Electronic Supplementary Material (ESI) for Analyst.

Rapid and simultaneous analyzing twelve virulence factor genes by microfluidic-CFPA chip for identifying diarrheagenic Escherichia *coli*

Bin Yang[1], Yang Li[2], Jun Yan[2], Yiling Fan[3], Xueen Fang[1*], Jilie Kong[1*]

1. Department of Chemistry and Institutes of Biomedical Sciences, Fudan University, Shanghai, 200433, PR China

2. Shanghai Suxin Biotechnology Co. Ltd, Shanghai, 201321, PR China

3. Shanghai Institute for Food and Drug Control, Shanghai 201203, PR China

[*]fxech@fudan.edu.cn (X. E. Fang); jlkong@fudan.edu.cn (J. L. Kong)

1. CFPA Primers used in the experiments

Table S1. The sequences of CFPA primers of twelve DEC virulence factor genes.

Target	Item	Sequence (5'-3')
stx1	F	FAM-CAGGACAAAT/iTAMdT/CTGATTTTTCACATGTTACCTTTC
	R	FAM-GGTTCCAC/iTAMdT/ATAAGAAGTAGTCAACGAAT
uida	F	FAM-CCGCTTTGG/iTAMdT/GTCCGTAATAACGGTTCAGGC
	R	FAM-CCCGTCCG/iTAMdT/GGAATATTTCGCGCCACT
stx2	F	FAM-TTCAGGCAGA/iTAMdT/CTGTCTGAAACTGCTCCT
	R	FAM-CCCTGATGA/iTAMdT/GACAGTCCCCAGTATCGCTGA
it	F	FAM-GGCAGAGG/iTAMdT/CTTCCAGATTAGCAGGTTTCCCA
	R	FAM-GCCTCTTAAC/iTAMdT/TATATTTCCTGAGATATATTGTGC
bfpB	F	FAM-ATCATGGAG/iTAMdT/TGTACATGGAATTATTCTGAGATC
	R	FAM-CACTGTTCA/iTAMdT/TTGTAAGTTGCGGCCAGACTT
pic	F	FAM-TCCCCCT/iTAMdT/ATCGGCACTCCAGGAAACCT
	R	FAM-TTGTTTTGCC/iTAMdT/ATTACTGTCTGAACTGCTTTGT
aggr	F	FAM-TTGAGAGAG/iTAMdT/CTTAGAAGAAATCAACAGTAAATCCA
	R	FAM-GATCGATACT/iTAMdT/GATGCTTGCGTCAGCATCAGCTACA
inve	F	FAM-CGACTTGTTAAG/iTAMdT/CCAAAAGAAAGAGTTTACACTC
	R	FAM-ATAGTTTT/iTAMdT/TGATTCTTCTGTTAGGGATT
sth	F	FAM-TGAGAGT/iTAMdT/GCCTCCGCAACTTGGGTGA
	R	FAM-CCGAAAAAAA/iTAMdT/TGTTTAATGACTGTTTTTGTGG
stp	F	FAM-GCACTTACT/iTAMdT/TTATTATGATTTTCTCAGCACC
	R	FAM-ACATTAGA/iTAMdT/TCATGTTGTAAAAAACAACAGTGAA
escv	F	FAM-CGTAATGGC/iTAMdT/CAACAGGCTTTCCTACATTGGTCT
	R	FAM-TACTTAA/iTAMdT/AGCTCCCGGTGAGATGTTTTGG
astA	F	FAM-GAACGATAT/iTAMdT/CTGTAAATGGACTGAAAGGCTTC
	R	FAM-GCCTCTT/iTAMdT/CTTTCAGGTCGCGAGTGACG

* F is forward primer; R is reverse primer.

