGGTGCGGTGGGGTGGG	33
EA27	23	28	GGGTTGGAAGGGTGGCTGGGACAGCGGG	41
EA28	11	18	GGTATGAGAGGCTGGGGG	15
EA29	32	21	GGGAGCGGGATAGGGCAGGGG	42
EA30	20	28	GGGTGTGCCACGGCAGTTGCGCGGGGGG	14
EA31	43	19	GGATTGAAAGGTTAGGGGG	15
EA32	24	21	GGAGCAAGGACAGGCCAAGGG	19
	54	20	GGGGAGGGAAGTTATCGCGG	11
EA33	23	27	GGGGAGGAAGGGTTGGGGCTTGTTGGG	39
	51	21	GGTCTGGGGAAGTTATCGCGG	11
EA34	33	29	GGGAAACGGGGGGAAAAGGGTAGGAGGGG	41
EA35	20	14	GGCAGGGTAGGAGG	19
	36	16	GGGGGGTTGGGCTGGG	40
EA36	20	17	GGTCAGGGCCGGGGCGG	21
	52	21	GGTGGGGGGAAGTTATCGCGG	12
EA37	30	30	GGGGGAGGCGGGGGTTTGGGGCAGGGTGGG	42
EA38	10	24	GGGTATGAGAGGGGCGGTGGGGGG	35
	43	15	GGGAGGATGGGCTGG	20
EA39	31	29	GGGAGGCTCGTGGCACGGGCGGGTAGGGG	30
EA40	11	18	GGTATGAGAGGGGTCCGG	14
	35	16	GGGTTGGGAGGGGGGG	41

The table lists all QGRS mapped to sequences of selected aptsamers, including information about the position of the QGRS, its distance from 3' and 5' splice sites, the actual sequence (underlining the G-groups) and its G-score.

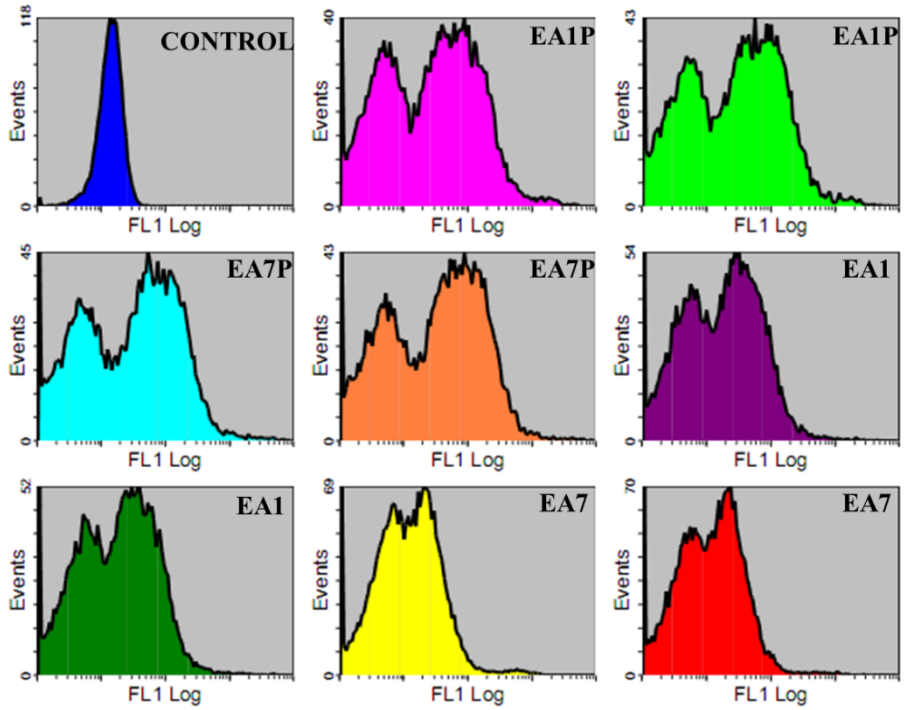

Fig. S1. Flow-cytometric analysisof *E.coli* 11775 binding with aptamers EA1P, EA7P, EA1, EA7. Duplicate analyses were carried out.

