1   **Rapid Detection of 2019 Novel Coronavirus SARS-CoV-2 Using a CRISPR-based**

2   **DETECTR Lateral Flow Assay**

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26   Short title: CRISPR-Cas12 detection of SARS-CoV-2

27

28   **KEYWORDS:** coronavirus, COVID-19, 2019-nCoV, SARS-CoV-2, betacoronavirus,

29   outbreak, Wuhan, CRISPR, CRISPR-Cas12, DETECTR, loop-mediated isothermal

30   amplification (LAMP), isothermal amplification, lateral flow, zoonotic, outbreak,

31   epidemic, pandemic, diagnostic testing, molecular testing

32

33   **ABSTRACT**

34   An outbreak of novel betacoronavirus, SARS-CoV-2 (formerly named 2019-

35   nCoV), began in Wuhan, China in December 2019 and the COVID-19 disease

36   associated with infection has since spread rapidly to multiple countries. Here we report

37   the development of SARS-CoV-2 DETECTR, a rapid (~30 min), low-cost, and accurate

38   CRISPR-Cas12 based lateral flow assay for detection of SARS-CoV-2 from respiratory

39   swab RNA extracts. We validated this method using contrived reference samples and

40   clinical samples from infected US patients and demonstrated comparable performance

41   to the US CDC SARS-CoV-2 real-time RT-PCR assay.

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43   Over the past 40 years, there have been recurrent large-scale epidemics from

44   novel emerging viruses, including human immunodeficiency virus (HIV), SARS and

45   MERS coronaviruses, 2009 pandemic influenza H1N1 virus, Ebola virus (EBOV), Zika

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46 virus (ZIKV), and most recently SARS-CoV-2[1,2]. All of these epidemics presumably

47 resulted from an initial zoonotic animal-to-human transmission event, with either

48 clinically apparent or occult spread into vulnerable human populations. Each time, a

49 lack of rapid, accessible, and accurate molecular diagnostic testing has hindered the

50 public health response to the emerging viral threat.

51 In early January 2020, a cluster of cases of pneumonia from a novel coronavirus,

52 SARS-CoV-2 (with the disease referred to as COVID-19), was reported in Wuhan,

53 China[1,2]. This outbreak has spread rapidly, with over 90,000 reported cases and 3,000

54 deaths as of March 4th, 2020[3]. Person-to-person transmission from infected individuals

55 with no or mild symptoms has been reported[4,5]. Assays using quantitative reverse

56 transcription-polymerase chain reaction (qRT-PCR) approaches for detection of the

57 virus in 4-6 hours have been developed by several laboratories, including an

58 Emergency Use Authorization (EUA)-approved assay developed by the US CDC[6].

59 However, the typical turnaround time for screening and diagnosing patients with

60 suspected SARS-CoV-2 has been >24 hours given the need to ship samples overnight

61 to reference laboratories. To accelerate clinical diagnostic testing for COVID-19 in the

62 United States, the FDA on February 28th, 2020 permitted individual clinically licensed

63 laboratories to report the results of in-house developed SARS-CoV-2 diagnostic assays

64 while awaiting results of an EUA submission for approval[7].

65 Here we report the development and initial validation of a CRISPR (clustered

66 regularly interspaced short palindromic repeats)-Cas12 based assay[8-11] for detection of

67 SARS-CoV-2 from extracted patient sample RNA in ~30 min, called SARS-CoV-2

68 DETECTR. This assay performs simultaneous reverse transcription and isothermal

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69   amplification using loop-mediated amplification (RT-LAMP)[12] from RNA extracted from

70   nasopharyngeal or oropharyngeal swabs in universal transport media (UTM), followed

71   by Cas12 detection of predefined coronavirus sequences, after which cleavage of a

72   reporter molecule confirms detection of the virus. We first designed primers targeting

73   the E (envelope) and N (nucleoprotein) genes of SARS-CoV-2 (**Fig. 1a**). The primers

74   amplify regions that overlap the WHO assay (E gene region) and US CDC assay (N2

75   region in the N gene)[6,13], but are modified to meet design requirements for LAMP. We

76   did not target the N1 and N3 regions used by the US CDC assay, as these regions

77   lacked suitable protospacer adjacent motif (PAM) sites for the Cas12 guide RNAs

78   (gRNAs). Next, we designed Cas12 gRNAs to detect three SARS-like coronaviruses

79   (SARS-CoV-2 accession NC_045512, bat SARS-like coronavirus (bat-SL-CoVZC45,

80   accession MG772933), and SARS-CoV, accession NC_004718)) in the E gene and

81   specifically detect SARS-CoV-2 only in the N gene **(Supplementary Fig. 1).**

82   Using synthetic, *in vitro* transcribed (IVT) SARS-CoV-2 RNA gene targets in

83   nuclease-free water, we demonstrated that the CRISPR-Cas12 based detection can

84   distinguish SARS-CoV-2 with no cross-reactivity for related coronavirus strains **(Fig. 1b,**

85   **Supplementary Fig. 2)**. We then optimized the conditions for the SARS-CoV-2

86   DETECTR assay on the E gene, N gene and the human RNase P gene as a control,

87   which consists of an RT-LAMP reaction at 62°C for 20 min and Cas12 detection

88   reaction at 37°C for 10 min. The DETECTR assay can be run in approximately 30 min

89   and visualized on a lateral flow strip **(Fig. 1c, d).** The SARS-CoV-2 DETECTR assay

90   requires detection of both the E and N genes to confirm a positive test **(Fig. 1e),** and

91   interpretation is consistent with that for the CDC assay N1 and N2 genes (the N3 gene

92   target region for the CDC assay is no longer being used due to concerns regarding

93   flaws in manufacturing reagents and potential decreased sensitivity)[14].