2. Performance of the microfluidic-CFPA chip in real clinical samples

Table S2 Comparison between the microfluidic CFPA chip and the PCR commercial kit for the detection of DEC among real clinical samples

	Bacterial strain	Microfluidic CFPA	PCR
1	EPEC	+	+
2	EPEC	+	+
3	ETEC	-	-
4	ETEC	+	-
5	EPEC	+	-
6	EIEC	-	+
7	EPEC	+	+
8	EAEC	+	+
9	EHEC	+	+
10	ETEC	+	+
11	EIEC	+	+
12	EPEC	+	+
13	EHEC	+	+
14	EHEC	+	+
15	EHEC	+	+
16	EHEC	+	+
17	EHEC	+	+
18	EHEC	+	+
19	EHEC	+	+
20	EHEC	+	+
21	EHEC	+	+
22	EHEC	+	+
23	/	-	-
24	EHEC	+	+

25	/	-	-
26	EHEC	+	+
27	EHEC	+	+
28	EHEC	+	+
29	EHEC	+	+
30	EHEC	+	+
31	EHEC	+	+
32	EHEC	+	+
33	EHEC,EAEC	+	+
34	EHEC,EIEC	+	+
35	EHEC	+	+
36	EHEC	+	+
37	EHEC,EIEC,ETEC	+	+
38	EHEC	+	+
39	EHEC,EAEC	+	+
40	EHEC	+	+
41	ETEC	+	+
42	EHEC	+	+
43	EHEC,ETEC	+	+
44	EHEC	+	+
45	EHEC	+	+
46	EHEC,ETEC	+	+
47	EHEC,ETEC	+	+
48	EHEC,EAEC,ETEC	+	+
49	EHEC,ETEC	+	+
50	EHEC	+	+
51	EHEC,EIEC,ETEC	+	+
52	EHEC,EIEC	+	+
53	EHEC,ETEC	+	+

54	EHEC,EIEC,ETEC	+	+
55	EHEC,ETEC	+	+
56	EHEC,EIEC,ETEC	+	+
57	EHEC,ETEC	+	+
58	EHEC,ETEC	+	+
59	EHEC	+	+
60	EHEC	+	+
61	EHEC	+	+
62	EHEC,EAEC	+	+
63	EHEC,EIEC	+	+
64	EHEC,EIEC,ETEC	+	+
65	EHEC	+	+
66	EHEC,EIEC,EAEC	+	+
67	EHEC,EAEC,ETEC	+	+

* +, Positive; -, Negative.

3. PCR Primers used in the experiments

Table S3. The sequences of PCR primers of twelve DEC virulence factor genes.

Target	Item	Sequence (5'-3')	Product size
stx1	F	CGATGTTACGGTTTGTTACTGTGACAGC	224 bp
	R	AATGCCACGCTTCCCAGAATTG	
uida	F	ATGCCAGTCCAGCGTTTTTGC	1487 bp
	R	AAAGTGTGGGTCAATAATCAGGAAGTG	
stx2	F	GTTTTGACCATCTTCGTCTGATTATTGAG	324 bp
	R	AGCGTAAGGCTTCTGCTGTGAC	
it	F	GAACAGGAGGTTTCTGCGTTAGGTG	655 bp
	R	CTTTCAATGGCTTTTTTTTGGGAGTC	
bfpB	F	GACACCTCATTGCTGAAGTCG	910 bp
	R	CCAGAACACCTCCGTTATGC	
pic	F	AGCCGTTTCCGCAGAAGCC	1111 bp
	R	AAATGTCAGTGAACCGACGATTGG	
aggr	F	ACGCAGAGTTGCCTGATAAAG	400 bp
	R	AATACAGAATCGTCAGCATCAGC	
inve	F	CGATAGATGGCGAGAAATTATATCCCG	766 bp
	R	CGATCAAGAATCCCTAACAGAAGAATCAC	
sth	F	TGTCTTTTTCACCTTTCGCTC	171 bp
	R	CGGTACAAGCAGGATTACAACAC	
stp	F	CCTCTTTTAGCAGACACTGAATCATTG	157 bp
	R	CAGGCAGGATTACAACAAAGTTCACAG	
escv	F	ATTCTGGCTCTCTTCTTCTTTATGGCTG	544 bp
	R	CGTCCCCTT TTACAAACTTCATCGC	
astA	F	TGCCATCAACACAGTATATCCG	102 bp
	R	ACGGCTTTGTAGTCCTTCCAT	

* F is forward primer; R is reverse primer.