ADVANCED MATERIALS TECHNOLOGIES

Supporting Information

for *Adv. Mater. Technol.,* DOI: 10.1002/admt.202100602

SARS-CoV-2 RNA Detection by a Cellphone-Based Amplification-Free System with CRISPR/CAS-Dependent Enzymatic (CASCADE) Assay

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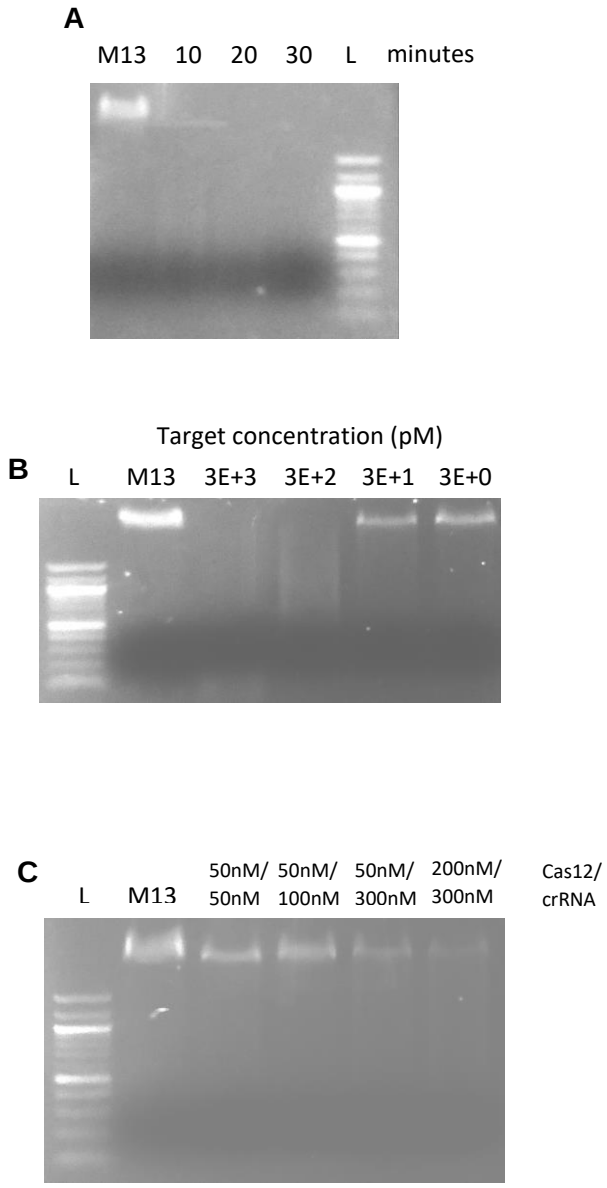

Figure S1. Gel electrophoresis of 1.1 nM M13mp18 Single-stranded DNA (NEB, N4040S) plasmid reacted with Cas12 activated by synthetic Orf1ab gene. A) A complete degradation of M13 is observed at 10 minutes of Cas12 reaction using 1 nM of SARS-CoV-2 ORF1ab synthetic DNA in the reaction. **B)** A decrease of intact M13 is observed in a target concentration of 300 pM after 30 minutes of Cas12 reaction. **C)** The highest ssDNA degradation at 30 pM was observed in 200 nM of Cas12 and 300 nM of crRNA. A 100 bp ladder (L) (Invitrogen™ 15628019), was inserted in each gel.