94   We next compared the analytic limits of detection (LoD) of the RT-LAMP/Cas12

95   DETECTR assay relative to the US FDA Emergency Use Authorization (EUA)-approved

96   CDC assay for detection of SARS-CoV-2 **(Table 1; Fig. 2d).** A standard curve for

97   quantitation was constructed using 7 dilutions of a control IVT viral nucleoprotein RNA

98   ("CDC VTC nCoV Transcript")[6], with 3 replicates at each dilution (**Fig. 2d, left;**

99   **Extended Data 1).** Ten two-fold serial dilutions of the same control nucleoprotein RNA

100   were then used to run the DETECTR assay, with 6 replicates at each dilution (**Fig. 2d,**

101   **right; Supplementary Fig. 3).** The estimated LoD for the CDC assay tested by

102   California Department of Public Health was 1 copy/µL reaction, consistent with the

103   analytic performance in the FDA package insert, versus 10 copies/µL reaction for the

104   DETECTR assay.

105   We then assessed the capability of the RT-LAMP assay to amplify SARS-CoV-2

106   nucleic acid directly from raw sample matrix consisting of nasopharyngeal swabs from

107   asymptomatic donors placed in universal transport medium (UTM) or phosphate

108   buffered saline (PBS) and spiked with SARS-CoV-2 IVT target RNA. Assay

109   performance was degraded at reaction concentration of ≥10% UTM and ≥20% PBS by

110   volume, with estimated limits of detection decreasing to 500 and 1,500 copies/µL,

111   respectively (**Supplementary Fig. 4).**

112   Finally, we tested extracted RNA from 11 respiratory swab samples collected

113   from 6 PCR-positive COVID-19 patients (COVID19-1A/B to COVID19-5A/B, where

114   A=nasopharyngeal swab and B=oropharyngeal swab and COVID19-6, a single

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115   nasopharyngeal swab) and 12 nasopharyngeal swab samples from patients with

116   influenza (n=4), common human seasonal coronavirus infections (n=3, representing

117   OC43, HKU1, NL63), and healthy donors (n=5) **(Fig. 2e, f; Supplementary Fig. 5).**

118   Relative to the CDC qRT-PCR, SARS-CoV-2 DETECTR was 90% sensitive and 100%

119   specific for detection of the coronavirus in respiratory swab samples, corresponding to

120   positive and negative predictive values of 100% and 91.7%, respectively **(Fig. 2g).**

121   Here we combined isothermal amplification with CRISPR-Cas12 DETECTR

122   technology to develop a rapid (~30 min) and low-cost test for detection of SARS-CoV-2

123   in clinical samples. The use of existing qRT-PCR based assays is hindered by the need

124   for expensive lab instrumentation, and availability is currently restricted to public health

125   laboratories. Importantly, the DETECTR assays developed here have comparable

126   accuracy to qRT-PCR and are broadly accessible, as they use routine protocols and

127   commercially available, "off-the-shelf" reagents. Key advantages of our approach over

128   existing methods such as qRT-PCR include (1) isothermal signal amplification for rapid

129   target detection obviating the need for thermocycling, (2) single nucleotide target

130   specificity (guide RNAs at the N2 site can distinguish SARS-CoV-2 from SARS-CoV

131   and MERS-CoV), (3) integration with portable, low-cost reporting formats such as lateral

132   flow strips, and (4) quick development cycle to address emerging threats from novel

133   zoonotic viruses (<2 weeks for SARS-CoV-2, **Supplementary Fig. 6**).

134   Although most of the cases of COVID-19 infection during the first month of the

135   epidemic were traced to the city of Wuhan and Hubei province in China, the ongoing

136   rise in cases now appears to be driven to local community transmission[15,16]. For a

137   number of reasons, there is an urgent public health need for rapid diagnostic tests for

138   SARS-CoV-2 infection. The documented cases of asymptomatic infection and

139   transmission in COVID-19 patients[4,5] greatly expand the pool of individuals who need to

140   be screened. Viral titers in hospitalized patients can fluctuate day-to-day with lack of

141   correlation to disease severity[17-19], and thus a single negative qRT-PCR test for SARS-

142   CoV-2 does not exclude infection. The virus has also been shown to be shed in stool[20],

143   raising the possibility of environmental contamination contributing to local disease

144   outbreaks. Low testing platforms such as the DETECTR CRISPR-Cas12 assay

145   developed here may be useful for periodic repeat testing of patient samples. Clinical

146   validation of this assay in response to recent draft guidance from the US FDA[7] is

147   currently ongoing in a CLIA (Clinical Laboratory Improvement Amendments)-certified

148   microbiology laboratory.

149   The major pandemics and large-scale epidemics of the past half century have all

150   been caused by zoonotic viruses. Despite these recurrent outbreaks, we still do not

151   have a programmable point of care (POC) diagnostic platform that can be used to

152   promptly address any emerging viral threat. The CRISPR-based DETECTR technology

153   provides such a platform, which we have reconfigured within days to detect SARS-CoV-

154   2 (**Supplementary Fig. 6**). Here we developed a SARS-CoV-2 DETECTR assay,

155   described its performance characteristics and demonstrated compatibility with lateral

156   flow strips. The future development of portable microfluidic-based cartridges to run the

157   assay and use of lyophilized reagents will enable POC testing outside of the clinical

158   diagnostic laboratory, such as airports, local emergency departments and clinics, and

159   other decentralized locations.

