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RSVAB WGS and GF protocols V.3

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Manuscript citation:
Generic novel system for genomic characterization of Respiratory Syncytial Virus obtaining whole genome sequencing and a full-length G and F sequences.

Protocol status: Working
We use this protocol and it's working

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Abstract

This SOP describes the procedure for generating cDNA from RSV viral nucleic acid extracts and subsequently producing amplicons tiling the viral genome using. We propose two systems for genomic characterization of RSV. First, a novel RSV amplicon-based system for WGS, and second, a method focused on obtaining the specific sequences of the main antigens, G and F.

Troubleshooting

dsCDNA generation:

1

During this step three master mixes will be prepared: MMI, MMII and MMIII.

Materials: **Kit Superscript III First Strand (Invitrogen)**
100% DMSO
RNAseH (Invitrogen)
Klenow fragment 3' →5' exo (New England Biolabs)
Primer FR26RV-N : 5′GCC GGA GCT CTG CAG ATA TCNNNNNN 3′

Note

This step must be performed in a RNase free, pre-PCR environment in which post PCR RSV amplicons are not present, to minimise risk of sample contamination.

Citation

Díez-Fuertes F, Iglesias-Caballero M, García-Pérez J, Monzón S, Jiménez P, Varona S, Cuesta I, Zaballos Á, Jiménez M, Checa L, Pozo F, Pérez-Olmeda M, Thomson MM, Alcamí J, Casas I
(2021). A Founder Effect Led Early SARS-CoV-2 Transmission in Spain..

https://doi.org/10.1128/JVI.01583-20 LINK

2 **MMI Preparation:**

	A	B
	FR26RV-N (10uM)	2
	DMSO	0,5
	Total	2,5 ul

Mix thoroughly by vortexing.

3 **MMII Preparation:**

	A	B
	10x First Strand Buffer	2
	DTT 100 mM	2
	MgCl2 25mM	4
	dNTPs	1
	RNaseOUT	0,5
	SSIII RT	0,5
	Total	10 ul

Kit Superscript III First Strand (Invitrogen)

4 **MMIII Preparation:**

	A	B
	Klenow 5'-3'	1
	RNAseH	0,5
	Total	1,5 ul

5 Defrost extracted RNA.

Maintain **on ice** the MMI,MMII and MMIII mixes.

6 **MMI Amplification:**

Add 5 µL Sample in MMI mix

Place the tube on a thermocycler and run the following program:

	A	B
	65ºC	5 min
	4ºC	2 min

Briefly tube centrifugation

7 **MMII Amplification:**
Addition of 10 µL from MMII in the tube with the MMI and the viral extraction.

Place the tube on a thermocycler and run the following program:

	A	B
	25ºC	10 min
	50ºC	50 min
	85ºC	10 min
	4ºC	∞

Briefly tube centrifugation

8 **MMIII Amplification:**
Addition of 1.5 µL of MMIII into the tube with the previous mixes and the viral extraction

Place the tube on a thermocycler and run the following program:

	A	B
	37ºC	60 min
	75ºC	15 min

Briefly tube centrifugation

9 **STOP POINT**: cDNA can be stored at 4°C (same day) or -20°C (up to a week).

RSVAB WGS protocol

10

Materials:

2x MyTaqRed mix (Bioline)
Primers:

	A	B
	Primer ID	**Sequence (5′-3′)**
	Mix 1	
	RSVCombinitial	ACGCGAAAAAATGCGTACWACA
	RSVWGS4R	CATGWTGWYTTATTTGCCCC
	RSVWGS2F	CACTWACAATATGGGTGCC
	RSVWGS1R	TCCATKGTTATTTGCCCC
	RSVWGS3.2F	ACATGGAAAGAYATYAGCC
	RSVWGS2R.2	CRTTYCTTAARGTRGGCC
	RSVWGS3.2R	TTGCATCTGTAGCAGGAATGG
	OG1-21	GGGGCAAATGCAACCATGTCC
	RSVGF-R	TTCGYGACATATTTGCCCC
	RSVCombending	ACGAGAAAAAAAGTGTCAAAAACTAA
	Mix 2	
	RSVCombinitial	ACGCGAAAAAATGCGTACWACA
	RSVWGS1R	TCCATKGTTATTTGCCCC