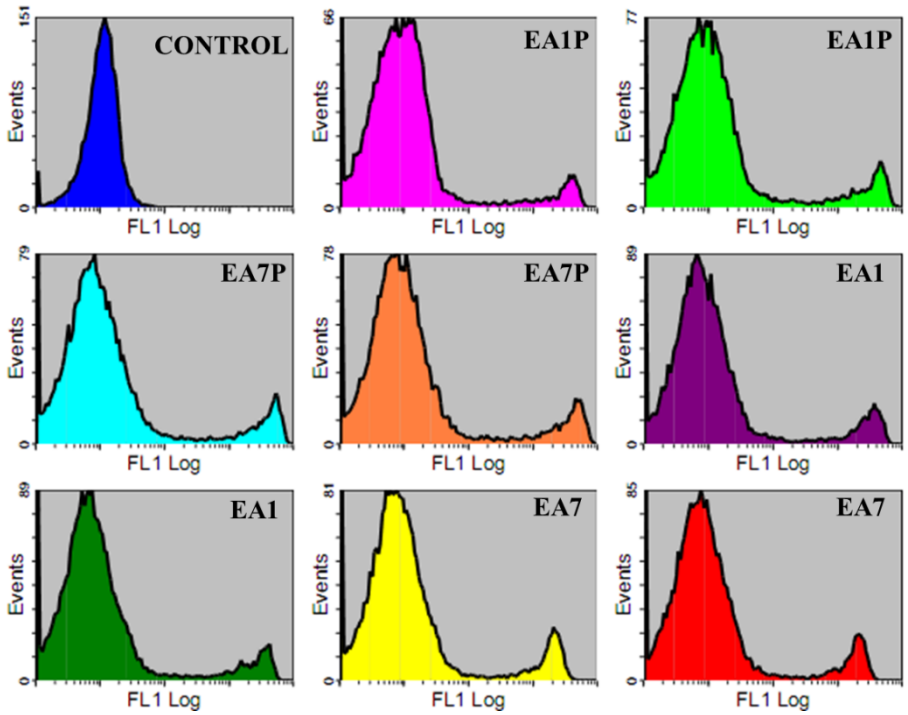

Fig. S2. Flow-cytometric analysisof *P.vulgaris* binding with aptamers EA1P, EA7P, EA1, EA7. Duplicate analyses were carried out.

