

Four thermostatic steps(4TS): A novel CRISPR-Cas12-based system for the rapid detection at home of respiratory pathogens

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Table S1. Sequences of primers used in the 4TS system

Gene		Sequence (5'-3')	Length
<i>SARS-CoV-2 N</i>	Primer1	F : GCTACTACCGAAGAGCTACCAGACGAA	219bp
		R: CGATTGCAGCATTGTTAGCAGGATT	
	Primer2	F: AGCCTCTTCTCGTTCCTCATCACGT	189bp
		R:TGGCCTTGTTGTTGTTGGCCTTTAC	
	Primer3	F: CTTCGGGAACGTGGTTGACCTACAC	167bp
		R: GGCTTGAGTTTCATCAGCCTTCTTCTT	
<i>MF Tuf</i>	Primer1	F: GCTAAACCAGGTTCAATTATCCCTCAC	187bp
		R: TTTCTCCAGGCATAACCATTTCACG	
	Primer2	F: GACAAGTTGGTGTTCCTCGTATCGT	123bp
		R: CAAATCCGTATTCTGAAAGAAGTGA	
	Primer3	F: TAAACCAGGTTCAATTATCCCTCAC	185bp
		R: TTTCTCCAGGCATAACCATTTCACG	
<i>CF OmpA</i>	Primer1	F: CTCAATGTAACCTCTAGCCCAGCAC	182bp
		R: ATGTTCAATCTGTATGAAAGAGCCAATC	
	Primer2	F: CTCAATGTAACCTCTAGCCCAGCAC	230bp
		R: GCATCGAAAGTAGCTCTTGACCAAT	
	Primer3	F: TAAGTACCATGAATGGCAAGTAGGATTG	215bp
		R: CAGCATATTTGTTGGAGGTGTCTAAAGT	
<i>FCV Orf1</i>	Primer1	F: GGGAAATCAAATCCTACGAGTACAACA	158bp
		R: GTCAATAATCATTGCCAGGTTTCAGG	
	Primer2	F: CTGGCAATGATTATTGACAACCACG	191bp
		R: AACCTGTGAAGAGGGCTTTGACTT	
	Primer3	F: CAAAGCCACCAAATTCTACAGCCAAGT	203bp
		R: GTGTTGCCACAATCCCTGAGACGAG	
<i>FHV TK</i>	Primer1	F: TCAAGCAAGATTTGCCGCACCATAC	241bp
		R:TACAACCTAGATTTCCACCAGGAGGTT	
	Primer2	F:GAGTTTAAACGGCGAAGTACCTGGTCAGAGCG	223bp
		R:AAGAAGGTATGGTGCGGCAAATCTTGCTTG	
	Primer3	F: GTCCGCATTTACATAGATGGTGCCT	241bp
		R: ATCTTGCTTGATAGTGGGCGGTGAT	

F, forward; R, reverse.

Table S2. Sequences of crRNA used in the 4TS system

Gene			crRNA (5'-3')
<i>SARS-CoV-2 N</i>	primer2	crRNA1	UAAUUUCUACUAAGUGUAGAUCAUCACCGCCAUUGCCA GCC
		crRNA2	UAAUUUCUACUAAGUGUAGAUUUGCUGCUGCUUGACAG AUU
		crRNA3	UAAUUUCUACUAAGUGUAGAUUCUGCUGCUUGACAGAUU GAA
<i>MF Tuf</i>	primer3	crRNA1	UAAUUUCUACUAAGUGUAGAUAAAACUAUAAACCACAA UUC
		crRNA2	UAAUUUCUACUAAGUGUAGAUUGUUCUAAAAAAGAA GAAG
		crRNA3	UAAUUUCUACUAAGUGUAGAUCAAGAAUUCGAAGCUGCA AUU
<i>CF OmpA</i>	primer3	crRNA1	UAAUUUCUACUAAGUGUAGAUUGGAGUGGCCACAACUUU AGA
		crRNA2	UAAUUUCUACUAAGUGUAGAUUACAGAUUGAACAUGC UUG
		crRNA3	UAAUUUCUACUAAGUGUAGAUAAUUGGUCAAGAGCUAC UUU
<i>FCV Orf1</i>	primer2	crRNA1	UAAUUUCUACUAAGUGUAGAUCCAAUUUUUGUCAUAGAA GCC
		crRNA2	UAAUUUCUACUAAGUGUAGAUUGGCUUUGCCAAUUUUG UCA
		crRNA3	UAAUUUCUACUAAGUGUAGAUUGCCGGGUGGGUGAUA ACCCU
<i>FHV TK</i>	primer3	crRNA1	UAAUUUCUACUAAGUGUAGAUACGGCGAAGUACCUGGU CAG
		crRNA2	UAAUUUCUACUAAGUGUAGAUAAACUGAUGUUGUCGG UGGU
		crRNA3	UAAUUUCUACUAAGUGUAGAUUGGAAUAGGUAAGAGU UUA

Table S3. The stability of the 4TS device test

Number	RT-qPCR	4TS		RT-qPCR	4TS
FCV1	+	 +	2	-	 +
3	-	 -	4	-	 -
5	+	 +	6	+	 +
7	-	 -	8	-	 -

9	+	 +	10	+	 +
11	+	 +	12	-	 -
13	-	 -	14	+	 +
15	+	 +	16	-	 -

17	-	 -	18	+	 -
19	+	 +	20	+	 +
FHV1	+	 +	2	+	 +
3	-	 -	4	-	 -

5	+	 +	6	+	 +
7	+	 +	8	+	 +
9	+	 +	10	-	 -
11	-	 -	12	-	 -

13	+	 -	14	+	 +
15		 +	16		 +
17	+	 +	18	+	 +
19	+	 +	20	+	 +

MF1	+	 +	2	+	 +
3	+	 +	4	+	 +
5	+	 +	6	-	 -
7	+	 +	8	-	 -

9	+	 +	10	+	 +
11	+	 +	12	+	 +
13	-	 -	14	+	 +
15	+	 +	16	-	 -

17	+	 +	18	+	 +
19	-	 -	20	+	 +
CF1	+	 +	2	-	 -
3	+	 +	4	-	 -

5	-	 -	6	-	 -
7	-	 -	8	+	 +
9	-	 -	10	+	 +
11	+	 +	12	-	 -

13	+		14	+	
		+			+
15	+		16	+	
		+			+
17	+		18	+	
		+			+
19	+		20	-	
		+			-

The accuracy rates of FCV, FHV, MF and CF tested by 4TS device are 95, 95, 100, 100% respectively (The result of FCV No. 2 and FHV No. 13 were inconsistent with that of RT-qPCR); +, positive; -, negative.