

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

v1: February 15, 2020

v2: February 18, 2020: contact email provided, corrected RNase P gRNA sequence, updates to test interpretation matrix, corrections to LoD and assay reaction time for CDC SARS-CoV-2 qRT-PCR assay

v3: March 2, 2020: updates to minimum sample equipment and test interpretation matrix

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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.

Please contact diagnostics@mammothbiosci.com with any questions or feedback.

*****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.

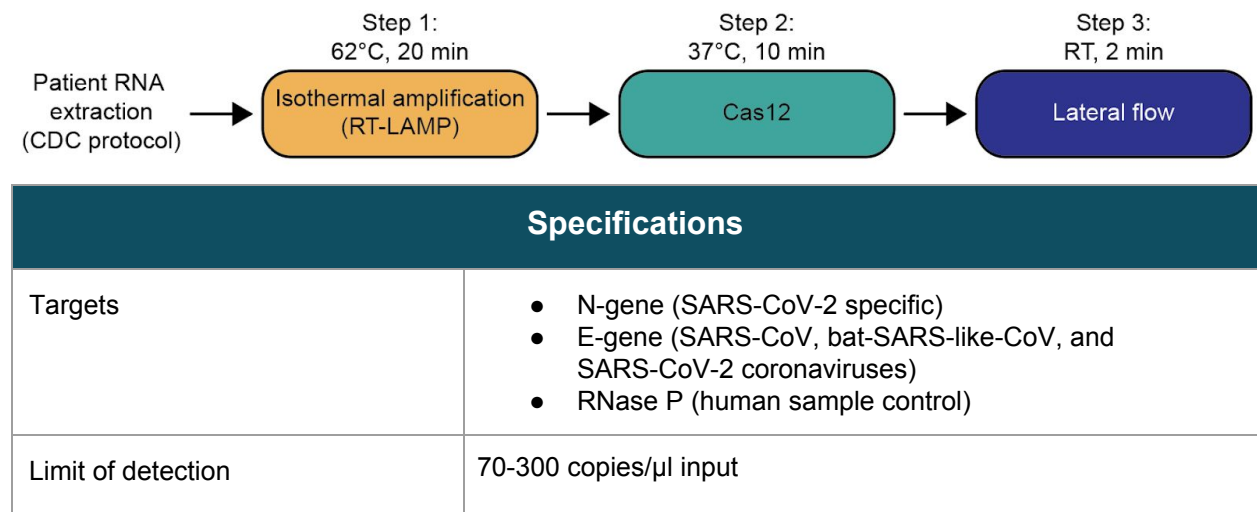


Table 1 | SARS-CoV-2 DETECTR assay workflow and specifications.

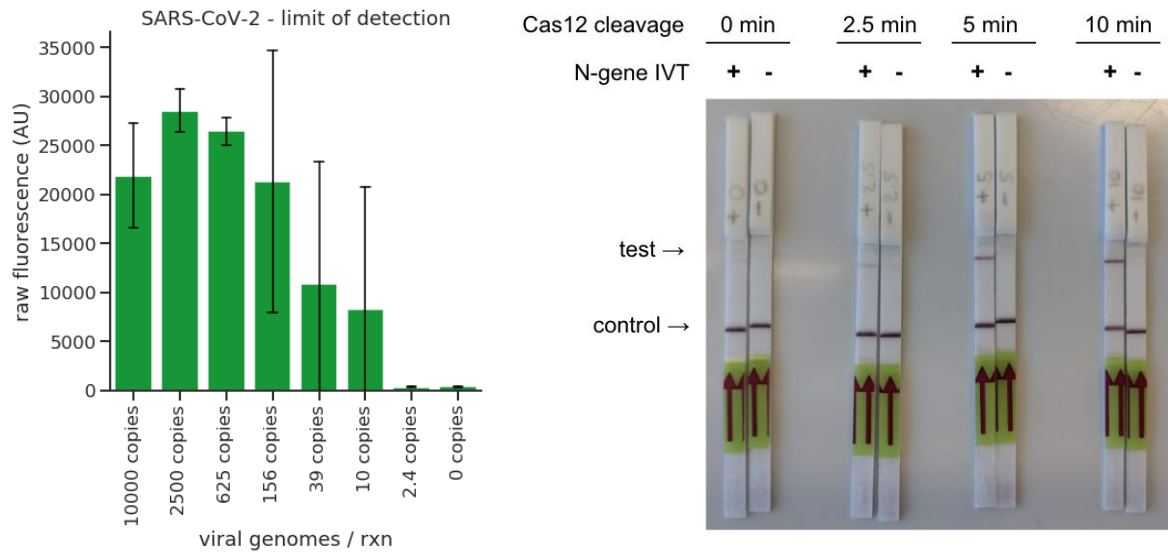


Figure 1 | SARS-CoV-2 DETECTR has a limit of detection (n=7) of 156-625 copies per 20 μ l reaction (or 70-300 copies per μ l input) and generates a clear visible signal on lateral flow strips within 30 minutes sample to result.

