Supplementary Materials

A Thermostable Cas12b from *Brevibacillus* Leverages One-pot Detection of SARS-CoV-2 Variants of Concern

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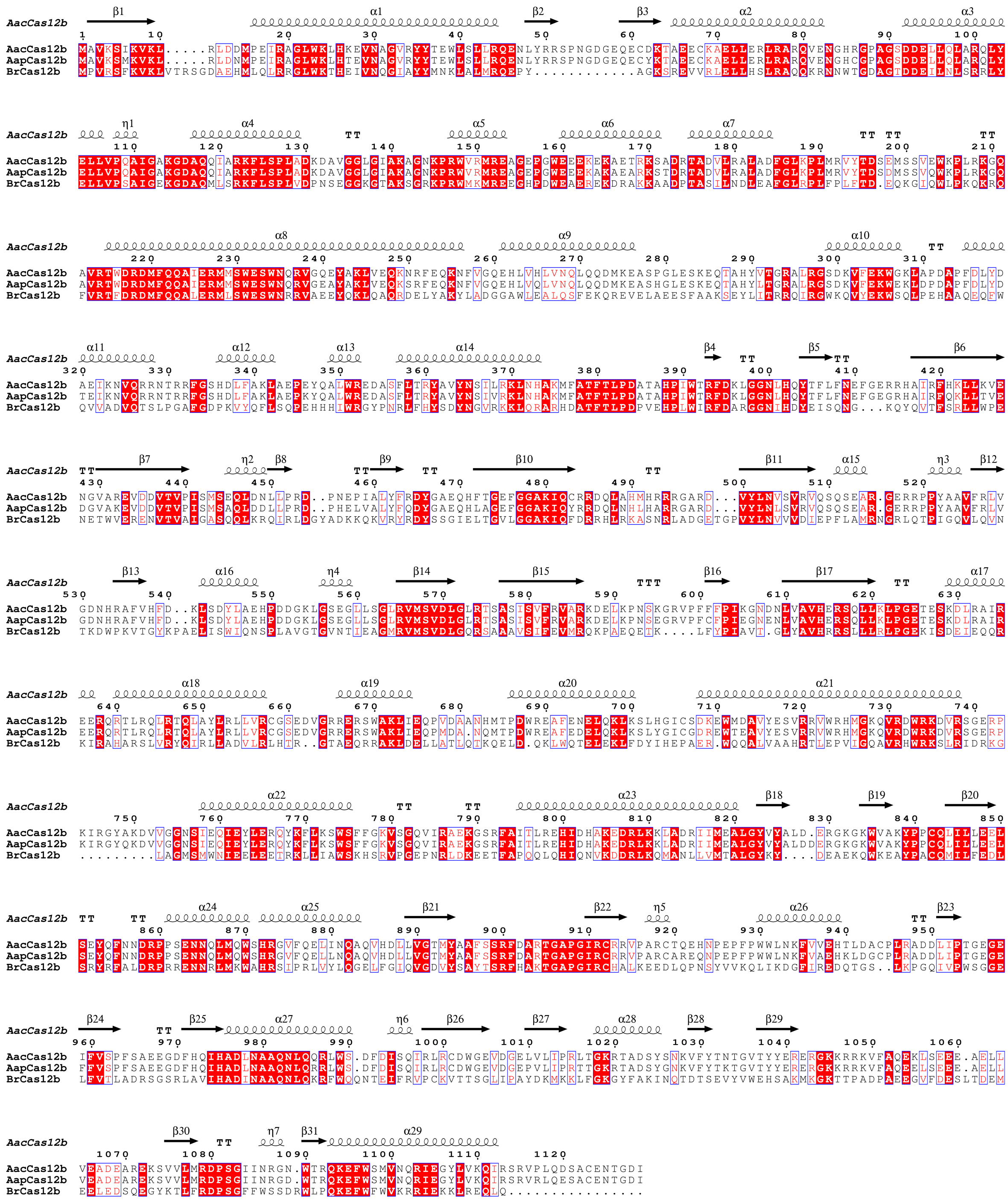

Figure S1. Sequence alignment of AapCas12 and BrCas12b against the structure of AacCas12b. The sequences of the above effectors were aligned using MultiAlin with Blosum62-12-2. The aligned sequence file was imported into Espript 3.0(1) and aligned against the AacCas12b structure (PDBID: 5U30)(2).

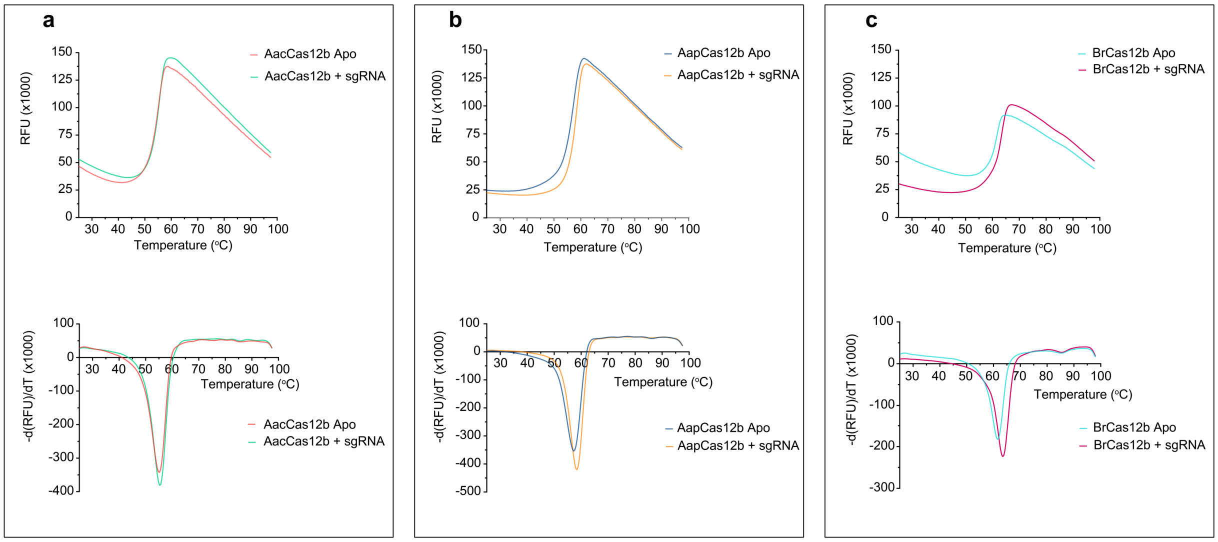

Figure S2. **Differential scanning fluorimetry of Apo and binary complexes of AacCas12b, AapCas12b, and BrCas12b**. The reaction was performed in duplicates and repeated twice (n = 2 biological replicates). The melting temperature was determined based on the global minimum of derivative fluorescence with respect to temperature.

