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RdRpSeq assay and NSeq assay

RT-PCR for generating amplicons for sequencing targeting the Betacoronavirus (MERS-CoV) RdRp gene (RdRpSeq assay) and N gene (NSeq assay):

Example formulation: Invitrogen SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase

First round	<u>25µl</u>	<u>Cycler:</u>	
<u>MasterMix:</u>	single rxn, µl	50°C 20'	
H ₂ O (RNase free)	4.1	94°C 3'	
MgSO ₄ (50mM)	0.4		
2x Reaction mix*	12.5	94°C 15"	45x
Fwd primer (10 µM)	1	56°C 15"	
Rev primer (10 µM)	1	72°C 30"	
SSIII/Taq EnzymeMix*	1		
	<hr/> 20	72°C 2'	
+ Template RNA	5	' = minutes; " = seconds	

*Invitrogen: SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase

Example formulation: Invitrogen Platinum® Taq DNA Polymerase

Second round	<u>50µl</u>	<u>Cycler:</u>	
<u>MasterMix:</u>	single rxn, µl	94°C 3'	
H ₂ O (RNase free)	36.8		
10xRx Buffer*	5		
MgCl ₂ (50mM)*	2	94°C 15"	45x
dNTPs(10mM each)	1	56°C 15"	
Fwd-Primer(10 µM)	2	72°C 30"	
Rev-Primer (10 µM)	2		
P.Taq Enzyme	0.2	72°C 2'	
	<hr/> 49	' = minutes; " = seconds	
Template DNA received from the first round (µl)	1		

* Invitrogen : Platinum® Taq DNA Polymerase

Primers:

RdRpSeq assay:

EMC-SeqRdRP-Rev: GCA TWG CNC WGT CAC ACT TAG G
EMC-SeqRdRP-Fwd: TGC TAT WAG TGC TAA GAA TAG RGC
EMC-SeqRdRP-Rnest: CAC TTA GGR TAR TCC CAW CCC A
W=A/T, N=A/C/T/G, R=A/G

Fragment size: first round : 242bp (including primers),
second round 228 bp (including primers)

Nseq assay:

EMC-SeqN-Fwd CCT TCG GTA CAG TGG AGC CA
EMC-SeqN-Rev GAT GGG GTT GCC AAA CAC AAA C
EMC-SeqN-Fnest TGA CCC AAA GAA TCC CAA CTA C

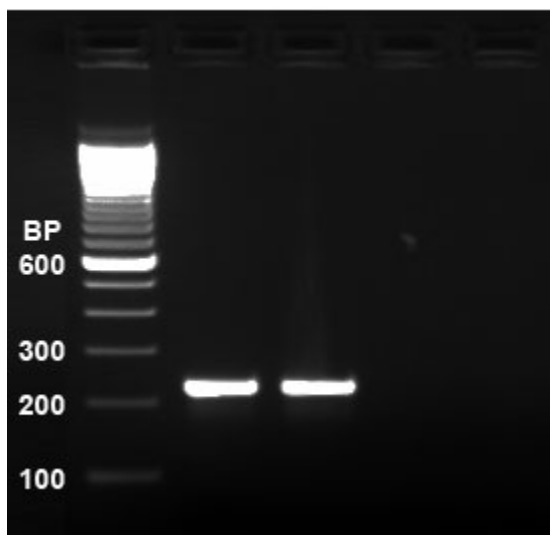
Fragment size: first round : 306-312 bp (including primers),
second round 279-285 bp (including primers)

Reference:

Corman/Müller et al.; Eurosurveillance, Volume 17, Issue 49, 06 December 2012

Example pictures of gel electrophoresis of the resulting DNA of both assays:

RdRpSeq assay



NSeq assay