SARS-CoV-2 DETECTR Reagents

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

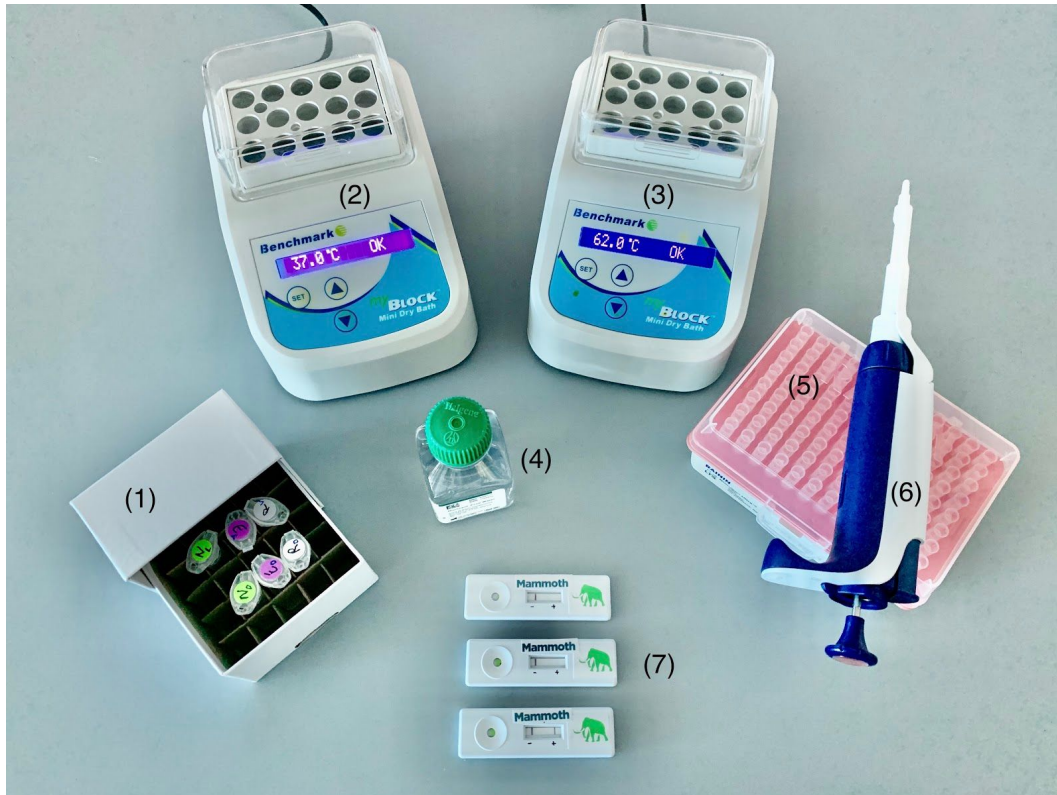
[RT-LAMP Master Mix \(Supplier: NEB\)](#)

[DNA oligos \(Supplier: IDT\)](#)

Primer sequences:

Name	Sequence (5' → 3')
N-gene F3	AACACAAGCTTTTCGGCAG
N-gene B3	GAAATTTGGATCTTTGTCATCC
N-gene FIP	TGCGGCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC
N-gene BIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
N-gene LF	TTCCTTGTCTGATTAGTTC
N-gene LB	ACCTTCGGGAACGTGGTT
E-gene F3	CCGACGACGACTACTAGC

Minimum sample equipment



- (1) DETECTR reagents
- (2) 37°C heat block
- (3) 62°C heat block
- (4) Nuclease-free water
- (5) Pipette tips
- (6) Pipette
- (7) Lateral flow strips

Experimental Protocol

A. 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 μ L	
10X NEBuffer 2.1	2 μ L	1X
1 μ M LbCas12a	1 μ L	50 nM
1 μ M gRNA	1.25 μ L	62.5 nM
TOTAL VOLUME	20 μL	

- Incubate LbCas12a with gRNA to generate RNP complexes for 30 minutes at 37°C.
- Add reporter substrate to final concentration of 500 nM.
- Place reactions on ice until ready to proceed.
 - Complexes are stable at 4°C for at least 24 hours.