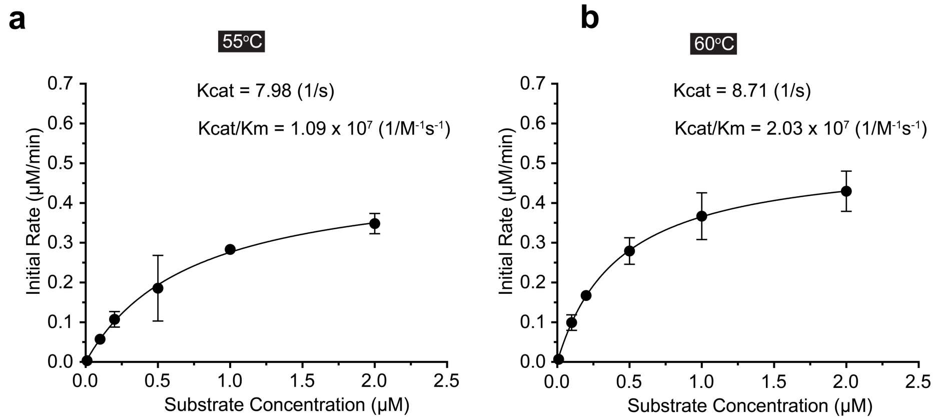

Figure S3. **Trans-cleavage kinetic analysis of BrCas12b.** (**a**) at 55^{o}C and (**b**) at 60^{o}C, (n = 2 biological replicates).

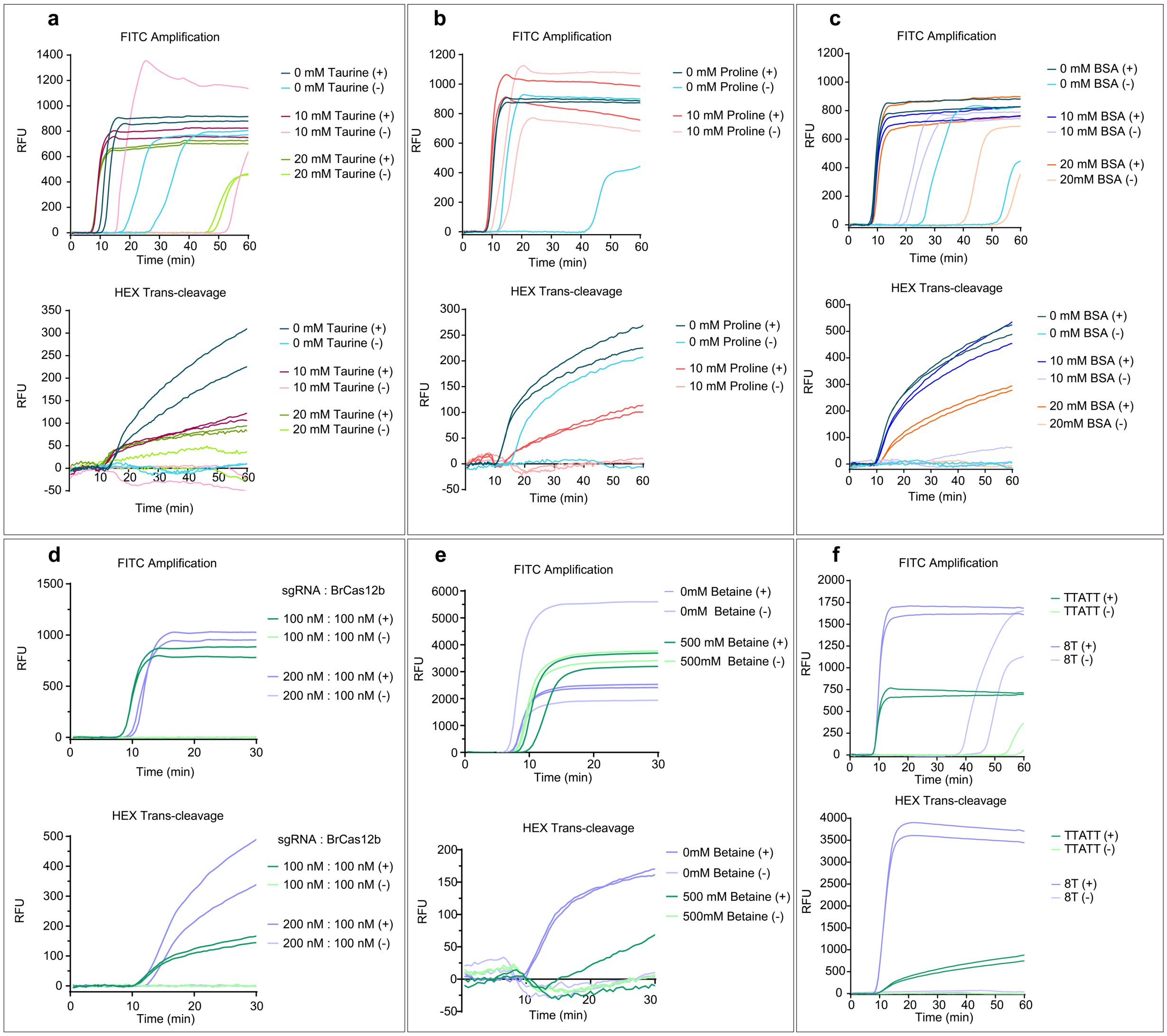

Figure S4. **BrCas12b one-pot reaction optimization**. (**a**) – (**f**) Various additives including taurine, proline, bovine serum albumin (BSA), and betaine were used in (a), (b), (c), and (d). Additionally, sgRNA, BrCas12b concentrations, and reporters were also optimized in (e) and (f). FAM channel indicates LAMP amplification detecting SYTO^{TM} 9, and HEX channel was used to detect BrCas12b trans-cleavage activity.

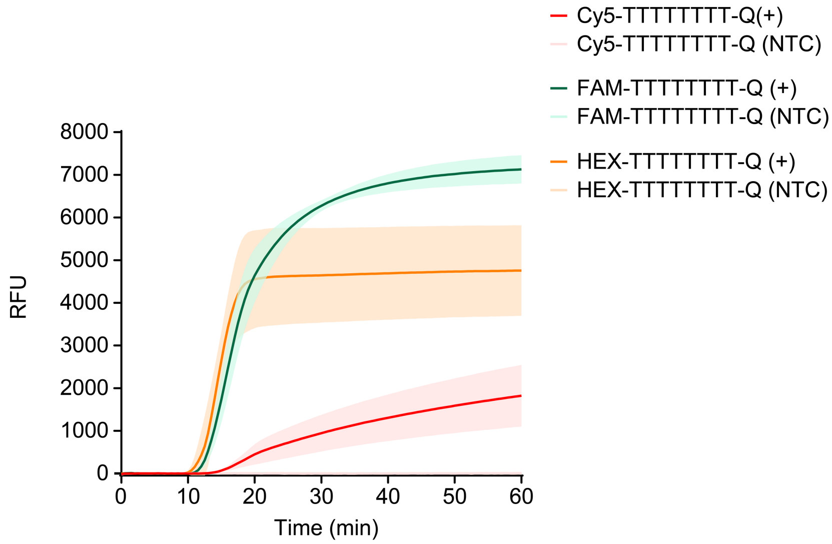

Figure S5. **Effects of reporters on BrCas12 one-pot detection reaction**. Different fluorophores (FAM, HEX, and Cy5) were placed on the 3'-end of the reporter that connects to a quencher via an 8-mer poly-T linker. FAM- and HEX-based reporters exhibited higher sensitivity compared to Cy5-based reporter at the same concentration. The shaded area represents standard deviation, n = 3 biological replicates).

