

## S1 File. Supporting Information Tables.

**Table A. Primers for polymerase chain reaction using to obtain DNA template for vRNA8 and mini-vRNA8.**

Name	Length (nt)	Sequence 5'→3' <sup>a</sup>
Rev	30	AGCAAAAGCAGGGTGACAAAAACATAATGG
For	61	AAAAATAATACGACTCACTATAGGGAGTAGAAACAAGGGTGTTTTTATCATTAAATAAGC

<sup>a</sup>The underlined nucleotides residues are the polymerase T7 promoter.

**Table B. Primers for polymerase chain reaction using to obtain mini-vRNA8.**

	Length (nt)	Sequence 5'→3' <sup>a</sup>
1	36	AAGAATTCAGTAGAAACAAGGGTGTTTTTATCATT
2	33	AAGGATCCAGAAAGTTTGAAGAAATAAGGTGGC
3	30	AAGGATCCGATGTCCAGACCAAGAGTGTTG
4	25	AACTGCAGAGCAAAAGCAGGGTGAC

<sup>a</sup>The underlined nucleotides residues are restriction sites: 1 - EcoR1, 2, 3 - BamH1, 4 - Pst1.

**Table C. Primers for reverse transcription.**

Name	Length (nt)	Complementary region in segment 8 RNA	Sequence 5'→3' <sup>a</sup>
Pr1	23	847-869	AGCaGGgTGaCAAAaACaTAATG
Pr2	22	719-740	GCCgAGaTCaGAAgTCCCTaAG
Pr3	23	572-591	CTGACATGACTCTCGAAGAAATG
Pr4	26	287-312	CTTGAATGGAATGATAACACAGTTCG
Pr5	22	429-450	GACCGGTTGGAAACCCTAATAC
Pr6	22	142-163	GGTGGCTGATTGAAGAAGTAAG

<sup>a</sup> DNA primers 1-6 were used to vRNA8; primers 1, 2, 6 to mini-vRNA8; in small letter (a, g) - LNA nucleotides. Each primer was labeled with 6-FAM at 5' end.

**Table D. Heptamer probes complementary to vRNA8.**

Complementary binding sites	Sequence and modifications of probes <sup>a</sup> 5'→3'
7	UgUuUcUPy
30	UdUuUdAPy
126	AdUuAcAPy
174	GuUuGdAPy
424	AcUuAgAPy
430	AdUdCuAPy
454	GdUuUuUPy
476	UdUuGdAPy
618	CuUdAdAPy
806	UuCuUuGPy

<sup>a</sup> nucleotides in capital letter (A, C, G, U, D) are 2'-O-methyl-RNA nucleotides, in small letter (a, c, g, u, d) - LNA nucleotides; D and d - 2,6 -diaminopurine (2'-O-methyl type or LNA, respectively). Py – pyrene (56-57).

**Table E. RNase H cleavage of vRNA8 in the presence of selected DNA oligonucleotides.**

Binding sites for vRNA8	Sequence of DNA oligonucleotide <sup>a</sup>	Predicted $\Delta G^{\circ}_{37}$ for DNA/RNA duplex <sup>b</sup> (kcal/mol)	Predicted $\Delta G^{\circ}_{37}$ of duplex calculated as RNA/RNA <sup>c</sup> (kcal/mol)	Predicted $\Delta G^{\circ}_{37}$ of mismatched duplex calculated as RNA/RNA <sup>c</sup> (kcal/mol)	RNase H cleavage site <sup>d</sup>
68 (534)	AAGTGG	-3.44	-6.3 (534) -6.2 (68)		70 (s) 535 (s)
194 (254, 390)	GAGAAG	-2.20	-5.7 (194) -5.0 (390) -5.0 (254)	-4.8 (148/149) -4.6 (729) -4.6 (179) -4.6 (407/408) -4.3 (57/58) -4.3 266/267	248-254 (s) 388 (w) 392 (s) 407 (s) 410 (s)
17 (118, 251, 684, 854)	AAACAG	-1.47	-4.2 (251) -3.9 (684) -3.9 (118) -1.8 (17) -1.8 (854)	-3.5 (107)	18-19 (w) 117 (s) 249 (s)
108 (192)	GAACAG	-2.63	-7.4 (108) -5.2 (192)	-4.6 (250/251) -4.3 (683/684) -4.3 (117/118)	249-250 (s)
143	AGTAAGACA	-6.43	-10.6	-6.1 (61/62) -6.1 (781/782) -5.9 (707) -5.5 (488) -5.4 (91) -5.4 (816/817)	143-145 (s)
163	ATAAGGTGG	-7.10	-12.0	-7.3 (818) -7.3 (534) -7.0 (626) -6.5 (67/68) -6.3 (197/198) -6.3 (352) -6.0 (766/767)	165-166 (s)