160   **(~1343 words, max 2000 words)**

161   **FIGURE LEGENDS**

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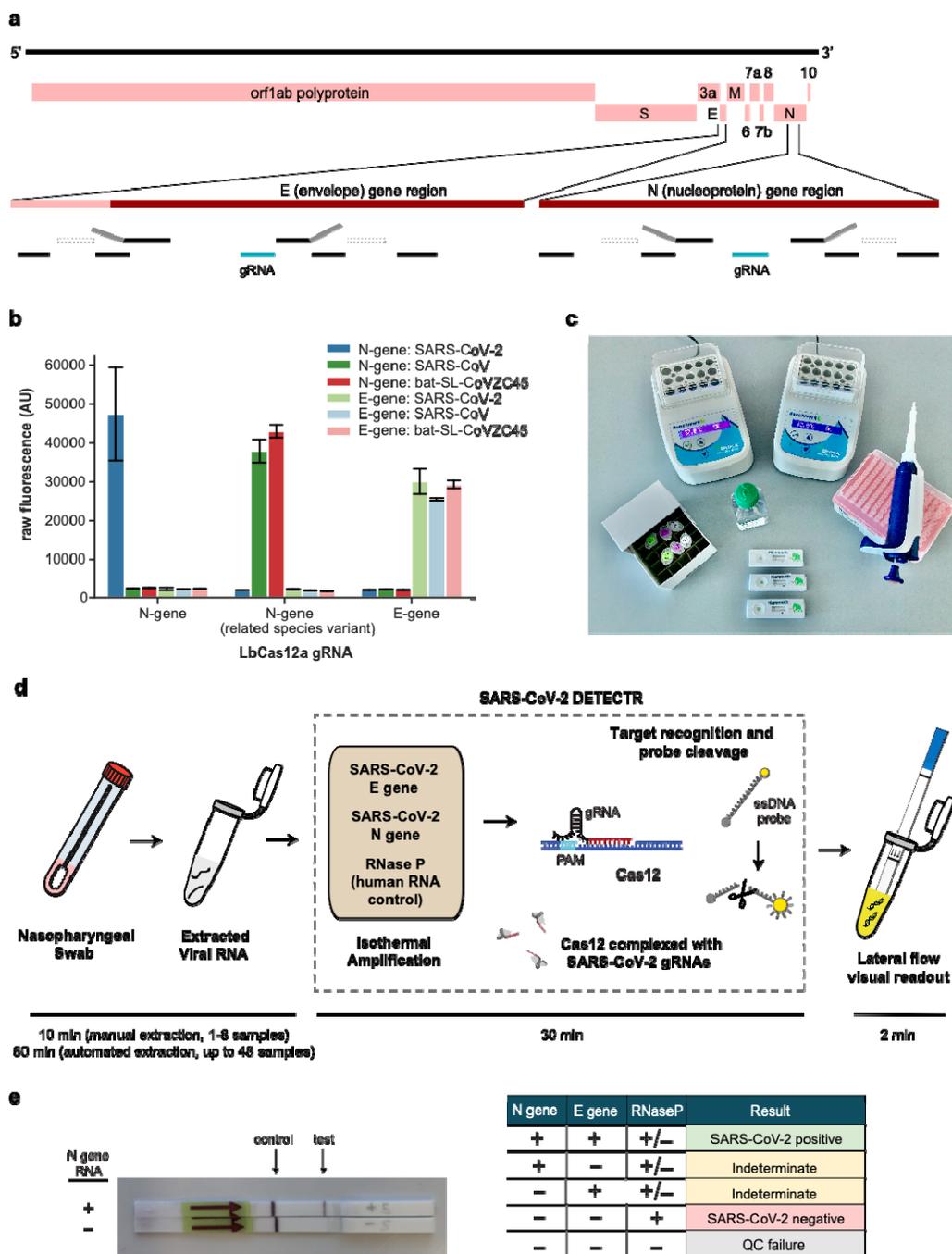
163   **Figure 1. A CRISPR-Cas12 based assay for detection of SARS-CoV-2. (a) Genome**

164   **map showing primers, probes and gRNAs.** Visualization of primers and probes on

165   the SARS-CoV-2 genome **(b) gRNA specificity.** Cas12 gRNAs are programmed to

166   specifically target SARS-CoV-2 or broadly detect related coronavirus strains. The N

167   gene gRNA used in the assay (left) is specific for SARS-CoV-2, whereas the E gene

168   gRNA is able to detect 3 SARS-like coronavirus (right). A separate N gene gRNA

169   targeting SARS-CoV and a bat coronavirus and differing by a single nucleotide from the

170   N gene gRNA used in the assay fails to detect SARS-CoV-2 (middle). **(c) Minimum**

171   **equipment needed to run protocol.** With appropriate biosafety level 2 requirements,

172   the minimum equipment required to run the protocol includes Eppendorf tubes with

173   reagents, heat blocks or water bath (37°C and 62°C), nuclease-free water, pipettes and

174   tips, and lateral flow strips. **(d) Schematic of SARS-CoV-2 DETECTR workflow.**

175   Conventional RNA extraction or sample matrix can be used as an input to DETECTR

176   (LAMP pre-amplification and Cas12-based detection for E gene, N gene and RNase P),

177   which is visualized by a fluorescent reader or lateral flow strip. **(e) Lateral flow strip**

178   **assay readout.** A positive result requires detection of at least the two SARS-CoV-2 viral

179   gene targets (N gene and E gene).

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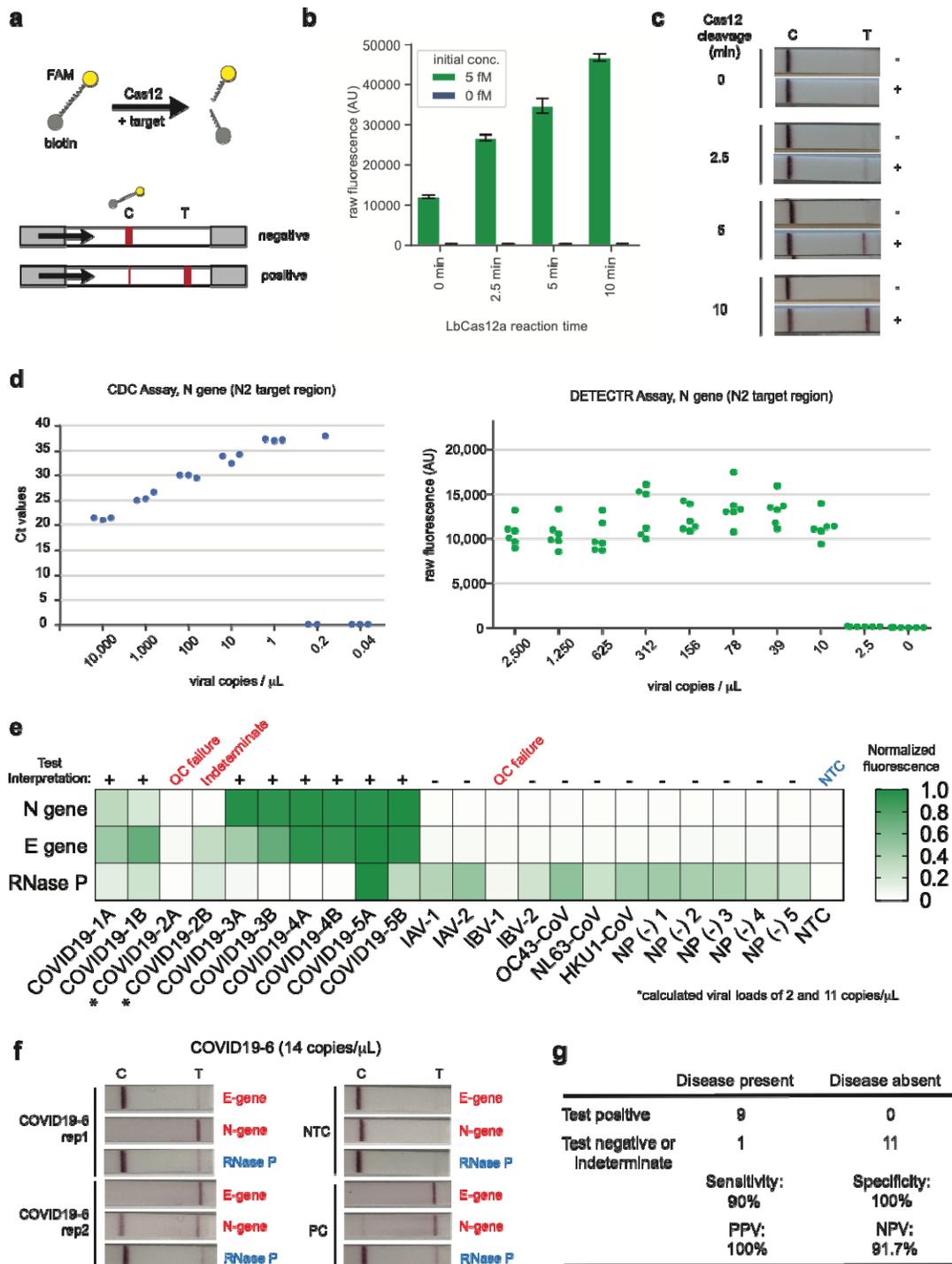
182 **Figure 2. Detection of SARS-CoV-2 in contrived and clinical nasopharyngeal or**

