SUPPLEMENTARY MATERIALS

Engineered CRISPR/Cas12a Enables Rapid SARS-CoV-2 Detection

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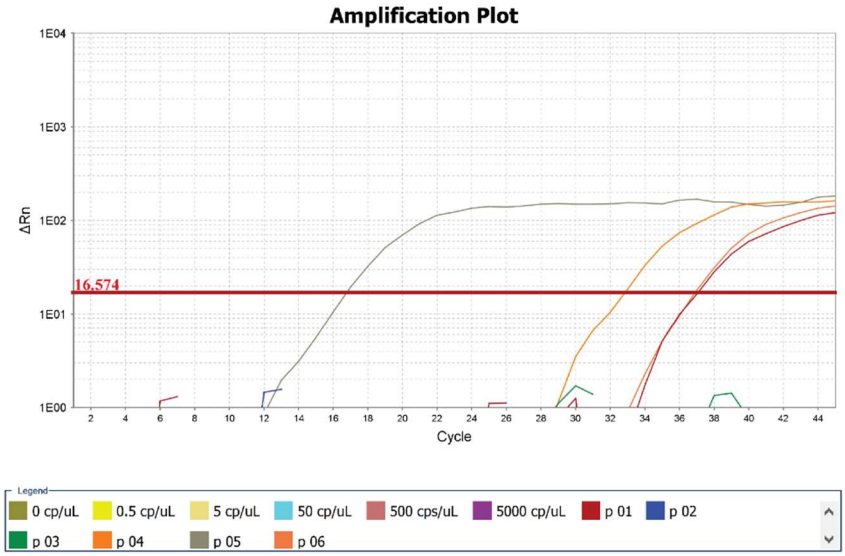
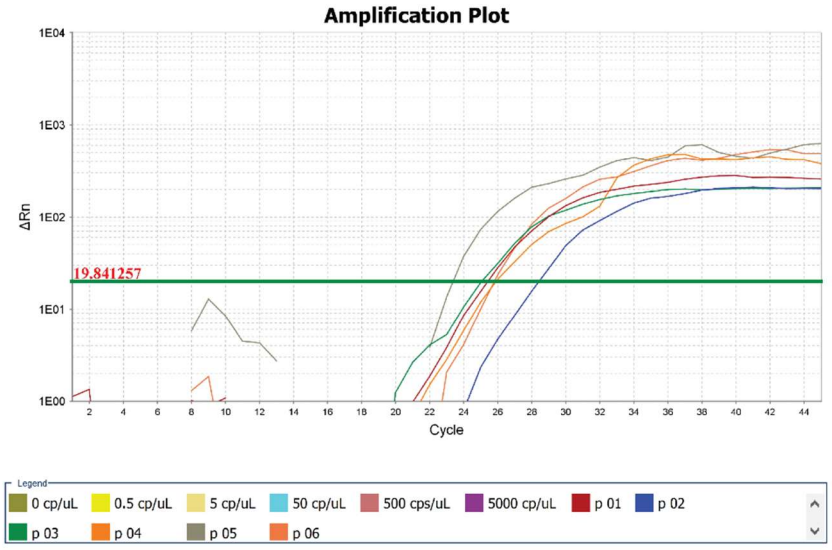


N1 Gene



RNase P Gene

Fig. S1. Representation of clinical validation of SARS-CoV-2 detection in 62 patient samples using RT-qPCR. Obtained patient samples were re-tested with RT-qPCR following CDC-recommended protocol to determine the Ct values.

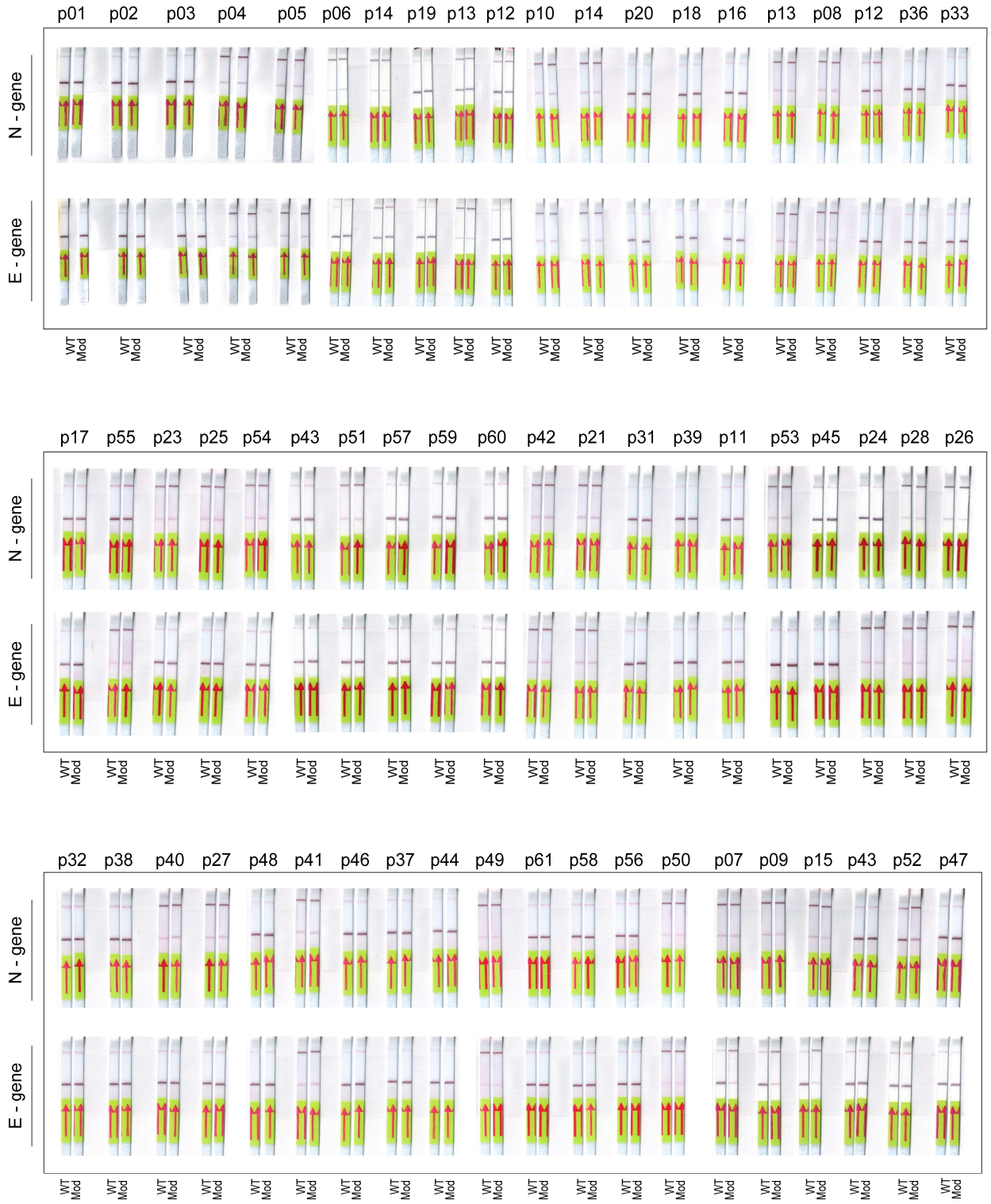


Fig. S2. Clinical validation of ENHANCE for the detection of SARS-CoV-2 in 62 patient samples using lateral flow assay. The patient samples were not in numerical order due to blind testing.

