

_____ → *Renilla Reniformis*
 MDTSKVYDPE... - Globin
 MEYSKVYDPE... - ORF-1ab
 MEYSKVYDPE... - ORF-S / ORF-6
 MDTSKVYDPE... - ORF-3a
 MYTSKVYDPE... - ORF-E
 MAYSKVYDPE... - ORF-M
 MKYSKVYDPE... - ORF-7a / ORF-8
 MIYSKVYDPE... - ORF-7b
 MSYSKVYDPE... - ORF-N

Figure S1. N-terminal modification of the Renilla luciferase coding region. Sequence alignment of the beginning of the Renilla coding region from the SARS-CoV-2 5'UTRs construct used in the figure 3. As the nucleotide context of the AUG codon is conserved; this only induced a modification of the second amino acid (indicated in bold).

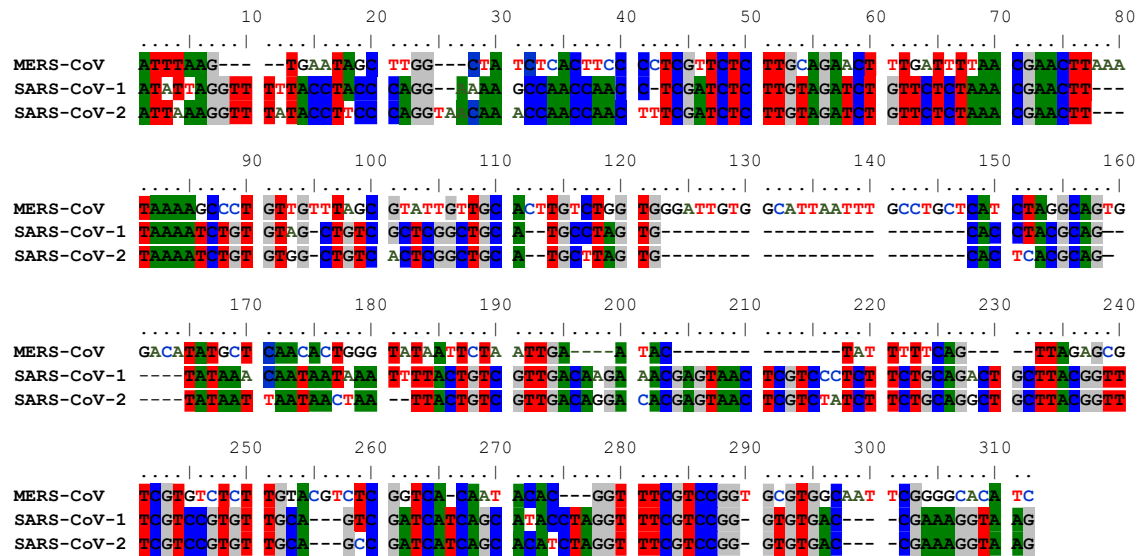


Figure S2. SARS CoV-1/2 and MERS CoV gRNA 5'UTRs conservation. The sequences of the 5'UTR from the SARS-CoV type 1 and 2 and MERS-CoV gRNA have been analyzed with a Multiple Sequence Comparison by Log- Expectation (MUSCLE) using the default parameters. Nucleotide identity has been highlighted in green for adenosine, blue for cytosine, red for tyrosine and grey for guanosine.



Figure S3. 5'UTR^{ORF1ab} uORFs conservation. **(A)** The sequence of the 5'UTR^{ORF1ab} uORFs from SARS-CoV-1, SARS-CoV-2 and MERS-CoV were analyzed with a Multiple Sequence Comparison by Log- Expectation (MUSCLE) using the default parameters. **(B)** Percentage of identity between the uORFs from 5'UTRs of MERS-CoV, SARS-CoV-1 and SARS-CoV-2 gRNAs resulting from a MUSCLE alignment.

