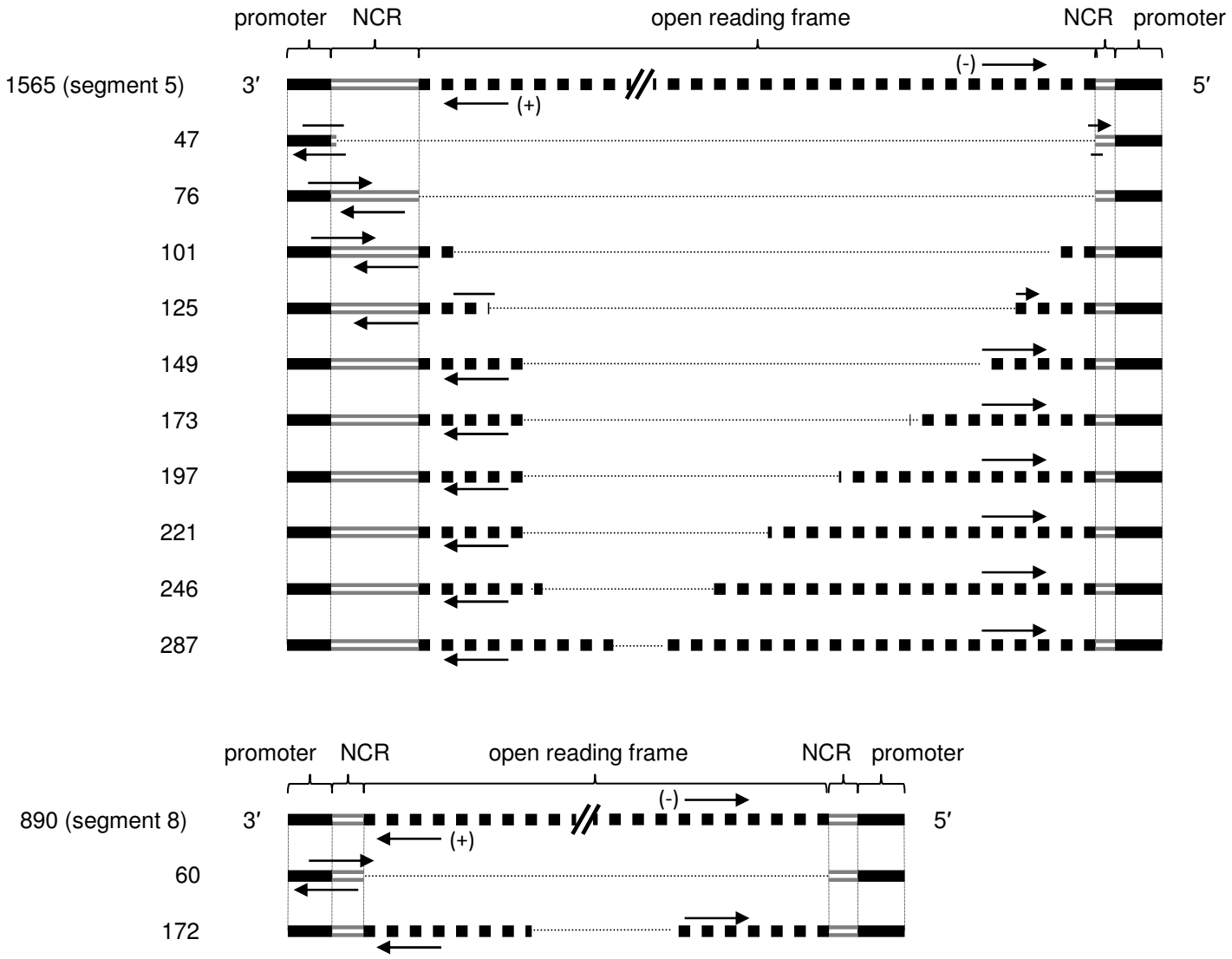
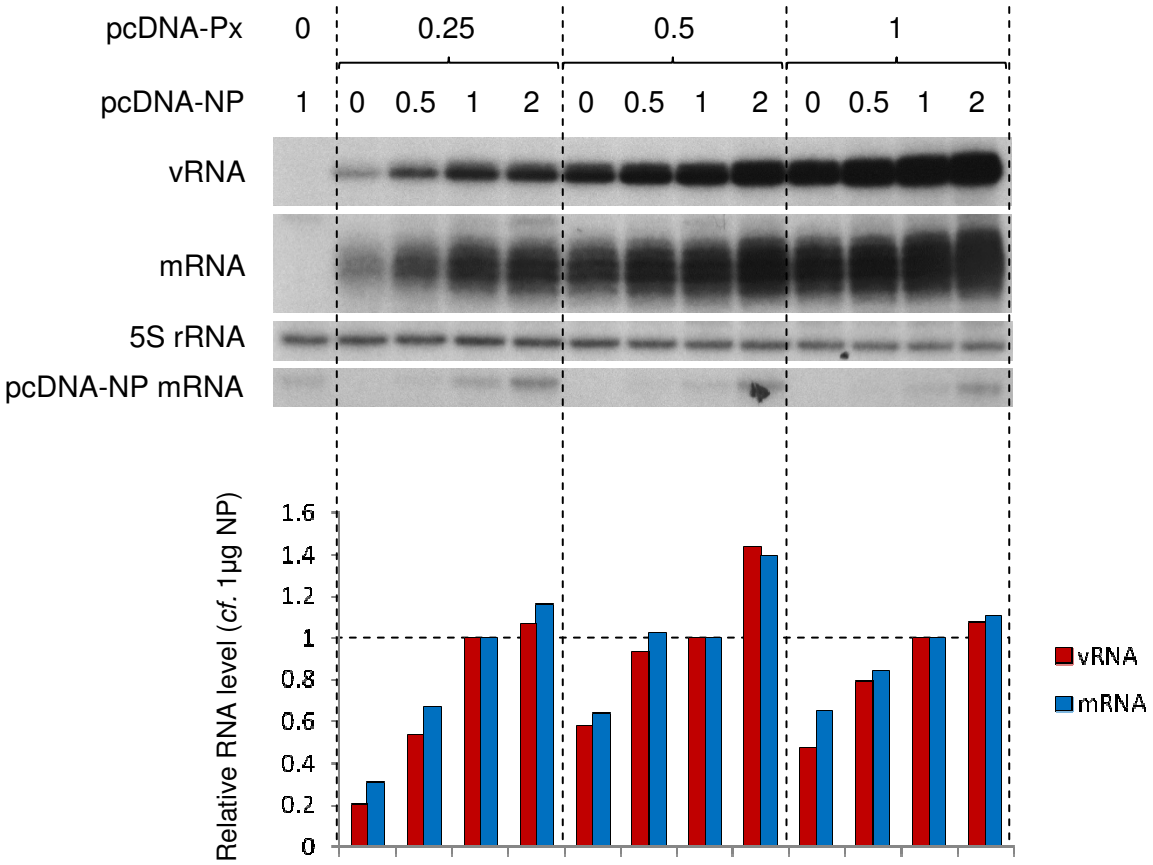
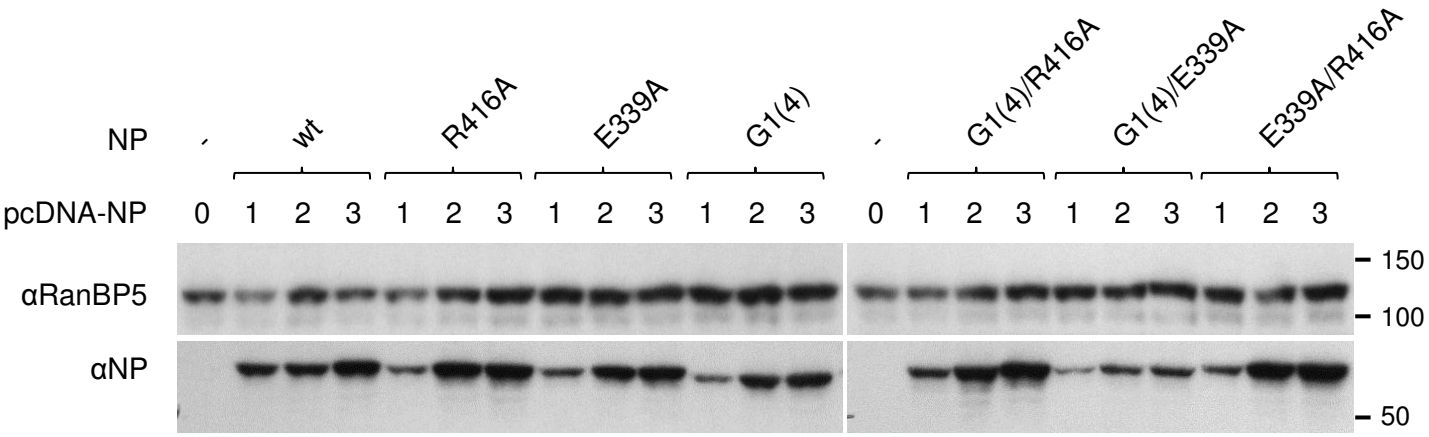


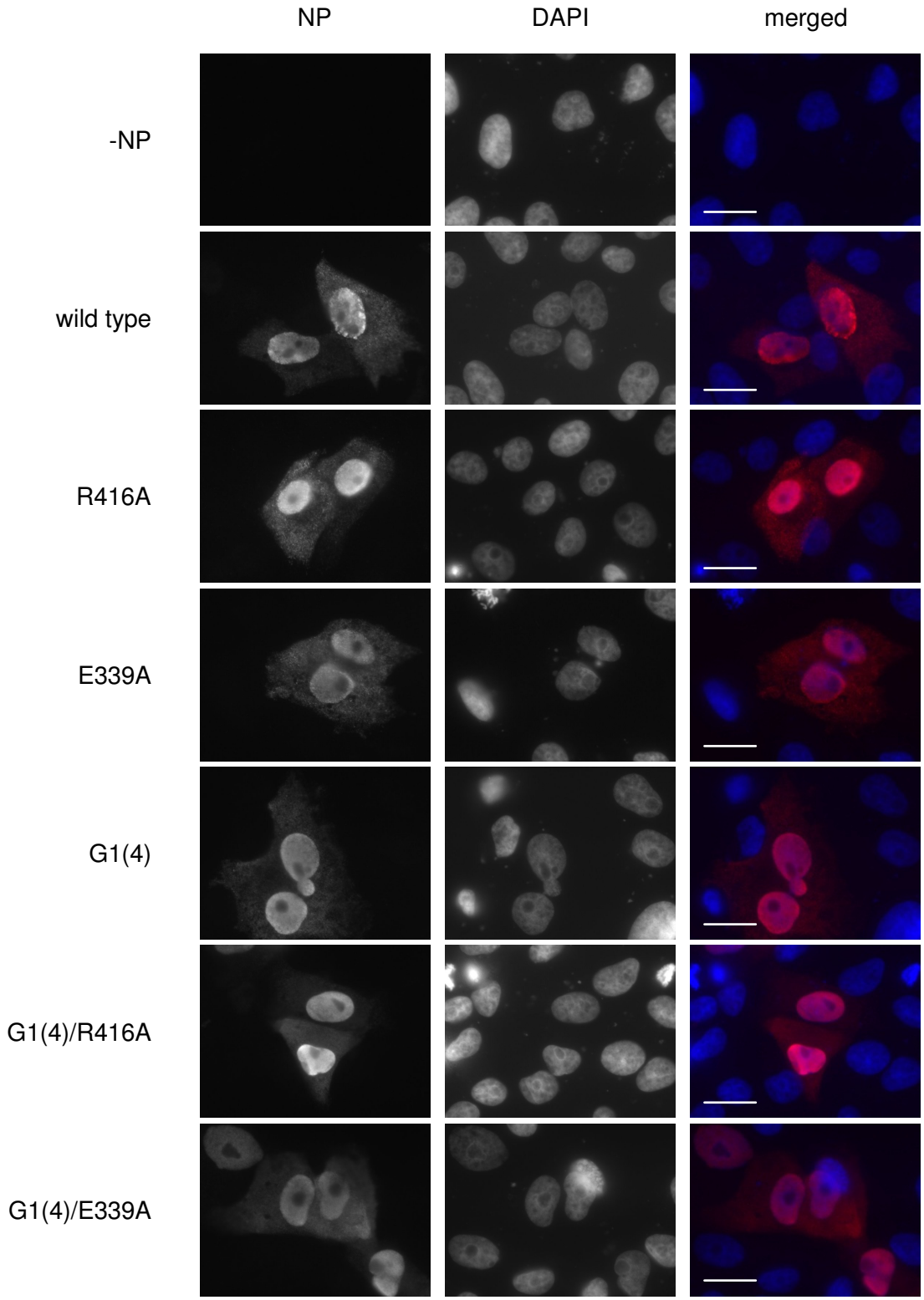
Supplementary Fig. S1. Schematic diagram of minigenome segments. Plasmids expressing short virus-like RNA genome segments were constructed by internal deletions of the non-coding regions and/or the open reading frames of segment 5 and segment 8. Each construct was designed to be approximately 24 nucleotides longer than the previous, representing the number of nucleotides believed to be associated with NP in the RNP structure. Sizes of the vRNAs are shown in nucleotides. Arrows indicate the approximate positions for binding of the primers to negative (above) and positive (below) sense RNAs during primer extension assays (see Supplementary Table S1). NCR = non-coding region.



