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A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR

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1 Works for me dx.doi.org/10.17504/protocols.io.bcmtiu6n

Coronavirus Method Development Community

Mammoth Biosciences

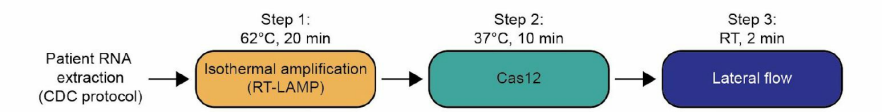
ABSTRACT

DISCLAIMER: This protocol has not been approved by the FDA and should not be used as a clinical diagnostic

Introduction

Given the global health emergency, rapid transmission, and severe respiratory disease associated with the outbreak of the 2019 novel coronavirus (SARS-CoV-2), Mammoth Biosciences has reconfigured our DETECTR platform to rapidly and accurately detect SARS-CoV-2 using a visual lateral flow strip format within 30 minutes from sample to result. To ensure specificity of detection, we selected a high-fidelity CRISPR detection enzyme and designed sets of gRNAs that can either 1) differentiate SARS-CoV-2 or 2) provide multi-coronavirus strain detection. SARS-CoV-2 DETECTR couples CRISPR detection with isothermal pre-amplification using primers based on protocols validated by the US Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO). Currently in the United States, the CDC SARS-CoV-2 real-time RT-PCR diagnostic panel has a laboratory turnaround time of approximately 4-6 hours, with results that can be delayed for >24 hours after sample collection due to shipping requirements. In addition, these tests are only available in CDC-designated public health laboratories certified to perform high-complexity testing.

Mammoth is working to enable point of care testing (POCT) solutions that can be deployed in areas at greatest risk of transmitting SARS-CoV-2 infection, including airports, emergency departments, and local community hospitals, particularly in low-resource countries. Leveraging an “off-the-shelf” strategy to enable practical solutions within a short time frame, we describe here a protocol that is fast (<30 min), practical (available immediately from international suppliers), and validated using contrived samples.



Specifications	
Targets	● N-gene (SARS-CoV-2 specific) ● E-gene (SARS-CoV, bat-SARS-like-CoV, and SARS-CoV-2 coronaviruses) ● RNase P (human sample control)

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Limit of detection	70-300 copies/µl input

Table 1: SARS-CoV-2 DETECTR assay workflow and specifications.

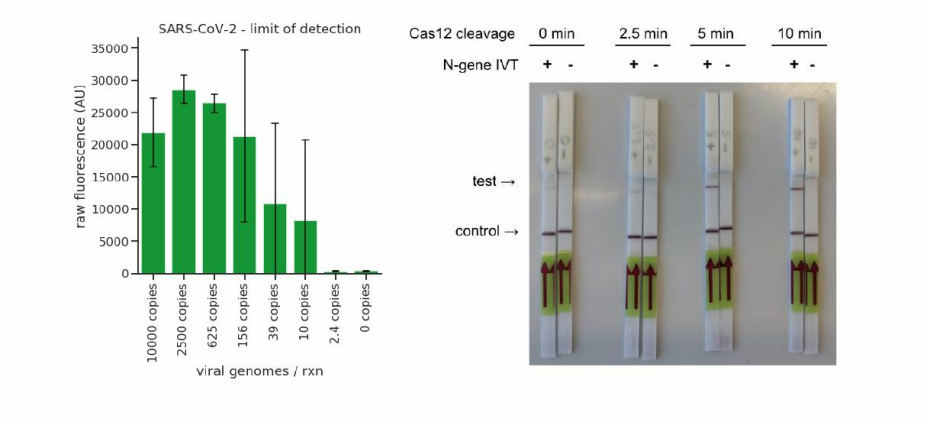

Figure 1: SARS-CoV-2 DETECTR has a limit of detection (n=7) of 156-625 copies per 20 µl reaction (or 70-300 copies per µl input) and generates a clear visible signal on lateral flow strips within 30 minutes sample to result.

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Conflicts of Interest: JPB, CLF, JS and JSC are employees of Mammoth Biosciences, CYC is on the Scientific Advisory Board of Mammoth Biosciences, and JSC is a co-founder of Mammoth Biosciences. JPB, CLF, JS, CYC and JSC are co-inventors on CRISPR-related technologies.

EXTERNAL LINK

https://mammoth.bio/2020/02/15/white-paper-a-protocol-for-rapid-detection-of-sars-cov-2-using-crispr-sars-cov-2-detectr/