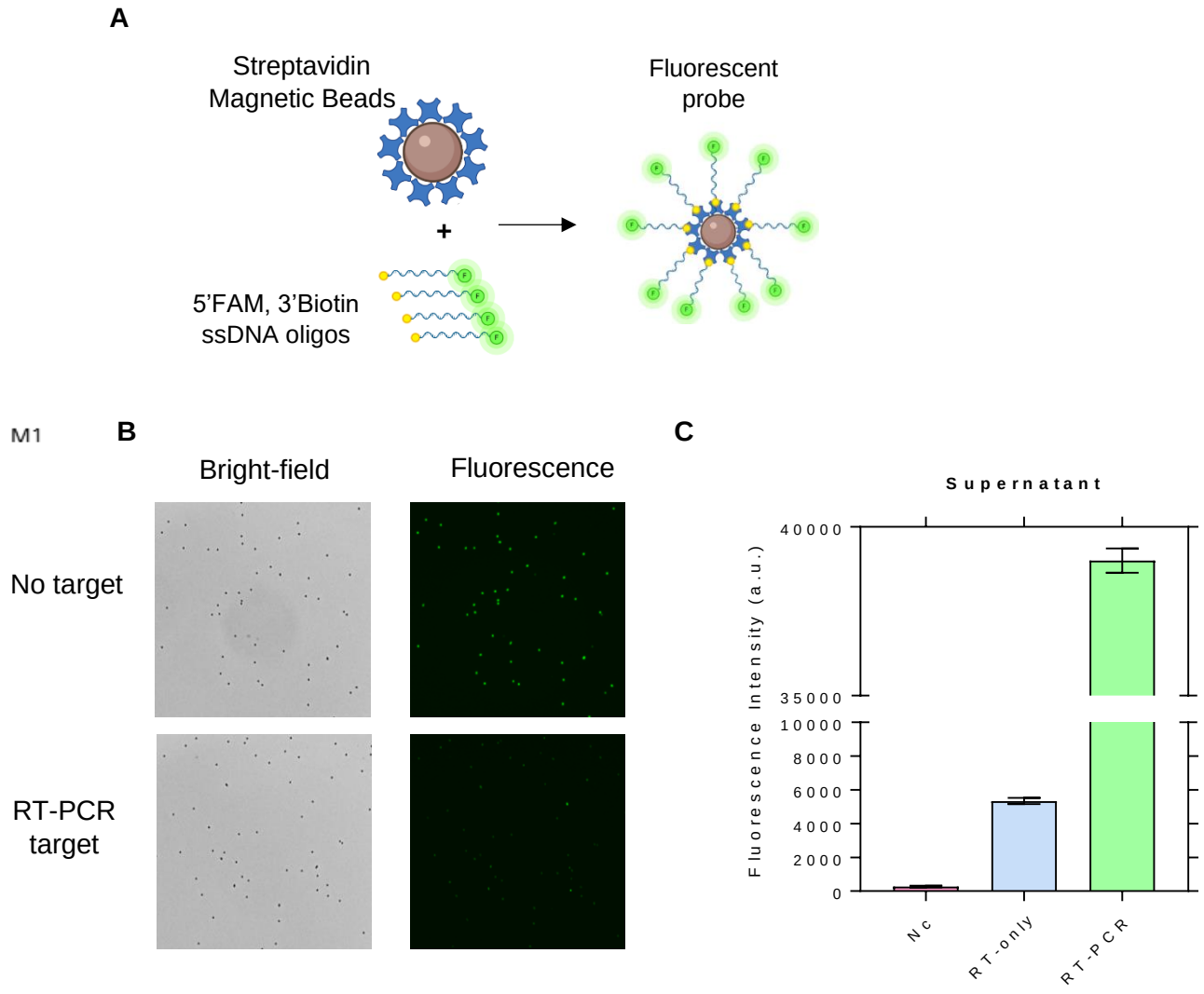

Figure S2. Fluorescence analysis of Cas12 activity using ssDNA-FAM coated microbeads. A concentration of 200 nM Lba Cas12a with 300 nM of crRNA targeting ORF1ab was used to demonstrate whether the ribonucleoprotein complex (RNP) could act on ssDNA on the surface of microbeads. **A)** Fluorescein-labeled ssDNA was bound to Dynabeads™ M-280, via biotin-streptavidin interaction to produce the fluorescent probe. **B)** Fluorescent beads were incubated in a Cas12 reaction with and without orf1ab amplified from SARS-CoV-2 genomic RNA ($5x10^7$ copies/mL) using RT-PCR. After the incubation, the beads were separated from the supernatant and resuspended in 6 µl of TE buffer, the beads without the supernatant were placed on a slide and visualized under a fluorescence microscope (Keyence, BZ-X800). Incubation with Cas12 reaction resulted in significant decrease of fluorescence of the beads. **C)** The supernatant fluorescence (ex:488 nm/em:530 nm/gain:195) was also analyzed using a plate reader (Tecan infinite 200 Pro). We observed significant release of FAM in the supernatant of the magnetic beads samples when SARS-CoV-2 genomic RNA ($5x10^7$ copies/mL) was reversely transcribed (RT-only) or amplified in a RT-PCR reaction.

