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An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection

method based on CRISPR-Cas12

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Short running title: Coronavirus sequence detection by CRISPR-Cas12

Keywords: CRISPR-Cas12, emerging virus, SARS-CoV2, COVID-19, 2019-nCoV,

diagnosis.

19 **Abstract**

20

21 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has received global

22 attention due to the recent outbreak in China. In this work, we report a CRISPR-Cas12 based

23 diagnostic tool to detect synthetic SARS-CoV-2 RNA sequences in a proof-of-principle

24 evaluation. The test proved to be sensitive, rapid, and potentially portable. These key traits of

25 the CRISPR method are critical for virus detection in regions that lack resources to use the

26 currently available methods.

27

28 **Introduction**

29

30 The rapid spread of the novel coronavirus is clearly a major concern for countries across the

31 world. This virus is a single-stranded RNA virus with high sequence identity to SARS-CoV

32 and has, therefore, been named SARS-CoV-2. This coronavirus variant is capable of

33 transmission from person to person, which makes it a dangerous virus that can cause a

34 pandemic.

35

36 Several SARS-CoV-2 detection assays have been reported to be currently under

37 development. The WHO website provides information on several virus detection protocols

38 that have been used in different countries such as China, Germany, Japan, and the US, among

39 others (*1*). All these are real-time reverse transcription PCR (rRT-PCR) based assays, and

40 despite their established efficiency, require highly specialized personnel and expensive

41 equipment for implementation. In such a backdrop, any development toward ultrasensitive,

42 cheaper, and portable diagnostic tests for the assessment of suspected cases, regardless of the

43 presence of qualified personnel or sophisticated equipment for virus detection, could help

44 advance the diagnosis of COVID-19.

45

46 CRISPR is a biotechnological technique well-known for its use in gene editing. Notably,

47 CRISPR has been recently used for the *in vitro* detection of nucleic acids, thereby emerging

48 as a powerful and precise tool for molecular diagnosis (*2-4*). Within the CRISPR-Cas effector

49 family, Cas12 is a RNA-guided DNase belonging to the class II type V-A system that induces

50 indiscriminate single-stranded DNA (ssDNA) collateral cleavage after target recognition.

51 This leads to the degradation of ssDNA reporters that, emit a fluorescence signal on cleavage

52 or alternatively, could be detected on a paper strip (by lateral flow) in a portable manner (5).

53 Therefore, CRISPR-Cas12 based tools possess the potential to emerge as an *in situ* diagnostic

54 tool for rapid detection of the SARS-CoV-2 virus. In this work, we employed CRISPR-

55 Cas12a and its unspecific collateral ssDNAse activity to generate a fast, accurate, and

56 portable SARS-CoV-2 sequence detection method.

57

58 **Materials and methods**

59

60 For the detection assays, we included synthetic RNA fragments of SARS-CoV-2

61 corresponding to the *RdRp*, *ORF1b* and *ORF1ab* genes, using the WH-human1 sequence

62 (GenBank MN908947) as a reference. Briefly, the SARS-CoV-2 fragments were synthesized

63 as complementary DNA oligonucleotides and treated with fill-in PCR (NEBNext® High-

64 Fidelity 2X PCR Master Mix) to generate the DNA templates. These DNA templates were

65 transcribed into RNA using an *in vitro* transcription (IVT) Kit (Ambion, Invitrogen) under

66 the control of a T7 promoter.

67

68   A target amplification step was performed using the TwistAmp® Basic recombinase

69   polymerase amplification (RPA) kit (TwistDx, Cambridge, United Kingdom) and RT was

70   carried out by the addition of 5 µL of synthetic SARS-CoV-2-RNA input, 2.5 µL of M-

71   MuLV reverse transcriptase (NEB), and 1 µL of murine RNase inhibitor (NEB) in a 50 µL

72   final reaction volume. Reactions were run for 30 min at 42°C. The RT-RPA was carried out

73   in one step, with the same pair of primers for both reactions.

74

75   To generate the CRISPR-detection complex, we mixed 75 nM of the commercially available

76   LbCas12a endonuclease (NEB) with the same amount of single guide RNA (sgRNA)

77   synthesized in-house by hybridization of DNA oligonucleotides followed by fill-in PCR and

78   IVT. The complexation reaction was carried out in a solution containing 1X NEBuffer 2.1

79   (NEB) (composed of 100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9)

80   at room temperature for 10 min.