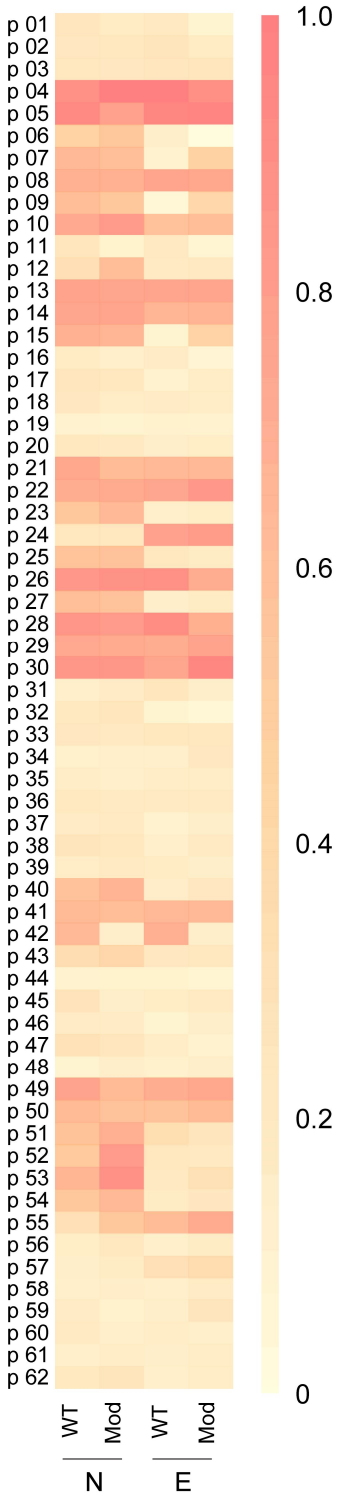


Fig. S3. Quantitative analysis of lateral flow assay using ENHANCE on 62 patient samples. The heat map shows ImageJ quantification of band intensity ratio of positive line (top band) to highest signal obtained in all 31 positive patient samples.

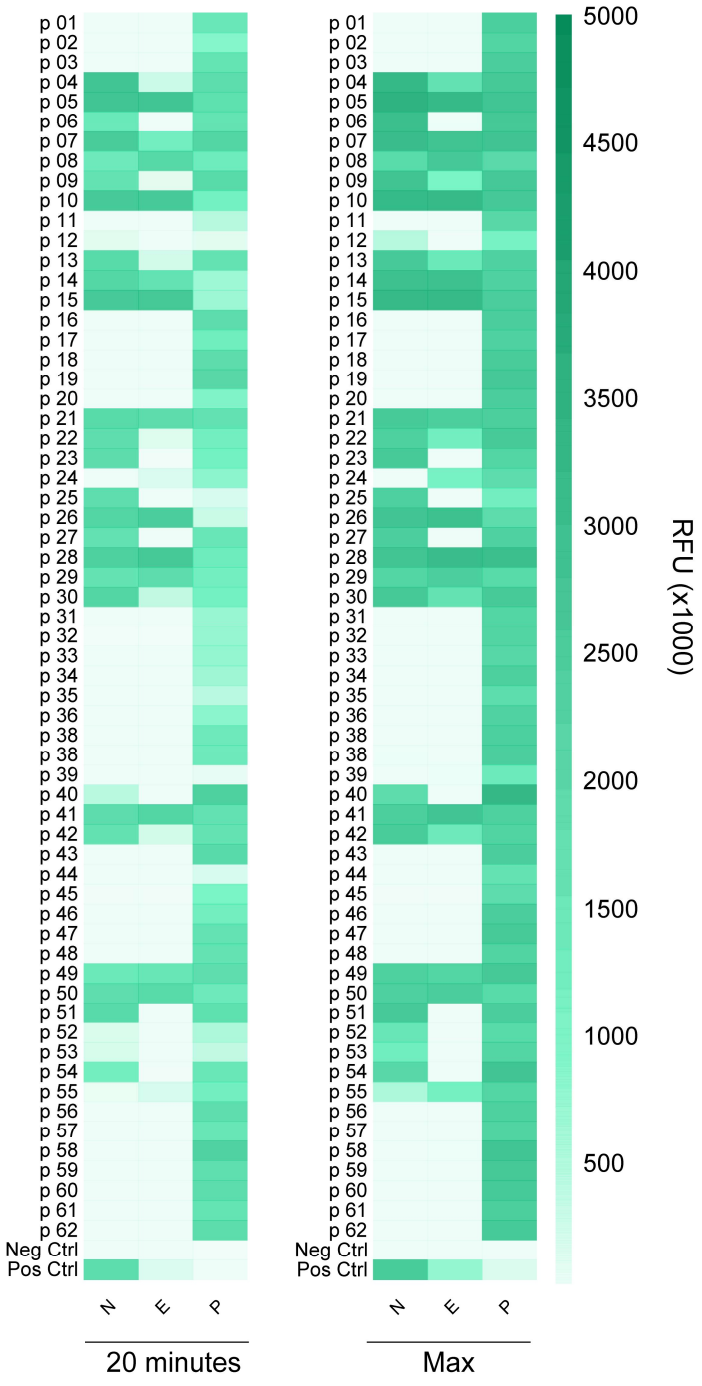


Fig. S4. Clinical validation of ENHANCEv2 for the detection of SARS-CoV-2 in 62 patient samples using fluorescence-based reporter assay. The heat map shows fluorescence intensities taken at t = 20 minutes (left) and maximum fluorescence intensities within an hour. The heat map is supplemental to fig. 4e in the main text whose fluorescence intensities were taken at t = 2.5 minutes.

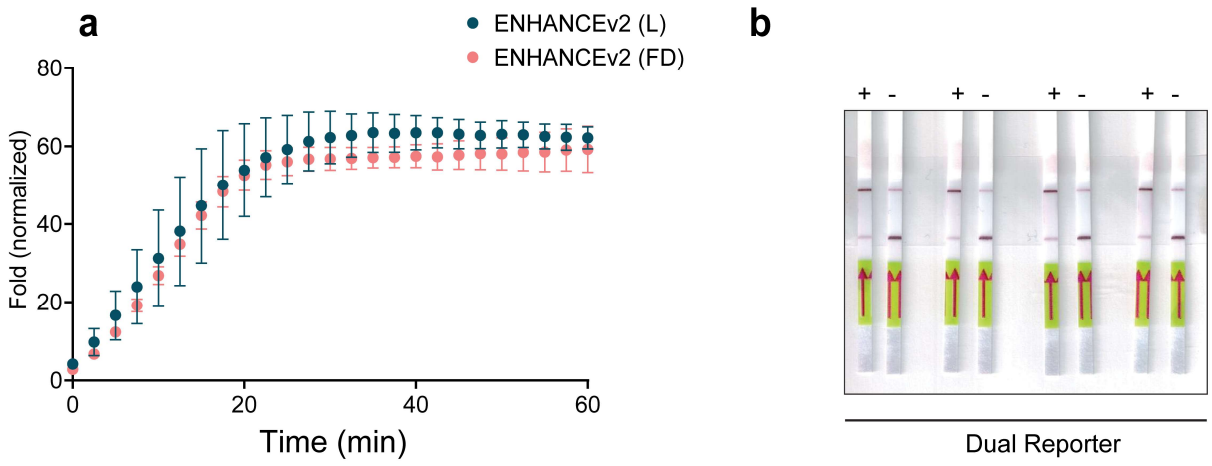


Fig. S5. Comparison between liquid version and lyophilized version of ENHANCEv2. (a) Fold change in fluorescence intensity normalized to corresponding NTC reactions targeting N2gene between liquid ENHANCEv2 (L) and freeze-dried ENHANCEv2 (FD) in one hour. (**b**) Representation of lateral flow paper strips showing compatibility of the dual reporter. Sample reactions from the fluorescence-based reporter assay using dual reporter in ENHANCEv2 was diluted down to a final concentration of 125 nM reporter followed by the lateral flow assay.

Table S1. Clinical comparison of prominent CRISPR-based detection methods with ENHANCE

	DETECTR[13]	SHERLOCK[10, 29]	StopCOVID.v2[17]	ENHANCE
Target genes	N, E	N, ORF1ab	N	N, E
Enzyme	Cas12a	Cas13a	Cas12b	Cas12a
LoD copies/µL **(Total copies)**	10 cp/µL (20 copies input)	1.35 cp/µL (10.8 copies input)	0.033 cp/µL (100 copies input in dry swabs)	0.2 cp/µL (1 copy input)
Clinical validation using Lateral Flow Assay (LFA) or Fluorescence (F)	LFA	LFA, F	F	LFA, F
Total time (RNA to detection) **Pre-amplification** **CRISPR reaction**	30-40 min 20-30 min 10 min	50 min 40 min 10 min	45 min One-pot reaction	33 min 30 min 3 min
Positive Prediction Rate	95%	100%, 100%	98.4%	96.7%, 96.7%
Negative Prediction rate	100%	88.0%, 96.0%	93.4%	96.7%, 96.7%
FDA EUA	Yes	Yes	No	No