Constructs	# PCR	5'-3' Primers	Template	
pSARS-CoV-2 Leader_Renilla	PCR #1	ATCTCTTGTAGATCTGTTCTCTAAACGAACATGGATCCTACTTCGAAAGTTTATG	pGlo-Renilla	
		AGATGTAAAGCTTGCATAAATGTTAT		
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAA AGATGTAAAGCTTGCATAAATGTTAT	PCR #1	
pSARS-CoV-2 5'UTR ^{ORF1ab} Renilla	PCR #1	TTCGTCCTGTTTGCAGCCGATCATCAGCACATCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGATCCTACTTCGAAAGTTTATG AGATGTAAAGCTTGCATAAATGTTAT	pGlo-Renilla	
		AAATTAATACTAATTACTGTCGTTGACAGGACACGAGTAACCTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCG AGATGTAAAGCTTGCATAAATGTTAT		PCR #1
	PCR #3	GTTCTCTAAACGAACTTTAAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACCTACGCAGTATAATTAATAACTAATTACTGTCGTTGACAG AGATGTAAAGCTTGCATAAATGTTAT	PCR #2	
		TAAGCAGAATTAGATCTATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGAGATCTGTTCTCTAAACGAACTTTAAAAATCTGTG AGATGTAAAGCTTGCATAAATGTTAT		PCR #3
	PCR #4	TAAGCAGGATCCTAATACGACTCACTATAGGCTAGCATTAAAGGTTTATACCTTCCCAGG AGATGTAAAGCTTGCATAAATGTTAT	PCR #4	
pSARS-CoV-1 Leader_Renilla	PCR #1	ATATTAGGTTTTTACCTACCCAGGAAAAGCCAAACCAACCTCGATCTCTTGAGATCTGTTCTCTAAACGAACATGGATCCTACTTCGAAAGTTTATG AGATGTAAAGCTTGCATAAATGTTAT	pGlo-Renilla	
		TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATATTAGGTTTTTACCTACCCAGG AGATGTAAAGCTTGCATAAATGTTAT		PCR #1
pSARS-CoV-1 5'UTR ^{ORF1ab} Renilla	PCR #1	TGCTTACGGTTTCGTCCGTGTTGCAGTCGATCATCAGCATACCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGATCCTACTTCGAAAGTTTATG AGATGTAAAGCTTGCATAAATGTTAT	pGlo-Renilla	
		ACCTACGCAGTATAAAACAATAATAAATTTTACTGTCGTTGACAAGAAACGAGTAACCTCGTCCCTCTTCTGCAGACTGCTTACGGTTTCGTCCGT AGATGTAAAGCTTGCATAAATGTTAT		PCR #1
	PCR #3	TCGATCTCTTGAGATCTGTTCTCTAAACGAACTTTAAAAATCTGTGTAGCTGTGCTCGGCTGCATGCCTAGTGCACCTACGCAGTATAAAACAATAATAA AGATGTAAAGCTTGCATAAATGTTAT	PCR #2	
		TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATATTAGGTTTTTACCTACCCAGGAAAAGCCAAACCAACCTCGATCTCTTGAGATCTGTTCT AGATGTAAAGCTTGCATAAATGTTAT		PCR #3
	pMERS-CoV Leader_Renilla	PCR #1	ATTTAAGTGAATAGCTTGGCTATCTCACTTCCCCTCGTTCTCTTGAGAACTTTGATTTTAACGAACATGGATCCTACTTCGAAAGTTTATG AGATGTAAAGCTTGCATAAATGTTAT	pGlo-Renilla
TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATTTAAGTGAATAGCTTGGCTATC AGATGTAAAGCTTGCATAAATGTTAT			PCR #1	
pMERS-CoV 5'UTR ^{ORF1ab} Renilla	PCR #1	TAGAGCGTCGTGTCTCTTGACGTCTCGGTACACAATACACGGTTTCGTCCGGTGCGTGGCAATTCGGGGCACATCATGGATCCTACTTCGAAAGTTTATG AGATGTAAAGCTTGCATAAATGTTAT	pGlo-Renilla	
		AATTTCCTGCTCATCTAGGCAGTGGACATATGCTCAACACTGGGTATAATTCTAATTGAATACTATTTTCAGTTAGAGCGTCGTGTCTCTTGTA AGATGTAAAGCTTGCATAAATGTTAT		PCR #1
	PCR #3	TAAAAGCCCTGTGTTTAGCGTATGTGCACTGTCTGGTGGGATGTGGCATTAAATTGCCTGCTCATCTAGGC AGATGTAAAGCTTGCATAAATGTTAT	PCR #2	
		TCACTTCCCCTCGTTCTCTTGACAGAACTTTGATTTTAAACGAACCTTAAATAAAAGCCCTGTGTTAGCG AGATGTAAAGCTTGCATAAATGTTAT		PCR #3
	PCR #4	TACGAAGGATCCTAATACGACTCACTATAGATTTAAGTGAATAGCTTGGCTATCTCACTTCCCCTCGTTCTCTTG AGATGTAAAGCTTGCATAAATGTTAT	PCR #4	

Table S1. List of primers used for the cloning of the SARS-CoV 1/2 and MERS-CoV leader and 5'UTR^{ORF1ab}.