81

82   For plate reader-based assays, ssDNA reporters labeled with FAM were included in the

83   detection mix; while for portable detection with paper strips, ssDNA reporters labeled with

84   biotin were included. The reaction was initiated by diluting the LbCas12a complexes to a

85   final concentration of 37 nM LbCas12a : 37 nM sgRNA in a solution containing 1X

86   NEBuffer 2.1 (NEB) and 1 µM custom ssDNA reporter substrates in a 40 µL reaction

87   volume. A 2 µL aliquot of RT-RPA input was used. Reactions were incubated for up to 90

88   min at 37°C. Fluorescence measurements were acquired at 10 min intervals (λ_{ex}: 485 nm and

89   λ_{em}: 535 nm) in a SpectraMax M2 fluorescence plate reader (Molecular Devices) operated in

90   the 384-well microplate format. For paper-based measurements, we used the Milenia

91   HybriDetect 1 (TwistDx) lateral flow system, as per the manufacturer's instructions. Finally,

92   we simulated a clinical sample by adding synthetic SARS-CoV-2 RNA fragments (10^5

93   copies/µL) to saliva samples from a healthy donor and measuring the results on the test strips.

94   The saliva was treated with heat and chemicals before using 2 µL as input for RT-RPA (5).

95

96   Details about the target sequences, sgRNA, primers for RT-RPA, and ssDNA reporters are

97   listed in the accompanying table 1. The general scheme of target synthesis, amplification,

98   detection with CRISPR, and visualization of the result are shown in the figure 1.

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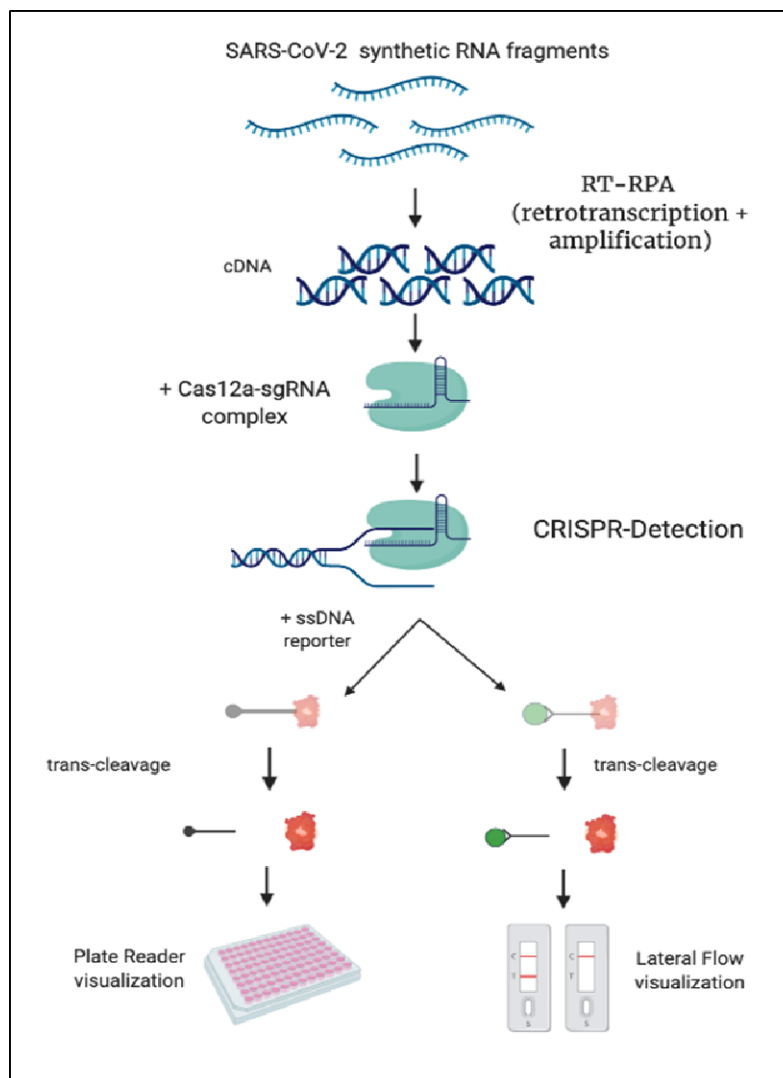
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101   **Figure 1**. CRISPR-based detection method for novel coronavirus. General scheme of

102   CRISPR detection procedure. In the two strategies all the process takes less than 60 min.

103

104 **Table. List of DNA oligonucleotides and ssDNA reporters used in this work**

