Supplementary Materials

Identification of *Cis*-Acting Elements on Positive-Strand Subgenomic mRNA Required for the Synthesis of Negative-Strand Counterpart in Bovine Coronavirus

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Table S1. Oligonucleotides used in this study

Oligonucleotide[a]	Polarity[b]	Sequence (5' → 3')	Position
pGEMNDEI(−)[c]	+	GAGAGTGCACCATATG	
ΔNL (+)	−	GACATCCTTAAAGTTTAGACCCTATAGTGAGTCGTATTAC	
ΔSLI (+)	−	TAAAAAGATCTAACAAGAGACTATAGTGAGAGTCGTATTACA	
ΔSLII (+)	−	CCTTAAAGTTTAGATCAGTGAAGCGGGATG	
sΔB(+)	−	CAAGACATCCATTCTGAATATTTCTGAGGTGTC	
sΔP (+)	−	CTAATTGATACAGGGTTGTCCTATCCCGACTTTC	
s3'Δ15 (+)	−	ACGCGTTTTTTTTTTTTTTTTTTTTTTTTTTGGCCATGATCAACTTC	
s3'Δ55 (+)	−	ACGCGTTTTTTTTTTTTTTTTTTTTTTTTTTTAATAGTACCCTGATGTG	
s3'Δ55-45 (+)	−	CATTCATTTACTAGGAATAGTACCCTGATGTGAGC	
s3'Δ55-40 (+)	−	CTTCATTCATTTAAATAGTACCCTGATGTGAGC	
s3'Δ55-30 (+)	−	GGCCATGATCAACTTAATAGTACCCTGATGTGAGC	
s3'Δ30-15 (+)	−	GTGATTCTTCCAATTCATTCATTTACTAGGGC	
sgmA' (+)	−	ACGCGTTTTTTTTTTTTTTTTTTTTTTTTTTTGATTCTTCCAATTGGCC	
sgmU' (+)	−	ACGCGTTTTTTTTTTTTTTTTTTTTTTTTTTTATGATTCTTCCAATTGGCC	
sgmG' (+)	−	ACGCGTTTTTTTTTTTTTTTTTTTTTTTTTTTCTGATTCTTCCAATTGGCC	
leader20(–)	+	GATTGTGAGCGATTTGCGTG	1-20[d]
M(+)	−	GTTCCATTCCTTTAGGAATTTAATAG	28746-28771[d]
M3 (–)	+	GGGTTCTGGCATGGACACCGC	29345-29365[d]
MHV3'UTR 3 (–)	+	GTCCTACGTCTAACCATAAGAACG	31213-31236[e]
MHV3'UTR 6 (–)	+	CCTGGGAAGAGCTCACATCAGG	31250-31271[e]
BCV 23-40 (+)	−	CCCGCTTCACTGATCTCT	23-40[d]
BCV 29-54 (+)	−	GATCTAACAAGAGTCAGTGAAGCG	29-54[d]
TGEV 7 (-)	+	TCTGGGTTGCCAAGGATGGTGCCATG	1098-1123[f]
TGEV 8 (+)	−	CATGGCACCATCCTTGGCAACCCAGA	1098-1123[f]
DI reverse (+)[c]	−	CACAGGAAACAGCTATGACC	
BCVN (+)	−	CCAGAACGATTTCCAAAGGACGCTCT	29430-29455[d]
18SrRNA (+)	−	GCCTGCTGCCTTCCTTGGATCTGGTAGCC	552-580[g]
18SrRNA (–)	+	CCCATTCGAACGTCTGCCCTATC	440-463[g]
MHV3'UTR2 (+)	−	CCTATCGCCGTTCTTATGGTTAGACG	31219-31244[e]
Taqman probe-5 (–)	+	TGAGCGATTTGCGTGCG	6-22[d]
Taqman probe-3 (–)	+	TAACCATAAGAACGGCGATAGGCGC	31223-31247[e]
MHV3'UTR-DR(–)	+	GATTGCAAAATAGAGAATGTG	31175-31197[e]
MHV3'UTR-DR(+)	−	AATAGTACCCTGATGTGA	31262-31279[e]