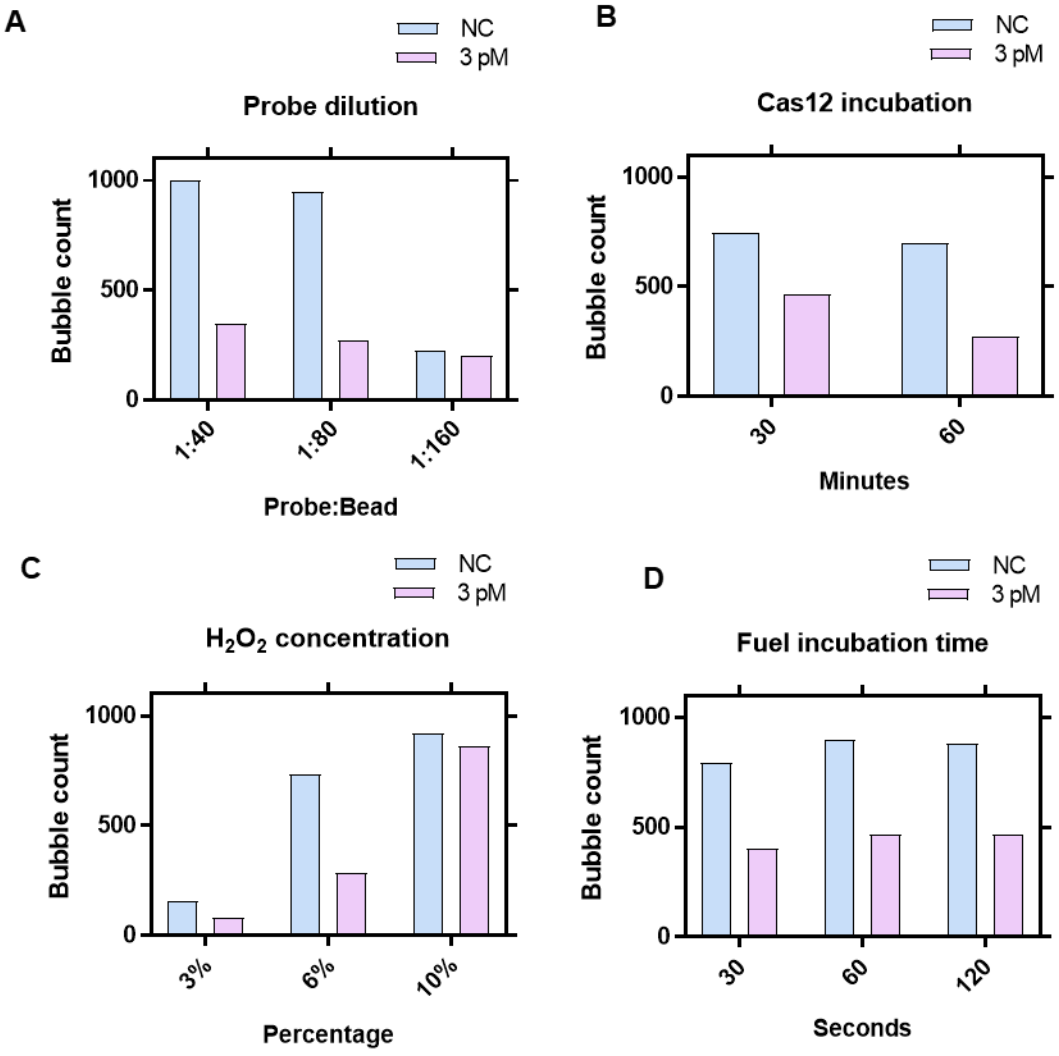

Figure S3. Standardization of steps involved in the CASCADE assay with 3 pM of ORF1ab synthetic DNA and 200 nM of RNP. A) The catalase-ssDNA probe was diluted in a solution containing a constant concentration of Dynabeads. The finest tuning was seen at 1:80 proportion of probe and bead. **B)** Cas12 incubation for 30 and 60 minutes. Longer incubations allow for increased differences between negative and positive samples. **C)** Bubble count with an H_2O_2 fuel solution at different concentrations. A more contrasting difference in bubble count was observed at 6% H_2O_2. **D)** Fuel incubation times in positive and negative samples. The Catalase produces bubbles at 30 seconds.