Constructs	# PCR	5'-3' Primers	Template
pSARS-CoV-2 5'UTR-ORF-1ab Renilla	PCR #1	TTCTGTCGGTGTTCAGCCGATCATCAGCACATCTAGGTTTCGTCGGGTGTGACCGAAAGGTAAGATGGAGACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	AATTAATAACTAATTACTGTCTGACAGGACACGAGTAACCTGCTATCTTCTGCAGGTGCTTACGGTTTCGTCCGTGTTCAGCCG TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
	PCR #3	GTTCTCTAAACGAACTTTAAAACTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCTGACAG TTCTCTGATATCTTCAGTTTGTGTC	PCR #2
	PCR #4	TAAGCAGAATTCAGATCTAATAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACTTAAAACTGTG TTCTCTGATATCTTCAGTTTGTGTC	PCR #3
	PCR #5	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAA TTCTCTGATATCTTCAGTTTGTGTC	PCR #4
pSARS-CoV-2 5'UTR-ORF-S Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACAATGTTTACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
pSARS-CoV-2 5'UTR-ORF-3a Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACCTATGGATACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
pSARS-CoV-2 5'UTR-ORF-E Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACCTATGTACACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
pSARS-CoV-2 5'UTR-ORF-M Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACCTAATATTATATTAGTTTTCTGTTTGGAACTTTAATTTTAGCCATGGCAACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
pSARS-CoV-2 5'UTR-ORF-6 Renilla	PCR #1	GACCATTCCAGTAGCAGTGACAATATGCTTTGCTTGTACAGTAAGTGACAACAGATGTTTACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	CAGGTGACTCAGGTTTGTCTGCATACAGTCGCTACAGGATTGGCAACTATAAATTAACACAGACCATTCAGTAGCAGTG TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
	PCR #3	TGTAGATCTGTTCTCTAAACGAACGCTTCTTATTACAAATGGGAGCTTCGCAGCGTGTAGCAGGTGACTCAGGTTTTC TTCTCTGATATCTTCAGTTTGTGTC	PCR #2
	PCR #4	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #3
pSARS-CoV-2 5'UTR-ORF-7a Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACATGAAACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
pSARS-CoV-2 5'UTR-ORF-7b Renilla	PCR #1	TTATTGTTGCGGCAATAGTGTTTATAACACTTTGCTTCACACTCAAAAGAAAGACAGAATGATTACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TGTAGATCTGTTCTCTAAAAGAACCTTACTCTCCAATTTTCTTATTGTGTCGGCAATAGTG TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
	PCR #3	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #2
pSARS-CoV-2 5'UTR-ORF-8 Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACATGAAACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1
pSARS-CoV-2 5'UTR-ORF-N Renilla	PCR #1	TGTAGATCTGTTCTCTAAACGAACAACATAAATGTCTACTTCGAAAGTTTATGATCCAG TTCTCTGATATCTTCAGTTTGTGTC	pGlo-Renilla
	PCR #2	TAAGCAGGATCCTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTGTAGATCTGTTCTCTAAACGAAC TTCTCTGATATCTTCAGTTTGTGTC	PCR #1

Table S2. List of primers used for the cloning of the different 5'UTRs of the SARS-CoV-2 gRNA and sgRNAs

Constructs	# PCR	5'-3' Primers	Template
pSARS-CoV-2 5'UTR_Renilla Mut-AUG-uORF	PCR #1.1	TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGT AAGCAGCCGAGTGACAGCCACACAGATTTTAAAGTTCGTTAGAGAACAGATCTAC	pSARS-CoV-2_5'UTR ^{ORF1ab} _Renilla
	PCR #1.2	TGTGTGGCTGTCACTCGGCTGCTTGCTTAGTGCACCTACGCAG ACATCAGGATCCATCTTACCTTTCGGTCACACCC	pSARS-CoV-2_5'UTR ^{ORF1ab} _Renilla
	PCR 2	TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGT ACATCAGGATCCATCTTACCTTTCGGTCACACCC	PCR #1.1 and PCR #1.2
pSARS-CoV-2 5'UTR_Renilla uORF-phase	PCR #1.1	TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGT GTAA TTATA TTGTAA TTATACTGCGTGAGTGC ACTAAGCATG	pSARS-CoV-2_5'UTR ^{ORF1ab} _Renilla
	PCR #1.2	AGTATAATTACAATATAATTACTGTCTGTGACAGGACACGAG ACATCAGGATCCATCTTTCCTTTCGGACACACCCGGAC	pSARS-CoV-2_5'UTR ^{ORF1ab} _Renilla
	PCR 2	TAAGCAGGATCCCTCGAGTAATACGACTCACTATAGATTAAGGTTTATACCTTCCCAGGT ACATCAGGATCCATCTTTCCTTTCGGACACACCCGGAC	PCR #1.1 and PCR #1.2
pMERS-CoV 5'UTR_Renilla Mut-AUG-uORF	PCR #1	TCACTTCCCCTCGTTCTCTTGCGAACTTTGATTTTAACGAACCTAAATAAAAGCCCTGTTGTTAGCG AGATGTAAAGCTTGCATAAATGTTAT	pMERS-CoV_5'UTR ^{ORF1ab} _Renilla
		TACGAAGGATCCTAATACGACTCACTATAGATTTAAGTGAATAGCTTGGCTATCTCACTTCCCCTCGTTCTCTTG AGATGTAAAGCTTGCATAAATGTTAT	
	PCR #3	TAAAAGCCCTGTGTTTAGCGTATTGTGCACTTGCTGTTGGGATTGTGGCAATAATTTGCTGCTCATCTAGGCAGTGGACATTGCTCAACACTGGG AGATGTAAAGCTTGCATAAATGTTAT	PCR #2

Table S3. List of primers used for the cloning of the uORFs mutations