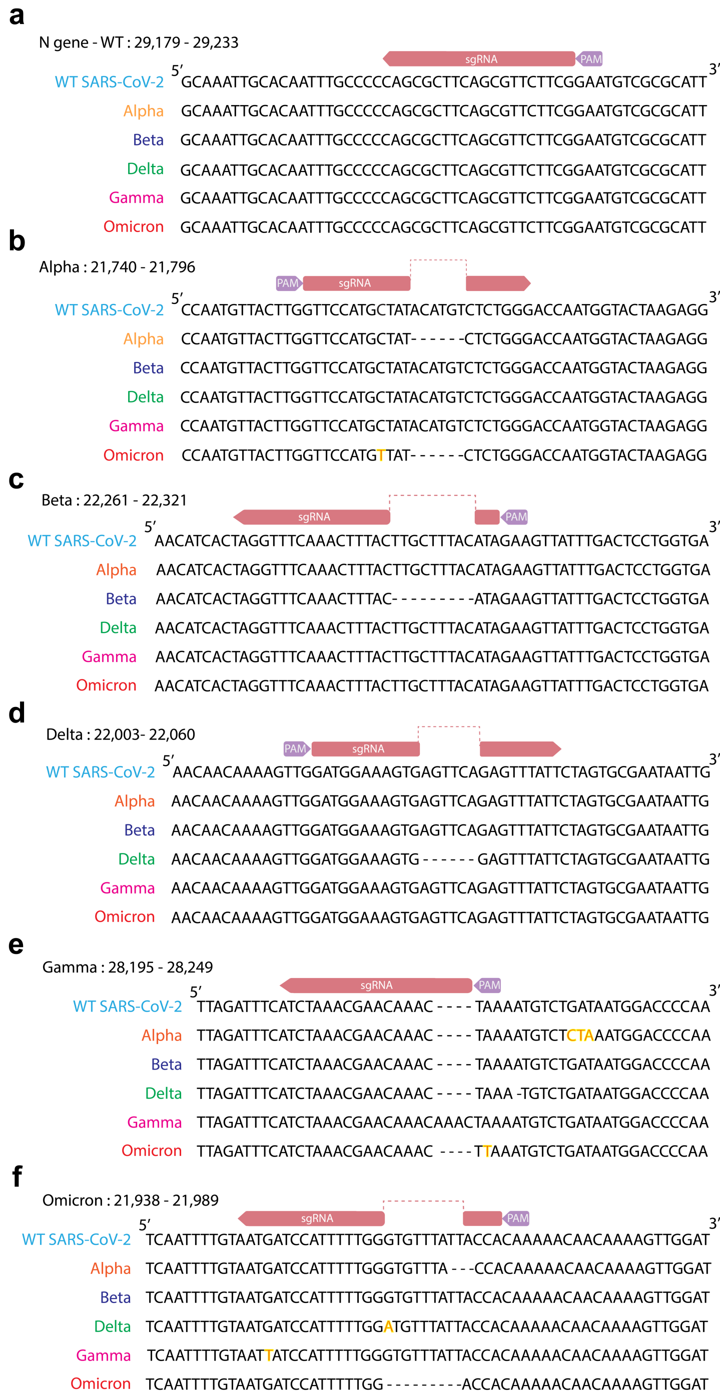

Figure S6. Specificity of single-guide RNA designs among the tested SARS-CoV-2 variants of concerns.

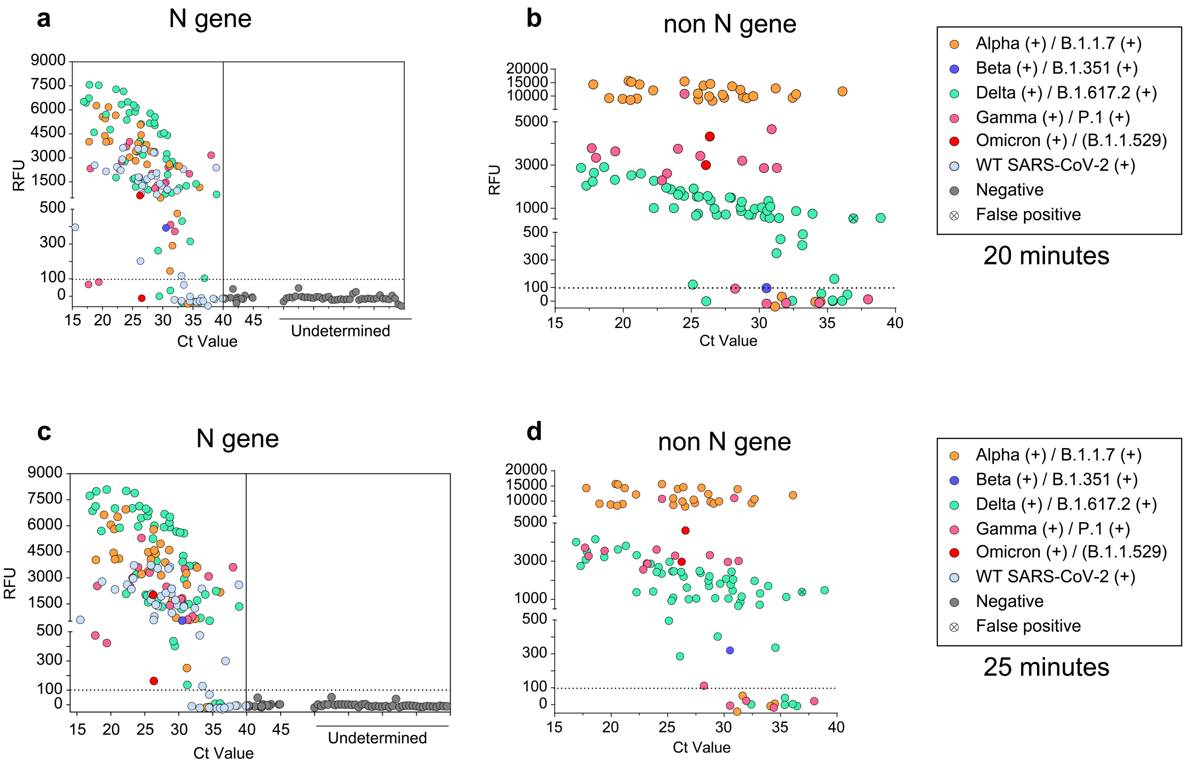

Figure S7. **Clinical validation of one-pot BrCa12b detection assay (supplement to figure 2 in the main text).** (**a**) and (**b**) One-pot patient sample detection with fluorescence measurements taken at t = 20 minutes. (**c**) and (**d**) The same assay as in (a) and (b) but at t = 25 minutes. The N gene target indicates the presence of SARS-CoV-2 while non-N gene targets indicates detection of variants.