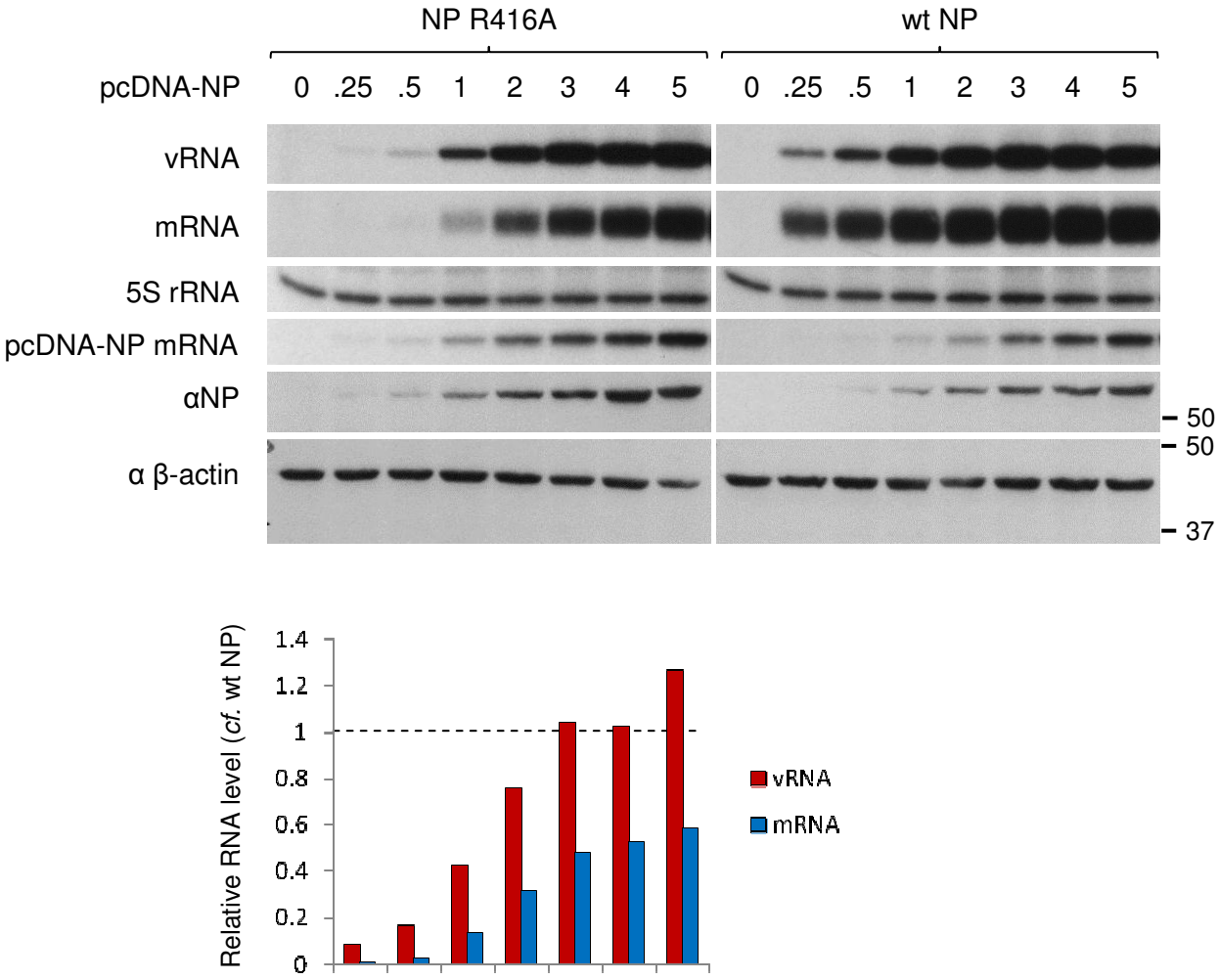
Supplementary Fig. S2. Replication and transcription of short vRNA-like gene segments *in vivo* is sensitive to the concentration of NP. Primer extension analysis of the accumulation of vRNA and mRNA following *in vivo* reconstitution of vRNPs from the 76 nucleotide long vRNA-like template in the presence of varying concentrations of polymerase and NP. The indicated amount in µg of each of the polymerase subunit-expressing plasmids (pcDNA-Px) and 0.5µg of the plasmid expressing the 76 nucleotide long RNA template were transfected together with the amount of NP-expressing plasmid (pcDNA-NP) shown in µg (in a total of 6µg). Polymerase was omitted as a negative control. Analysis of the 5S rRNA levels served as an internal control. The level of mRNA derived from the NP-expressing plasmid is also shown. Quantification was performed by phosphorimage analysis. RNA levels detected in the negative control were set to 0. Graphs depicting the vRNA and mRNA levels accumulating in the presence of varying concentrations of NP relative to those accumulating in the presence of 1µg NP at the same concentration of polymerase are shown.