[a] The positive and negative symbols in the oligonucleotide names indicate the polarities of the nucleic acids to which the oligonucleotides anneal. Oligonucleotides named with a negative symbol used for mutagenesis in the text have a sequence complementaery to a positive-sense oligonucelotide of the same name. [b] Polarity of the oligonucleotide relative to the positive-strand viral genome. [c] Indicates the oligonucleotide which anneals to pGEM3Zf(-) vector (Promega). [d] BCoV-Mebus: GenBank accession number U00735.2; [e] MHV-A59: GenBank accession number NC_001846.1; [f] Indicates the reporter sequence in BCoV DI RNA which anneals to oligonucleotide; [g] 18S rRNA: GenBank accession number M10098.1.

Figure S1. Comparison of the efficiency of the (–)-strand RNA synthesis between sgmRNA 7 and BCoV DI RNA without the strategy of head-to-tail ligation. (**A**) RT-PCR product was observed from BCoV-infected and BM25A- or sBM25A-transfected cells (lanes 2 and 3) but not from control groups: lane 4, total cellular RNA from mock-infected cells; lane 5, total cellular RNA from BCoV-infected and mock-transfected cells. RT-PCR products of ~100 bp were also observed from mock-infected and BM25A- or sBM25A-transfected cells (lanes 6 and 7). (**B**) The relative efficiency of (–)-strand RNA synthesis from constructs BM25A and sBM25A, as measured by RT-qPCR without the step of head-to-tail ligation. Control A: total cellular RNA from mock-infected cells. Control B: total cellular RNA from BCoV-infected cells. Control C: total cellular RNA from BM25A-transfected mock-infected cells. Control D: total cellular RNA from sBM25A -transfected mock-infected cells. M (lanes 1 and 8), ds DNA size markers in nt pairs. The values (**B**) represent the mean±SD of three individual experiments. * $p < 0.05$.

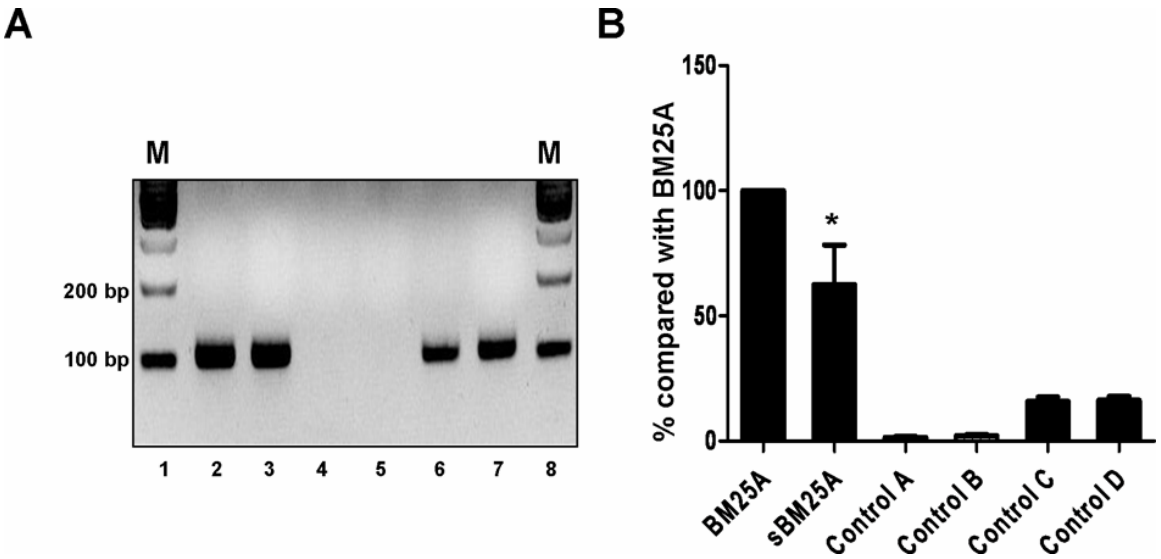

Figure S2. Analysis of the positive-strand RNA synthesis at 48 hpi of VP1 by Northern blot assay with 18S rRNA and M sgmRNA as internal controls.