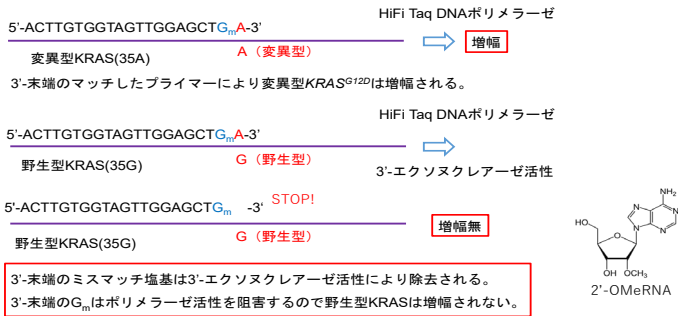


Abstract.

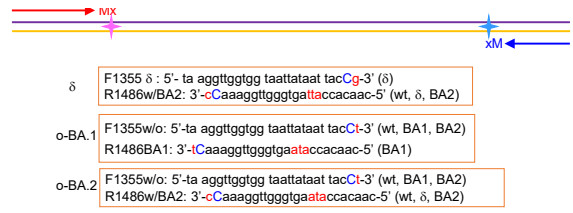
In our previous study, we reported highly specific and quantitative PCR analysis discriminating single base mutation between *KRAS<sup>wt</sup>*, *KRAS<sup>G12D</sup>*, *KRAS<sup>G12A</sup>* and *KRAS<sup>G12V</sup>* genes using chemically modified primers and proofreading polymerase. In the present study, highly sensitive and rapid detection of SARS-CoV-2  $\delta$ ,  $\alpha$ -BA1 and  $\alpha$ -BA2 variants by the PCR method. The forward primer was designed to discriminate the mutation at 1355 position of spike glycoprotein and the reverse primer was designed to discriminate the mutation at 1486 position. We could show that PCR using these sets of primers clearly discriminated these three variants of SARS-CoV-2 with high sensitivity.

変異型プライマーによる変異型・野生型KRASの増幅



Tx: KRAS(35x)  
Acttggtgtagttggagct(a/a/c/t)ggcgtaggcaagagtgccctgacgatacagtaatcagaatcatttgggacgaa  
tatgatccaacaataga-3'  
 Reverse Primer: 5'-tctattgttgatcattctg-3'  
 Forward Primers:  
 POM2c; 5'-acttggtgtagttggagct(G)<sup>c</sup>-3'  
 POM2a; 5'-acttggtgtagttggagct(G<sup>m</sup>)<sup>a</sup>-3'  
 POM2g; 5'-acttggtgtagttggagct(G<sup>m</sup>)<sup>g</sup>-3'  
 POM2t; 5'-acttggtgtagttggagct(G<sup>m</sup>)<sup>l</sup>-3'  
 PSM2c; 5'-acttggtgtagttggagct(G)<sup>c</sup>-3'  
 PSM2a; 5'-acttggtgtagttggagct(G<sup>m</sup>)<sup>a</sup>-3'  
 PSM2g; 5'-acttggtgtagttggagct(G<sup>m</sup>)<sup>g</sup>-3'  
 PSM2t; 5'-acttggtgtagttggagct(G<sup>m</sup>)<sup>l</sup>-3'

ウイルス株*	(野生型株)	アルファ株	ベータ株	ガンマ株	デルタ株	カッパ株	ラムダ株	ミュー株	オミクロン株
Target	EPL_ISL_402124	EPL_ISL_601443	EPL_ISL_700428	EPL_ISL_833137	EPL_ISL_2379631	EPL_ISL_1704611	EPL_ISL_2508582	EPL_ISL_2933813	EPL_ISL_6913953.2
L452L/L452R	452L	452L	452L	452L	L452R	L452R	L452Q	452L	452L <sup>ts</sup>
L452L/L452R/L452Q	452L	452L	452L	452L	L452R	L452R	L452Q	452L	452L
N501N/N501Y	501N	N501Y	N501Y	N501Y	501N <sup>ts</sup>	501N	501N	N501Y	N501Y
N501Y/E484K	501N	N501Y	N501Y	N501Y	501N	501N	501N	N501Y	N501Y
	484E	484E	E484K	E484K	484E	E484Q	484E	E484K	E484A
T478T/T478K	478T	478T	478T	478T	T478K	478T	478T	478T	T478K <sup>ts</sup>
P681P/P681R/P681H	681P	P681H	681P <sup>ts</sup>	681P	P681R	P681R	681P	P681H	P681H
E484E/E484K/E484Q	484E	484E	E484K	E484K	484E	E484Q	484E	E484K	E484A
E484E/E484K	484E	484E	E484K	E484K	484E	E484Q	484E	E484K	E484A
F490F/F490S	490F	490F	490F	490F	490F	490F	F490S	490F	490F
E484E/E484A	484E	484E	E484K	E484K	484E	E484Q	484E	E484K	E484A
G339G/G339D	339G	339G	339G	339G	339G	339G	339G	339G	G339D



	1355	1486	1451
Wuhan-Hu-1	Ct-3'	3'-cC	5'-gaa
$\delta$	Cg-3'	3'-cC	5'-gaa
$\alpha$ -BA.1	Ct-3'	3'-cC	5'-gaa
$\alpha$ -BA.2	Ct-3'	3'-cC	5'-gca

