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RSVAB WGS and GF protocols V.6

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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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Keywords: novel rsv amplicon, viral genome, cdna from rsv, rsvab wg, viral nucleic acid extract, rsv, specific sequences of the main antigen, systems for genomic characterization, generating cdna, producing amplicon, genomic characterization, main antigen

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.

Sample management

1 No prior treatment is necessary. However, in samples with high Cts, the use of DNAseI in the sample as a pretreatment can improve coverage.

dsCDNA generation:

2

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

3 **MMI Preparation:**

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

Mix thoroughly by vortexing.

4 **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)

5 **MMIII Preparation:**

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

6 Defrost extracted RNA.

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

7 **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

8 **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

9 **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

	A	B
	75ºC	15 min

Briefly tube centrifugation

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

RSVAB WGS protocol

11

Citation

Iglesias-Caballero M, Camarero-Serrano S, Varona S, Mas V, Calvo C, García ML, García-Costa J, Vázquez-Morón S, Monzón S, Campoy A, Cuesta I, Pozo F, Casas I (2023)
. Genomic characterisation of respiratory syncytial virus: a novel system for whole genome sequencing and full-length G and F gene sequences.

https://doi.org/10.2807/1560-7917.ES.2023.28.49.2300637 LINK

Materials:

2x MyTaqRed mix (Bioline)
Primers:

	A	B
	Primer ID	**Sequence (5'-3')**
	Mix A	
	RSVCombinitial	ACGCGAAAAAATGCGT

	A	B
		ACWACA
	RSVWGS4R	CATGWTGWYTTATTTGCCCC
	RSVWGS2F	CACTWACAATATGGGTGCC
	RSVWGS2R.2	CRTTYCTTAARGTRGGCC
	RSVGF-R	TTCGYGACATATTTGCCCC
	RSVWGS3.2F	ACATGGAAAGAYATYAGCC
	RSVWGS3.2R	TTGCATCTGTAGCAGGAATGG
	RSVWGS6F	TATAYAGATAYCAYATGGGTGG
	RSVWGS14R	CGATATAACAARTTRGGATCACC
	RSVWGS13F	AAAAGATTGGGG

	A	B
		AGAGGG
	RSVWGS13R	GGRCCTATTGTAAGGACTARGT
	Mix B	
	RSVWGS9F	GARCAACTCAAAGAAAATGG
	RSVWGS9R	AYTGRAACATRGGCACCC
	OG1-21	GGGGCAAATGCAACCATGTCC
	RSVWGS5F	CATAATTAYTTTGAATGGC
	RSVWGS5R.2	CAAACATTTAATCTRCTRAGGCTRA
	RSVWGS14F.2	TYAACAACYCKAATCAYGTGG
	RSVWGS6R	CCCTCTCCCCAATCTTTTTC

	A	B
	RSVWGS13F	AAAAGATTGGGGAGAGGG
	RSVWGS3.2R	TTGCATCTGTAGCAGGAATGG
	RSVCombEnding	ACGAGAAAAAAAGTGTCAAAAACTAA

Primer description by mix

	1	NC_038235.1	1	22	RSVCombinitial	1	+
	2	NC_038235.1	2202	2221	RSVWGS9F	1	+
	3	NC_038235.1	2329	2348	RSVWGS4R	2	-
	4	NC_038235.1	3352	3370	RSVWGS2F	1	+
	5	NC_038235.1	3364	3381	RSVWGS9R	2	-
	6	NC_038235.1	4672	4692	OG1-21	1	+

	7	NC_038235.1	7593	7611	RSVGF-R	2	-
	8	NC_038235.1	7674	7692	RSVWGS5F	1	+
	9	NC_038235.1	9304	9322	RSVWGS3.2F	1	+
	10	NC_038235.1	9317	9341	RSVWGS5R.2	2	-
	11	NC_038235.1	9932	9949	RSVWGS2R.2	2	-
	12	NC_038235.1	10313	10333	RSVWGS14F.2	1	+
	13	NC_038235.1	10799	10820	RSVWGS6F	1	+
	14	NC_038235.1	13036	13055	RSVWGS6R	2	-
	15	NC_038235.1	13038	13055	RSVWGS13F	1	+
	16	NC_038235.1	11455	11477	RSVWGS14R	2	-
	17	NC_038235.1	14213	14233	RSVWGS3.2R	2	-
	18	NC_038235.1	14508	14531	RSVWGS13R	2	-
	19	NC_038235.1	15192	15217	RSVCombEnding	2	-
	1	NC_001781.1	1	22	RSVCombinitial	1	+
	2	NC_001781.1	2202	2221	RSVWGS9F	1	+