Table S2. Sequences used in the study

Name	Sequence	Source
crRNAs		
crN1-WT	UAAUUUCUACUAAGUGUAGAUGUGGACCCUAGAUUCAACU	This study
crN1-Mod	UAAUUUCUACUAAGUGUAGAUGUGGACCCUAGAUUCAACUTATTATT	This study
crN2-WT	UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUC	J.P. Broughton et al.
crN2-Mod	UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUCTATTATT	This study

crE1-WT	UAAUUUCUACUAAGUGUAGAUGUGGUAUUCUUGCUAGUUAC	J.P. Broughton et al.
crE1-Mod	UAAUUUCUACUAAGUGUAGAUGUGGUAUUCUUGCUAGUUACTATTATT	This study
crE2-WT	UAAUUUCUACUAAGUGUAGAUUUGCUUUCGUGGUAUUCUUG	J.P. Broughton et al.
crE2-Mod	UAAUUUCUACUAAGUGUAGAUUUGCUUUCGUGGUAUUCUUGTATTATT	This study
crR1-WT	UAAUUUCUACUAAGUGUAGAUUCAAGCUGUCACGGCCAAUG	This study
crR1-Mod	UAAUUUCUACUAAGUGUAGAUUCAAGCUGUCACGGCCAAUGTATTATT	This study
crR2-WT	UAAUUUCUACUAAGUGUAGAUACAUUUGUCAAGCUGUCACG	This study
crR2-Mod	UAAUUUCUACUAAGUGUAGAUACAUUUGUCAAGCUGUCACGTATTATT	This study
crP-WT	UAAUUUCUACUAAGUGUAGAUAAUUACUUGGGUGUGACCCU	K.A Curtis et al[33].
crP-Mod	UAAUUUCUACUAAGUGUAGAUAAUUACUUGGGUGUGACCCUTATTATT	This study
LAMP Primers		
F3-N1	TCATGACGTTCGTGTTGT	This study
B3-N1	TTGAGTGAGAGCGGTGAA	This study
FIP-N1	TAATGCGGGGTGCATTTCGAGATTTCATCTAAACGAACAAAC	This study
BIP-N1	TAACCAGAATGGAGAACGCAAGTATTATTGGGTAAACCTTGG	This study
LF-N1	CTGATTTTGGGGTCCATTA	This study
LB-N1	GTGGGGCGCGATCAAAACAAC	This study
F3-N2	AACACAAGCTTTCGGCAG	J.P. Broughton et al.
B3-N2	GAAATTTGGATCTTTGTCATCC	J.P. Broughton et al.
FIP-N2	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC	J.P. Broughton et al.

BIP-N2	CGCATTGGCATGGAAGTCACTTTGATGGC ACCTGTGTAG	J.P. Broughton et al.
LF-N2	TTCCTTGTCTGATTAGTTC	J.P. Broughton et al.
LB-N2	ACCTTCGGGAACGTGGTT	J.P. Broughton et al.
F3-E1&E2	CCGACGACGACTACTAGC	J.P. Broughton et al.
B3-E1&E2	AGAGTAAACGTAAAAAGAAGGTT	J.P. Broughton et al.
FIP-E1&E2	CTAGCCATCCTTACTGCGCTACTCACGTTA ACAATATTGCA	J.P. Broughton et al.
BIP-E1&E2	ACCTGTCTCTTCCGAAACGAATTTGTAAGC ACAAGCTGATG	J.P. Broughton et al.
LF-E1&E2	TCGATTGTGTGCGTACTGC	J.P. Broughton et al.
LB-E1&E2	TGAGTACATAAGTTCGTAC	J.P. Broughton et al.
F3-R1&R2	TCAAGTATTGAGTGAAATGGTC	This study
B3-R1&R2	TCTATAGAGACACTCATAAAGTCT	This study
FIP-R1&R2	AAACACTATTAGCATAAGCACGGTTCACTA TATGTTAAACCA	This study
BIP-R1&R2	TGCACTTTTATCTACTGATGGTGTTGTAAAT TGCGGACAT	This study
LF-R1&R2	GTTGTGGCATCTCCTGATGA	This study
LB-R1&R2	GTAACAAAATTGCCGATAAG	This study
F3-RNaseP	TTGATGAGCTGGAGCCA	K.A Curtis et al.
B3-RNaseP	CACCCTCAATGCAGAGTC	K.A Curtis et al.
FIP-RNaseP	GTGTGACCCTGAAGACTCGGTTTTAGCCA CTGACTCGGATC	K.A Curtis et al.
BIP-RNaseP	CCTCCGTGATATGGCTCTTCGTTTTTTTCTT ACATGGCTCTGGTC	K.A Curtis et al.
LF-RNaseP	ATGTGGATGGCTGAGTTGTT	K.A Curtis et al.
LB-RNaseP	CATGCTGAGTACTGGACCTC	K.A Curtis et al.
Reporter		
Fluorescence-based	/56-FAM/TTATT/3IABkFQ/	J.S. Chen et al

reporter		
Lateral flow reporter	/56-FAM/TTATTATT/3Bio/	J.P. Broughton et al.
Dual reporter	/56-FAM/TTATTA/iBiodT/3IABkFQ/	This study
LbCas12a amino acid sequences		
LbCas12a	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENID NKRLLVEDEKRAEDYKGVKKLLDRYYLSFIN DVLHSIKLKNLNNYISLFRKKTRTEKENKELE NLEINLRKEIAKAFKGNEGYKSLFKKDIIETILP EFLDDKDEIALVNSFNGFTTAFTGFFDNREN MFSEEAKSTSIAFRCINENLTRYISNMDIFEKV DAIFDKHEVQEIKEKILNSDYDVEDFFEGEFF NFVLTQEGIDVYNAIIGGFVTESGEKIKGLNE YINLYNQKTKQKLPKFKPLYKQVLSDRESLSF YGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKL EKLFKNFDEYSSAGIFVKNGPAISTISKDIFGE WNVIRDKWNAEYDDIHLKKKAVVTEKYEDDR RKSFKKIGSFSLEQLQEYADADLSVVEKLKEII IQKVDEIYKVYGSSEKLFDADFVLEKSLKKND AVVAIMKDLLDSVKSFENYIKAFFGEGKETNR DESFYGDFVLAYDILLKVDHIYDAIRNYVTQK PYSKDKFKLYFQNPQFMGGWDKDKETDYR ATILRYGSKYYLAIMDKKYAKCLQKIDKDDVN GNYEKINYKLLPGPNKMLPKVFFSKKWMAYY NPSEDIQKIYKNGTFKKGDMFNLNDCHKLIDF FKDSISRYPKWSNAYDFNFSETEKYKDIAGF YREVEEQGYKVSFESASKKEVDKLVEEGKL YMFQIYNKDFSDKSHGTPNLHTMYFKLLFDE NNHGQIRLSGGAELFMRRASLKKEELVVHPA NSPIANKNPDNPKKTTTLSYDVYKDKRFSED QYELHIPIAINKCPKNIFKINTEVRVLLKHDDN PYVIGIDRGERNLLYIVVVDGKGNIVEQYSLN EIINNFNGIRIKTDYHSLLDKKEKERFEARQN WTSIENIKELKAGYISQVVHKICELVEKYDAVI ALEDLNSGFKNSRVKVEKQVYQKFEKMLIDK LNYMVDKKSNPCATGGALKGYQITNKFESFK SMSTQNGFIFYIPAWLTSKIDPSTGFVNLLKT KYTSIADSKKFISSFDRIMYVPEEDLFEFALDY KNFSRTDADYIKKWKLYSYGNRIRIFRNPKKN NVFDWEEVCLTSAYKELFNKYGINYQQGDIR ALLCEQSDKAFYSSFMALMSLMLQMRNSITG RTDVDFLISPVKNSDGIFYDSRNYEAQENAIL PKNADANGAYNIARKVLWAIGQFKKAEDEKL DKVKIAISNKEWLEYAQTSVKH	M.A. Moreno-Mateos et al
LbCas12a^{D156R}	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENID NKRLLVEDEKRAEDYKGVKKLLDRYYLSFIN	This study

