An improved rapid and sensitive long amplicon method for nanopore-based RSV whole genome sequencing

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Supplementary Tables

Supplementary Table 1. The information for primers used in the modified one-step multiplex RT-PCR. Changes highlighted in green have been made to PCR primer concentration and primers of amplifying fragment 3 and 6 compared to our previous publication (1)

Multiplex Tube	Primer Name	Primer Sequence (5'- 3')[b]	Position of first 5' base[a]	Amplicon length (bp)	Final concentration (μM)
Tube 1	1-1F	ACGCGAAAAAATGCGTACTACAAAC	1		0.16
	1-1R	CTG**M**ACCATAGGCATTCATAAACA	1898	1898 (fragment 1)	0.16
	1-3F3	GCATCACT**W**ACAATATGGGT**K**CC	3349		0.55
	1-3R6	CACTTTTGAT**Y**TTGTTCACTTC**Y**CC	6201	2876 (fragment 3)	0.55
	1-5F	TGATGCATCAATATCTCAAGTCA	7181		0.6
	1-5R	G**R**CCTAT**D**CCTGCATACTC	11116	3936 (fragment 5)	0.6
Tube 2	2-2F	ATGGGAGA**R**GT**R**GCTCCAGAATA	1562		0.25
	2-2R	CGTGTAGCTGT**R**TG**Y**TTCCAA	4004	2443 (fragment 2)	0.25
	2-4F	AGCAAATT**Y**TGGCC**Y**TA**Y**TTTAC	4334		0.6
	2-4R1	CTCATAGCAACACATGCTGATTG	7967	3634 (fragment 4)	0.3
	2-4R2	GAGTTTGCTCATGGCAACACAT	7974	3641 (fragment 4)	0.3
	2-6F	TGGACCAT**W**GAAGC**Y**ATATCA	10912		0.44
	2-6R	AGTGTCAAAAACTAAT**R**TCTCGT	15265	4354 (fragment 6)	0.44
	2-6R2	ACGAGAAAAAAAGTGTCAAAAACTAAT	15272	4365 (fragment 6)	0.44

[a]Primer nucleotide numbering was based on a human RSV-B strain (GISAID accession numbers 2584506)

[b]Degenerate bases are highlighted in bold.

Supplementary Table 2. Component for multiplex RT-PCR. SuperScript IV One-Step RT-PCR System with ezDNase (Invitrogen) and primers with concentration at 20μM are used for the preparation of multiplex PCR mixes below.

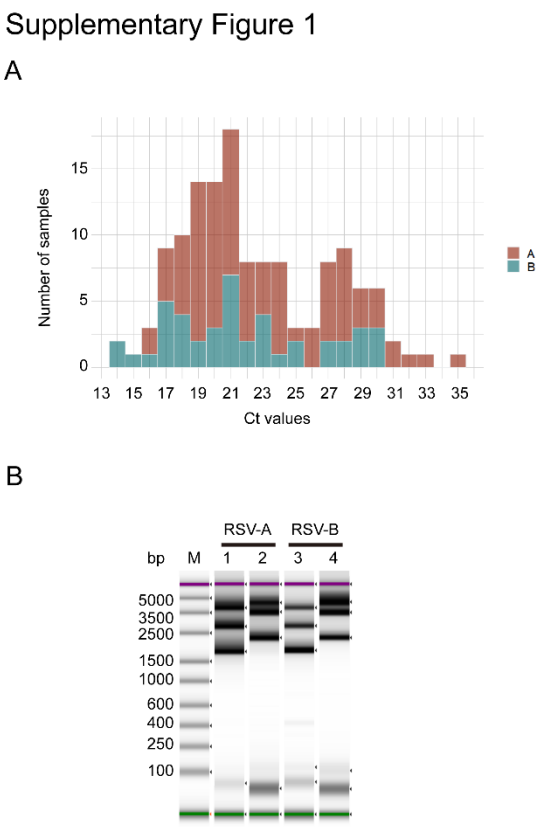
Component for multiplex PCR mix 1	Volumes (μL) per reaction
1-1F	0.2
1-1R	0.2
1-3F3	0.69
1-3R6	0.69
1-5F	0.75
1-5R	0.75
2X Platinum SuperFi RT-PCR Master Mix	12.5
SuperScript IV RT Mix	0.25
template RNA	8.97