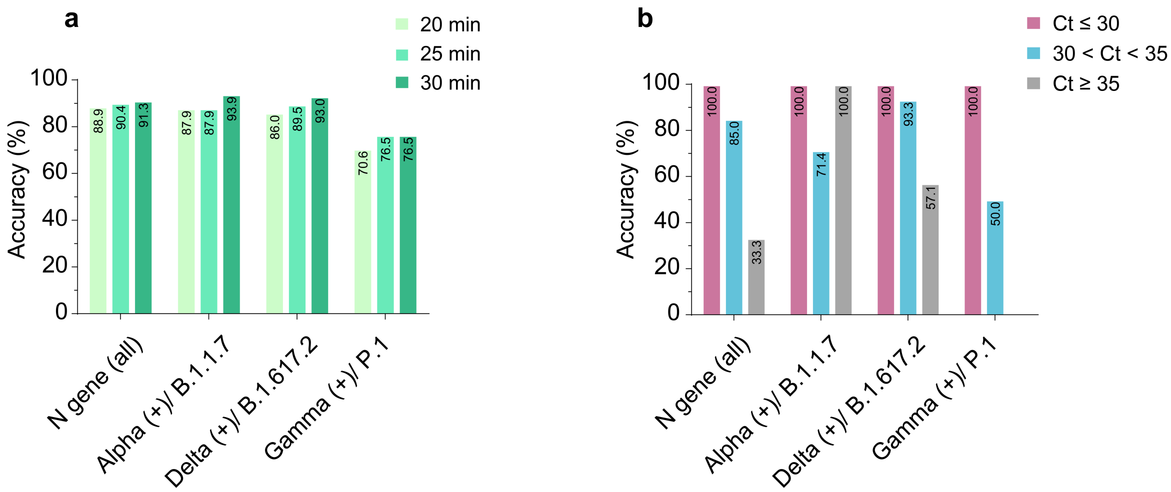

Figure S8. **Percent accuracy of patient sample validation.** (**a**) Percent accuracy of variant detection with respect to time. (**b**) Percent accuracy of variant detection with respect to Ct values.

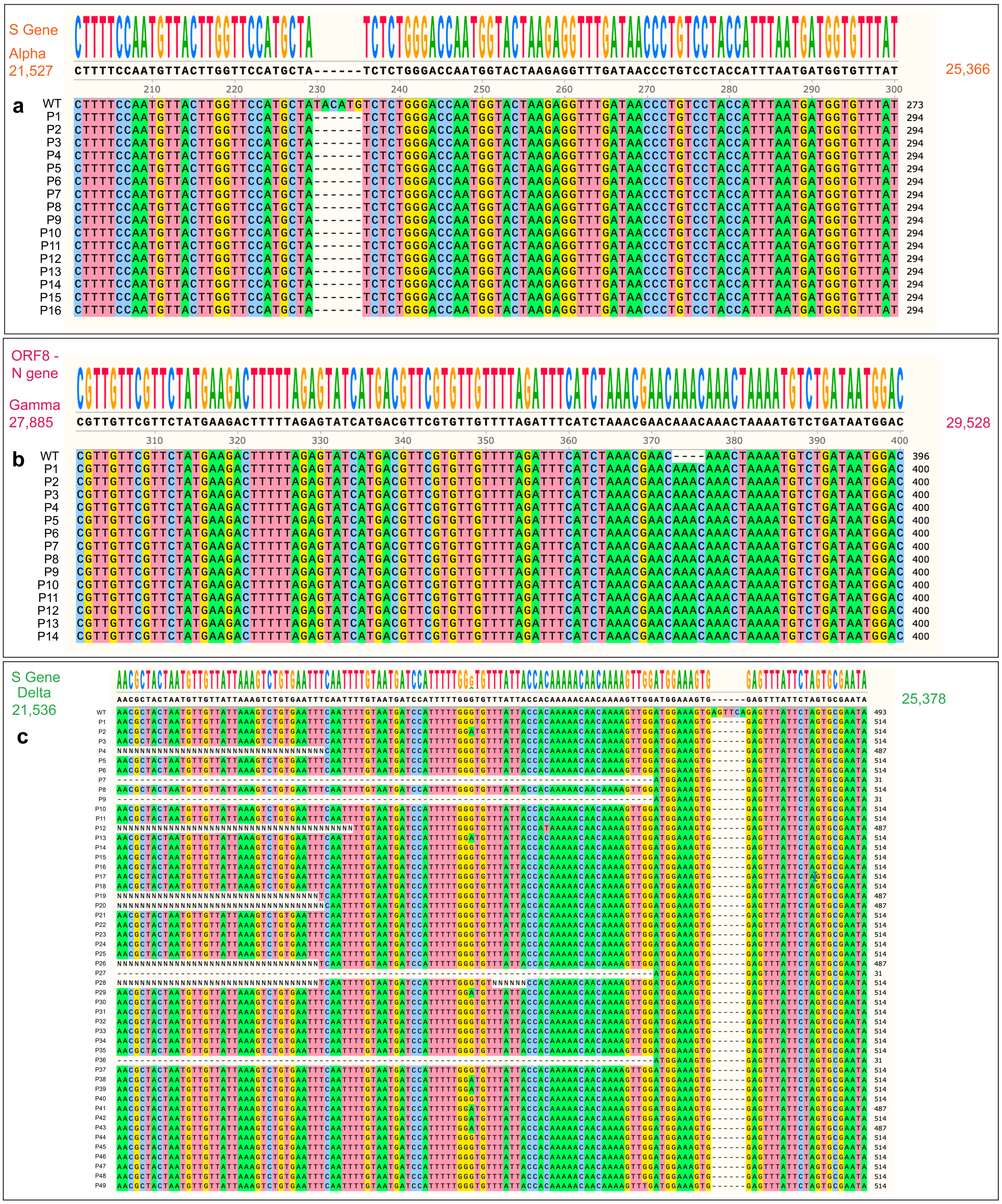

Figure S9. **Sequence alignment of patient samples in reference to wild-type SARS-CoV-2 genomic RNA.** Samples were confirmed through sequencing to identify SARS-CoV-2 variants. (**a**) Alpha. (**b**) Gamma. (**c**) Delta. “P” represents a sample. Sequences were imported into Snapgene and aligned with Clustal Omega(3, 4).

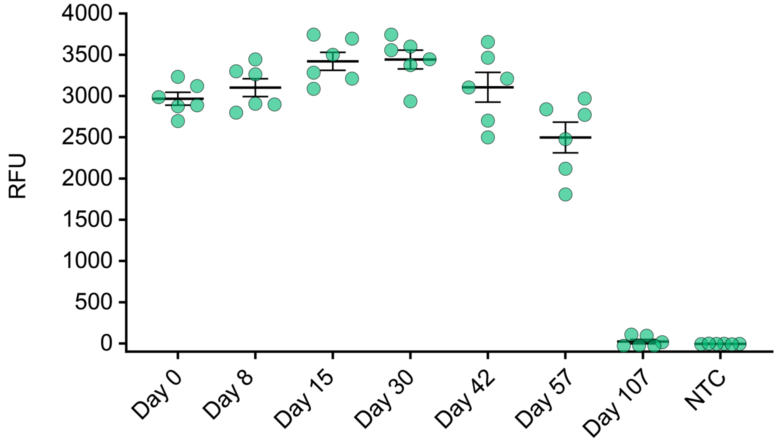

Figure S10. **Stability test of the lyophilized BrCas12b-based one-pot detection reagents.** Due to involvement of multiple proteins in the reagent, it was kept at 4^{o}C prior to testing. The fluorescence signals were taken at t = 60 minutes, (n = 6 technical replicates).