ATTACHMENTS

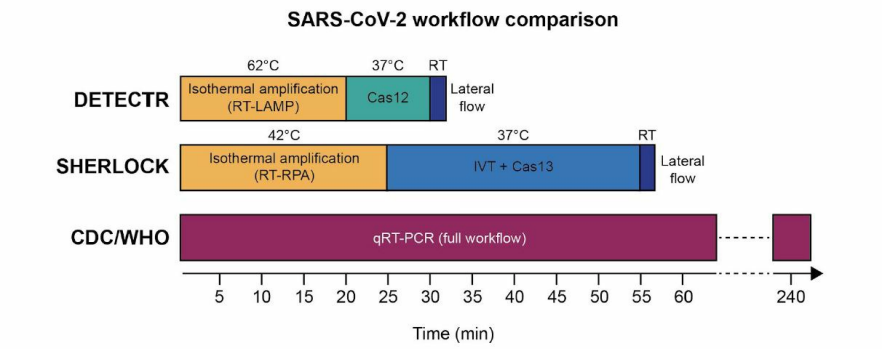
SARS-CoV-2.pdf

GUIDELINES

Appendix

While we were preparing this whitepaper, another protocol for SARS-CoV-2 detection using CRISPR diagnostics (SHERLOCK, v.20200214) was published. We compare the assay workflows and specifications between CRISPR diagnostics and established CDC/WHO protocols below. (Note: as of this publication, CRISPR diagnostics workflows have not yet been approved by the FDA)

Citation: Mammoth Biosciences, James P. Broughton, Wayne Deng, Clare L. Fasching, Jasmeet Singh, Charles Y. Chiu, Janice S. Chen (03/17/2020). A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR. https://dx.doi.org/10.17504/protocols.io.bcmtiu6n



Appendix Figure 1: Comparison of SARS-CoV-2 assay workflows for DETECTR, SHERLOCK, and CDC/WHO

	SARS-CoV-2 DETECTR	SARS-CoV-2 SHERLOCK	CDC SARS-CoV2 qRT-PCR
Target	N gene & E gene (N gene gRNA compatible with CDC N2 amplicon, E gene compatible with WHO protocol)	S gene & Orf1ab gene	N-gene (3 amplicons)
Sample control	RNase P	None	RNase P
Limit of Detection	70-300 copies/µl input	10-100 copies/µl input	1 copy/µL input
Assay reaction time	~30 min	~60 min	~45-60 minutes
Assay components	RT-LAMP (62 °C, 290 min), Cas12 (37 °C, 10 min), Lateral flow (RT, 2 min)	RT-RPA (42 °C, 25 min), IVT + Cas13 (37 °C, 30 min), Lateral flow (RT, 2 min)	UDG digestion (25 °C, 2 min), reverse transcription (50 °C, 15 min), denature (95 °C, 2 min), amplification (95 °C, 3 min; 55 °C 30 sec; 45 cycles)
Heavy instrumentation required	No	No	Yes
FDA EUA approval	No	No	Yes

Appendix Tavle 1: Comparison of SARS-CoV-2 specifications for CRISPR diagnostic protocols to the current CDC assay.

MATERIALS TEXT

SARS-CoV-2 DETECTR Reagents

Step 1: Isothermal amplification (62°C, 20 min)

- RT-LAMP Master Mix (Supplier: NEB)

WarmStart LAMP Kit (DNA and RNA) - 100 rxns
by New England Biolabs
Catalog #: E1700S

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- DNA oligos (Supplier: IDT)

- Primer sequences:

Name	Sequence (5' → 3')
N-gene F3	AACACAAGCTTTCGGCAG
N-gene B3	GAAATTTGGATCTTTGTCATCC
N-gene FIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC
N-gene BIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
N-gene LF	TTCCTTGTCTGATTAGTTC
N-gene LB	ACCTTCGGGAACGTGGTT
E-gene F3	CCGACGACGACTACTAGC
E-gene B3	AGAGTAAACGTAAAAAGAAGGTT
E-gene FIP	ACCTGTCTCTTCCGAAACGAATTTGTAAGCACAAGCTGATG
E-gene BIP	CTAGCCATCCTTACTGCGCTACTCACGTTAACAATATTGCA
E-gene LF	TCGATTGTGTGCGTACTGC
E-gene LB	TGAGTACATAAGTTCGTAC
RNaseP POP7 F3*	TTGATGAGCTGGAGCCA
RNaseP POP7 B3*	CACCCTCAATGCAGAGTC
RNaseP POP7 FIP*	GTGTGACCCTGAAGACTCGGTTTTAGCCACTGACTCGGATC
RNaseP POP7 BIP*	CCTCCGTGATATGGCTCTTCGTTTTTTTCTTACATGGCTCT GGTC
RNaseP POP7 LF*	ATGTGGATGGCTGAGTTGTT
RNaseP POP7 LB*	CATGCTGAGTACTGGACCTC

* RNaseP POP7 primers published in Curtis et al. , (2018) .

