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A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR V.1

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Coronavirus Method De...

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Protocol status: Working
We used this protocol in our group and it is working

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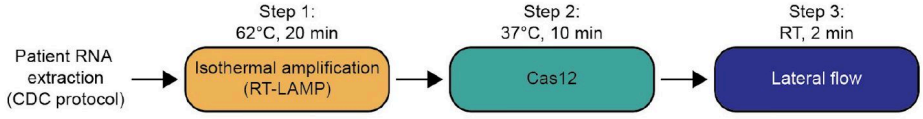
Abstract

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)
	Limit of detection	70-300 copies/µl input

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

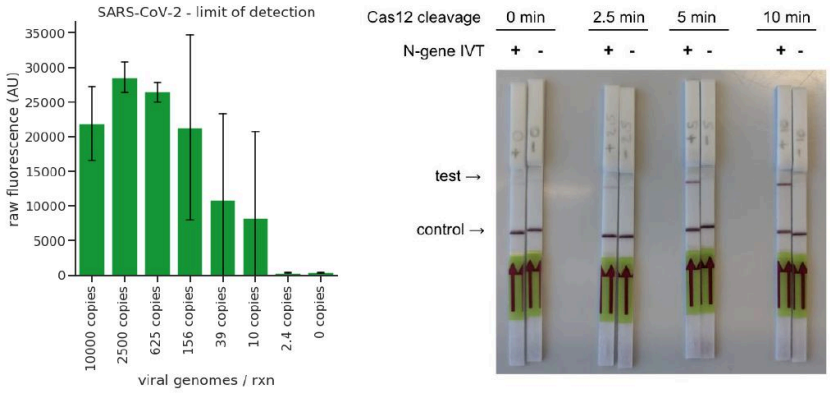


Figure 1: SARS-CoV-2 DETECTR has a limit of detection (n=7) of 156-625 copies per 20 µl reaction (or 70-300 copies per µl input) and generates a clear visible signal on lateral flow strips within 30 minutes sample to result.

Acknowledgements: We thank Vikram Joshi, Nefeli Tsaloglou and Xin Miao for advice and helpful discussions in the preparation of this whitepaper.

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.

Attachments

PDF

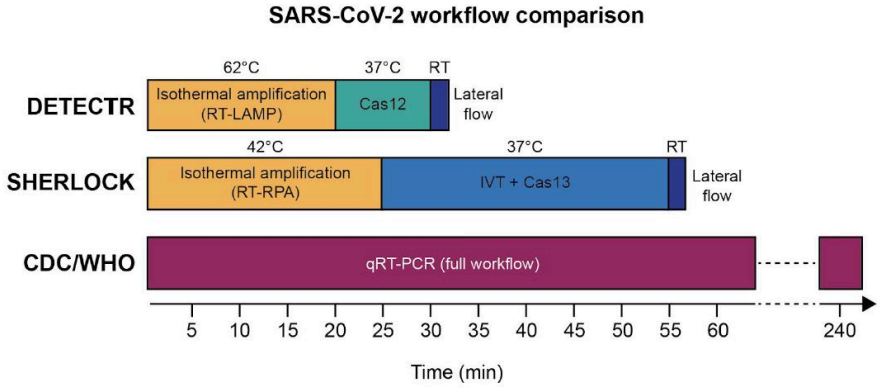
SARS-CoV-2.pdf

603KB

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)



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

STEP MATERIALS

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

-

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 **New England Biolabs Catalog #**E1700S

- **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	CGC ATT GGC ATG GAA GTC ACT TTG ATG GCA CCT GTG TAG
	N-gene LF	TTC CTT GTC TGA TTA GTT C
	N-gene LB	ACC TTC GGG AAC GTG GTT
	E-gene F3	CCG ACG ACG ACT ACT AGC
	E-gene B3	AGA GTA AAC GTA AAA AGA AGG TT
	E-gene FIP	ACC TGT CTC TTC CGA AAC GAA TTT GTA AGC ACA AGC TGA TG
	E-gen	CTA GCC

	e BIP	ATC CTT ACT GCG CTA CTC ACG TTA ACA ATA TTG CA
	E-gene LF	TCG ATT GTG TGC GTA CTG C
	E-gene LB	TGA GTA CAT AAG TTC GTA C
	RNaseP POP7 F3*	TTG ATG AGC TGG AGC CA
	RNaseP POP7 B3*	CAC CCT CAA TGC AGA GTC
	RNaseP POP7 FIP*	GTG TGA CCC TGA AGA CTC GGT TTT AGC CAC TGA CTC GGA TC

	RNaseP POP7 BIP*	CCT CCG TGA TAT GGC TCT TCG TTT TTT TCT TAC ATG GCT CTG GTC
	RNaseP POP7 LF*	ATG TGG ATG GCT GAG TTG TT
	RNaseP POP7 LB*	CAT GCT GAG TAC TGG ACC TC

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

Citation

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 LINK

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

- LbCas12a (Supplier: NEB)

EnGen Lba Cas12a (Cpf1) - 70 pmol **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

	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 NEB uffer 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 [M] 500 nanomolar (nM) .

5 Place reactions On ice until ready to proceed.

Note

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

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 .

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

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.

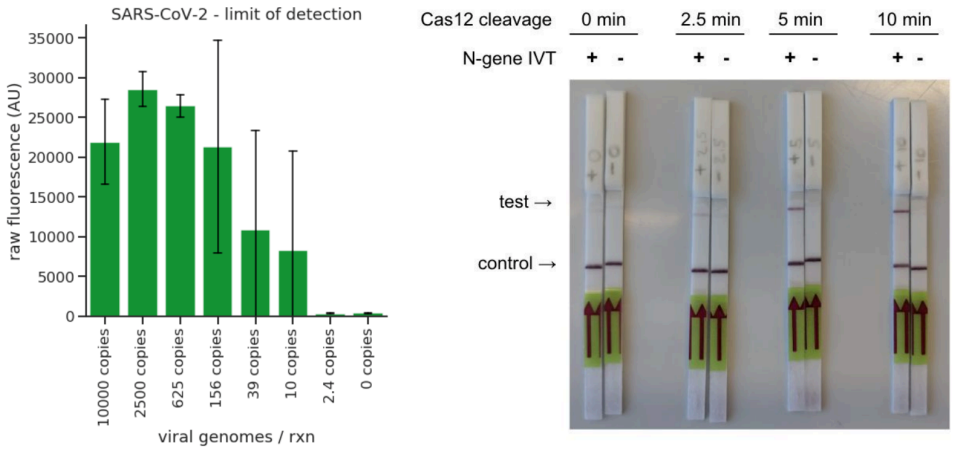


Figure 1 | SARS-CoV-2 DETECTR has a limit of detection (n=7) of 156-625 copies per 20 µl reaction (or 70-300 copies per µl input) and generates a clear visible signal on lateral flow strips within 30 minutes sample to result.

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

Citations

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