Table S1. Clinical characteristics of SPADE with S gene in 30 minutes

	Sensitivity (%)				Specificity (%)				Accuracy (%)				PPV				NPV			
	< 30	< 35	< 40	> 40	< 30	< 35	< 40	> 40	< 30	< 35	< 40	>40	< 30	< 35	< 40	> 40	< 30	< 35	< 40	> 40
α	100	93.8	93.9	··	100	100	100	100	100	96.2	96.8	100	100	100	100	··	100	90.9	93.5	100
β	··	100	100	··	100	100	100	100	100	100	100	100	··	100	100	··	100	100	100	100
γ	100	81.3	76.5	··	100	100	100	100	100	93.8	93.2	100	100	100	100	··	100	91.4	91.3	100
δ	100	98.0	94.6	··	100	100	97.1	100	100	98.7	95.6	100	100	100	98.1	··	100	96.3	91.7	100
o	100	100	100	··	··	··	··	··	100	100	100	··	100	100	100	··	··	··	··	··
Other	100	100	100	··	100	100	100	100	100	100	100	100	100	100	100	··	100	100	100	100
Total	100	94.5	92.7	··	100	100	99.4	100	100	97.6	96.7	100	100	100	99.1	··	100	95.8	95.1	100

Table S2. Portable multiplexing detection device (FISSH) specifications

Dichroic Beam Splitter (Mirror) 1 (close to both LEDs on the scheme)

Specification	Value
Reflection Band	R_{avg} > 98% 350 – 585 nm
Transmission Band	T_{avg} > 93% 601 – 950 nm

Dichroic Beam Splitter (Mirror) 1 (close to sensor and sample)

Reflection Band 1	R_{abs} > 94% 471 – 491 nm
Reflection Band 2	R_{abs} > 94% 541.5 – 544.5 nm
Reflection Band 3	R_{abs} > 94% 632.8 – 647.1 nm
Transmission Band 1	T_{avg} > 93% 503.5 – 526.5 nm
Transmission Band 2	T_{avg} > 93% 560 – 615.5 nm
Transmission Band 3	T_{avg} > 93% 665.5 – 800 nm

Excitation Filter

Transmission Band 1	T_{avg} > 85% 352 – 404 nm
Center Wavelength 1	377.5 nm
Guaranteed Minimum Bandwidth 1	52 nm
FWHM Bandwidth 1 (nominal)	57.3 nm
Transmission Band 2	T_{avg} > 93% 461 – 487.5 nm
Center Wavelength 2	474.1 nm
Guaranteed Minimum Bandwidth 2	26.5 nm
FWHM Bandwidth 2 (nominal)	30.1 nm
Transmission Band 3	T_{avg} > 93% 543 – 566 nm
Center Wavelength 3	554.5 nm
Guaranteed Minimum Bandwidth 3	23 nm
FWHM Bandwidth 3 (nominal)	27.6 nm
Transmission Band 4	T_{avg} > 93% 626 – 644 nm
Center Wavelength 4	635 nm
Guaranteed Minimum Bandwidth 4	18 nm

FWHM Bandwidth 4 (nominal)	22.9 nm

Emission Filter

Transmission Band 1	T_{avg} > 93% 414 – 450 nm
Center Wavelength 1	432 nm
Guaranteed Minimum Bandwidth 1	36 nm
FWHM Bandwidth 1 (nominal)	39.7 nm
Transmission Band 2	T_{avg} > 93% 499.5 – 530 nm
Center Wavelength 2	514.8 nm
Guaranteed Minimum Bandwidth 2	30.5 nm
FWHM Bandwidth 2 (nominal)	35.5 nm
Transmission Band 3	T_{avg} > 93% 580 – 611 nm
Center Wavelength 3	595.5 nm
Guaranteed Minimum Bandwidth 3	31 nm
FWHM Bandwidth 3 (nominal)	36.2 nm
Transmission Band 4	T_{avg} > 93% 661 – 800 nm
Center Wavelength 4	730.5 nm
Guaranteed Minimum Bandwidth 4	139 nm
FWHM Bandwidth 4 (nominal)	147.2 nm

Table S3. Sequences used in the study

3.1. RT-LAMP Primers

Primer target	Primer name	Sequence	1X Concentration
N gene (for presence of SARS-CoV-2) **62ºC RT-LAMP**	N_F3	AACACAAGCTTTCGGCAG	0.2 µM
	N_B3	GAAATTTGGATCTTTGTCATCC	0.2 µM
	N_FIP	TGCGGCCAATGTTTGTAATCAGCCAA GGAA ATTTTGGGGAC	1.6 µM
	N_BIP	CGCATTGGCATGGAAGTCACTTTGAT GGC ACCTGTGTAG	1.6 µM
	N_LF	TTCCTTGTCTGATTAGTTC	0.8 µM
	N_LB	ACCTTCGGGAACGTGGTT	0.8 µM
Alpha (B.1.1.7) **62ºC RT-LAMP**	Alpha_F3	ATACACTAATTCTTTCACACGT	0.2 µM
	Alpha_B3	CCTCTTATTATGTTAGACTTCTCAG	0.2 µM
	Alpha_FIP	GGAAAAGAAAGGTAAGAACAACCCTG ACAAAGTTTTCAGAT	1.6 µM
	Alpha_BIP	ATGGTACTAAGAGGTTTGATTGGAAG CAAAATAAACACCATC	1.6 µM
	Alpha_LF	CCTGAGTTGAATGTAAAACTGAGG	0.8 µM
	Alpha_LB	AACCCTGTCCTACCATTTAA	0.8 µM
Beta (B.1.351)	Beta_F3	ACACGCCTATTAATTTAGTGC	0.4 µM
	Beta_B3	TAGAAAAGTCCTAGGTTGAAGA	0.4 µM

62ºC RT-LAMP	Beta_FIP	CCTAGTGATGTTAATACCTATTGGCTC CCTCAGGGTTTTTCG	1.6 µM
	Beta_BIP	AAGTTATTTGACTCCTGGTCATAATAA GCTGCAGCACC	1.6 µM
	Beta_LF	CAAATCTACCAATGGTTCTAAAGC	0.8 µM
	Beta_LB	GATTCTTCTTCAGGTTGGACAGC	0.8 µM
Delta (B.1.617.2) **62ºC RT-LAMP**	Delta_F3	CCCTACTTATTGTTAATAACGCT	0.4 µM
	Delta_B3	ATTCTTAAACACAAATTCCCTAAG	0.4 µM
	Delta_FIP	TTTTTGTGGTAATAAACACCCAAAAAC TAATGTTGTTATTAAAGTCTGTG	1.6 µM
	Delta_BIP	CTAGTGCGAATAATTGCACTTTTGATG TTTTCCTTCAAGGTCCAT	1.6 µM
	Delta_LF	ATGGATCATTACAAAATTGAAA	0.8 µM
	Delta_LB	ATATGTCTCTCAGCCTTTTCTT	0.8 µM
Gamma (P.1) **62ºC RT-LAMP**	Gamma_F3	CAATTAATTGCCAGGAACCTAA	0.2 µM
	Gamma_B3	TACTGCCAGTTGAATCTGA	0.2 µM
	Gamma_FIP	CAACACGAACGTCATGATACTCTAAG GGTAGTCTTGTAGTG	1.6 µM
	Gamma_BIP	ACTAAAATGTCTGATAATGGACCCCG GGTCCACCAAACGTAAT	1.6 µM
	Gamma_LF	AAAGTCTTCATAGAACGAACAA	0.8 µM
	Gamma_LB	AAAATCAGCGAAATGCACCCC	0.8 µM
Omicron (B.1.1.529) **60ºC RT-LAMP**	Omicron_F3	ACTTTAGATTCGAAGACCCA	0.4 µM
	Omicron_B3	AGAAAAGGCTGAGAGACAT	0.4 µM
	Omicron_FIP	TCATTACAAAATTGAAATTCACAGTCC CTACTTATTGTTAATAACGC	1.6 µM
	Omicron_BIP	AACAACAAAAGTTGGATGGAAAGAAAA GTGCAATTATTCGCACTAG	1.6 µM
	Omicron_LF	GACTTTAATAACAACATTAGTA	0.8 µM
	Omicron_LB	AGTGAGTTCAGAGTTTATTC	0.8 µM
RNase P **62ºC RT-LAMP**	RNaseP_F3	TTGATGAGCTGGAGCCA	0.2 µM
	RNaseP_B3	CACCCTCAATGCAGAGTC	0.2 µM
	RNaseP_FIP	GTGTGACCCTGAAGACTCGGTTTTAG CCA CTGACTCGGATC	1.6 µM
	RNaseP_BIP	CCTCCGTGATATGGCTCTTCGTTTTTT TCTT ACATGGCTCTGGTC	1.6 µM
	RNaseP_LF	ATGTGGATGGCTGAGTTGTT	0.8 µM
	RNaseP_LB	CATGCTGAGTACTGGACCTC	0.8 µM

