

Supplemental files

Supplementary table 1. GISAID acknowledgment table including sequences to conduct this study (pdf).

Supplementary table 2. Amino acid substitutions detected in hCoV-19/Brazil/AM-20143138-R2/2020 and hCoV-19/Brazil/AM-20140055-R1/2020 in comparison to the SARS-CoV-2 reference sequence EPI_ISL_402124. Highlighted in blue are the 21 lineage-defining substitutions and amino acid changes. Common substitutions and mutations are in gray.

Strain details	Number of substitutions / mutations	Nucleotide substitutions	Amino acid substitutions
hCoV-19/Brazil/AM-20143138-R2/2020	33 nucleotides 25 amino acids	C241T T733C C2749T	NS3_S253P NSP3_S370L NSP3_K977Q
EPI_ISL_811149		C3037T C3828T A5648C	NSP6_F108del NSP6_G107del NSP6_S106del
30/12/2020		A6319G A6613G C12778T C13860T C14408T C16883A G17259T C21614T C21621A C21638T G21974T G22132T A22812C G23012A A23063T A23403G C23525T C24642T G25088T T26149C G28167A C28512G A28877T G28878C G28881A G28882A G28883C	NSP12_P323L NSP13_T216N NSP13_E341D Spike_L18F Spike_T20N Spike_P26S Spike_D138Y Spike_R190S Spike_K417T Spike_E484K Spike_H655Y Spike_N501Y Spike_D614G Spike_T1027I Spike_V1176F N_P80R N_R203K N_G204R Note: Insertion of 4 nucleotides and gap of 9 nucleotides when compared to the reference sequence.
		Deletion : 11288-11297 Insertion : 28269:AAAC	

hCoV-19/Brazil/AM-20140055-R1/2020	7 nucleotides 5 amino acids	C241T C3037T A6466G C9661T C14408T C20938T A23403G	NSP1_S142del NSP1_F143del NSP1_K141del NSP12_P323L Spike_D614G
EPI_ISL_811148			
24/03/2020		Deletion: 686-695	Note: Gap of 9 nucleotides when compared to the reference sequence.

PCR amplification for the whole SARS-CoV-2 genome.

We have previously published a protocol to generate high-quality near to full length genomes positions 47 to 29,835 or 99.6% of the SARS-CoV-2 reference sequence EPI_ISL_402124 ¹. We observed that some sequences were better amplified (e.g., stronger amplification of the final amplicon observed in agarose gel electrophoresis) using a combination of two reverse primers at the 3' end (**Supplementary table 3**). Additionally, some thermocycling conditions were adjusted (**Supplementary table 4**). Therefore, using this alternative protocol some sequences have 29,593bp, positions 47 to 29,644 (98.96%) of the EPI_ISL_402124.

Supplementary table 3. Primers strategy used for the whole-genome sequencing of the SARS-CoV-2 based on the strategy described previously ¹.

Amplicon	Primer Name	Sequence 5`-3	pb
1	24_F	GTAACAAACCAACCAACTTTTCGA	3962
	3986_R	TTGTAACTTCTTCAACACAAGCTTT	
2	3841_F	AATGAAGAGTGAAAAGCAAGTTGAA	4125
	7966_R	TTGACACATAAGCTGACTGTAGTAA	
3	7836_F	TAGACAACCTGAGAGCTAATAACAC	4153
	11989_R	AAAGGCTTCAGTAGTATCTTTAGCT	
4	11857_F	TGTAAAGTGACATCAGTAGTCTTA	4203
	16060_R	TAGGATGTTTAGTAAGTGGGTAAGC	
5	15911_F	ATGATTATGTGTACCTTCCTTACCC	4123

	20034_R	ACGGGCATTTCTAAATAAGTCTACT	
6	19865_F	ATACTGTGATCTGGGACTACAAAAG	4279
	24144_R	AAAACAGTAAGGCCGTTAAACTTTT	
7	23997_F	CAAGCAAGAGGTCATTTATTGAAGA	2036
	26032_R	GCTGGTAATAGTCTGAAGTGAAGTA	
8	25827_F	TTTTCTTTGCTGGCATACTAATTGT	3120
	W_HU_1_28946R	CAAGCAGCAGCAAAGCAAGA	
9	28155_F	ATTAATTGCCAGGAACCTAAATTGG	1515 / 1707
	29672_R	ATGTGAGATTAAAGTTAACTACATCT	
	29861_R	CTAAGAAGCTATTAATCAACATGGG	

Supplementary table 4. Thermocycling conditions to amplify the SARS-CoV-2 genomes.

Number of Cycles	Steps	Temperature	Time
1	Initial denaturation	98°C	30 seconds
	Denaturation	98°C	10 seconds
35	Hybridization	60°C or 55°C*	10 seconds
	Extension	72°C	2 minutes
1	Final extension	72°C	5 minutes
1	Hold	4°C	∞

*All the amplicons 1 to 8 are amplified under the hybridization temperature 60°C except amplicon 9 which is amplified under the hybridization temperature 55°C

Reference

1. Nascimento VAD, Corado ALG, Nascimento FOD, et al. Genomic and phylogenetic characterisation of an imported case of SARS-CoV-2 in Amazonas State, Brazil. Mem Inst Oswaldo Cruz 2020;115:e200310.