183 **oropharyngeal swab samples. (a) Schematic of DETECTR coupled with lateral**

184 **flow readout.** The intact FAM-biotinylated reporter molecule flows to the control capture

185 line. Upon recognition of the matching target, the Cas-gRNA complex cleaves the

186 reporter molecule, which flows to the target capture line. **(b-c) Comparison of**

187 **fluorescence to lateral flow. (b)** Fluorescence signal of LbCas12a detection assay on

188 RT-LAMP amplicon for SARS-CoV-2 N-gene saturates within 10 min. RT-LAMP

189 amplicon generated from 2 µL of 5 fM or 0 fM SARS-CoV-2 N-gene IVT RNA by

190 amplifying at 62°C for 20 min. **(c)** LbCas12a on the same RT-LAMP amplicon produces

191 visible signal through lateral flow assay within 5 min. **(d) Limit of detection for CDC**

192 **qPCR and DETECTR.** Ct values using the CDC qPCR assay (n=3) and fluorescence

193 values using SARS-CoV-2 DETECTR (n=6) using SARS-CoV-2 N2 gene IVT-RNA. **(e)**

194 **Patient sample DETECTR data.** DETECTR fluorescence values were normalized to

195 the highest value within the N gene, E gene or RNase P set, with a positive threshold at

196 five standard deviations above background. Final determination of the SARS-CoV-2 test

197 was based on the interpretation matrix in Fig. 1e, with results indicated above the heat

198 map. **(f) SARS-CoV-2 DETECTR assay identifies presence of SARS-CoV-2 viral**

199 **RNA from clinical sample.** Two replicate assays were performed using 2 µL of

200 extracted RNA for each reaction (titer 12 copies/µL). Positive controls used IVT RNA for

201 SARS-CoV-2 targets and total human RNA for RNase P. LbCas12a detection assays

202 were run on lateral flow strips (TwistDx) and imaged after 3 min. **(g) Performance**

203 **characteristics of the SARS-CoV-2 DETECTR assay.** Abbreviations: fM, femtomolar;

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204   NTC, no-template control; PPV, positive predictive value; NPV, negative predictive

205   value.

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207

208   **Table 1. Comparison of the DETECTR (RT-LAMP/Cas12) assay with the CDC qRT-**

209   **PCR assay for detection of SARS-CoV-2**

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	SARS-CoV-2 DETECTR, RT-LAMP/Cas12	CDC SARS-CoV-2 qRT-PCR
Target	E gene & N gene*	N-gene (3 amplicons, N1, N2, and N3)
Sample control	RNase P	RNase P
Limit of Detection	10 copies/µL input	3.2 copies/µL input
Assay reaction time (approximate)	30 min	120 min
Assay sample-to-result time (approximate)	45 min (with manual RNA extraction)	4 hr (including RNA extraction)
Assay components	RT-LAMP (62°C, 20 min) Cas12 (37°C, 10 min) Lateral flow strip (RT, 2 min; no additional time if using fluorescence readout)	UDG digestion (25°C, 2 min), reverse transcription (50°C, 15 min), denature (95°C, 2 min) amplification, (95°C, 3 sec; 55°C 30 sec; 45 cycles)
Bulky instrumentation required	No	Yes
FDA EUA approval	No	Yes

211
212   *E gene primers target same amplicon region as in the WHO protocol; N gene primers

213   target same N2 amplicon region as in the CDC protocol

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219   **METHODS**

220   *Nucleic acid preparation*

221   SARS-CoV-2 target sequences were designed using all available genomes

222   available from GISAID[21] as of January 27, 2020. Briefly, viral genomes were aligned

223   using Clustal Omega. Next, LbCas12a target sites on the SARS-CoV-2 genome were

224   filtered against SARS-CoV, two bat-SARS-like-CoV genomes and common human

225   coronavirus genomes. Compatible target sites were finally compared to those used in

226   current protocols from the CDC and WHO. LAMP primers for SARS-CoV-2 were

227   designed against regions of the N-gene and E-gene using PrimerExplorer v5

228   (https://primerexplorer.jp/e/). RNase P POP7 primers were originally published by

229   Curtis, et al. (2018) and a compatible gRNA was designed to function with these primer

230   sets.