Supplementary Fig. S3. The expression levels of wild type and mutant NPs are proportional to the amount of NP-expressing plasmid transfected. Western blot analysis of the expression levels of wild type and mutant NP from cell lysates following *in vivo* reconstitution of vRNPs from trimeric polymerase and the 221 nucleotide long vRNA-like template in the presence of varying concentrations of NP. 0.5μg of each of the polymerase subunit-expressing plasmids and the plasmid expressing the 221 nucleotide long RNA template were transfected together with the amount of NP-expressing plasmid shown in μg (in a total of 5μg). RanBP5 was detected as a loading control. Size markers are shown in kDa.



Supplementary Fig. S4. Mutant NPs display similar sub-cellular localisation patterns to that of wild type NP. Images of cellular distribution of wild type and mutant NPs in transfected Vero cells. Nuclei were stained with DAPI. Scale bar = 20μm.



Supplementary Fig. S5. Oligomerisation mutant NP is less efficient than wild type NP at supporting replication and transcription of intermediate length vRNA-like gene segments *in vivo*. Primer extension analysis of the accumulation of vRNA and mRNA following *in vivo* reconstitution of vRNPs from trimeric polymerase and the 221 nucleotide long vRNA-like template in the presence of varying concentrations of wild type or mutant (R416A) NP. 0.5μg of each of the polymerase subunit-expressing plasmids and the plasmid expressing the 221 nucleotide long RNA template were transfected together with the amount of NP-expressing plasmid shown in μg (in a total of 5μg). NP was omitted in the negative control. Analysis of the 5S rRNA levels served as an internal control. The levels of mRNA derived from the NP-expressing plasmid are also shown, as are the NP expression levels with β-actin detected as a loading control. Size markers are shown in kDa. Quantification of RNA was performed by phosphorimage analysis. RNA levels detected in the negative control were set to 0. A graph depicting the vRNA and mRNA levels accumulating in the presence of varying concentrations of mutant NP relative to those accumulating in the presence of the same concentration of wild type NP is shown.

Supplementary Table S1: Details of template-specific primers used for detecting different RNA species in primer extension following *in vivo* RNP reconstitution.

Template length (nucleotides)	Primer (length in nucleotides)	Primer binding site[1]	Viral RNA species[2]	Reverse transcript length (nucleotides)
1565 (segment 5)	149- (21)	1505 - 1525	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
47	47- (24)	6 - 29	vRNA	42
	47+ (23)	24 - 2	mRNA	34-37
			cRNA	24
76	76- (24)	8 - 31	vRNA	69
	76+ (23)	46 - 24	mRNA	56-59
			cRNA	46
101	76- (24)	8 - 31	vRNA	94
	76+ (23)	46 - 24	mRNA	56-59
			cRNA	46
125	125- (23)	59 - 81	vRNA	67
	76+ (23)	46 - 24	mRNA	56-59
			cRNA	46
149	149- (21)	89 - 109	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
173	149- (21)	113 - 133	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
197	149- (21)	137 - 157	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
221	149- (21)	161 - 181	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
246	149- (21)	186 - 206	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
287	149- (21)	227 - 247	vRNA	61
	149+ (21)	74 - 54	mRNA	84-87
			cRNA	74
890 (segment 8)	172- (20)	816 - 835	vRNA	75
	172+ (20)	51 - 32	mRNA	61-64
			cRNA	51
60	60- (20)	8 - 27	vRNA	53
	60+ (19)	21 - 3	mRNA	31-34
			cRNA	21
172	172- (20)	98 - 117	vRNA	75
	172+ (20)	51 - 32	mRNA	61-64
			cRNA	51

[1]based on 5′ to 3′ numbering of the template sequence in the positive sense.
[2]mRNA transcribed from pcDNA-NP is detected by primers 76+ and 149+ to yield reverse transcripts of 117 and 145 nucleotides, respectively.