Curtis KA, Morrison D, Rudolph DL, Shankar A, Bloomfield LSP, Switzer WM, Owen SM (2018). A multiplexed RT-LAMP assay for detection of group M HIV-1 in plasma or whole blood.. Journal of virological methods.
https://doi.org/10.1016/j.jviromet.2018.02.012

Step 2: Cas12 detection (37°C, 10 min)

- LbCas12a (Supplier: NEB)

EnGen Lba Cas12a (Cpf1) - 70 pmol
by New England Biolabs
Catalog #: M0653S

- crRNA (Supplier: Synthego)
-
- Reporter (Supplier: IDT)

Name	Sequence (5' → 3')
N gene gRNA (SARS-CoV-2 specific)	UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUC
E gene gRNA (pan-coronavirus)	UAAUUUCUACUAAGUGUAGAUGUGGUAUUCUUGCUAGUUAC
RNase P gRNA (Sample control)	UAAUUUCUACUAAGUGUAGAUGACCUGCGAGCGGGUUCUGA

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Reporter	/56-FAM/TTATTATT/3Bio/

Step 3: Lateral flow (RT, 2 min)

Milenia HybriDetect 1 lateral flow strips (Supplier: TwistDx

Sample Equipment

- Pipette tips
- 37 °C heat block
- 62°C heat block
- Microcentrifuge
- Eppendorf tubes
- Pipettes
- Lateral flow strips
- Sample collection device (nasopharyngeal swab)
- Timer

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Prepare nucleic acid sample and CRISPR reagents

1 ⚠

Extract patient RNA following CDC recommendations.

2 Prepare *LbCas12a RNP complexes* for the samples to be tested. **One** complex for N-gene, E-gene, and RNase P gRNAs is needed **for each sample**.

Reagent	*Volume*	*Final Concentration*
Nuclease-free water	15.75 µl	
10X NEBuffer 2.1	2 µl	1X
1 µM LbCas12a	1 µl	50 nM
1 µM gRNA	1.25 µl	62.5 nM
TOTAL VOLUME	**20 µl**	

3

Incubate LbCas12a with gRNA to generate RNP complexes for **00:30:00** at **37 °C** .

4

Add *reporter substrate* to final concentration of **500 Nanomolar (nM)** .

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5 Place reactions **On ice** until ready to proceed.

Complexes are stable at 4°C for at least 24 hours.

Run DETECTR reaction

6

On ice , prepare **three** *RT-LAMP reactions*, **one each** for N-gene, E-gene, and RNase P primer sets:

Reagent	*Volume*	*Final Concentration*
10X Isothermal Amplification Buffer (NEB)	2.5 µl	
100 mM MgSO 4 (NEB)	1.13 µl	6.5 mM (4.5 mM added, 2 mM in 1X IsoAmp Buffer)
10 mM dNTPs (NEB)	3.5 µl	1.4 mM
10X Primer Mix	2.5 µl	0.2 µM F3 / 0.2 µM B3 / 1.6 µM FIP / 1.6 µM BIP / 0.8 µM LF / 0.8 µM LB
Bst 2.0 polymerase (NEB)	1 µl	8 units / rxn
Warmstart RTx (NEB)	0.5 µl	7.5 units / rxn
Nuclease-free water	3.87 µl	
Nucleic acid sample	5 µl	
TOTAL VOLUME	**25 µl**	

7

Incubate at **62 °C** for **00:20:00** .

Note: Use precaution when opening amplification tubes to prevent amplicon contamination.

8

Combine **2 µl** of the *RT-LAMP reaction* with **20 µl** of the *LbCas12a RNP complex* with the appropriate gRNA.

9

Add **80 µl 1X NEBuffer 2.1** .

10

Incubate at **37 °C** for **00:10:00** .

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11 Insert *Milenia HybriDetect 1 (TwistDx) lateral flow strip* directly into reaction.

12 Allow the lateral flow strip to run for **00:02:00** at **Room temperature** and observe the result.

Test interpretation

13

Note: The *line closest to the sample pad* is the *control line* and the line that appears farthest from the sample pad is the *test line* (see Figure 1). A sample with complete cleavage of the reporter molecule may appear to have no signal at the control line.

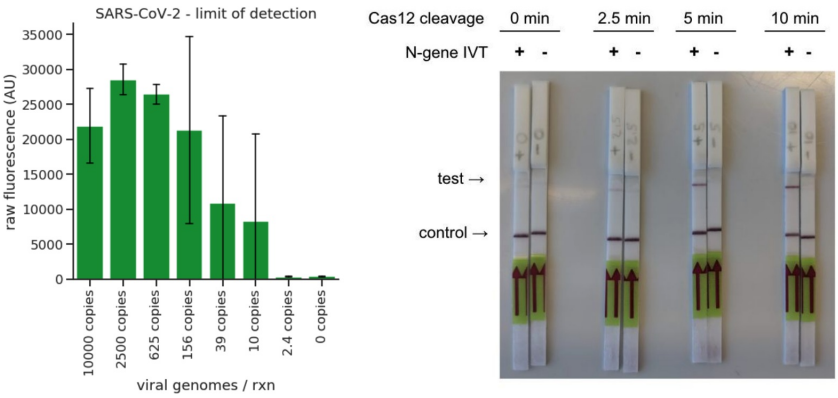

Figure 1 | SARS-CoV-2 DETECTR has a limit of detection (n=7) of 156-625 copies per 20 µl reaction (or 70-300 copies per µl input) and generates a clear visible signal on lateral flow strips within 30 minutes sample to result.

N-gene	E-gene	RNase P	Interpretation
+	+	+/-	SARS-CoV-2 positive
+	-	+/-	Indeterminate
-	+	+/-	Indeterminate
-	-	+	SARS-CoV-2 negative
-	-	-	QC failure

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