PROTOCOL FOR SEQUENCING PCR OF SARS-COV-2

This is a protocol for multiplex PCR reactions prior to the library preparation of the Oxford Nanopore sequencing.

Protocol version updated on *01 June 2021*

Materials

- primers pool A and B
- Micropipettes and tips
- Microcentrifuge tubes size 1,5mL
- PCR microtubes with attached caps size 0.2 mL
- Disposable lab coats
- Disposable powder-free lab gloves
- 0,2mL microtube racks
- Thermocycler

Mastermix

All primers pools should be diluted to a total of 10uM from the 100uM stock.

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Components	***Volume (uL)***		***Components***	***Reduced Volume (uL)***
Nuclease Free water	12,75		Nuclease Free water	5,33
Q5 reaction buffer (5x)	5		Q5 reaction buffer (5x)	2,5
dNTPs (10mM)	0,5		dNTPs (10mM)	0,25
Primers for the pool 10uM	4		Primers for the pool 10uM	1,8
Q5 DNA polymerase	0,25		Q5 DNA polymerase	0,125

Prepare ***2*** mastermixes with the above components for the appropriate number of samples. In one tube add the ***primers*** of the pool ***A*** and in the other tube, add primers of the pool ***B***.
After the mastermix is finished, pipette out ***22.5uL (or 10 uL if using the reduced reaction)*** of the ***mastermix*** into PCR microtubes, and then add in each tube ***2.5ul*** of the appropriate cDNA.

Thermocycling parameters (total time: ~2h37m)

Step	***Temperature***	***Time***	***Cycle number***
Initial denature	98ºC	30 seconds	1
Denature	98ºC	15 seconds	30
Annealing and extension	65ºC	5 minutes	
Hold	4ºC	∞	1