

Supplementary materials

Simultaneous detection of various pathogenic *Escherichia coli* in water by sequencing multiplex PCR amplicons

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Table S1. Target genes and its concentration of DNA in standard solution.

Strain codes	Target gene	DNA concentration* (ng/μL)
EAEC-1	aggR	207
OT-1	stx1	313
OT-4	stx2	153
SG-2	ipaH	254
OCM-012	eae	314
ETS-002	STh	367
ETS-018	LT	620

*The DNA concentrations were measured using a Quantus fluorometer (Promega, Madison, WI, USA).

Table S2. Target genes and those primers and probes, and information of PCR amplification.

Target bacteria	Target genes	primers, probes	Sequence (5'-3')	Length(bp)	Accession Number	References	Note
Enteroaggregative <i>E. coli</i> (EAEC)	<i>aggR</i>	<i>aggR</i> -f	TCAGTAAGATTGCAAAAGAAGAAATCA	310	LC122428	This study	
		<i>aggR</i> -r	AGCTTACAGCCGATATATAAAATTGACT				
		<i>aggR</i> -pro	FAM-TAGTTGTCCGAATTGGTC-MGB				
Enterohemorrhagic <i>E. coli</i> (EHEC)	<i>stx1</i>	<i>stx1</i> -f	TGATTGATAGTGGCACAGGGG	342	FR875150	This study	
		<i>stx1</i> -r	CAGACTGCGTCAGTGAGGTT				
		<i>stx1</i> -pro	FAM-TATCTGCATCCCCGTACGACTGATCCC-TAMRA				
	<i>stx2</i>	<i>stx2</i> -f	CGGACAGAGATATCGACCCC	301	KT356557	This study	
		<i>stx2</i> -r	CGACACGTTGCAGAGTGGTA				
		<i>stx2</i> -pro	FAM-ATTAAACACACCCACCGGGCAGT-TAMRA				
Enteroinvasive <i>E. coli</i> (EIEC)/ <i>Shigella</i> spp.	<i>ipaH</i>	<i>ipaH</i> -f	TGATGCCACTGAGAGCTGTG	349	FJ227542	This study	
		<i>ipaH</i> -r	CTCTTCACGGCTTC TGACCA				
		<i>ipaH</i> -pro	FAM-ACCGTGTGCGGCTCACATGGAA-TAMRA				
Enteropathogenic <i>E. coli</i> (EPEC)	<i>eae</i>	<i>eae</i> -f	GGAACGGCAGAGGTTAATCTGCAG	360	M58154	Kong et al., 2019	
		<i>eae</i> -r	CGAAGCCATTGTCTGGGCGCTC			This study	
		<i>eae</i> -pro	FAM-TCGGAGCGCGTTACATTGACTCCC-TAMRA				
Enterotoxigenic <i>E. coli</i> (ETEC)	<i>STh</i>	<i>STh</i> -f	TCACCTTTCGCTCAGGATGC	167	AY342059	This study	
		<i>STh</i> -r	CACCCGGTACAAGCAGGATT				
		<i>STh</i> -pro	FAM-TAGTCCTGAAAGCATGAAT-MGB				
	<i>LT</i>	<i>LT</i> -f	AGGAACACAAACCGGCTTTG	349	V00275	This study	
		<i>LT</i> -r	GTAACCATCCTCTGCGCGGAG				
		<i>LT</i> -pro	FAM-AGCGTATACAGCCCTCACCCATATGAACAG-TAMRA				
<i>E. coli</i>	<i>uidA</i>	<i>uidA</i> -277F	CATTACGGCAAAAGTGTGGGTCAAT	658	unknown	Walk et al., 2009	This was used in the Single PCR and Multiplex PCR.
		<i>uidA</i> -934R	TCAGCGTAAGGGTAATGCGAGGTA				
		<i>uidA</i> -f	CGGAAGCAACGCGTAAACTC	90	unknown	silkie et al., 2008	This was used in the Digital PCR.
		<i>uidA</i> -r	TGAGCGTCGCAGAACATTACA				
		<i>uidA</i> -pro	FAM-CTTGTACACACCGCCCGTC-TAMRA				

Table S3. Sampling rivers, and its infomations in this study.

River	Length(km)	Land use	Latitude and Longitude	Sampling date
Oyodo	107	Urban	31°54'27.5"N 131°25'02.9"E	2018/12/17
Oka	9.6	Urban	31°51'10.8"N 131°23'27.4"E	2018/12/17
Yae	8.4	Urban	31°52'25.8"N 131°24'05.5"E	2018/12/17