



06-07-2003 4:08 PM

CoV Wuhan 2019-2020

1554

Newly discovered betacoronavirus, Wuhan 2019-2020

A previously unknown betacoronavirus was detected in patients during an outbreak of respiratory illnesses, including atypical pneumonia, that started mid-December 2019 in the city of Wuhan, the capital of Central China's Hubei Province.

The newly discovered coronavirus is similar to some of the betacoronaviruses detected in bats, but it is distinct from SARS-CoV and MERS-CoV.

The genome of the newly discovered CoV consists of a single, positive-stranded RNA that is approximately 30k nucleotides long. The overall genome organization of the newly discovered CoV is similar to that of other coronaviruses. The newly sequenced virus genome encodes the open reading frames (ORFs) common to all betacoronaviruses, including ORF1ab that encodes many enzymatic proteins, the spike-surface glycoprotein (S), the small envelope protein (E), the matrix protein (M), and the nucleocapsid protein (N), as well as several nonstructural proteins.

Virus name	Accession ID	Passage details/host	Collection date	Host	Originating lab	Submitting lab
BetaCoV/Wuhan/WH-01/2019	EPI_ISL_402119	Var. Echovirus Passaged	2019-12-30	Human	National Institute for Viral	National Institute for Viral
BetaCoV/Wuhan/IVDC-HB-04/2020	EPI_ISL_402120	Original	2020-01-01	Human	National Institute for Viral	National Institute for Viral
BetaCoV/Wuhan/IVDC-HB-05/2019	EPI_ISL_402121	Original	2019-12-30	Human	National Institute for Viral	National Institute for Viral
BetaCoV/Wuhan/IPBCAMS-WH-01/2019	EPI_ISL_402123	Original	2019-12-24	Human	Institute of Pathogen Bio	Institute of Pathogen Bio
BetaCoV/Wuhan/WIV04/2019	EPI_ISL_402124	Original	2019-12-30	Human	Wuhan Jinyintan Hospital	Wuhan Institute of Virology

FIG. 1

Postulated PCT amplification sites that specifically detect BetaCov/Wuhan

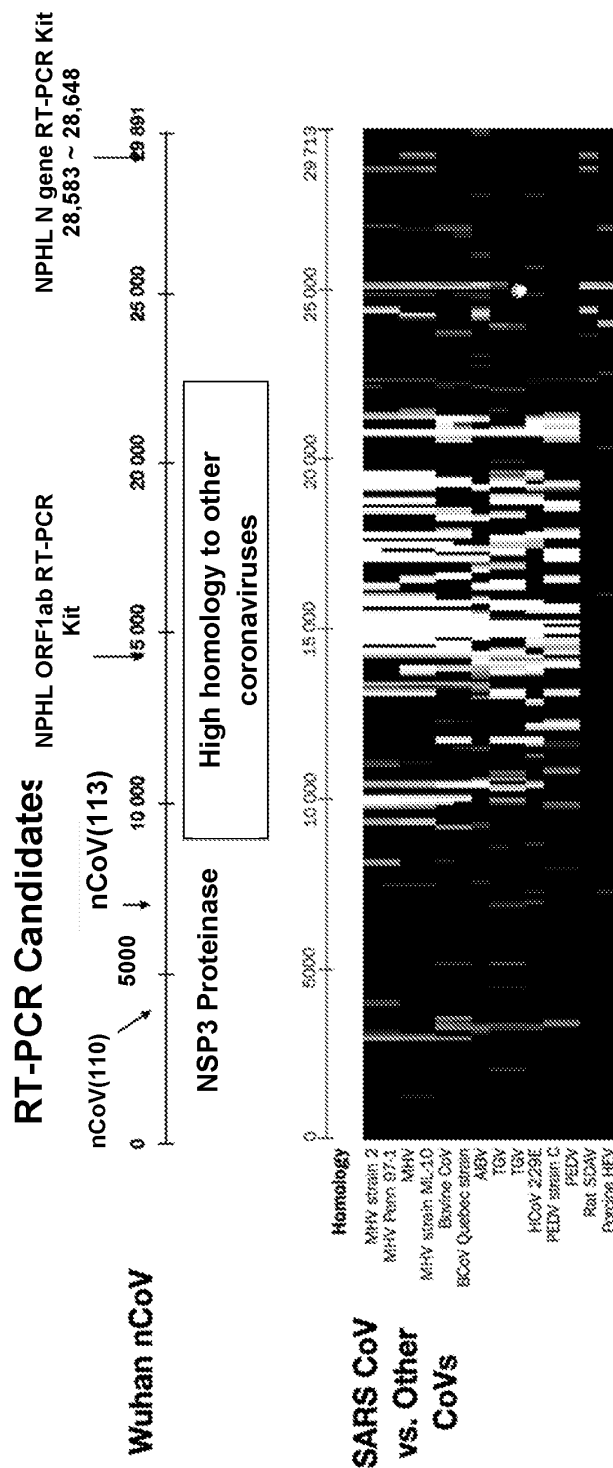


Figure 2. Homology of SARS-CoV genome sequence (SIN2500) to other coronaviruses

YiJun Ruan, et al. The Lancet Vol. 361. May 2003 1779-1785

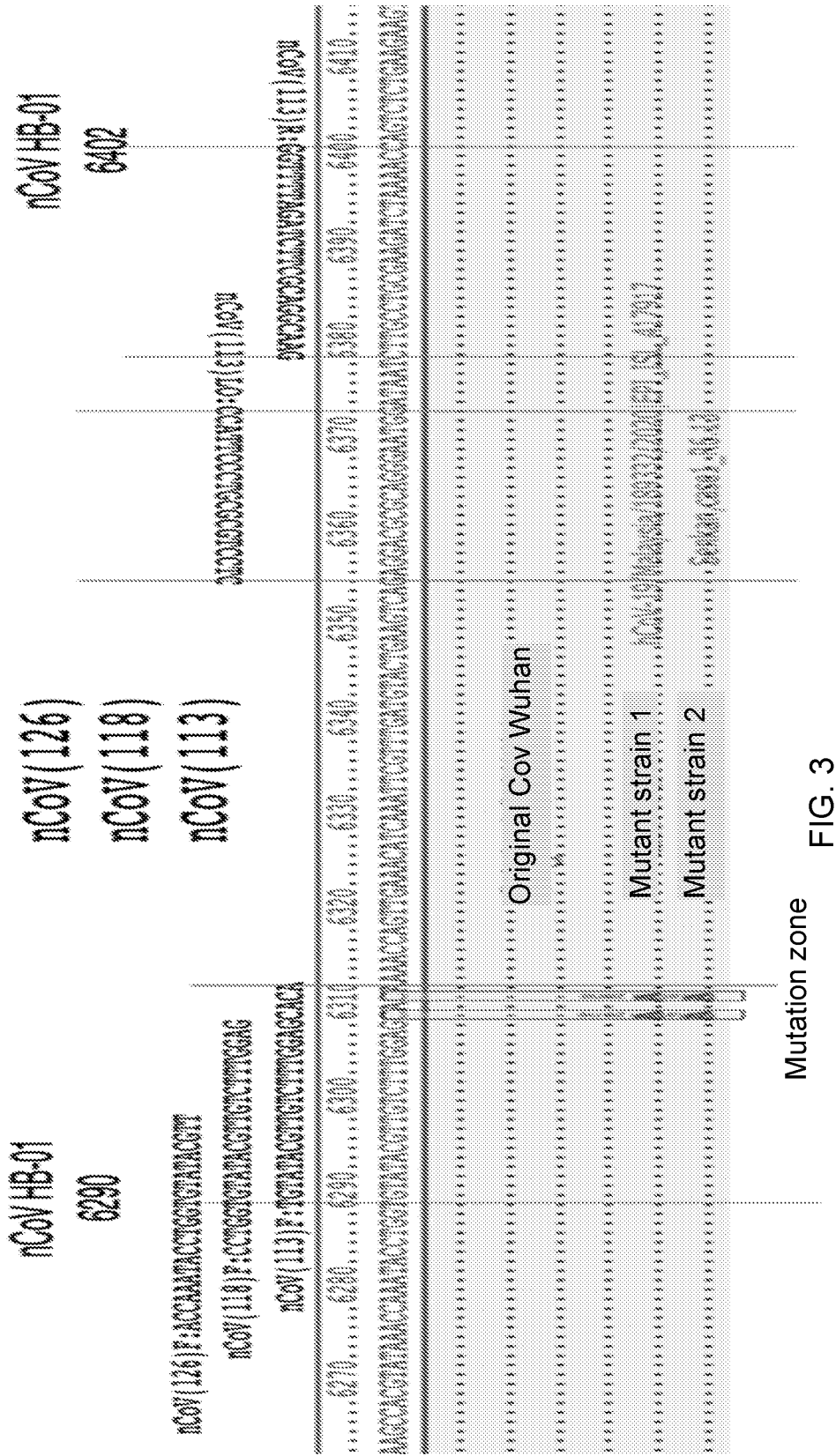
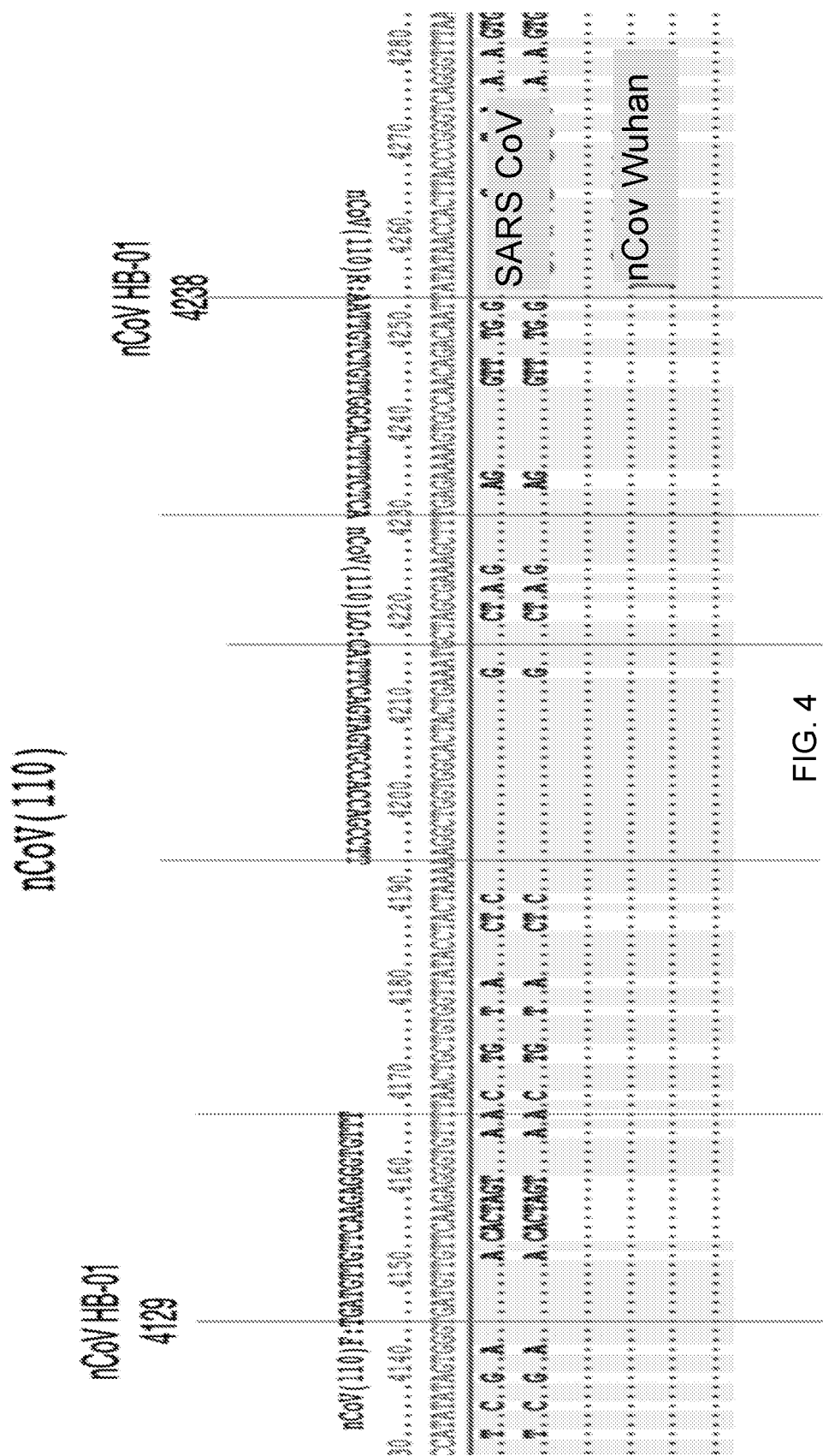