Name	Sequence	Source
nCoV-RdRp-sgRNA R	ATGTGTGGCGGTTCACTATATGTatctacacttagtagaaatta	This paper
nCoV-RdRp-Template PCR fill-in F1	CCCTAATACGACTCACTATAGGCAAGTATTGAGTGAAATGGTCATGTGTGGCGGTTCACT	GenBank MN908947
nCoV-RdRp-Template PCR fill-in R2	GCAGTTGTGGCATCTCCTGATGAGGTTCCACCTGGTTTAACATATAGTGAACCGCCACAC	GenBank MN908947
nCoV-RdRp-Template PCR fill-in F3	AGATGCCACAACTGCTTATGCTAATAGTGTTTTTAACATTTGTCAAGCTGTC	GenBank MN908947
nCoV-RdRp-Primer RT-RPA F	GTGARATGGTCATGTGTGGCGG	Charité, Berlin, Germany
nCoV-RdRp-Primer RT-RPA R	CARATGTTAAASACACTATTAGCATA	Charité, Berlin, Germany
nCoV-ORF1b-sgRNA R	TGGTTGCTTTGTAGGTTACCTGTatctacacttagtagaaatta	This paper
nCoV-ORF1b- Template PCR fill-in F1	CCCTAATACGACTCACTATAGGCAATGGGGTTTTACAGGTAACCTACAAAGCAACCATGA	GenBank MN908947
nCoV-ORF1b- Template PCR fill-in R2	TCACAACTAGCTACATGTGCATTACCATGGACTTGACAATACAGATCATGGTTGCTTTGT	GenBank MN908947
nCoV-ORF1b- Template PCR fill-in F3	AGCTAGTTGTGATGCAATCATGACTAGGTGTCTAGCTGTCCACGAGTGCTTTGTTAAGCG	GenBank MN908947
nCoV-ORF1b-Primer RT-RPA F	TGGGGYTTTACRGGTAACCT	Hong Kong University
nCoV-ORF1b-Primer RT-RPA R	AACRCGCTTAACAAAGCACTC	Hong Kong University
nCOV-ORF1ab-sgRNA R	GTCTGTACCGTCTGCGGTATGTGatctacacttagtagaaatta	This paper
nCOV-ORF1ab- Template PCR fill-in F1	CCCTAATACGACTCACTATAGGCCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCG	GenBank MN908947
nCOV-ORF1ab- Template PCR fill-in R2	GGAGTTGATCACAACTACAGCCATAACCTTTCCACATACCGCAGACGGTACAGACTGTGT	GenBank MN908947
nCOV-ORF1ab- Template PCR fill-in F3	GTTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATCGTT	GenBank MN908947
nCOV-ORF1ab-Primer RT-RPA F	CCCTGTGGGTTTTACACTTAA	China CDC
nCOV-ORF1ab-Primer RT-RPA R	ACGATTGTGCATCAGCTGA	China CDC
T7-Cas12scaffold F	CCCTAATACGACTCACTATAGGTAATTTCTACTAAGTGTAGAT	This paper
ssDNA biotin	/56-FAM/TTATT/3Bio	Chen *et al.*, 2018
ssDNA FAM	/56-FAM/TTATT/3IABkFQ	Chen *et al.*, 2018

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106

107 **Results and Discussion**

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109   The best results were obtained in attempts to detect the *ORF1ab* region with primers

110   suggested by the China CDC (*6*). Based on visualization of these results in a fluorescence

111   spectrophotometer, the limit of detection (LOD) for *ORF1ab* coronavirus sequences was

112   estimated to be up to 10 copies/µL (figure 2 panel A of the accompanying figure), which is 4

113 orders of magnitude lower than the viral load found for the patient reported in Berlin (10^5

114 copies/µL) (*7*).

115

116 When assays were performed using a paper strip, we obtained a similar LOD, demonstrating

117 that the detection system could be rendered portable without loss in sensitivity (figure 2 panel

118 B of the accompanying figure).

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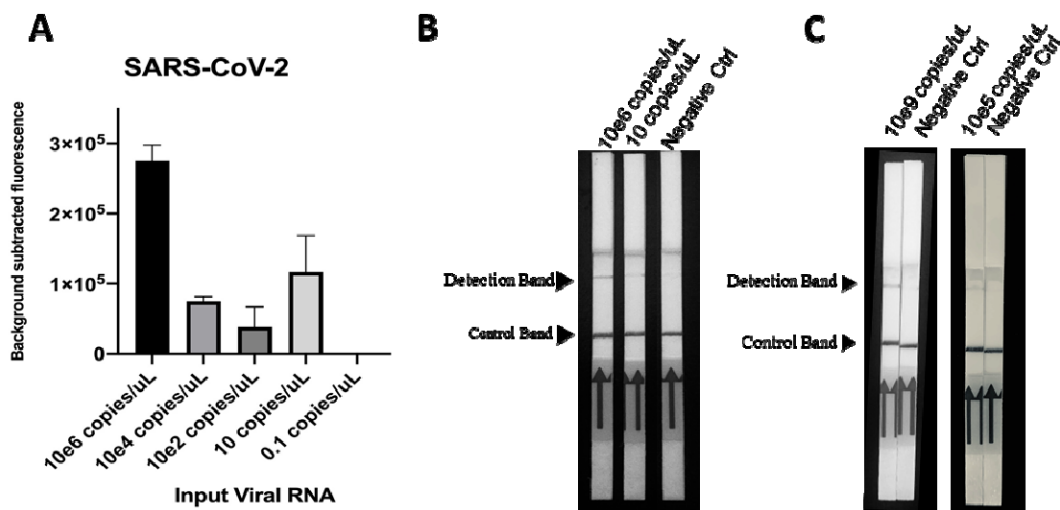
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121 **Figure 2**. A) Assessment of the detection limit of synthetic SARS-CoV-2 by the CRISPR-