Component for multiplex PCR mix 2	Volumes (μL) per reaction
2-2F	0.31
2-2R	0.31
2-4F	0.75
2-4R1	0.38
2-4R2	0.38
2-6F	0.55
2-6R	0.55
2-6R2	0.55
2X Platinum SuperFi RT-PCR Master Mix	12.5
SuperScript IV RT Mix	0.25
template RNA	8.47

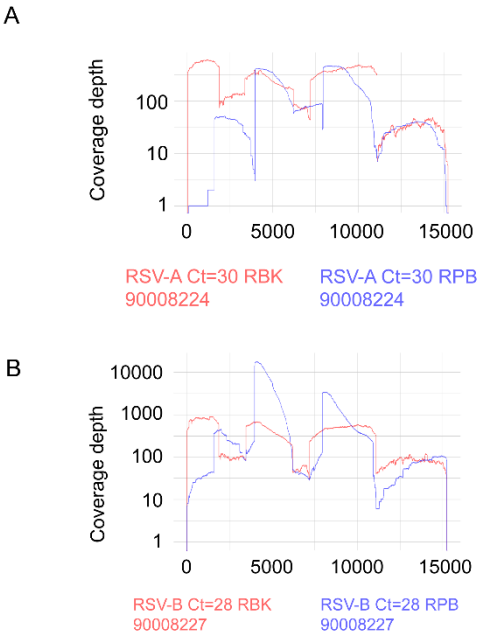
Supplementary Table 3. Comparison of sequencing samples with insufficient amplicons by using rapid barcoding (RBK) and rapid PCR barcoding (RPB) kits. NGS results were characterised into four groups including Full-length (RSV whole genome sequence obtained), G (Full G sequence only), GF (Full F and G sequence obtained) and other (no full G and F sequences obtained).

Sample ID	RSV Ct	RSV type	RPB NGS	RBK NGS
90007809	29	A	GF	Full-length
90007814	30	B	Other	Full-length
90007817	27	B	G	Full-length
90007819	30	B	G	Other
90008162	25	B	G	Full-length
90008175	24	A	GF	Full-length
90008179	26	A	GF	Full-length
90008178	28	A	G	Full-length
90008183	23	B	GF	Full-length
90008191	27	A	GF	Full-length
90008187	27	A	GF	Full-length
90008196	27	A	GF	Full-length
90008203	33	A	G	Other
90008227	28	B	G	G
90008229	32	A	G	G
90008225	27	A	GF	Full-length
90008228	28	A	G	Full-length
90008224	30	A	GF	G
90008231	26	A	G	Full-length
90008380	35	A	Other	Other

Supplementary Figure



Supplementary Figure 1. The performance of modified RSV mRT-PCR for generating RSV genomic amplicons in 135 clinical samples. (A) Histograms of the Ct value distribution for clinical samples with two RSV subgroups, RSV-A in dark red and RSV-B in dark green. (B) Tapestation images of RSV amplicons generated from one RSV-A and one RSV-B positive clinical samples. DNA marker (M); multiplex RT-PCR products of pool 1 (lane 1) and pool 2 (lane 2) from an RSV-A sample; multiplex RT-PCR products of pool 1 (lane 3) and pool 2 (lane 4) from an RSV-B sample.



Supplementary Figure 2. RSV NGS achieved by ONT rapid barcoding (RBK) and rapid PCR barcoding (RPB) for samples with insufficient amplicons for NGS library preparation. Coverage depth of sequenced representative RSV-A (A) and RSV-B (B) in genomic position with RBK (red) and RPB (blue) for NGS library preparation

References

1. Dong X, Deng YM, Aziz A, Whitney P, Clark J, Harris P, et al. A simplified, amplicon-based method for whole genome sequencing of human respiratory syncytial viruses. J Clin Virol. 2023;161:105423.