	3	NC_001781.1	2331	2350	RSVWGS4R	2	-
	4	NC_001781.1	3354	3372	RSVWGS2F	1	+
	5	NC_001781.1	3366	3383	RSVWGS9R	2	-
	6	NC_001781.1	4673	4693	OG1-21	1	+
	7	NC_001781.1	7607	7624	RSVGF-R	2	-
	8	NC_001781.1	7687	7705	RSVWGS5F	1	+
	9	NC_001781.1	9316	9334	RSVWGS3.2F	1	+
	10	NC_001781.1	9329	9353	RSVWGS5R.2	2	-
	11	NC_001781.1	9944	9961	RSVWGS2R.2	2	-
	12	NC_001781.1	10325	10345	RSVWGS14F.2	1	+
	13	NC_001781.1	10811	10832	RSVWGS6F	1	+
	14	NC_001781.1	13048	13067	RSVWGS6R	2	-
	15	NC_001781.1	13050	13067	RSVWGS13F	1	+
	16	NC_001781.1	11467	11489	RSVWGS14R	2	-
	17	NC_001781.1	14225	14245	RSVWGS3.2R	2	-

	18	NC_001781.1	14522	14543	RSVWGS13R	2	-
	19	NC_001781.1	15197	15222	RSVCombEnding	2	-

Primer scheme

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.

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

	A	B
	Mezcla A	**1x (µl)**
	MyTaqRed 2x	15
	H2O	7,8
	RSV CombInit (10µM)	0,2
	WGS 4R (10µM)	0,2
	WGS 2F (10µM)	0,2
	WGS 2R (10µM)	0,2
	RSV GF_R (10µM)	0,2

	A	B
	WGS 3.2F (10µM)	0,2
	WGS 3.2R (10µM)	0,2
	WGS 6F (10µM)	0,2
	WGS 14R (10µM)	0,2
	WGS 13F (10µM)	0,2
	WGS 13R (10µM)	0,2
	Volumen total	**25**

13 **Preparation of RSV Amplification Mix B:**

	A	B
	Mezcla B	**1x (µl)**
	MyTaqRed 2x	15
	H2O	8
	WGS 9F (10µM)	0,2
	WGS 9R (10µM)	0,2
	RSV OG1_21 (10µM)	0,2

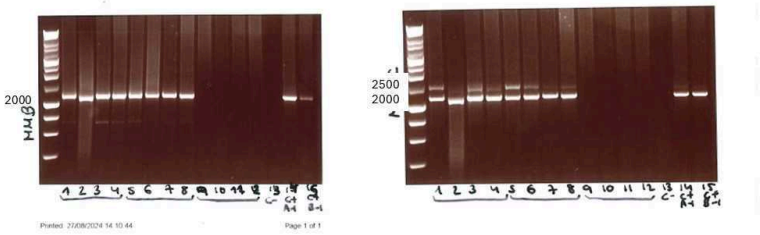
	A	B
	WGS 5F (10µM)	0,2
	WGS 5R.2 (10µM)	0,2
	WGS 14F.2 (10µM)	0,2
	WGS 6R (10µM)	0,2
	WGS 13F (10µM)	0,2
	WGS 3.2R (10µM)	0,2
	CombEnd (10µM)	0,2
	Volumen total	**25**

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

15 **Amplification protocol:**

	A	B	C
	95ºC	1 min	
	95ºC	30 seg	x45
	55ºC	7 min	
	72ºC	2 min	
	72ºC	5 min	
	12ºC	∞	

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



1-4: <22
5-8: 22-28
9-12: >30

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

RSVAB GF protocol starting from viral extraction

18 Materials:
Qiagen OneStep RT-PCR kit.

19 **Preparation of GF amplification mix:**

	A	B
	H20	10
	5xQ PCR MM	6
	dNTPs	1
	RSVWGS_2F (10uM)	1
	RSVGF-R (10 uM)	1
	RT-PCR mix	1
	Total	20 ul

20 Addition of 10 µL of the viral extraction

21 **Amplification protocol GF:**

	A	B	C
	48ºC	60 min	
	95ºC	15 min	
	95ºC	30 seg	x 45
	55ºC	2 min	
	72ºC	1 min	
	72ºC	5 min	
	12ºC	∞	

Citations

Step 2

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