FIG. 3



1 PCR reagent
iTaq Universal Probes One-Step Kit

Cat#172-5140 (100rxns)
Cat#172-5141 (500rxns)

2 Master Mix preparation

200227K

Reaction vol (ul)= 25

		MM1		Final Conc. (uM)	No.	Sample	copies/ul	Date	Source
	Description	25 ul/Rxn (ul)	17						
<u>From</u> <u>Bio-Rad</u> <u>Kti</u>	iScript advanced reverse transcriptase	0.50	8.50		1				
	iTaq universal probes reaction mix (2x) (Buffer containing hot-start Taq DNA pol, dNTPs, MgCl2, enhancers, stabilizers, passive dyes and ROX)	12.50	212.50		2	SARS-CoV-2 synthetic plasmid	10 ⁶	24-Feb-20	EGT
	H2O	8.75	148.75		3				
Test sample	RNA sample	2.50	42.50		4				
	Total Volume	24.25	412.25		5				

MM3			
	Description	25 ul/Rxn (ul)	6
	MM1	25.00	150.00
Primers & Probes	2335/2336-nCoV(13)-F/R, 10uM	0.50	3.00
	2343-nCoV(13)-C (FAM), 10uM	0.25	1.50
	Total Volume	25.75	154.50

0.2 FR
0.1 P

FIG. 5a

MM4			
	Description	25 ul/Rxn (ul)	6
	MM4	25.75	154.50
Primers & Probes	2339/2340 nCoV(110) F/R, 10uM	0.50	3.00
	2341 nCoV(110)LO (FAM), 10uM	0.25	1.50
Total Volume			159.00

0.2 FR
0.1 P

3 RT-PCR cycling

conditions			
		BioRad CFX96	
Step	Temp (°C) net (sec)	Duration	No. of Cycle(s)
1	48	15 mins	1
2	95	2 min 30 s	1
3	95	20 secs	42
	51.5-60.5*	42 secs	

* Perform gradient temperature and fluorescence data collection here.