122 based assay, inferred from fluorescence values after 30 min. Background subtracted

123 fluorescence represents sample minus control fluorescence without target. Detection of

124 synthetic SARS-CoV-2 RNA using commercially available paper strips (lateral-flow) in

125 buffer (B) or in saliva (C). The optimization in saliva }continues in process to obtain more

126 intense signal on paper strip. Microsoft Excel 2016 was used to arrange the data for analysis.

127 GraphPad Prism v.8.1.2 (San Diego, USA) was used to plot the graph. In all cases, data

128 represent mean ± SD (n=3).

129

130   Finally, we simulated a clinical sample by adding synthetic SARS-CoV-2 RNA fragments to

131   saliva samples collected from a healthy person. There are advantages using saliva for the

132   diagnosis of COVID-19. Saliva specimens can be provided by the patient easily without any

133   invasive procedures (8). Positive results for this artificial saliva-sample were obtained using

134   the paper strip-based assay (figure 2 panel C). These latest results indicate that the CRISPR

135   detection system reported here was not inhibited by naturally occurring molecules in the

136   saliva and can reach real saliva specimens concentration (8), therefore, emerging to be a

137   promising method got the rapid and portable detection of clinical cases of COVID-19.

138

139   **Conclusions**

140

141   The current rRT-PCR-based COVID-19 diagnostic approaches have been shown to be very

142   efficient and accurate for virus detection. However, the virus can spread to regions where the

143   equipment required to perform rRT-PCR is not available. We demonstrated that the CRISPR-

144   Cas12 based detection method is characterized by a LOD value lower than the minimum

145   levels needed to presently detect the virus in clinical samples. The main advantage of the

146   CRISPR-diagnostic method reported here is its portability and low cost (USD 1-2/reaction)

147   (*2*). A possible criticism for this study is that the method has not been tested using patient

148   samples. This is because clinical cases have not yet been reported in our region (South

149   America). Nevertheless, our work represents a proof-of-principle study of the usefulness of

150   the CRISPR-Cas12 detection technology that could be deployed worldwide without the

151   requirement of sophisticated instrumentation.

152

153   **Note**
154   While we were preparing this paper, another two protocols for SARS-CoV-2 detection using
155   CRISPR diagnostics (SHERLOCK, v.20200214; DETECTER v.20200215) was published,
156   confirming the potential of the technique.

157

Disclosure statement

159   CG and PBF are shareholders in CASPR Biotech.

160

Funding

162   CASPR Biotech is a privately funded company. CL, PBF are supported by the National

163   Scientific and Technical Research Council (CONICET) of Argentina.

164

Acknowledgments

166   The authors thanks Guillermo Repizo from IBR-CONICET for his critical suggestion on this

167   manuscript.

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References

170   1. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-

171   guidance/laboratory-guidance.

172   2. Gootenberg JS, Abudayyeh OO, Lee JW, et al. Nucleic acid detection with CRISPR-

173   Cas13a/C2c2.2017 Science.;356(6336):438–442.

174   3. Chen JS, Ma E, Harrington LB, et al. CRISPR-Cas12a target binding unleashes

175   indiscriminate single-stranded DNase activity.2018. Science.;360(6387):436–439.

176   4. Li SY, Cheng QX, Wang JM, et al. CRISPR-Cas12a-assisted nucleic acid detection.

177   2018. Cell Discov.;4:20.

178   5. Myhrvold C, Freije CA, Gootenberg JS, et al. Field-deployable viral diagnostics using

179   CRISPR-Cas13. 2018. Science;360(6387):444-448.

180   6. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia

181   in China, 2019. N Engl J Med. DOI: 10.1056/NEJMoa2001017.

182   7. Camilla Rothe, Mirjam Schunk.Transmission of 2019-nCoV Infection from an

183   Asymptomatic Contact in Germany, 2019. N Engl J Med. DOI:

184   10.1056/NEJMc2001468.

185   8. Kelvin Kai-Wang To, Owen Tak-Yin Tsang, Cyril Chik-Yan Yip, et al. Consistent

186   Detection of 2019 Novel Coronavirus in Saliva. 2020. Clin Infect Dis, pii:ciaa149.

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