3.2. Single-guide RNA (sgRNA)

Target	Sequence
N gene (for presence of SARS-CoV-2)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**CGAAGAACGCUGAAGCGCUG**
Alpha (B.1.1.7)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG

	CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**GUUCCAUGCUAUCUCUGGGA**
Beta (B.1.352)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**UAUGUAAAGUUUGAAACCUA**
Delta (B.1.617.2)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**GAUGGAAAGUGGAGUUUAUU**
Gamma (P1)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**GUUUGUUUGUUCGUUUAGAU**
Omicron (B.1.1.529)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**UGGUCCAAAAAUGGAUCAUU**
RNase P	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**AAUUACUUGGGUGUGACCCU**
sgGFP (for Michaelis-Menten Kinetics)	GAAGGUGGUUAGCUACAGGCUGACCAGUGCAGUUGUGUC AUGUGCUACGGUGACCUAACACGUCACUCAGUCACAACGG CUAUCUAUAUUUCCACUAACCAAAGUUAGUGGAAAUGUAG AUGGUUAGCAC**CUCAGGGCGGACUGGGUGCU**

3.3. Reporters

Reporter name	Sequence
Reporter 1	/5HEX/TTTTTTTT/3IABkFQ/
Reporter 2	/5HEX/TTTTTTTT
Reporter 3	/56FAM/TTATT/3IABkFQ/
Reporter 4	/56FAM/TTTTTTTT/3IABkFQ/
Reporter 5	5Cy5/TTTTTTTT/3BHQ_2/

3.5. Oligos

Name	Sequence
Act_GFP_T (for Michaelis-Menten Kinetics)	CCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACC CC
Act_GFP_NT (for Michaelis-Menten Kinetics)	GGGGTCTTTGCTCAGGGCGGACTGGGTGCTCAGGTAG TGG

3.5. Protein Sequences

Name	Sequence
AacCas12b	MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYR RSPNGDGEQECDKTAEECKAELLERLRARQVENGHRGPAGSDDELLQLA RQLYELLVPQAIGAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKPRWV RMREAGEPGWEEEKEKAETRKSADRTADVLRALADFGLKPLMRVYTDSE MSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKL VEQKNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGR ALRGSDKVFEKWGKLAPDAPFDLYDAEIKNVQRRNTRRFGSHDLFAKLAE PEYQALWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDATAHPIWTRFD KLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDDVTVPISMSE QLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRR GARDVYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSD YLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNS KGRVPFFFPIKGNDNLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQL RTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPVDAANHMTPDWREAFEN ELQKLKSLHGICSDKEWMDAVYESVRRVWRHMGKQVRDWRKDVRSGER PKIRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRF AITLREHIDHAKEDRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLI LLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELINQAQVHDLLVGTMY AAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPWWLNKFVVEHTLDA CPLRADDLIPTGEGEIFVSPFSAEEGDFHQIHADLNAAQNLQQRLWSDFDI SQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYERERG KKRRKVFAQEKLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQK EFWSMVNQRIEGYLVKQIRSRVPLQDSACENTGDI
AapCas12b	MAVKSMKVKLRLDNMPEIRAGLWKLHTEVNAGVRYYTEWLSLLRQENLY RRSPNGDGEQECYKTAEECKAELLERLRARQVENGHCGPAGSDDELLQL ARQLYELLVPQAIGAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKPRW VRMREAGEPGWEEEKAKAEARKSTDRTADVLRALADFGLKPLMRVYTDS DMSSVQWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGEAYAK LVEQKSRFEQKNFVGQEHLVQLVNQLQQDMKEASHGLESKEQTAHYLTG RALRGSDKVFEKWEKLDPDAPFDLYDTEIKNVQRRNTRRFGSHDLFAKLA EPKYQALWREDASFLTRYAVYNSIVRKLNHAKMFATFTLPDATAHPIWTRF DKLGGNLHQYTFLFNEFGEGRHAIRFQKLLTVEDGVAKEVDDVTVPISMSA QLDDLLPRDPHELVALYFQDYGAEQHLAGEFGGAKIQYRRDQLNHLHARR GARDVYLNLSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSD YLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNS EGRVPFCFPIEGNENLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQL RTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPMDANQMTPDWREAFEDE LQKLKSLYGICGDREWTEAVYESVRRVWRHMGKQVRDWRKDVRSGERP KIRGYQKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFA ITLREHIDHAKEDRLKKLADRIIMEALGYVYALDDERGKGKWVAKYPPCQLI LLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELLNQAQVHDLLVGTMY AAFSSRFDARTGAPGIRCRRVPARCAREQNPEPFPWWLNKFVAEHKLDG CPLRADDLIPTGEGEFFVSPFSAEEGDFHQIHADLNAAQNLQRRLWSDFDI SQIRLRCDWGEVDGEPVLIPRTTGKRTADSYGNKVFYTKTGVTYYERERG KKRRKVFAQEELSEEEAELLVEADEAREKSVVLMRDPSGIINRGDWTRQK EFWSMVNQRIEGYLVKQIRSRVRLQESACENTGDI