Annealing Temperature (°C)
60.5
60.0
59.0
57.2
55.1
53.3
52.1
51.5

4 Data Analysis			
No.	Target	Dye	Bio-Rad CFX 96
1	nCoV	FAM	Channel FAM Threshold 50

FIG. 5b

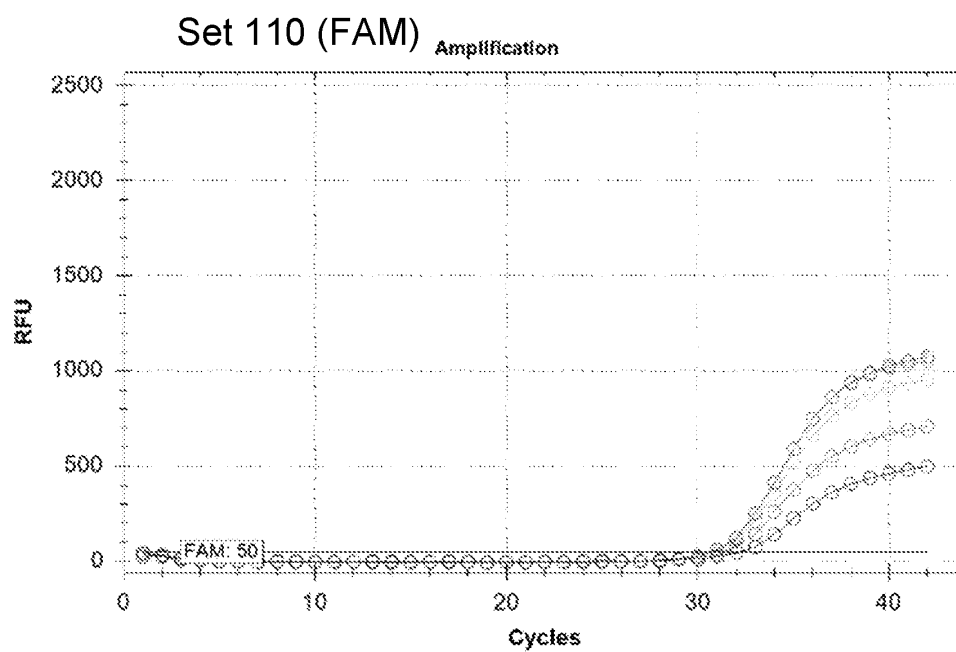


FIG. 6a

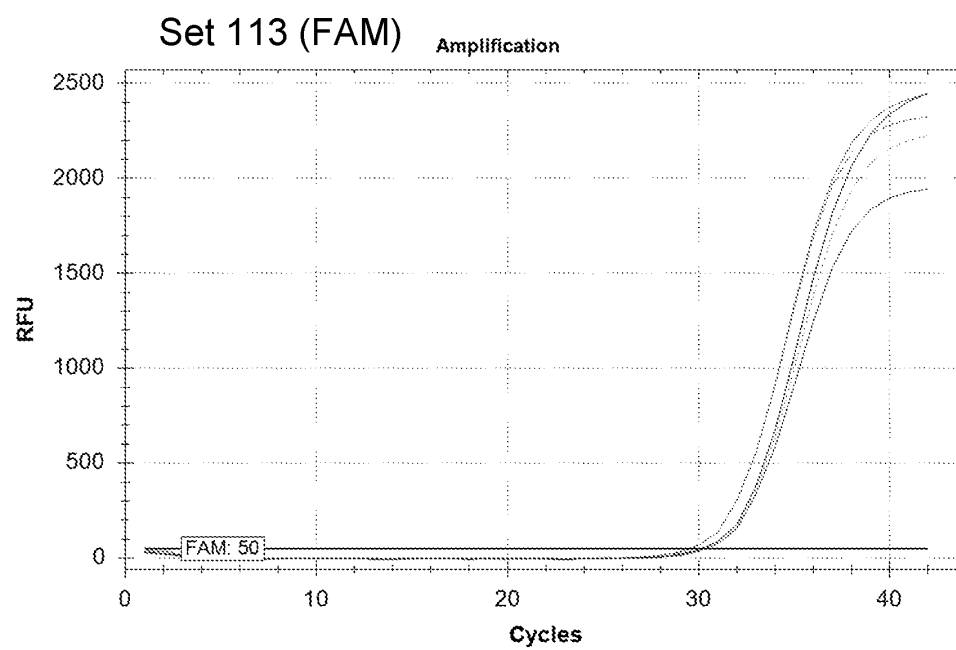


FIG. 6b

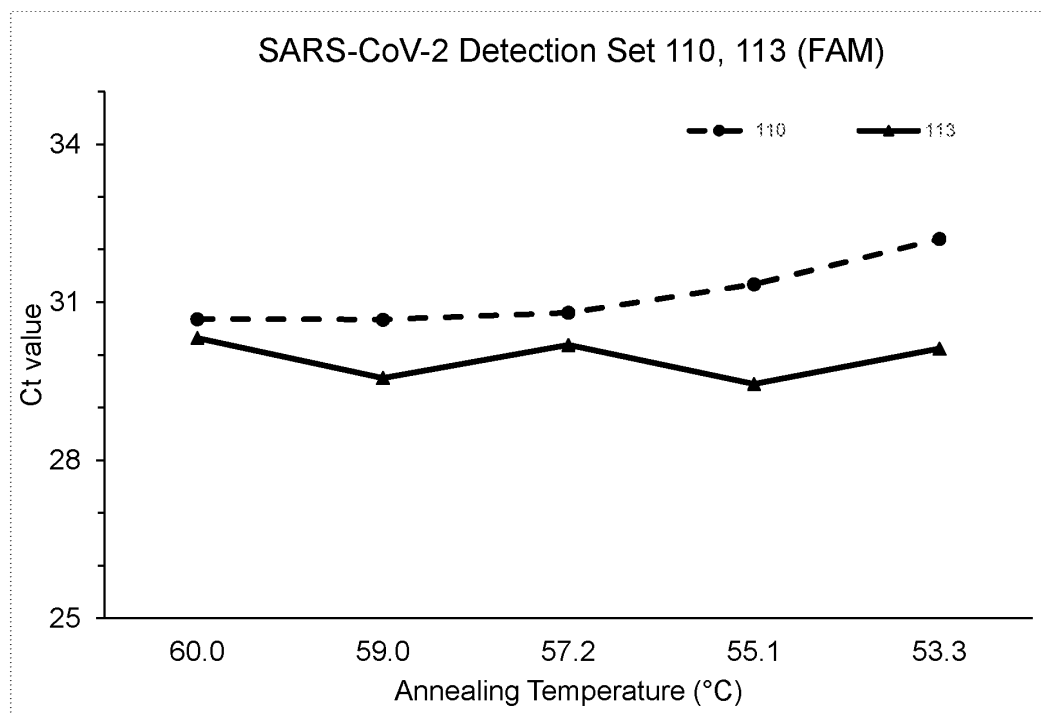


Fig. 7

						Total number of sequences	
						268,011	
	Case	Sense SEQ (5'-3')	Length	Priming Efficiency (P.E. #)	Number of cases	% of cases	
113 Forward primer		TGTATACGTTGTCCTTTGGAGCACA	24	492 (Above the threshold): Perfect match	265,212	98.96%	
Targ Single Mut	1	TGCATACGTTGTCCTTTGGAGCACA	24	472(Above the threshold)	5	0.002%	
Targ Single Mut	2	TGTGTACGTTGTCCTTTGGAGCACA	24	472(Above the threshold)	43	0.016%	
Targ Single Mut	3	TGTACACGTTGTCCTTTGGAGCACA	24	471(Above the threshold)472	115	0.043%	
Targ Single Mut	4	TGTATGCGTTGTCCTTTGGAGCACA	24	463(Above the threshold)471	10	0.004%	
Targ Single Mut	5	TGTATACATTGTCCTTTGGAGCACA	24	446(Above the threshold)463	7	0.003%	
Targ Single Mut	6	TGTATACGCTGTCCTTTGGAGCACA	24	450(Above the threshold)446	4	0.001%	
Targ Single Mut	7	TGTATACGTAGTCCTTTGGAGCACA	24	439(Above the threshold)450	1	0.000%	
Targ Single Mut	8	TGTATACGTTGTCCTTTGGAGCACA	24	411(Above the threshold)439	37	0.014%	
Targ Single Mut	9	TGTATACGTTGTCCTTAGGAGCACA	24	401(Above the threshold)411	2	0.001%	
Targ Single Mut	10	TGTATACGTTGTCCTTTGAGCACA	24	376(Above the threshold)401	2	0.001%	
Targ Single Mut	11	TGTATACGTTGTCCTTTGAGCACA	24	356(Above the threshold)376	6	0.002%	
Targ Single Mut	12	TGTATACGTTGTCCTTTGGGCACA	24	375(Above the threshold)356	26	0.010%	
Targ Single Mut	13	TGTATACGTTGTCCTTTGGCGACA	24	349(Above the threshold)375	30	0.011%	
Targ Single Mut	14	TGTATACGTTGTCCTTTGGAACACA	24	353(Above the threshold)349	12	0.004%	
Targ Single Mut	15	TGTATACGTTGTCCTTTGGATCACA	24	312(Above the threshold) was 353	24	0.009%	
Targ Single Mut	16	TGTATACGTTGTCCTTTGGAGTACA	24	<i>489(Above the threshold)118F substitutes</i>	553	0.206%	
Targ Single Mut	17	TGTATACGTTGTCCTTTGGAGCGCA	24	<i>489(Above the threshold)118F substitutes</i>	3	0.001%	
Targ Single Mut	18	TGTATACGTTGTCCTTTGGAGCCCA	24	<i>489(Above the threshold)118F substitutes</i>	1	0.000%	
Targ Single Mut	19	TGTATACGTTGTCCTTTGGAGCATA	24	<i>489(Above the threshold)118F substitutes</i>	287	0.107%	