	A	B
	RSVWGS8R.2	TCMAWYTCWGCAGCTCC
	RSVWGS5R	CAAACATTTAATCTRCTAAGGC
	RSVWGS6F	TTATAYAGATATCAYATGGGTGG
	RSVWGS6R	CCCTCTCCCCAATCTTTTTC
	RSVWGS9F	GARCAACTCAAAGAAAATGG
	RSVWGS9R	AYTGRAACATRGGCACCC
	RSVCombending	ACGAGAAAAAAAGTGTCAAAAACTAA

	A	B	C	D	E	F
	NC_001781.1	1	22	RSVCombinitial	1	+
	NC_001781.1	2202	2221	RSVWGS9F	1	+
	NC_001781.1	2331	2350	RSVWGS4R	2	-
	NC_001781.1	3324	3342	RSVWGS_2F	1	+
	NC_001781.1	3366	3383	RSVWGS9R	2	-
	NC_001781.1	4675	4695	OG121	1	+
	NC_001781.1	5619	5636	RSVWGS_1R	2	-
	NC_001781.1	7609	7627	RSVGF-R	2	-
	NC_001781.1	7759	7775	RSVWGS_8R	2	-
	NC_001781.1	9294	9315	RSVWGS_5R	2	-
	NC_001781.1	9278	9296	RSVWGS_3.2F	1	+
	NC_001781.1	9906	9923	RSVWGS_2R	2	-
	NC_001781.1	10772	10794	RSVWGS_6F	1	+
	NC_001781.1	13010	13029	RSVWGS_6R	2	-
	NC_001781.1	14187	14207	RSVWGS_3.2R	2	-

	A	B	C	D	E	F
	NC_001781.1	15200	15225	RSVCombEnding	2	-
	NC_038235.1	1	22	RSVCombinitial	1	+
	NC_038235.1	2202	2221	RSVWGS9F	1	+
	NC_038235.1	2329	2348	RSVWGS4R	2	-
	NC_038235.1	3322	3340	RSVWGS_2F	1	+
	NC_038235.1	3364	3381	RSVWGS9R	2	-
	NC_038235.1	4673	4693	OG121	1	+
	NC_038235.1	5648	5665	RSVWGS_1R	2	-
	NC_038235.1	7597	7615	RSVGF-R	2	-
	NC_038235.1	7789	7805	RSVWGS_8R	2	-
	NC_038235.1	9324	9345	RSVWGS_5R	2	-
	NC_038235.1	9308	9326	RSVWGS_3.2F	1	+
	NC_038235.1	9936	9953	RSVWGS_2R	2	-
	NC_038235.1	10802	10824	RSVWGS_6F	1	+
	NC_038235.1	13040	13059	RSVWGS_6R	2	-
	NC_038235.1	14217	14237	RSVWGS_3.2R	2	-
	NC_038235.1	15201	15226	RSVCombEnding	2	-

Primer scheme with RSVA and RSVB RefSeq

Note

The protocol is based in the RSV genome amplification in two separate mixes with an unique amplification program. The mixes that will be mixed at the end of cycling.

11 **Preparation of RSV Amplification Mix 1:**

	A	B
	MyTaq Red 2x	15
	H20	8,4
	RSV Combinitial (5 uM)	0,2
	RSVWGS1R (5uM)	0,2
	RSVWGS2F (5 uM)	0,2
	RSVWGSW2R.2 (5 uM)	0,2
	RSVWGS4R (5 uM)	0,2
	RSVWGS3.F (5 uM)	0,2
	RSVWGS3.2R (5 uM)	0,2
	OG1-21 (5uM)	0,2
	RSVGF-R (5 uM)	0,2
	RSV Combending (5uM)	0,2
	Total	25

12 **Preparation of RSV Amplification Mix 2:**

	A	B
	2x My Taq Red	15
	H2O	8,6
	RSV Combinitial (10uM)	0,2
	RSVWGS1R (10 uM)	0,2
	RSVWGS5R (10 uM)	0,2
	RSVWGS8R.2 (10 uM)	0,2
	RSVWGS6F (10 uM)	0,2
	RSVWGS6R (10 uM)	0,2
	RSVWGS9F (10 uM)	0,2
	RSVWGS9R (10 uM)	0,2
	RSV Combending (10 uM)	0,2

	A	B
	Total	25 ul

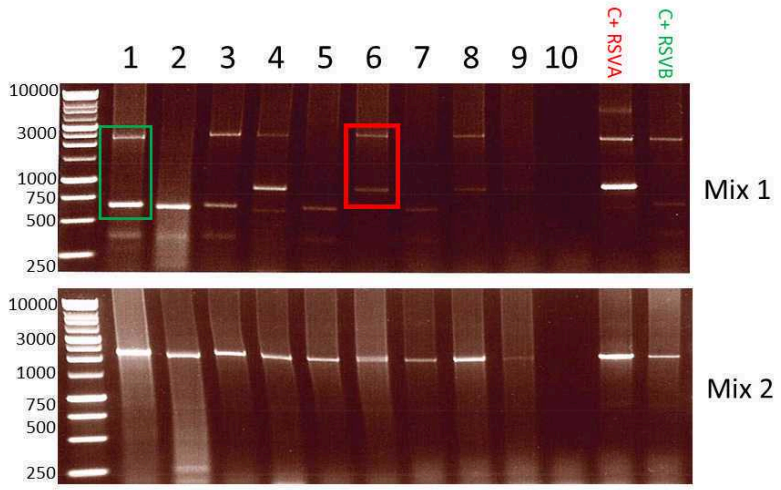
13 Addition of 5 µL of the previous prepared double stranded cDNA on each mix.

14 **Amplification protocol:**

	A	B	C
	95ºC	1 min	
	95ºC	30 seg	x45
	55ºC	8 min	
	72ºC	2 min	
	72ºC	5 min	
	12ºC	∞	

15 To assess PCR performance, the amplicons can be loaded onto a 1% agarose gel for electrophoresis.

Expected result



16 Finally, mix in one single tube both mixes and proceed to purification and library preparation.

RSVAB GF protocol starting from ds cDNA

17 Due to the significance of achieving accurate RSV genomic characterization, it was developed the RSVAB-GF PCR to complement the genomic coverage of both antigenic major proteins in cases where WGS encounters difficulties, and to provide a simpler and more cost-effective method of obtaining the sequences of both antigens.

18 Materials:

2x MyTaqRed mix (Bioline)

Primers:

	A	B
	OG1-21	GGGGCAAATGCAACCATGTCC
	RSVGF-R	TTCGYGACATATTTGCCCC

19 **Preparation of cDNA GF amplification mix:**

	A	B
	H20	5,5
	2X MyTaqRed	12,5
	OG1-21 (10 uM)	1
	RSVGF-R (10 uM)	1
	Total	20 ul

20 Addition of 5 µL of the previous prepared double stranded cDNA on the mix.

21 **Amplification protocol cDNA GF:**

	A	B	C
	95ºC	1 min	
	95ºC	30 seg	x35
	60ºC	3 min	
	72ºC	2 min	
	72ºC	5 min	
	12 ºC	∞	

RSVAB GF protocol starting from viral extraction

22 Materials:
Qiagen OneStep RT-PCR kit.
Glycerolised 1% H20

23 **Preparation of GF amplification mix:**

	A	B
	H20gly	10
	5xQ PCR MM	6
	dNTPs	1
	OG1-21 (10uM)	1
	RSVGF-R (10 uM)	1
	RT-PCR mix	1
	Total	20 ul

24 Addition of 10 µL of the viral extraction

25 **Amplification protocol GF:**

	A	B	C
	48ºC	60 min	
	95ºC	15 min	
	95ºC	30 seg	x 35
	60ºC	3 min	
	72ºC	2 min	
	72ºC	5 min	
	12ºC	∞	

Citations

Step 1

Díez-Fuertes F, Iglesias-Caballero M, García-Pérez J, Monzón S, Jiménez P, Varona S, Cuesta I, Zaballos Á, Jiménez M, Checa L, Pozo F, Pérez-Olmeda M, Thomson MM, Alcamí J, Casas I. A Founder Effect Led Early SARS-CoV-2 Transmission in Spain.
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