BrCas12b	MPVRSFKVKLVTRSGDAEHMLQLRRGLWKTHEIVNQGIAYYMNKLALMR QEPYAGKSREVVRLELLHSLRAQQKRNNWTGDAGTDDEILNLSRRLYELL VPSAIGEKGDAQMLSRKFLSPLVDPNSEGGKGTAKSGRKPRWMKMREE GHPDWEAEREKDRAKKAADPTASILNDLEAFGLRPLFPLFTDEQKGIQWL PKQKRQFVRTFDRDMFQQALERMLSWESWNRRVAEEYQKLQAQRDELY AKYLADGGAWLEALQSFEKQREVELAEESFAAKSEYLITRRQIRGWKQVY EKWSQLPEHAAQEQFWQVVADVQTSLPGAFGDPKVYQFLSQPEHHHIW RGYPNRLFHYSDYNGVRKKLQRARHDATFTLPDPVEHPLWIRFDARGGNI HDYEISQNGKQYQVTFSRLLWPENETWVERENVTVAIGASQQLKRQIRLD GYADKKQKVRYRDYSSGIELTGVLGGAKIQFDRRHLRKASNRLADGETGP VYLNVVVDIEPFLAMRNGRLQTPIGQVLQVNTKDWPKVTGYKPAELISWIQ NSPLAVGTGVNTIEAGMRVMSVDLGQRSAAAVSIFEVMRQKPAEQETKLF YPIAVTGLYAVHRRSLLLRLPGEKISDEIEQQRKIRAHARSLVRYQIRLLADV LRLHTRGTAEQRRAKLDELLATLQTKQELDQKLWQTELEKLFDYIHEPAER WQQALVAAHRTLEPVIGQAVRHWRKSLRIDRKGLAGMSMWNIEELEETR KLLIAWSKHSRVPGEPNRLDKEETFAPQQLQHIQNVKDDRLKQMANLLVM TALGYKYDEAEKQWKEAYPACQMILFEDLSRYRFALDRPRRENNRLMKW AHRSIPRLVYLQGELFGIQVGDVYSAYTSRFHAKTGAPGIRCHALKEEDLQ PNSYVVKQLIKDGFIREDQTGSLKPGQIVPWSGGELFVTLADRSGSRLAVI HADINAAQNLQKRFWQQNTEIFRVPCKVTTSGLIPAYDKMKKLFGKGYFA KINQTDTSEVYVWEHSAKMKGKTTPADPAEEGVFDESLTDEMEELEDSQ EGYKTLFRDPSGFFWSSDRWLPQKEFWFWVKRRIEKKLREQLQ

Table S4. GISAID information for clinical samples

Virus name	Accession ID	Lineage
hCoV-19/USA/FL-Path-Saliva-705b/2021	EPI_ISL_3655932	AY.119
hCoV-19/USA/FL-Path-Saliva-707b/2021	EPI_ISL_3655934	AY.26
hCoV-19/USA/FL-Path-Saliva-709b/2021	EPI_ISL_3655936	AY.44
hCoV-19/USA/FL-Path-Saliva-746b/2021	EPI_ISL_3655964	AY.3.1
hCoV-19/USA/FL-Path-Saliva-747b/2021	EPI_ISL_3655965	AY.25.1
hCoV-19/USA/FL-Path-Saliva-749b/2021	EPI_ISL_3655967	AY.47
hCoV-19/USA/FL-Path-Saliva-750b/2021	EPI_ISL_3655968	B.1.617.2
hCoV-19/USA/FL-Path-Saliva-752b/2021	EPI_ISL_3655969	AY.47
hCoV-19/USA/FL-Path-Saliva-753b/2021	EPI_ISL_3655970	AY.75
hCoV-19/USA/FL-Path-Saliva-755b/2021	EPI_ISL_3655971	AY.3
hCoV-19/USA/FL-Path-Saliva-756b/2021	EPI_ISL_3655972	B.1.617.2
hCoV-19/USA/FL-Path-Saliva-758b/2021	EPI_ISL_3655974	AY.3
hCoV-19/USA/FL-Path-Saliva-759b/2021	EPI_ISL_3655975	AY.103
hCoV-19/USA/FL-Path-Saliva-760b/2021	EPI_ISL_3655976	AY.110
hCoV-19/USA/FL-Path-Saliva-761b/2021	EPI_ISL_3655977	AY.25
hCoV-19/USA/FL-Path-Saliva-763b/2021	EPI_ISL_3655978	B.1.617.2
hCoV-19/USA/FL-Path-Saliva-764b/2021	EPI_ISL_3655979	AY.44
hCoV-19/USA/FL-Path-Saliva-765b/2021	EPI_ISL_3655980	AY.110
hCoV-19/USA/FL-Path-Saliva-767b/2021	EPI_ISL_3655981	AY.119

hCoV-19/USA/FL-Path-Saliva-768b/2021	EPI_ISL_3655982	AY.110
hCoV-19/USA/FL-Path-Saliva-769b/2021	EPI_ISL_3655983	AY.110
hCoV-19/USA/FL-Path-Saliva-770b/2021	EPI_ISL_3655984	AY.47
hCoV-19/USA/FL-Path-Saliva-771b/2021	EPI_ISL_3655985	AY.103
hCoV-19/USA/FL-Path-Saliva-772b/2021	EPI_ISL_3655986	AY.119
hCoV-19/USA/FL-Path-Saliva-773b/2021	EPI_ISL_3655987	AY.47
hCoV-19/USA/FL-Path-Saliva-774b/2021	EPI_ISL_3655988	AY.103
hCoV-19/USA/FL-Path-Saliva-776b/2021	EPI_ISL_3655989	B.1.617.2
hCoV-19/USA/FL-Path-Saliva-777b/2021	EPI_ISL_3655990	B.1.617.2
hCoV-19/USA/FL-Path-Saliva-778b/2021	EPI_ISL_3655991	AY.44
hCoV-19/USA/FL-Path-Saliva-779b/2021	EPI_ISL_3655992	AY.113
hCoV-19/USA/FL-Path-Saliva-780b/2021	EPI_ISL_3655993	AY.110
hCoV-19/USA/FL-Path-Saliva-781b/2021	EPI_ISL_3655994	AY.122
hCoV-19/USA/FL-Path-Saliva-793b/2021	EPI_ISL_3656005	AY.3
hCoV-19/USA/FL-Path-Saliva-1296/2021	EPI_ISL_3656270	AY.47
hCoV-19/USA/FL-Shands-VTM-2422/2021	EPI_ISL_3657454	AY.114
hCoV-19/USA/FL-Shands-VTM-2442/2021	EPI_ISL_3657471	AY.47
hCoV-19/USA/FL-Shands-VTM-2444/2021	EPI_ISL_3657472	AY.110
hCoV-19/USA/FL-Shands-VTM-2451/2021	EPI_ISL_3657478	AY.47
hCoV-19/USA/FL-Shands-VTM-2452/2021	EPI_ISL_3657479	AY.47
hCoV-19/USA/FL-Shands-VTM-2455/2021	EPI_ISL_3657481	AY.3.1
hCoV-19/USA/FL-Shands-VTM-2456/2021	EPI_ISL_3657482	AY.47
hCoV-19/USA/FL-Shands-VTM-2459/2021	EPI_ISL_3657485	AY.3
hCoV-19/USA/FL-Shands-VTM-2460/2021	EPI_ISL_3657486	AY.47
hCoV-19/USA/FL-Path-Saliva-757b/2021	EPI_ISL_3655973	None
hCoV-19/USA/FL-Path-Saliva-845b/2021	EPI_ISL_3656039	B.1
hCoV-19/USA/FL-Path-Saliva-1066b/2021	EPI_ISL_3656155	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1067b/2021	EPI_ISL_3656156	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1068b/2021	EPI_ISL_3656157	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1070b/2021	EPI_ISL_3656158	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1071b/2021	EPI_ISL_3656159	B.1.1.519
hCoV-19/USA/FL-Path-Saliva-1074b/2021	EPI_ISL_3656160	B.1.1
hCoV-19/USA/FL-Path-Saliva-1075b/2021	EPI_ISL_3656161	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1077b/2021	EPI_ISL_3656162	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1078b/2021	EPI_ISL_3656163	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1080b/2021	EPI_ISL_3656165	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1084b/2021	EPI_ISL_3656168	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1088b/2021	EPI_ISL_3656171	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1091b/2021	EPI_ISL_3656173	B.1.526
hCoV-19/USA/FL-Path-Saliva-1092b/2021	EPI_ISL_3656174	AY.47
hCoV-19/USA/FL-Path-Saliva-1285/2021	EPI_ISL_3656260	P.1.10