Fig. 8a

Targ Single Mut	20	TGTATACGTTGTCTTTTGGAGCAGA	24	489(Above the threshold)118F substitutes	2	0.001%
Targ Single Mut	21	TGTATACGTTGTCTTTTGGAGCAAA	24	489(Above the threshold)118F substitutes	696	0.260%
Targ Single Mut	22	TGTATACGTTGTCTTTTGGAGCACG	24	489(Above the threshold)118F substitutes	1	0.000%
Targ Double Mut	23	TGTATACGTTGTCTTTGGCGCAAA	24	147:118F' substitutes	1	0.000%
Targ Double Mut	24	TGTATACGTTGTCTTTGGACCATA	24	163:118F' substitutes	1	0.000%
Targ Double Mut	25	TGTATACGTTGTCTTTGGAGAAAA	24	489(Above the threshold)118F substitutes	870	0.325%
Targ Double Mut	26	TGTATACGTTGTCTTTGGAGGAAA	24	489(Above the threshold)118F substitutes	1	0.000%
Targ Double Mut	27	TGTATACGTTGTCTTTGGAGMAAA	24	489(Above the threshold)118F substitutes	43	0.016%
Targ Double Mut	28	TGTATACGTTGTCTTTGGAGNAAA	24	489(Above the threshold)118F substitutes	4	0.001%
Targ Triple Mut	29	TGTATACGTTGTCTTTGGAAAAAA	24	147:118F' substitutes	3	0.001%
Targ Triple Mut	30	TGTATACGTTGTCTTTGGARAAAA R=(A/G)	24	489(Above the threshold)118F substitutes	1	0.000%
Targ Single Mut	31	TGTATATGTTGTCTTTGGAGCACA	24	448(Above the threshold)	1	0.000%
Targ Single Mut	32	TGTATACGTTGCCCTTTGGAGCACA	24	436(Above the threshold)	2	0.001%
Targ Single Mut	33	TGTATACGTTGGCTTTGGAGCACA	24	427(Above the threshold)	1	0.000%
Targ Single Mut	34	TGTATACGTTGTCTATGGAGCACA	24	411(Above the threshold)	3	0.001%
Targ Single Mut	35	TGTATACGTTGTCTTTGGAGAAAA	24	304(Above the threshold)	1	0.000%
				TOTAL	2,799	1.044%

Fig. 8b

	Case	Sense SEQ (5'-3')	Length	Priming Efficiency (P.E. #)	Number of cases	% of cases
118 Forward primer		CCTGGTGTATACGTTGTCCTTTGGAG	25	489(Above the threshold):Perfect match	201,598	75.22%
Targ Single Mut	1	TCTGGTGTATACGTTGTCCTTTGGAG	25	492(Above the threshold)113F substitutes	523	0.195%
Targ Single Mut	2	CATGGTGTATACGTTGTCCTTTGGAG	25	492(Above the threshold)113F substitutes	5	0.002%
Targ Single Mut	3	CGTGGTGTATACGTTGTCCTTTGGAG	25	492(Above the threshold)113F substitutes	6	0.002%
Targ Single Mut	4	CTTGGTGTATACGTTGTCCTTTGGAG	25	492(Above the threshold)113F substitutes	64,476	24.057%
Targ Single Mut	5	CCTGGTGCATACGTTGTCCTTTGGAG	25	472(Above the threshold)113F substitutes	3	0.001%
Targ Single Mut	6	CCTGGTGTACGTTGTCCTTTGGAG	25	472(Above the threshold)113F substitutes	33	0.012%
Targ Single Mut	7	CCTGGTGTACACGTTGTCCTTTGGAG	25	471(Above the threshold)113F substitutes	113	0.042%
Targ Single Mut	8	CCTGGTGTATGCGTTGTCCTTTGGAG	25	463(Above the threshold)113F substitutes	8	0.003%
Targ Single Mut	9	CTTGGTGTATRCGTTGTCCTTTGGAG	25	R=(A/G) no issue	1	0.000%
Targ Single Mut	10	CCTGGTGTATACATTGTCCTTTGGAG	25	446(Above the threshold)113F substitutes	1	0.000%
Targ Single Mut	11	CCTGGTGTATACGCTGTCCTTTGGAG	25	450(Above the threshold)113F substitutes	4	0.001%
Targ Single Mut	12	CCTGGTGTATACGTTGTCCTTTGGAG	25	411(Above the threshold)113F substitutes	36	0.013%
Targ Single Mut	13	CCTGGTGTATACGTTGTCCTTTAGGAG	25	401(Above the threshold)113F substitutes	2	0.001%
Targ Single Mut	14	CCTGGTGTATACGTTGTCCTTTGGAG	25	376(Above the threshold)113F substitutes	2	0.001%
Targ Single Mut	15	CCTGGTGTATACGTTGTCCTTTGTAG	25	356(Above the threshold)113F substitutes	4	0.001%
Targ Single Mut	16	CCTGGTGTATACGTTGTCCTTTGGCG	25	349(Above the threshold)113F substitutes	31	0.012%
Targ Single Mut	17	CCTGGTGTATACGTTGTCCTTTGGGG	25	375(Above the threshold)113F substitutes	25	0.009%
Targ Single Mut	18	CCTGGTGTATACGTTGTCCTTTGGAT	25	353(Above the threshold)113F substitutes	24	0.009%
Targ Single Mut	19	CCTGGTGTATACGTTGTCCTTTGGAC	25	353(Above the threshold)113F substitutes	1	0.000%
Targ Single Mut	20	CCTGGTGTATACGTTGTCCTTTGGAA	25	349(Above the threshold)113F substitutes	15	0.006%
Targ Double Mut	21	CTTGGTGTATACGTTGTCCTTTGTAG	25	356(Above the threshold)113F substitutes	2	0.001%
Targ Double Mut	22	CTTGGTGTATACGTTGTCCTTTGGAG	25	411(Above the threshold)113F substitutes	1	0.000%
Targ Double Mut	23	CTTGGTGTATACGTTGTCCTTTGGAG	25	439(Above the threshold)113F substitutes	1	0.000%

