First-strand synthesis and touchdown PCR for SARS-CoV-2 V.2

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Annealing primer to RNA

Note: Pre-warm5X SS IV Buffer at room temperature before using

Combine the following in a tube:

A	B
	(13 µl)
Random primers 6 (NEB)	1 µl (60ng/µl)
10 mM dNTP mix (10 mM each)	1 µl
Template RNA	11 µl

1. Mix by pipetting and briefly centrifuge thecomponents.

2. Heat the RNA-primer mix at 65°C for 5 minutes, and then incubate on ice for at least 1minute.

Prepare RT reaction mix

1.Vortex and briefly centrifuge the 5× SSIV Buffer.
2.Combine the following into a reaction tube:
(7 µl)

5× First Strand buffer 4 µl
100 mM DTT 1 µl
RNAse out 1 µl
SS IV Reverse Transcriptase (200 U/µL) 1 µl
Cap the tube, mix, and then briefly centrifuge the contents.

Reverse Transcriptase step

1. Add RT reaction mix to the annealed RNA, mix well

2. Incubate under the following conditions:

23 °C for 10 minutes
50°C for 10 minutes
55°C for 10 minutes
80°C for 10 minutes
4 °C ∞

Multiplex PCR

Set up two multiplex PCR reaction mix as follows:

Q5 High-Fidelity 2X Master Mix	12.5 µl
H2O	6.75 µl
Primers pool (1 or 2) at 10 µM	1.75 µl
cDNA	4 µl
TOTAL	**25 µl**

Cycling conditions for **Q5 High-Fidelity 2X Master Mix (~1500bp)**

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A	B	C
Temp.	Time	Number of cycles
98	30 sec	1
98	10 sec	4
65	2 min	
72	3 min	
98	10 sec	3
64	2 min	
72	3 min	
98	10 sec	2
63	2 min	
72	3 min	
98	10 sec	2
62	2 min	
72	3 min	
98	10 sec	2
61	2 min	
72	3 min	
98	10 sec	22
60	2 min	
72	3 min	
72	3 min	1
4	∞	

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