hCoV-19/USA/FL-Path-Saliva-1286/2021	EPI_ISL_3656261	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1287/2021	EPI_ISL_3656262	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1288/2021	EPI_ISL_3656263	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1289/2021	EPI_ISL_3656264	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1290/2021	EPI_ISL_3656265	P.1.10
hCoV-19/USA/FL-Path-Saliva-1291/2021	EPI_ISL_3656266	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1292/2021	EPI_ISL_3656267	P.1.10
hCoV-19/USA/FL-Path-Saliva-1293/2021	EPI_ISL_3656268	B.1.1.7
hCoV-19/USA/FL-Path-Saliva-1294/2021	EPI_ISL_3656269	B.1
hCoV-19/USA/FL-Path-Saliva-1090b/2021	EPI_ISL_3656172	B.1.575
hCoV-19/USA/FL-Path-Saliva-1083b/2021	EPI_ISL_3656167	B.1.575
hCoV-19/USA/FL-Path-Saliva-1085b/2021	EPI_ISL_3656169	B.1.575

Table S5. Detailed Resource Information

Resources	Identifier	Supplier
Plasmids		
pMBP-AacCas12b	Addgene plasmid # 113433	Addgene
BPK2014-AapCas12b	Addgene plasmid # 121949	Addgene
PET28a+_BrCas12b	Addgene plasmid # 170819	Addgene
Bacterial Strains		
NEB® 5-alpha Competent E. coli (High Efficiency)	Cat NO: C2987H	New England Biolabs
BL21(DE3) Competent E. coli	Cat NO: C2527H	New England Biolabs
Chemicals and Reagents		
HiScribe™ T7 Quick High Yield RNA Synthesis Kit	Cat NO: E2050S	New England Biolabs
RNA Clean & Concentrator-25	Cat NO: R1017	ZYMO Research
L-Proline, 99%	Cat NO: A10199	Alfa Aesar
Taurine, 99%	Cat NO: A12403	Alfa Aesar
Bovine Serum Albumin, Molecular Biology Grade	Cat NO: B9000S	New England Biolabs
Betaine 5M Solution, MB Grade	LOT: 00955041	ThermoFisher Scientific
WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	Cat NO: M1708S	New England Biolabs
Lyo-ready WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	Under Development	New England Biolabs
SYTO™ 9	Cat NO: S34854	ThermoFisher Scientific
Lysozyme	Cat NO: 100831	MP Biomedicals, LLC

Starchyose Tetrahydrate	Cat NO: 153946	MP Biomedicals, LLC
Deoxyribonuclease I	Cat NO: LS002145	Worthington Biochemical Corporation
Phenylmethylsulfonly Fluoride	Cat NO: 195381	MP Biomedicals, LLC
NEBuffer™ 2.1	Cat NO: B7202	New England Biolabs
Sodium Dodecyl Sulfate (SDS), White Powder, Electrophoresis, Fisher BioReagents™	Cat NO: BP166-100	Fisher Scientific
Ethylenediaminetetraacetic Acid (0.5M Solution/pH 8.0), Fisher BioReagents	CAT: BP2482-500	Fisher Scientific
Glycerol (Certified ACS), Fisher Chemical™	CAT: G33-1	Fisher Scientific
GelRed® 10,000X in water	Cat NO: 41003	Biotium
Agarose LE (Molecular Biology Grade)	Cat NO: A-201-100	Gold Biotechnology
Protein Thermal Shift™ Dye Kit	Cat NO: 4461146	ThermoFisher Scientific
Dithiothreitol (White Crystals or Powder/Electrophoresis), Fisher BioReagents™	Cat NO: BP172-25	ThermoFisher Scientific
QIAamp 96 Viral RNA Kit	Cat NO: / ID: 52904	Qiagen
Illumina COVIDSeq Test kit	Cat NO: 20049393, 20051772	Illumina
Qubit dsDNA HS Assay Kit	Cat NO: Q33230	Thermofisher Scientific
NovaSeq 6000 Sequencing System SP Reagent Kit	Cat NO: 20040326	Illumina
NovaSeq Xp 2-Lane Kit	Cat NO: 20021664	Illumina
SARS-CoV-2 RUO qPCR Primer & Probe Kit	Cat NO: 10006713	Integrated DNA Technologies
ProtoScript® II Reverse Transcriptase	Cat NO: M0368L	New England Biolabs
PrimeSTAR® HS DNA Polymerase	Cat NO:R010B	Takara
TaqMan Fast Advanced Master Mix	Cat NO: 4444556	ThermoFisher Scientific
D-(+)-Trehalose, anhydrous 99%	Cat NO: AC30987-0250	Thermo Scientific Chemicals
Isopropyl β-d-1-thiogalactopyranoside	Cat NO: I2481C	Gold Biotechnology
LB Broth, Miller	Cat NO: BP9723-500	Fisher Scientific
Sodium Chloride	Cat NO:7647-14-5	Fisher Scientific
Tris-HCl	Cat NO:BP153-500	Fisher Scientific
Pierce™ TCEP-HCl	Cat NO: 20490	ThermoFisher Scientific
HEPES	Cat NO:75277-39-3	Fisher Scientific
Sodium acetate	Cat NO: S220-1	Fisher Scientific
Consumables		
0·22 µm filter	Cat NO: SLGP033RS	Millipore Sigma

30 kDa MWCO Vivaspin® 20	Cat NO: Z614637	Satorius
HiTrap® Heparin High Performance 1 mL HP column	Cat NO: GE17-0406-01	Cytiva
Dialysis tubing	Cat NO: 470231-460	Ward's Science
Histrap 5 mL FF column	Cat NO: 17525501	Cytiva
MicroAmp Fast Optical 96-well Reaction Plate with Barcode	Cat NO: 4346906	Applied Biosystems
MicroAmp 8-Cap Strip	Cat NO: N8010535	Applied Biosystems
MicroAmp 8-tube Strip (0.2 mL)	Cat NO: N8010580	Applied Biosystems
Microplate, PS, 96 Well, F-bottom, Black, Fluotrac, Medium binding	Cat NO: 6550076	Greiner Bio-one
Equipment		
Mosquito HV Genomics Liquid Handler	Cat NO: G2991BA	sptlabtech
4200 TapeStation System		Agilent
Bio-rad CFX96 Real-Time system with C1000 Thermal Cycler module	Cat NO: 4376600	Bio-rad
qPCR StepOne Plus system		ThermoFisher Scientific
HPLC 1100 system		Agilent

References

1. Robert X, Gouet P. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res. 2014;42(Web Server issue):W320-4.
2. Yang H, Gao P, Rajashankar KR, Patel DJ. PAM-Dependent Target DNA Recognition and Cleavage by C2c1 CRISPR-Cas Endonuclease. Cell. 2016;167(7):1814-28 e12.
3. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol. 2011;7:539.
4. Goujon M, McWilliam H, Li WZ, Valentin F, Squizzato S, Paern J, et al. A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Research. 2010;38:W695-W9.