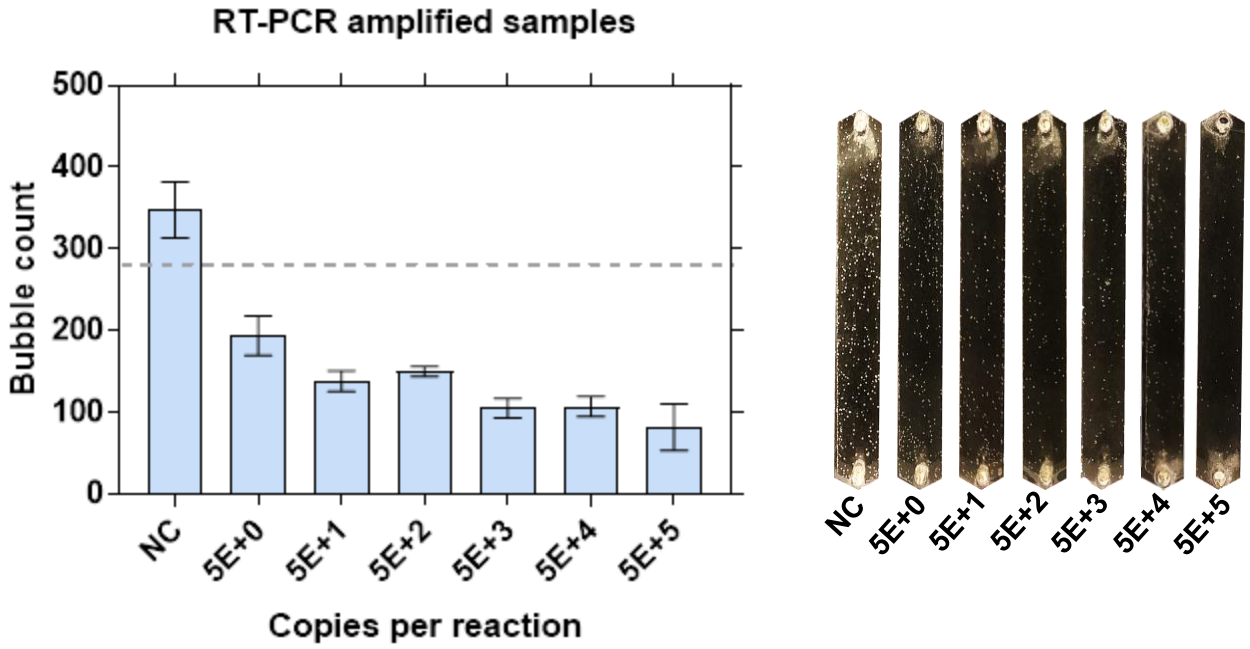

Figure S4. Bubble based assay of RT-PCR amplified samples. Samples were Reverse transcribed and amplified with polymerase chain reaction by SuperScript™ IV One-Step RT-PCR System. Cycling was performed as follows: 52 °C for 10 min; 98 °C for 2 min; 35 cycles at 98 °C for 10 s, 52 °C for 10 s, and 72 °C for 30s, and final elongation at 72 °C for 5 min. Y axis shows the absolute bubble count. The cut-off was calculated as the negative control (NC) mean subtracted the standard deviation of the lowest concentrated group (5E+0).