Fig. 9a

Targ <u>Double</u> Mut	24	CTTGGTGTACACGTTGCTCTTTGGAG	25	471(Above the threshold)113F substitutes	2	0.001%
Targ <u>Double</u> Mut	25	CTTGGTGTGTACGTTGCTCTTTGGAG	25	472(Above the threshold)113F substitutes	10	0.004%
Targ <u>Double</u> Mut	26	CTTGGTGCATACGTTGCTCTTTGGAG	25	473(Above the threshold)113F substitutes	2	0.001%
Targ <u>Double</u> Mut	27	ATTGGTGTATACGTTGCTCTTTGGAG	25	489(Above the threshold)113F substitutes	5	0.002%
Targ <u>Double</u> Mut	28	TTTGGTGTATACGTTGCTCTTTGGAG	25	489(Above the threshold)113F substitutes	1,047	0.391%
Targ <u>Single</u> Mut	29	CCTGGTGTATACGTTGCCCTTTGGAG	25	436(Above the threshold)113F substitutes	2	0.001%
Targ <u>Single</u> Mut	30	CCTGGTGTATACGTTGGCTTTGGAG	25	427(Above the threshold)113F substitutes	1	0.000%
Targ <u>Single</u> Mut	31	CCTGGTGTATACGTTGCTCTATGGAG	25	411(Above the threshold)113F substitutes	3	0.001%
Targ <u>Double</u> Mut	32	YTTGGTGTATACGTTGCTCTTTGGAG	25	489(Above the threshold)113F substitutes	12	0.004%
Targ <u>Double</u> Mut	33	CTTGGTGTATACGTTGCTCTTTGGAG	25	489(Above the threshold)113F substitutes	1	0.000%
Targ <u>Double</u> Mut	34	CTTGGTGYATACGTTGCTCTTTGGAG	25	489(Above the threshold)113F substitutes	1	0.000%
Targ <u>Double</u> Mut	35	CTTGGTGTATATGTTGCTCTTTGGAG	25	448(Above the threshold)113F substitutes	1	0.000%
Targ <u>Double</u> Mut	36	CTTGGTGTATACATTGCTCTTTGGAG	25	446(Above the threshold)113F substitutes	6	0.002%
Targ <u>Double</u> Mut	37	CTTGGTGTATACGTTGCTCTTTGGGG	25	375(Above the threshold)113F substitutes	1	0.000%
Targ <u>Double</u> Mut	38	CTTGGTGTATGCGTTGCTCTTTGGAG	25	463(Above the threshold)113F substitutes	2	0.001%
		Y=(C/T)		TOTAL	66,413	24.780%

Fig. 9b

	Case	SENSE SEQ(5'-3')	Length	REV primer antisense (5'-3')	Number of cases	% of cases
113/118 Reverse primer		<u>CTTGCCTGCGAAGATCTATAAAC</u>	23	GGTTTAGATCTTCGACGGCAAG	266,094	99.28%
Targ Single Mut	1	TTTGCCTGCGAAGATCTATAAAC	23	211(Above the threshold)	137	0.051%
Targ Single Mut	2	ATTGCCTGCGAAGATCTATAAAC	23	189(Above the threshold)	1	0.000%
Targ Single Mut	3	CTTGTCTGCGAAGATCTATAAAC	23	328(Above the threshold)	20	0.007%
Targ Single Mut	4	CTTGCCTGCGAAGATCTATAAAC	23	336(Above the threshold)	2	0.001%
Targ Single Mut	5	CTTGCATGCGAAGATCTATAAAC	23	350(Above the threshold)	12	0.004%
Targ Single Mut	6	CTTGCCTGCGAAGATCTATAAAC	23	361(Above the threshold)	57	0.021%
Targ Single Mut	7	CTTGCCAGCGAAGATCTATAAAC	23	364(Above the threshold)	1	0.000%
Targ Single Mut	8	CTTGCCTACGAAGATCTATAAAC	23	392(Above the threshold)	50	0.019%
Targ Single Mut	9	CTTGCCTGTGAAGATCTATAAAC	23	387(Above the threshold)	56	0.021%
Targ Single Mut	10	CTTGCCTGCGACGATCTATAAAC	23	437(Above the threshold)	19	0.007%
Targ Single Mut	11	CTTGCCTGCGAGGATCTATAAAC	23	448(Above the threshold)	76	0.028%
Targ Single Mut	12	CTTGCCTGCGATGATCTATAAAC	23	440(Above the threshold)	1	0.000%
Targ Single Mut	13	CTTGCCTGCGAAAAATCTATAAAC	23	453(Above the threshold)	4	0.001%
Targ Single Mut	14	CTTGCCTGCGAATATCTATAAAC	23	441(Above the threshold)	17	0.006%
Targ Single Mut	15	CTTGCCTGCGAAGGTCTATAAAC	23	457(Above the threshold)	13	0.005%
Targ Single Mut	16	CTTGCCTGCGAAGCTCTATAAAC	23	449(Above the threshold)	4	0.001%
Targ Single Mut	17	CTTGCCTGCGAAGACCTATAAAC	23	464(Above the threshold)	136	0.051%