231   Target RNAs were generated from synthetic gene fragments of the viral genes of

232   interest. First a PCR step was performed on the synthetic gene fragment with a forward

233   primer that contained a T7 promoter. Next, the PCR product was used as the template

234   for an in-vitro transcription (IVT) reaction at 37°C for 2 hours. The IVT reaction was then

235   treated with TURBO DNase (Thermo) for 30 min at 37°C, followed by a heat-

236   denaturation step at 75°C for 15 min. RNA was purified using RNA Clean and

237   Concentrator 5 columns (Zymo Research). RNA was quantified by Nanodrop and Qubit

238   and diluted in nuclease-free water to working concentrations.

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240 *DETECTR assays*

241   DETECTR assays were performed using RT-LAMP for pre-amplification of viral

242 or control RNA targets and LbCas12a for the *trans*-cleavage assay. RT-LAMP was

243 prepared as suggested by New England Biolabs

244 (https://www.neb.com/protocols/2014/10/09/typical-rt-lamp-protocol) with a $MgSO_4$

245 concentration of 6.5 mM and a final volume of 10 µL. LAMP primers were added at a

246 final concentration of 0.2 µM for F3 and B3, 1.6 µM for FIP and BIP, and 0.8 µM for LF

247 and LB. Reactions were performed independently for N-gene, E-gene, and RNase P

248 using 2 µL of input RNA at 62°C for 20 min.

249   LbCas12a *trans*-cleavage assays were performed similar to those described in

250 Chen, et al. (2018). 50 nM LbCas12a (available from NEB) was pre-incubated with 62.5

251 nM gRNA in 1X NEBuffer 2.1 for 30 min at 37°C. After formation of the RNA-protein

252 complex, the lateral flow cleavage reporter (/56-FAM/TTATTATT/3Bio/, IDT) was added

253 to the reaction at a final concentration of 500 nM. RNA-protein complexes were used

254 immediately or stored at 4°C for up to 24 hours before use.

255

256 *Lateral flow readout*

257   After completion of the pre-amplification step, 2 µL of amplicon was combined

258 with 18 µL of LbCas12a-gRNA complex and 80 µL of 1X NEBuffer 2.1. The 100 µL

259 LbCas12a trans-cleavage assay was allowed to proceed for 10 min at 37°C.

260 A lateral flow strip (Milenia HybriDetect 1, TwistDx) was then added to the reaction tube

261 and a result was visualized after approximately 2 min. A single band, close to the

15

262   sample application pad indicated a negative result, whereas a single band close to the

263   top of the strip or two bands indicated a positive result.

264

265   *Optimized DETECTR method for patient samples*

266   The patient optimized DETECTR assays were performed using RT-LAMP

267   method as described above with the following modifications: A DNA binding dye,

268   SYTO9 (Thermo Fisher Scientific), was included in the reaction to monitor the

269   amplification reaction and the incubation time was extended to 30 min to capture data

270   from lower titre samples.

271   The fluorescence based patient optimized LbCas12a *trans*-cleavage assays

272   were performed as described above with modifications; 40nM LbCas12a was pre-

273   incubated with 40nM gRNA, after which 100nM of a fluorescent reporter molecule

274   compatible with detection in the presence of the SYTO9 dye

275   (/5Alex594N/TTATTATT/3IAbRQSp/) was added to the complex. 2 µL of amplicon was

276   combined with 18 µL of LbCas12a-gRNA complex in a black 384-well assay plate and

277   monitored for fluorescence using a Tecan plate reader.

278

279   *Contrived sample preparation.*

280   In-vitro transcribed RNA (gift from California Department of Public Health

281   (CDPH)), with a titer of 10,000 copies/µL (Ct value of 21) was diluted into 2,500

282   copies/µL first, then serially diluted in water to concentration of 1 250, 625, 312, 156, 78,

283   39, 10 and 2.5 copies per microliter.

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286   *Human clinical sample collection and preparation*

287   Negative nasopharyngeal swabs were acquired from healthy donors in Chiu lab

288   with the approval of the University of California, San Francisco (UCSF) IRB. Clinical

289   nasopharyngeal and oropharyngeal swab samples of SARS-CoV-2 patients were

290   collected in UTM and transported to the CDPH or UCSF lab. Sample RNA of SARS-

291   CoV-2 was extracted following instructions as described in the CDC EUA-approved

292   protocol[6] (input 120 µL, elution of 120 µL) using Qiagen DSP Viral RNA Mini Kit

293   (Qiagen) at CDPH and the MagNA Pure 24 instrument (Roche Life Science) at UCSF.

294   Nasopharyngeal swab samples of influenza and common coronavirus were extracted at

295   UCSF using the MagNA Pure 24 instrument.

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297   *CDC real-time qRT-PCR assay*

298   The CDC assay was performed using the ABI 7500 Fast DX instrument (Applied

299   Biosystems) according the CDC EUA-approved protocol[6].

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305 **SUPPLEMENTARY FIGURES**