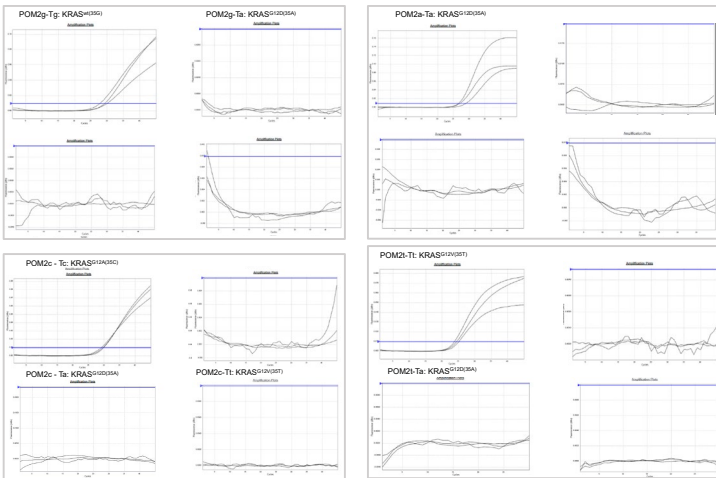
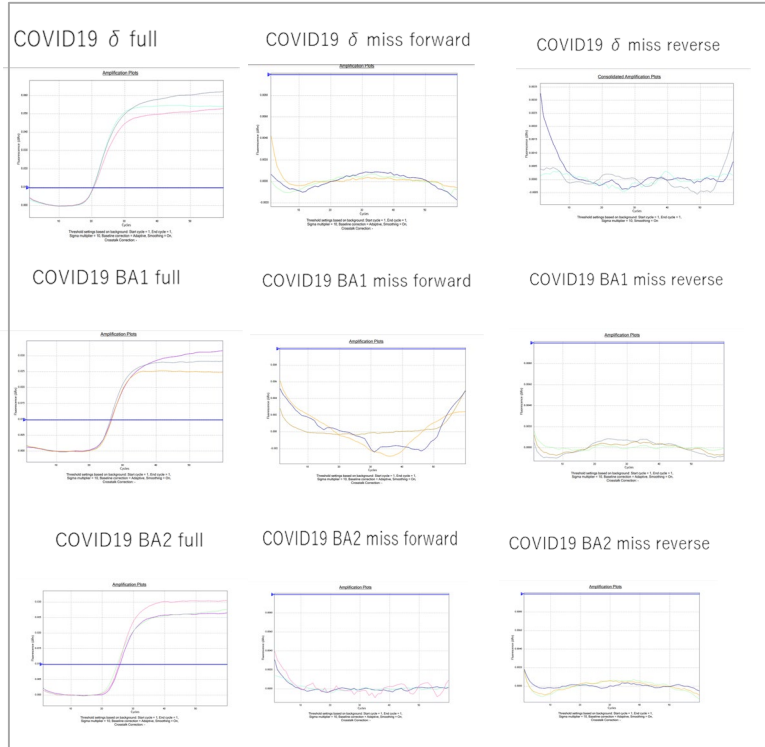
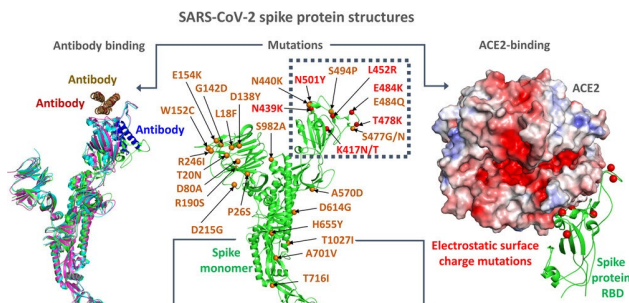


Table 1. qPCR by TaKaRa Ex Taq Polymerase and 2'-OMeRNA Modified Primers

Primers	Ct/Tg (wt)	Ct/Ta (G12D)	Ct/Tc(G12A)	Ct/Tt(G12V)	$\Delta$ Ct
POM2t	NA	NA	NA	24.77 $\pm$ 0.81	$\infty$
POM2c	NA	NA	25.95 $\pm$ 0.20	NA	$\infty$
POM2a	NA	22.67 $\pm$ 0.92	NA	NA	$\infty$
POM2g	25.41 $\pm$ 0.84	NA(G-T)	NA	NA	$\infty$
PSM2t	NA/	NA	NA	24.69 $\pm$ 0.15	$\infty$
PSM2c	NA	NA	25.31 $\pm$ 0.30	NA	$\infty$
PSM2a	NA	23.63 $\pm$ 0.19	NA	NA	$\infty$
PSM2g	24.02 $\pm$ 0.85	NA(G-T)	NA	NA	$\infty$



	SARS-CoV-2 $\delta$	SARS-CoV-2 $\alpha$ .BA.1	SARS-CoV-2 $\alpha$ .BA.2
F1355 $\delta$ R1486w/BA2	20.57 $\pm$ 0.07	NA	NA
F1355w/o R1486BA1	NA	25.32 $\pm$ 0.35	NA
F1355w/o R1486w/BA2	NA	NA	26.48 $\pm$ 0.19



結論  
 2つのプライマーの3'-末端で同時に1塩基変異を識別することにより、SARS-CoV-2の3つの変異株を Real Time PCR により識別することを可能にした。