B. Run DETECTR reaction

- 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 μ L	
100 mM MgSO ₄ (NEB)	1.13 μ L	6.5 mM (4.5 mM added, 2 mM in 1X IsoAmp Buffer)
10 mM dNTPs (NEB)	3.5 μ L	1.4 mM
10X Primer Mix	2.5 μ L	0.2 μ M F3 0.2 μ M B3 1.6 μ M FIP 1.6 μ M BIP 0.8 μ M LF 0.8 μ M LB
Bst 2.0 polymerase (NEB)	1 μ L	8 units / rxn
Warmstart RTx (NEB)	0.5 μ L	7.5 units / rxn
Nuclease-free water	3.87 μ L	

Nucleic acid sample	5 μ L	
TOTAL VOLUME	25 μL	

2. Incubate at 62°C for 20 minutes.
 - a. Note: Use precaution when opening amplification tubes to prevent amplicon contamination.
3. Combine 2 μ L of the RT-LAMP reaction with 20 μ L of the LbCas12a RNP complex with the appropriate gRNA.
4. Add 80 μ L of 1X NEBuffer 2.1.
5. Incubate at 37°C for 10 minutes.
6. Insert Milenia HybriDetect 1 (TwistDx) lateral flow strip directly into reaction.
7. Allow the lateral flow strip to run for 2 minutes at room temperature and observe the result.

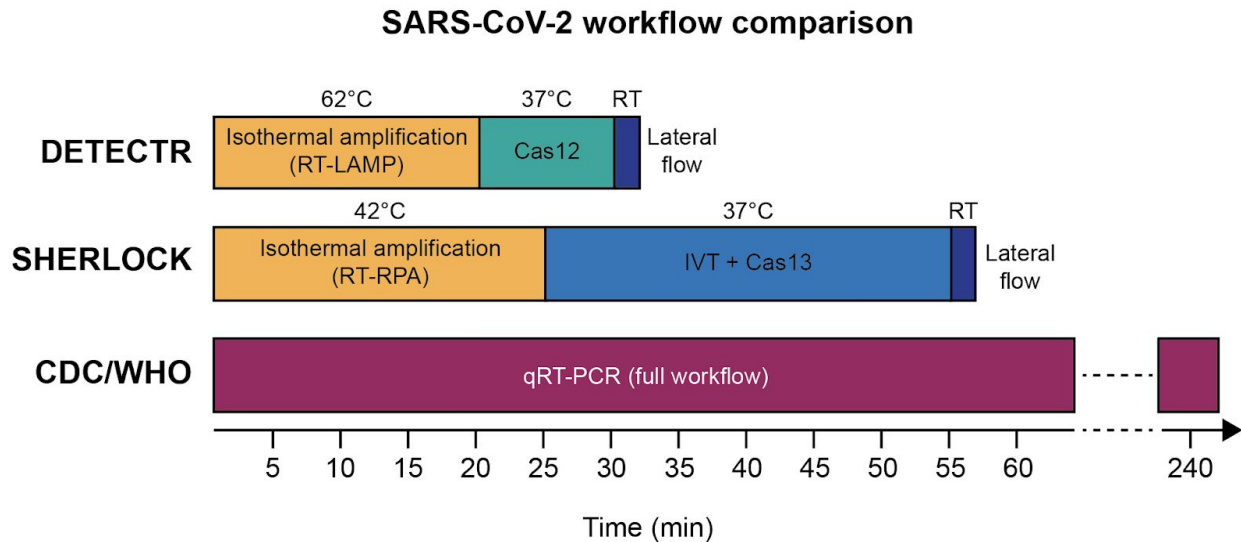
C. Test interpretation

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.

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

Appendix

While we were preparing this white paper, 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)



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/ μ l input	10-100 copies/ μ l input	3.16-10 copies/ μ L input
Assay reaction time	~30 min	~60 min	~120 minutes

Assay components	RT-LAMP (62°C, 20 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 sec; 55°C 30 sec; 45 cycles)
Heavy instrumentation required	No	No	Yes
FDA EUA approval	No	No	Yes

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