Table S1. Comparison of methods for SARS-CoV-2 detection with CRISPR assays.

	RNA treatment	Enzyme	Reported LOD	Readout	Test time (min)[α]	Additional information	Citation
CRISPR-FDS	RT-RPA	Cas12	0.38 copies/µL	Mobile-assisted Fluorescence	15	Images are transferred to a computer for processing.	[1]
AIOD-CRISPR	RT-RPA	Cas12	5 copies/ sample	Fluorescence	20	A more prolonged incubation time is necessary to consistently detect 5 copies in all replicates.	[2]
deCOViD	RT-RPA	Cas12	1 (RNA) and 20 (extraction-free) gEq*/µL input	Fluorescence	<30	An RT-PCR machine is required for lowering the LOD. Although most concentrations were detected before 30 minutes using the real-time system, the one-pot reaction took 60 minutes to finish.	[3]
SHERLOCK (STOPCovid)	RT-LAMP	Cas12	100 copies input	Fluorescence/ Lateral Flow	40/70	The STOPCovid reaction (LAMP+Cas12 only) can take up to 1 hour, and the visual result takes 2 minutes to complete.	[4,5]
CASdetec	RT-RAA	Cas12	$5x10^3$ copies/mL input	Fluorescence	40-60	It requires an RT-PCR system, and the LOD using the POCT device is not reported.	[6]
CRISPR-COVID	RT-RPA	Cas13	7.5 copies/ reaction	Fluorescence	40	Time for sample preparation or signal analysis is not taken under consideration. A portable fluorescence reader was applied (Qubit- Thermo Fisher Scientific).	[7]
DETECTR	RT-LAMP	Cas12	10 copies/ µl input	Fluorescence/ Lateral Flow	30-40	An estimated turnaround for the visual result is between 45 and 55 minutes when the extraction and the lateral flow readout are added. A 60	[8]

						minutes automated extraction was also reported for a higher number of samples.	
ITP-CRISPR	RT-LAMP	Cas12	10 copies/ µl of reaction	Image-based fluorescence	35	In order to improve the sensitivity in patient samples, the RT-LAMP time had to be increased to 30 minutes. The images need to be analyzed in ImageJ.	[9]
SHINE	RT-RPA	CAS13	10 copies/ µL	Fluorescence	55	Longer incubation of up to 3 hours was reported to detect the lowest RNA concentrations (10 copies/ µL). Smartphone-taken images are transmitted to a server for analysis.	[10]
Fozouni *et al*.	None	Cas13	50 copies/µL input	Mobile-assisted Fluorescence	<30	A mobile phone microscope is required. Longer incubation times are needed for lower viral loads. An accuracy of 100% was only achieved with concentrations above 200 copies/µL. Accuracy of 50% was reported in 50 copies/µL.	[11]
Ooi *et al.*	RT-LAMP	Cas12	50 (RNA) 10^3 (extraction-free) copies/reaction	Fluorescence/ Lateral Flow	30	The assay time is reported for RNA extraction-free protocol only. RNA purification will add time to the test.	[12]
CASCADE	RT	Cas12	50 cp/µL	Mobile-assisted Bubble count	71	RNA extraction is required. Cas12 incubation takes 60 min, but development of bubble signal takes only 1 min. Smartphone-taken image analysis is very simple.	This study

[a]Most studies report the times required for ribonucleoprotein complex incubation and for results readout. Some assays still require additional time for RNA pre-processing and for analysis of the fluorescence outputs.