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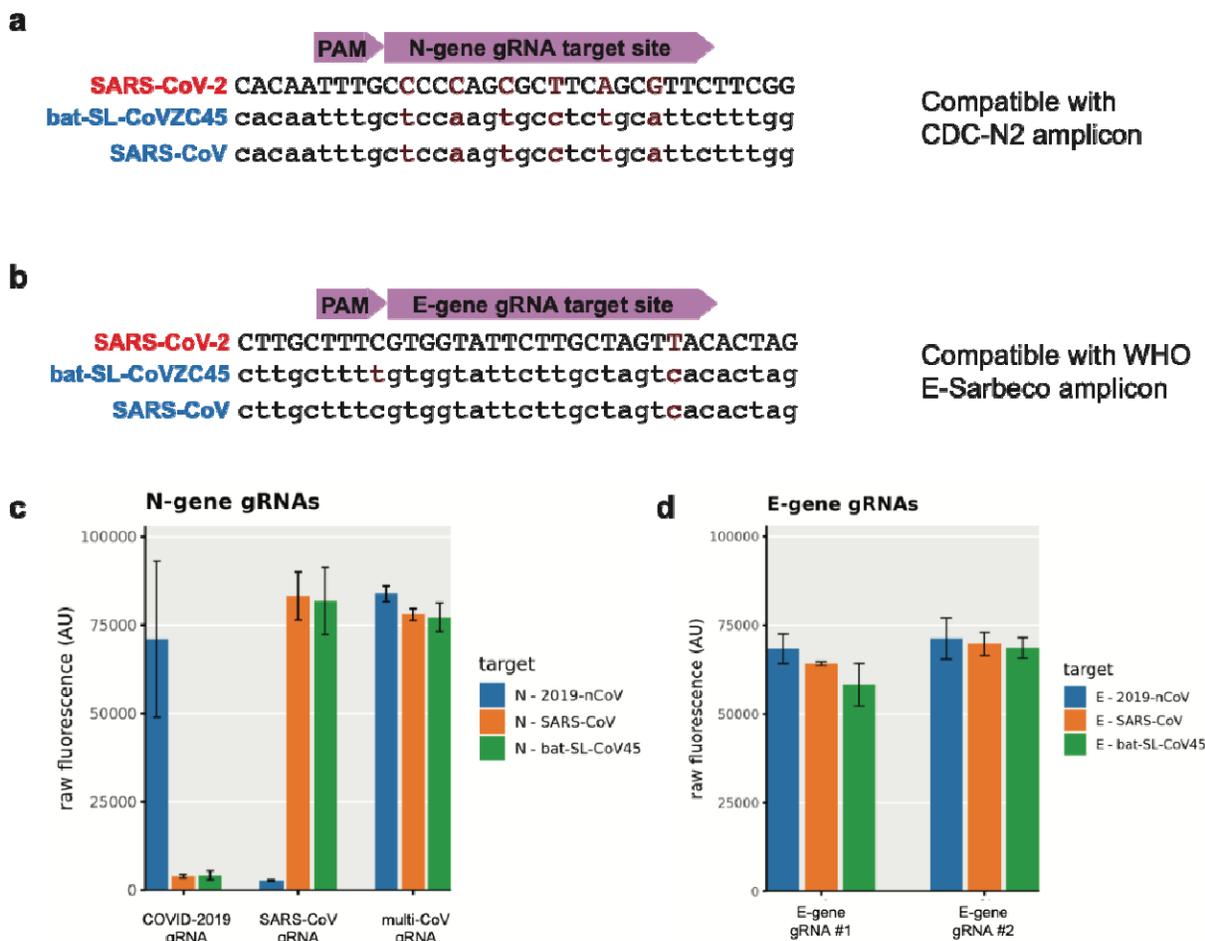
307

308 **Supplementary Figure 1.** Comparison of sequences between SARS-CoV-2, SARS-

309 CoV, and bat-SL-CoVZC45 at the sites targeted by the gRNAs used in this study. **(a)**

310 The N-gene gRNA is compatible with the CDC-N2 amplicon, and **(b)** the E-gene gRNA

311 is compatible with the WHO E-Sarbeco amplicon. **(c-d)** DETECTR fluorescence values

312 using **(c)** N gene gRNAs and **(d)** E gene gRNAs.

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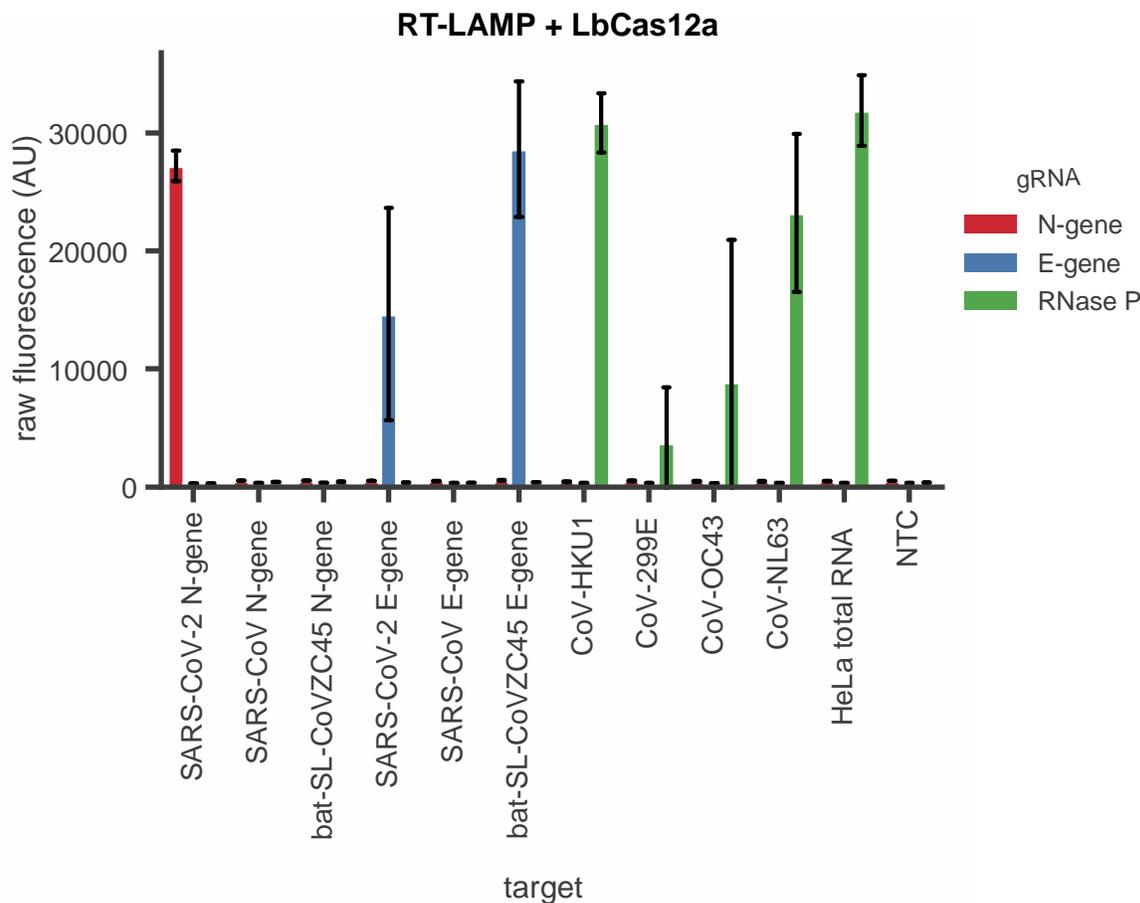
Supplementary Figure 2. Cross-reactivity of DETECTR to common human

