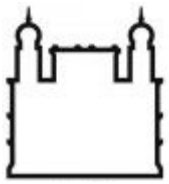

cDNA synthesis and PCR protocol for Metagenomics

Reagents and Supplies

Q5® High-Fidelity DNA Polymerase
SuperScript™ IV First-Strand Synthesis
dNTP
Micropipette set
Primers
Tips
Lab coat
Powder-free gloves
PCR tubes 0.2ml
Tube racks 0.2ml
Thermocycler
Laminar flow chapel

Procedures to cDNA synthesis

a) Add in a 0.2mL tube for each reaction:

RLB RT **9N** (2 uM) - 1 µL

dNTPs (10 mM) - 1µL

Treated RNA - 10 µL

b) Place the tube to incubate in a heat block for **5 minutes at 65 ºC.**

c) Then place the tube **immediately on the ice** to prevent the formation of secondary structures.

d) On each tube of the previous reaction, add **8 µL of** the Master Mix below:

SSIV buffer (5x) -4 µL

DTT (100 mM) -1 µL

RNase OUT (inhibitor) -1µL

SS IV RTase (200U/ul) -1 µL

RLB **TSO** (2 uM) -1 µL

TOTAL = 20µL

e) Then place the tubes in the thermocycler following the configuration represented in the table below:

Number of cycles	Temperature (ºC)	Time
1	42	1h30 minutes
1	70	10 mins
1	4	∞

Procedures to Metagenomics PCR

a) Prepare the PCR Mix by following the volumes for 1 reaction shown in the table below:

Component	**Amount for 1 reaction**
Ultra pure water	15.75 µL
Q5 reaction buffer (5x)	5 µL
dNTPs (10uM)	0.5 µL
RLB **PCR 20 uM primer**	1 µL

b) After calculating the number of reactions to be prepared for the Master mix, **transfer 22.5** µL of the mix to the 0.2mL PCR tubes and then, in another physically separated work area, **add 2.5 µL** of cDNA to each PCR tube containing the Master mix.

c) Place the tubes in the thermocycler, following the configuration represented in the table below:

Number of cycles	Temperature (ºC)	Time
1	98	45 seconds
26	98	15 seconds
	56	15 seconds
	65	5 mins
1	65	6 mins
	4	∞

References

Chiu C, Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, Stryke D, Bouquet J, Somasekar S, Linnen JM. Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis.