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Version 2

RSVAB WGS and GF protocols V.2

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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.



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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
	MgCl ₂ 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
	65°C	5 min
	4°C	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
	25°C	10 min
	50°C	50 min
	85°C	10 min
	4°C	∞

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
	37°C	60 min
	75°C	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	ACGAGAAAAAAAGTGTCAAAAATAA
Mix 2	
RSVCombinitial	ACGCGAAAAAATGCGTACWACA
RSVWGS1R	TCCATKGTTATTTGCCCC

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

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
H2O	8,4
RSV Combinitial	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	0,2



A	B
RSVGF-R	0,2
RSV Combending	0,2
Total	25

12 Preparation of RSV Amplification Mix 2:

A	B
2x My Taq Red	15
H2O	8,6
RSV Combinital (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	0,2
RSVWGS9R	0,2
RSV Combending	0,2
Total	25 ul

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

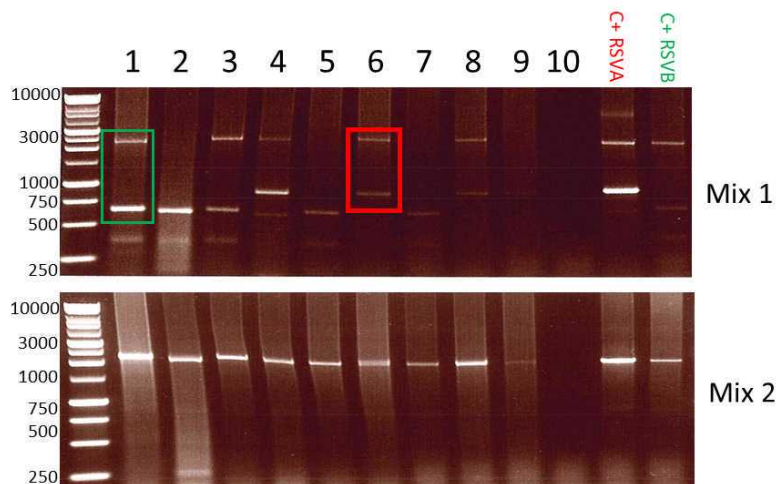
14 Amplification protocol:

A	B	C
95°C	1 min	
95°C	30 seg	x45

	A	B	C
	55°C	8 min	
	72°C	2 min	
	72°C	5 min	
	12°C	∞	

- 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	GGGGCAAATGCAACCATGTC C
	RSVGF-R	TTCGYGACATATTTGCCCC

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

	A	B
	H2O	5,5
	2X MyTaqRed	12,5
	OG1-21 (10 uM)	1
	RSVGF-R (10 uM)	1
	Total	20 ul

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

21 **Amplification protocol cDNA GF:**

	A	B	C
	95°C	1 min	
	95°C	30 seg	x35
	60°C	3 min	
	72°C	2 min	
	72°C	5 min	



	A	B	C
	12 °C	∞	

RSVAB GF protocol starting from viral extraction

22 Materials:

Qiagen OneStep RT-PCR kit.

Glycerolised 1% H₂O

23 **Preparation of GF amplification mix:**

	A	B
	H ₂ Ogly	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
	48°C	60 min	
	95°C	15 min	
	95°C	30 seg	x 35
	60°C	3 min	



	A	B	C
	72°C	2 min	
	72°C	5 min	
	12°C	∞	

Citations

Step 1

Díez-Fuertes F, Iglesias-Caballero M, García-Pérez J, Monzón S, Jiménez P, Varona S, Cuesta I, Zaballo 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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