coronaviruses. SARS-CoV-2 DETECTR assay (RT-LAMP + Cas12a) was evaluated on

IVT RNA products from SARS-CoV-2, SARS-CoV, bast-SL-CoVZC45, and clinical

samples from common human coronaviruses. As expected, the N-gene is only detected

in SARS-CoV-2, whereas the E-gene is detected only in SARS-CoV-2 and bat-SL-

CoVZC45. SARS-CoV E-gene was not detected as the RT-LAMP primer set is not

capable of amplifying the SARS-CoV E-gene, even though the E-gene gRNA is capable

of detecting the SARS-CoV E-gene target site. RNase P is detected in common human

coronaviruses because these samples are RNA extracted from clinical samples. Result

shown at 15 min of LbCas12a detection assay signal on fluorescent plate reader.
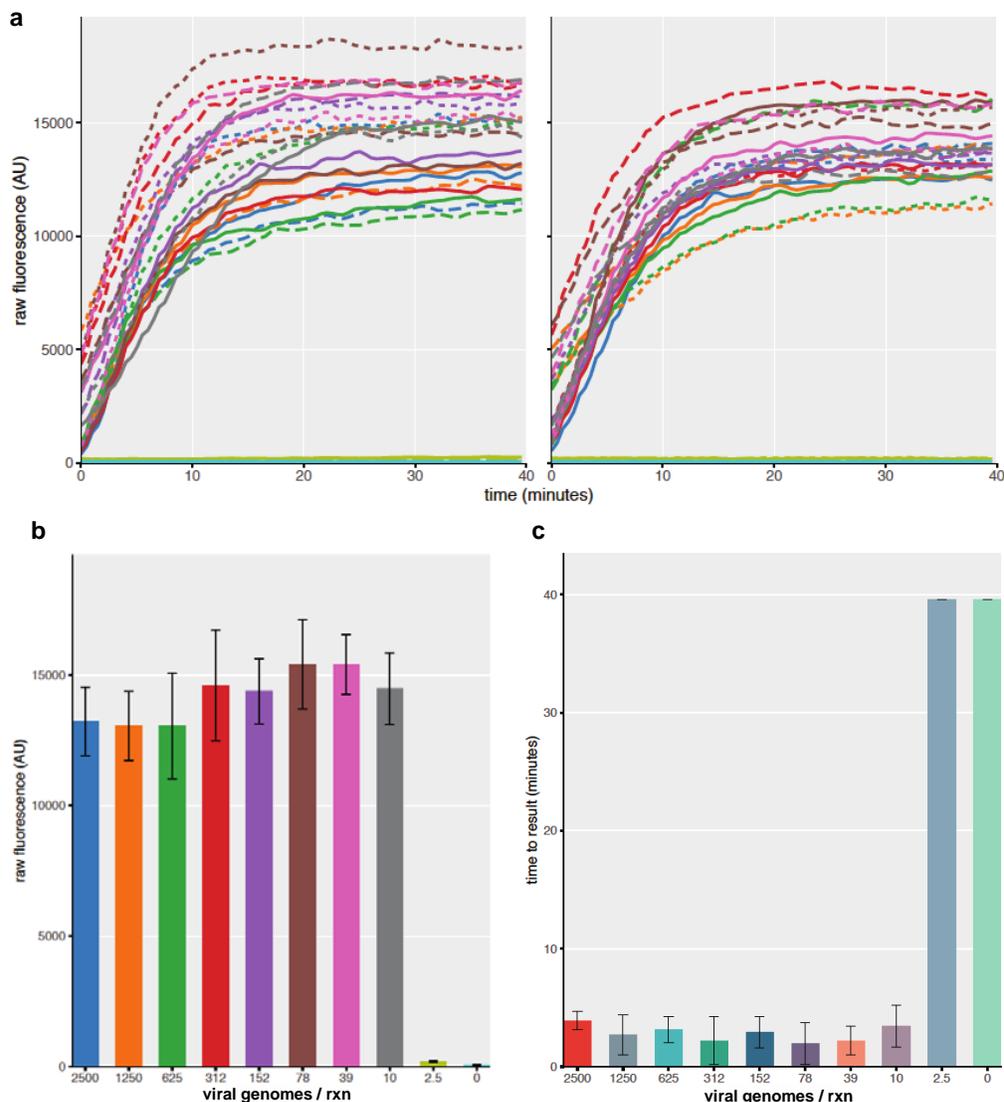
Supplementary Figure 3. DETECTR analysis of SARS-CoV-2 identifies down to 10 viral genomes in approximately 30 min. Duplicate LAMP reactions were amplified for twenty min followed by LbCas12a DETECTR analysis. **(a)** Raw fluorescence curves generated by LbCas12a detection of SARS-CoV-2 N-gene (n=6) show saturation in less than 20 min. **(b)** Further analysis reveals the limit of detection of the SARS-CoV-2 N-gene to be 10 viral genomes per reaction (n=6). **(c)** Evaluation of the time to result of these reactions highlights detection of 10 viral genomes of SARS-CoV-2 in under 5 min (n=6).

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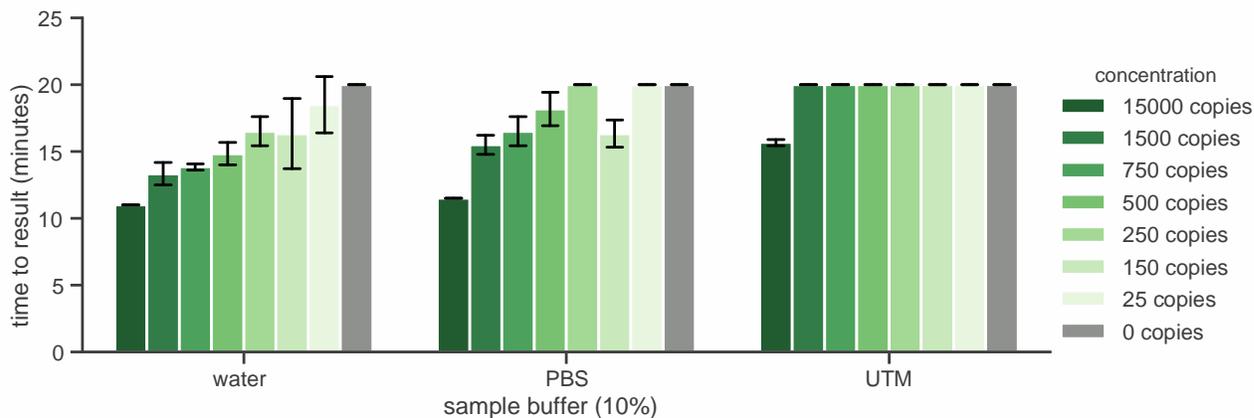
335
336 **Supplementary Figure 4.** Impact of sample buffers on performance of RT-LAMP pre-