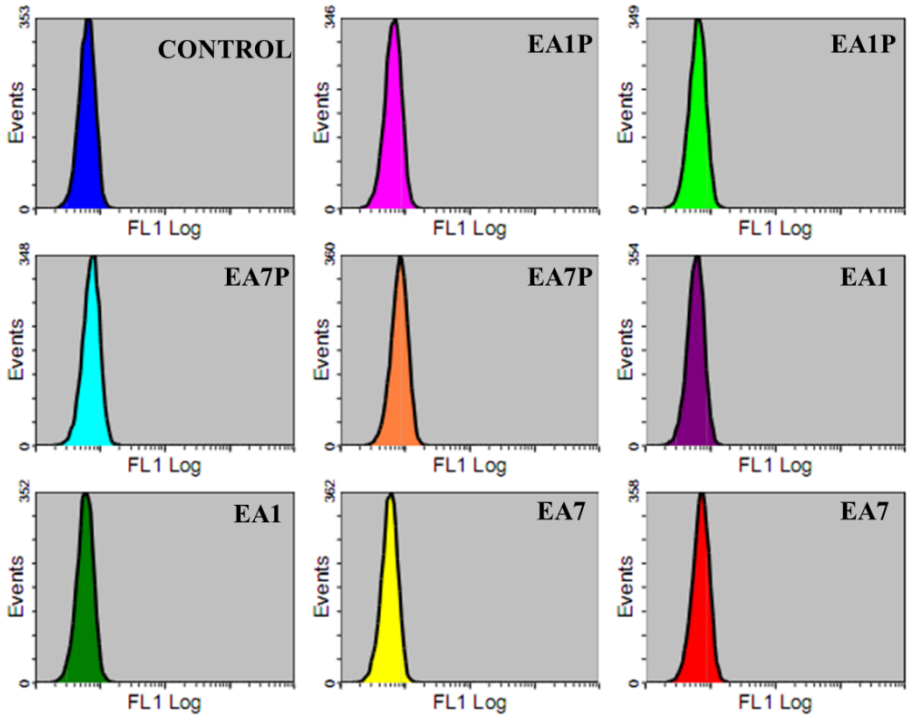

Fig. S3. Flow-cytometric analysisof *B.subtilis* binding with aptamers EA1P, EA7P, EA1, EA7. Duplicate analyses were carried out.

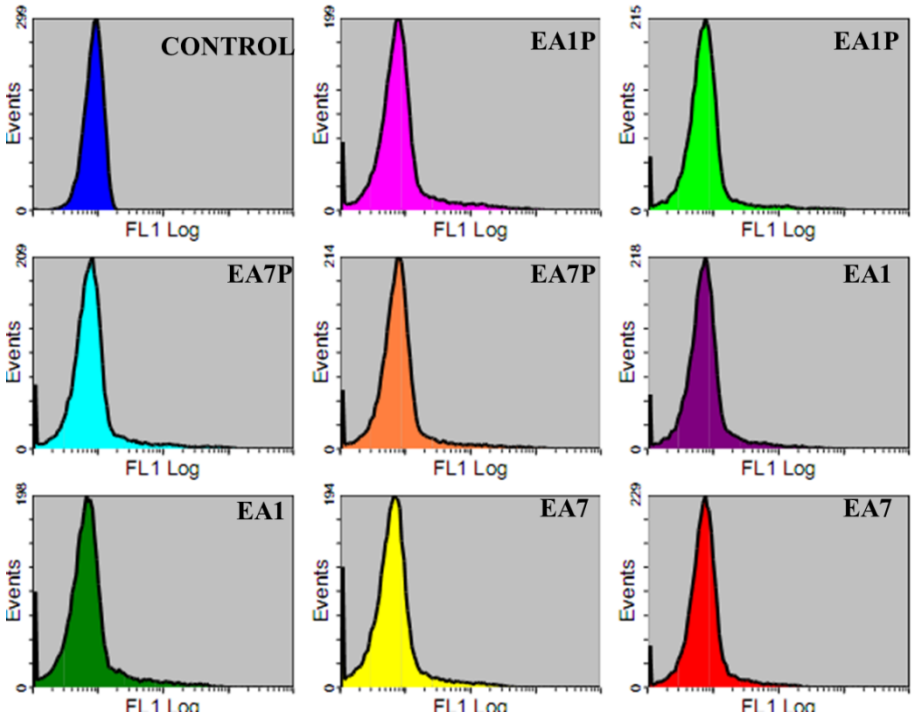

Fig. S4. Flow-cytometric analysisof *E.Coli* DH5α binding with aptamers EA1P, EA7P, EA1, EA7. Duplicate analyses were carried out.