Fig. 10a

Targ Single Mut	18	CTTGCCTGCGAAGATATAAAACC	23	459(Above the threshold)	1	0.000%
Targ Single Mut	19	CTTGCCTGCGAAGATGTAAACC	23	456(Above the threshold)	1	0.000%
Targ Single Mut	20	CTTGCCTGCGAAGATTAAACC	23	462(Above the threshold)	322	0.120%
Targ Single Mut	21	CTTGCCTGCGAAGATCAAAACC	23	468(Above the threshold)	3	0.001%
Targ Single Mut	22	CTTGCCTGCGAAGATCTCAAACC	23	472(Above the threshold)	4	0.001%
Targ Single Mut	23	CTTGCCTGCGAAGATCTACAACC	23	472(Above the threshold)	3	0.001%
Targ Single Mut	24	CTTGCCTGCGAAGATCTAAAGCC	23	479(Above the threshold)	14	0.005%
Targ Single Mut	25	CTTGCCTGCGAAGATCTAAAACC	23	477(Above the threshold)	3	0.001%
Targ Single Mut	26	CTTGCCTGCGAAGATCTAAAAGC	23	477(Above the threshold)	9	0.003%
Targ Single Mut	27	CTTGCCTGCGAAGATCTAAAATC	23	477(Above the threshold)	365	0.136%
Targ Single Mut	28	CTTGCCTGCGAAGATCTAAAACCT	23	489(Above the threshold)	549	0.205%
Targ Double Mut	29	CTTGCCTGCGAAGATCTATTACC	23	462(Above the threshold)	1	0.000%
Targ Double Mut	30	CTTGCCTGCAAGATTAAACC	23	378(Above the threshold)	2	0.001%
Targ Double Mut	31	CTTGCCCAACGAAGATCTAAACC	23	323(Above the threshold)	1	0.000%
Targ Double Mut	32	ATGGCCTGCGAAGATCTAAACC	23	101	1	0.000%
Targ Single Mut	33	CCTGCCTGCGAAGATCTAAACC	23	201(Above the threshold)	2	0.001%
Targ Single Mut	34	CATGCCTGCGAAGATCTAAACC	23	168(Above the threshold)	2	0.001%
Targ Single Mut	35	CGTGCCTGCGAAGATCTAAACC	23	168(Above the threshold)	2	0.001%
Targ Single Mut	36	CTAGCCTGCGAAGATCTAAACC	23	282(Above the threshold)	3	0.001%
Targ Single Mut	37	CTCGCCTGCGAAGATCTAAACC	23	317(Above the threshold)	1	0.000%
Targ Single Mut	38	CTTTCCTGCGAAGATCTAAACC	23	277(Above the threshold)	4	0.001%

Fig. 10b

Targ Single Mut	39	CTTGCCTGCAAAAGATCTTAAACC	23	409(Above the threshold)	6	0.002%
Targ Single Mut	40	CTTGCCTGCGCAGATCTTAAACC	23	429(Above the threshold)	2	0.001%
Targ Single Mut	41	CTTGCCTGCGGAGATCTTAAACC	23	441(Above the threshold)	3	0.001%
Targ Single Mut	46	CTTGCCTGCGAAGAGCTTAAACC	23	458(Above the threshold)	1	0.000%
Targ Single Mut	42	CTTGCCTGCGAAGATCTTAAACC	23	473(Above the threshold)	1	0.000%
Targ Single Mut	45	CTTGCCTGCGAAGATCTAGAAC	23	475(Above the threshold)	1	0.000%
Targ Single Mut	43	CTTGCCTGCGAAGATCTTAAATCC	23	478(Above the threshold)	1	0.000%
Targ Double Mut	44	CTTGCCTGCGAAGGTCTTAAACT	23	446(Above the threshold)	3	0.001%
				TOTAL	1917	0.715%

Fig. 10c

	Number	SENSE SEQ(5'-3')	Length	REV Probe antisense (5'-3')	Number of cases	% of cases
113/118 Probe		GAGGACGCGCAGGGAATGG	19	CCATTCCCTGCGCGTCCTC	267,973	99.99%
Targ Single Mut	1	AAGGACGCGCAGGGAATGG	19	559(Above the threshold): Perfect match	22	0.008%
Targ Single Mut	28	GAGGACGCGCAGGGAATGT	19	247 (Above the threshold)	16	0.006%
				TOTAL	38	0.014%

Fig. 11