337 amplification. Time-to-result for RT-LAMP amplification (lower value indicates faster

338 amplification) with 10% universal transport medium (UTM), 10% PBS, or 10% water

339 final volume for the SARS-CoV-2 N-gene on a standard curve of the 2019-nCoV

340 positive control plasmid (IDT) in 10% reaction volume. Results indicate that 10% PBS

341 inhibits the assay less than 10% UTM.

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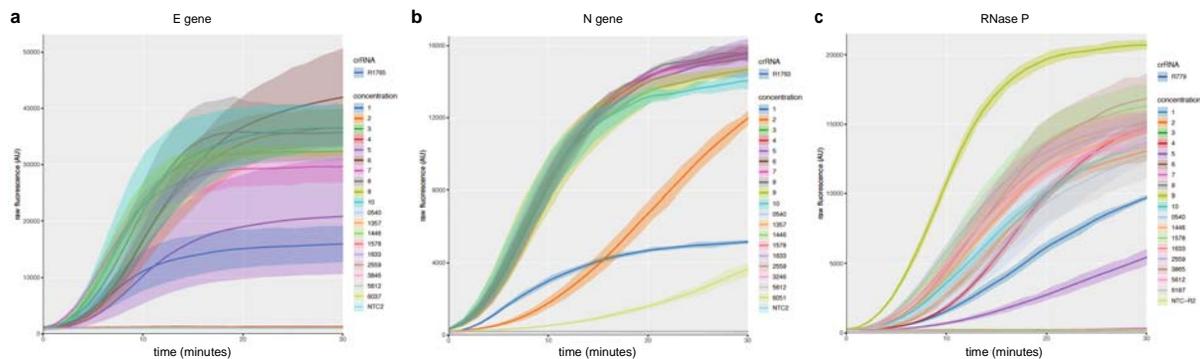
Supplementary Figure 5. DETECTR kinetic curves on COVID-19 infected patient

samples. Ten nasopharyngeal/oropharyngeal swab samples from 5 patients (COVID19-

1 to COVID19-5) were tested for SARS-CoV-2 using two different genes, N2 and E as

well as a sample input control, RNase P. **(a)** Using the standard amplification and

detection conditions, 9 of the 10 patient samples resulted in robust fluorescence curves

indicating presence of the SARS-CoV-2 E-gene (20-minute amplification, signal within

10 min). **(b)** The SARS-CoV-2 N-gene required extended amplification time to produce

strong fluorescence curves (30-minute amplification, signal within 10 min) for 8 of the 10

patient samples. **(c)** As a sample input control, RNase P was positive for 17 of the 22

total samples tested (20-minute amplification, signal within 10 min).

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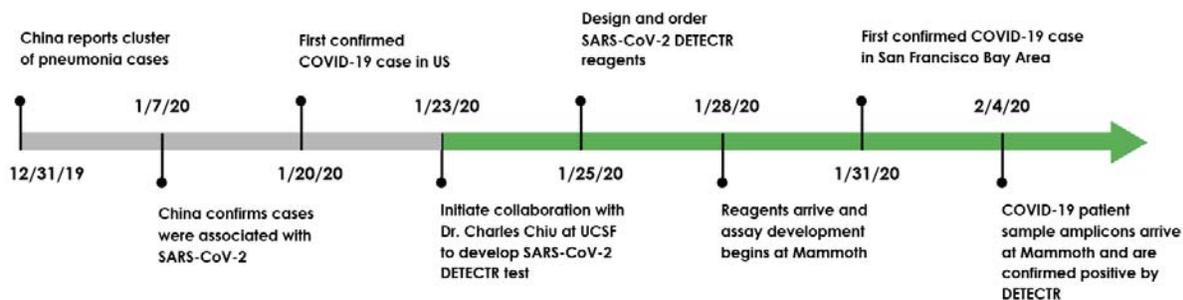
357
358 **Supplementary Figure 6.** Timeline showing major events in the progression of COVID-

359 19 detection and assay development.

360

361   **Extended Data 1 ("Extended_Data_1.xlsx").** Standard curve generated by running

362   seven 5- or 10-fold dilutions of the CDC N2 qRT-PCR assay, with 3 replicates each

363   dilution. The R-squared measure corresponding to the regression line is 0.9981.

364

365   **Extended Data 2 ("Extended_Data_2.xlsx").** Primer, reporter molecules, target gene

366   fragments, and guide RNAs used in this study.

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378 **AUTHOR CONTRIBUTIONS**

379 CYC and JSC conceived the study. JPB conceived, designed and validated DETECTR

380 reagents and protocols. XD and GY validated RT-PCR and LAMP on patient samples.

381 JPB, XD, CLF performed experiments and analyzed data. GY, JS, AG and AS

382 performed experiments. KZ, SM, EH and WG coordinated the study, consented UCSF

383 patients and collected samples. JS, CYP, HG and DW collected samples from patients

384 and extracted the viral RNA. CYC, JPB, XD, and JSC wrote and edited the manuscript.

385 All authors read the manuscript and agree to its contents.

386

387 **COMPETING INTERESTS**

388 CYC is the director of the UCSF-Abbott Viral Diagnostics and Discovery Center

389 (VDDC), receives research support funding from Abbott Laboratories, and is on the

390 Scientific Advisory Board of Mammoth Biosciences, Inc. JSC is a co-founder of

391 Mammoth Biosciences, Inc. JPB, CLF, and JS are employees of Mammoth

392 Biosciences, Inc. CYC, JPB, XD, CLF, JS and JSC are co-inventors of CRISPR-related

393 technologies.

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