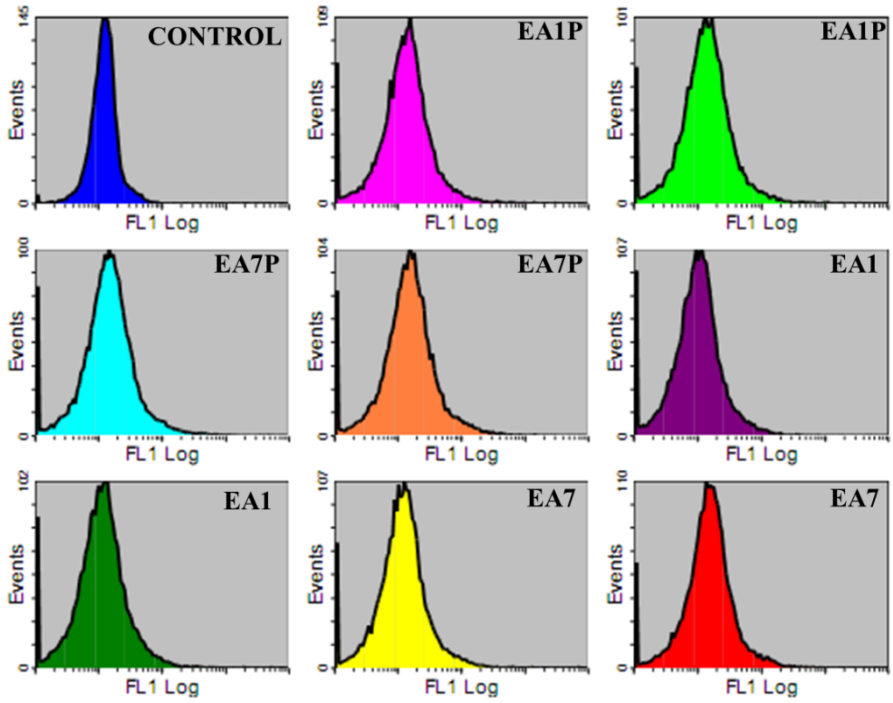

Fig. S5. Flow-cytometric analysisof *E.aerogenes* binding with aptamers EA1P, EA7P, EA1, EA7. Duplicate analyses were carried out.

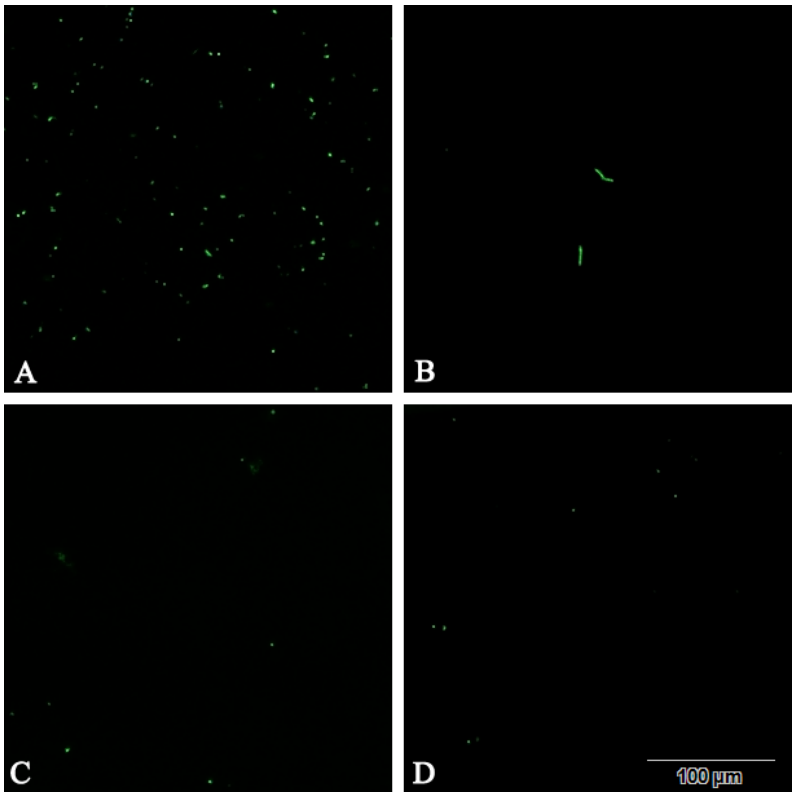

Fig. S6. Image of detection of bacterial cells in drinking water in microfluidic device: Representative images of *E.Coli* 11775 cells captured (A) and control bacterial cells *B.subtilis* (B) on EA1P aptamer immobilized surface. Representative images of *E.Coli* 11775 cells captured with the random DNA (C) and without DNA immobilized surface (D).