	DVLHSIKLKNLNNYISLFRKKTRTEKENKELE NLEINLRKEIAKAFKGNEGYKSLFKKDIIETILP EFLDDKDEIALVNSFNGFTTAFTGFF**R**NREN MFSEEAKSTSIAFRCINENLTRYISNMDIFEKV DAIFDKHEVQEIKEKILNSDYDVEDFFEGEFF NFVLTQEGIDVYNAIIGGFVTESGEKIKGLNE YINLYNQKTKQKLPKFKPLYKQVLSDRESLSF YGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKL EKLFKNFDEYSSAGIFVKNGPAISTISKDIFGE WNVIRDKWNAEYDDIHLKKKAVVTEKYEDDR RKSFKKIGSFSLEQLQEYADADLSVVEKLKEII IQKVDEIYKVYGSSEKLFDADFVLEKSLKKND AVVAIMKDLLDSVKSFENYIKAFFGEGKETNR DESFYGDFVLAYDILLKVDHIYDAIRNYVTQK PYSKDKFKLYFQNPQFMGGWDKDKETDYR ATILRYGSKYYLAIMDKKYAKCLQKIDKDDVN GNYEKINYKLLPGPNKMLPKVFFSKKWMAYY NPSEDIQKIYKNGTFKKGDMFNLNDCHKLIDF FKDSISRYPKWSNAYDFNFSETEKYKDIAGF YREVEEQGYKVSFESASKKEVDKLVEEGKL YMFQIYNKDFSDKSHGTPNLHTMYFKLLFDE NNHGQIRLSGGAELFMRRASLKKEELVVHPA NSPIANKNPDNPKKTTTLSYDVYKDKRFSED QYELHIPIAINKCPKNIFKINTEVRVLLKHDDN PYVIGIDRGERNLLYIVVVDGKGNIVEQYSLN EIINNFNGIRIKTDYHSLLDKKEKERFEARQN WTSIENIKELKAGYISQVVHKICELVEKYDAVI ALEDLNSGFKNSRVKVEKQVYQKFEKMLIDK LNYMVDKKSNPCATGGALKGYQITNKFESFK SMSTQNGFIFYIPAWLTSKIDPSTGFVNLLKT KYTSIADSKKFISSFDRIMYVPEEDLFEFALDY KNFSRTDADYIKKWKLYSYGNRIRIFRNPKKN NVFDWEEVCLTSAYKELFNKYGINYQQGDIR ALLCEQSDKAFYSSFMALMSLMLQMRNSITG RTDVDFLISPVKNSDGIFYDSRNYEAQENAIL PKNADANGAYNIARKVLWAIGQFKKAEDEKL DKVKIAISNKEWLEYAQTSVKH	

Table S3. Interpretation of results

Fluorescence-based reporter detection assay

Table S3.1 Assay Controls

	Positive Control	Negative Control	Interpretation
SARS-CoV-2 N2 Gene ($N2_{t=20}/N2_{t=0}$)	≥ 5	< 5	Valid for N2 gene.
	< 5	< 5	Invalid for positive control. Indicates QC failure.
	≥ 5	≥ 5	Invalid for negative control. Indicates N2 gene contamination
SARS-CoV-2 E2 Gene ($E2_{t=20}/E2_{t=0}$)	≥ 5	< 5	Valid for E2 gene.
	< 5	< 5	Invalid for positive control. Indicates QC failure.
	≥ 5	≥ 5	Invalid for negative control. Indicates E2 gene contamination
Human RNASE-P Gene ($RNASE\text{-}P_{t=20}$ / $RNASE\text{-}P_{t=0}$)	N/A	< 5	Valid for RNASE-P.
		≥ 5	Invalid. Indicates RNASE-P gene contamination.

Table S3.2 Fluorescence Fold-Change Interpretation

SARS-CoV-2 N2 Gene ($N2_{t=20}$ /$NTC_{t=20}$)	**SARS-CoV-2 E2 Gene ($E2_{t=20}$ /$NTC_{t=20}$)**	**Human RNASE-P Gene ($RNASE\text{-}P_{t=20}$ /$NTC_{t=20}$)**	**Interpretation**
≥ 5	≥ 5	N/A	Positive for SARS-CoV-2
≥ 5	< 5	N/A	
< 5	≥ 5	N/A	
< 5	< 5	≥ 5	Negative for SARS-CoV-2

Lateral Flow assay

Table S3.3 Assay Controls

	Positive Control	Negative Control	Interpretation
SARS-CoV-2 N2 Gene Visual readout	+	-	Valid for N2 gene.
	-	+	Invalid for positive control. Indicates QC failure.
	+	+	Invalid for negative control. Indicates N2 gene contamination
SARS-CoV-2 E2 Gene Visual readout	+	-	Valid for E2 gene.
	-	+	Invalid for positive control. Indicates QC failure.
	+	+	Invalid for negative control. Indicates E2 gene contamination
Human RNASE-P Gene Visual readout	N/A	-	Valid for RNASE-P.
		+	Invalid. Indicates RNASE-P gene contamination.

Table S3.4 Lateral Flow Sample Interpretation

SARS-CoV-2 N2 Gene Visual Readout	SARS-CoV-2 E2 Gene Visual Readout	Human RNASE-P Gene Visual Readout	Interpretation
+	+	N/A	Positive for SARS-CoV-2
+	-	N/A	
-	+	N/A	
-	-	+	Negative for SARS-CoV-2