Table S2. Comparison of patient sample testing in different platforms.

	RT-qPCR		Normalized Bubble count		SURF keypoints	
Patient	**Copies/mL**	**True Classification**	**Result**	**Predicted class**	**Result**	**Predicted class**
1	0	Negative	-0.070	Negative	-0.143	Negative
2	0	Negative	0.059	Negative	-1.428	Negative
3	15000000	Positive	0.445	Positive	0.129	Positive
4	28600	Positive	0.407	Positive	0.384	Positive
5	47500000	Positive	0.477	Positive	0.512	Positive
6	230000000	Positive	0.529	Positive	0.398	Positive
7	87700000	Positive	0.445	Positive	0.393	Positive
8	142000000	Positive	0.422	Positive	0.377	Positive
9	17200000	Positive	0.455	Positive	0.376	Positive
10	46300000	Positive	0.434	Positive	0.418	Positive
11	750000	Positive	0.233	Positive	0.052	Positive

Table S3. List of synthetic nucleic acids used in this study.

Name	Sequence (5' – 3')	Source
Orf1ab forward primer	CCCTGTGGGTTTTACACTTAA	China CDC
Orf1ab reverse primer	ACGATTGTGCATCAGCTGA	China CDC
Synthetic Orf1ab DNA target	AATGACCCTGTGGGTTTTACACTTAAAAACACA GTCTGTACCGTCTGCGGTATGTGGAAAGGTTAT GGCTGTAGTTGTGATCAACTCCGCGAACCCATG CTTCAGTCAGCTGATGCACAATCGTTTTTA	GenBank MN908947
Orf1ab crRNA	mC*mA*mC* rArUrA rCrCrG rCrArG rArCrG rGrUrA rCrArG rArCrG rUrUrU rUrArG rArGrC rUrArG rArArA rUrArG rCrArA rGrUrU rArArA rArUrA rArGrG rCrUrA rGrUrC rCrGrU rUrArU rCrArA rCrUrU rGrArA rArArA rGrUrG rGrCrA rCrCrG rArGrU rCrGrG rUrGrC mU*mU*mU* rU	Our design
Fluorescent Reporter	/5'FAM/AAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAA/3'BioTEG/	Our design
Phosphorylated ssDNA	/5'PHOS/AAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAA/3'BioTEG/	Our design

Table S4. List of samples obtained from BEI resources

Source	Sample Name	Catalog Number	Genome Copy Number
BEI Resources [α]	Genomic RNA from SARS-Related Coronavirus 2, Isolate Hong Kong/VM20001061/2020	NR-52388	4.8E+7 genome equivalents/mL
BEI Resources[β]	Quantitative PCR (qPCR) Control RNA from Heat-Inactivated SARS-Related Coronavirus 2, Isolate USA-WA1/2020	NR-52347	5.0E+7 genome equivalents/mL
BEI Resources[β]	Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020	NR-52285	4.8E+7 genome equivalents/mL
BEI Resources [α]	Quantitative PCR (qPCR) Control RNA from Inactivated SARS Coronavirus, Urbani	NR-52346	2.1E+7 genome equivalents/mL
BEI Resources [α]	Genomic RNA from Middle East Respiratory Syndrome Coronavirus (MERS-CoV), EMC/2012	NR-45843	7.6E+7 genome equivalents/mL

[α] The following reagent was obtained through BEI Resources, NIAID, NIH.

[β]The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH.

Table S5. Patient samples used in this study.

	Donor code	Viral Load (cps/mL) (qPCR)
1	S000551975	Negative
2	S000551976	Negative
3	S00552273	1.50E+07
4	S00552275	2.86E+04
5	S00552275	4.75E+07
6	S00552279	2.30E+08
7	S00552282	8.77E+07
8	S00552283	1.42E+08
9	S00552284	1.72E+07
10	S00552286	4.63E+07
11	S00552293	7.50E+05

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