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SPOT2 protocol

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XPRIZE Rapid Covid Tes...

stlane2

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Protocol status: Working
We use this protocol and it's working

Created: September 08, 2020

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Protocol Integer ID: 41792

Materials

STEP MATERIALS

- Magnesium Sulfate (MgSO4) Solution - 6.0 ml **New England Biolabs** **Catalog #**B1003S
- E gene primer mix **Catalog #**N/A
- Non-primer oligos **Catalog #**N/A
- Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England Biolabs** **Catalog #**M0538L
- WarmStart RTx Reverse Transcriptase - 250 rxns **New England Biolabs** **Catalog #**M0380L
- Isothermal Amplification Buffer - 6.0 ml **New England Biolabs** **Catalog #**B0537S
- Nuclease-free Water - 25 ml **New England Biolabs** **Catalog #**B1500S
- Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs** **Catalog #**N0447S
- N gene primer mix **Catalog #**N/A
- Saliva sample **Catalog #**N/A
- Manganese(II) chloride tetrahydrate **Sigma Aldrich** **Catalog #**M3634
- Reporter probe 1 **Catalog #**N/A
- Reporter Probe 2 **Catalog #**N/A
- Non-CRISPR nuclease **Catalog #**N/A

Protocol materials

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- WarmStart RTx Reverse Transcriptase - 250 rxns **New England Biolabs Catalog #**M0380L
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- Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England Biolabs Catalog #**M0538L

1 Label test kit bag with patient's name and a unique sample number for the test run. Provide a 20 uL microcap and PCR tube and have patient collect a saliva sample and place it into then seal the PCR tube. Label PCR tube with sample number matching the test kit bag.

2 Place PCR tubes into floating rack and insert into sous vide water bath at 95C. Incubate for 5 minutes for sample pretreatment.

Equipment

Thermal Immersion Cooker	NAME
Sous Vide - low cost waterbath	TYPE
Wancle	BRAND
P00252	SKU

95 °C 00:05:00

2.1 During incubation, scan barcodes on test kits and enter patient identifying information into the SPOT2 device.

Equipment

SPOT2 Device	NAME
Data logging fluorometer	TYPE
University of Illinois	BRAND
N/A	SKU

Equipment	
USB Laser Barcode Scanner	NAME
Barcode Scanner	TYPE
WoneNice	BRAND
WN3300	SKU

3 After completion of pretreatment, use the second provided 20 uL microcap to transfer a small volume of saliva from the PCR tube to the capillary tube, containing SPOT assay mastermix. Place capillary tubes into floating rack and insert into sous vide water bath at 63C for 30 minutes.

63 °C 00:30:00

SPOT assay mastermix:

	Initial concentration	Final concentration	Amount (µL)
Upper compartment			
WarmStart® Bst 2.0	8000 units/mL	160 units/mL	2
WarmStart® RTx	15,000 units/mL	150 units/mL	1
Isothermal amplification buffer	10X	0.5X	8
dNTPs	10 mM	0.7 mM	5.6
MgSO4	100 mm	4 mM	3.2
N gene primer mix	10X	0.25X	2
E gene primer mix	10X	0.25X	2
Saliva samples			5
Non-CRISPR nuclease	5 mg/mL or 55 µM	1.375 uM	2
MnCl2	50 mM	0.5 mM	0.8
Non-primer oligos (total 6 oligos)	100 µM	625 nM	3

	Reporter probe 1	100 µM	156.25 nM	0.125
	Reporter probe 2	100 µM	312.5 uM	0.25
	Nuclease-free water			44.025
	Total			80

Bst 2.0 WarmStart DNA Polymerase - 8,000 units **New England Biolabs Catalog #**M0538L

2 µL

WarmStart RTx Reverse Transcriptase - 250 rxns **New England Biolabs Catalog #**M0380L

1 µL

Isothermal Amplification Buffer - 6.0 ml **New England Biolabs Catalog #**B0537S

8 µL

Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #**N0447S

5.6 µL [M] 0.7 millimolar (mM)

Magnesium Sulfate (MgSO4) Solution - 6.0 ml **New England Biolabs Catalog #**B1003S

3.2 µL [M] 4 millimolar (mM)

N gene primer mix **Catalog #**N/A

E gene primer mix **Catalog #**N/A

Saliva sample **Catalog #**N/A

Nuclease-free Water - 25 ml **New England Biolabs Catalog #**B1500S

Non-CRISPR nuclease **Catalog #**N/A

Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3634

0.8 µL [M] 0.5 millimolar (mM)

Non-primer oligos **Catalog #**N/A

Reporter probe 1 **Catalog #**N/A

Reporter Probe 2 **Catalog #**N/A

4 Transfer floating capillary rack to sous vide at 95C for 5 minutes.

95 °C 00:05:00

5 Individually transfer capillary tubes to SPOT2 device and press "Run" on the SPOT software. An integrated RFID chip on each capillary will automatically link test result to the patient information entered previously.

Equipment

SPOT2 Device	NAME
Data logging fluorometer	TYPE
University of Illinois	BRAND
N/A	SKU