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SPOT2 protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bk28kyhw

stlane2

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STEPS MATERIALS

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

EQUIPMENT

NAME	CATALOG #	VENDOR
SPOT2 Device	N/A	
USB Laser Barcode Scanner	WN3300	Amazon
Thermal Immersion Cooker	P00252	

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.

Thermal Immersion Cooker
Sous Vide - low cost waterbath
Wancle **P00252**

95 °C **00:05:00**

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

SPOT2 Device
Data logging fluorometer
University of Illinois **N/A**

USB Laser Barcode Scanner
Barcode Scanner
WoneNice **WN3300**

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

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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
by New England Biolabs
Catalog #: M0538L

2 µl

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

1 µl

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

8 µl

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

5.6 µl [M]0.7 Milimolar (mM)

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

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3.2 µl [M]**4 Milimolar (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
by New England Biolabs
Catalog #: B1500S

Non-CRISPR nuclease
Catalog #: N/A

Manganese(II) chloride tetrahydrate
by Sigma Aldrich
Catalog #: M3634

0.8 µl [M]**0.5 Milimolar (mM)**

Non-primer oligos
Catalog #: N/A

Reporter probe 1
Catalog #: N/A

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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.

SPOT2 Device
Data logging fluorometer
University of Illinois **N/A**

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