170	TGAAGAAAT	-3.83	-8.7	-8.1 (575) -7.5 (150) -6.3 (408/409) -5.0 (714) -4.8 (70) -4.5 (253)	172 (s) 251 (s) 253 (s) 149 (w) 73 (w)
408	AGAAGAAGG	-5.72	-12.4	-10 (149) -7.2 (170) -7.2 (576) -6.9 (713)	411-415 (s)
436	AAACCCTAA	-7.25	-10.2	-7.9 (13) -7.5 (722/723) -7.0 (759) -6.3 (221/222) -6.2 (522/523)	438 (w) 221-223 (w) 522-523 (w) 724 (w)
535	AGAAAGTGG	-6.36	-12.3	-8.5 (657) -7.6 (664/665) -7.5 (68) -6.7 (472)	535-536 (s)
576	CGAAGAAAT	-4.43	-9.9	-8.0 (169/170) -6.8 (149/150) -6.5 (408/409) -5.3 (714)	578 (w) 172 (w)
721	CCCTAAGAG	-8.78	-14.8	-7.8 425	
729	TCAGAAGTC	-7.65	-12.6	-10.3 (179/180) -6.9 (410/411) -6.6 (211)	730 (w) 182 (w)

*a* – Sequences of DNA 6-mers were the same as probe for certain site, sequences of DNA 9-mer were specific for designated site; *b* - calculated for DNA/RNA duplex in 300 mM NaCl, as in experiment (<http://ozone3.chem.wayne.edu>); *c* - calculated in RNAstructure5.3 program as RNA/RNA duplex for standard condition (1 M NaCl) to show the difference in  $\Delta G^{\circ}_{37}$  between complementary and predicted mismatched duplexes, in parenthesis - site of possible DNA oligonucleotide binding, denoted by the middle nucleotide of the complementary RNA region (or two nucleotides for duplex with an even number of nucleotides); *d* – nucleotides preceding RNase H cleavage site; s-strong cut, w-weak cut.

**Table F. Isoenergetic microarrays probes that bind strongly and moderately to vRNA8 and mini-vRNA8 and their thermodynamic properties<sup>a</sup>.**

Probe name <sup>b</sup>	Binding sites for vRNA8 <sup>c</sup>	Probe sequence <sup>d</sup>	Strength of probe binding <sup>e</sup>		$\Delta G^{\circ}_{37}$ of duplex for complementary binding site <sup>f</sup> (kcal/mol)	Nucleotide of RNA target complementary to 3'g of hexamer probe	$\Delta G^{\circ}_{37}$ of duplex for possible mismatched sites <sup>f,g</sup> (kcal/mol)
			vRNA8	mini-vRNA8			
1p	149 (170, 408, 576, 774)	dDgDdg	1.000 (S)	0.4391 (S)	-4.4 (-11.37) 149 -4.2 (-11.37) 408 -2.7 (-9.24) 774 -2.3 (-9.24) 170 -2.3 (-9.24) 576	149C 170U 408C 576U 774G	-4.5 (-9.34) <b>179</b> -4.5 (-9.34) <b>729</b> -4.1 (-6.0) <b>69/70</b>
2p	69 (147, 178, 728)	GdDgUg	0.7241 (S)	-	-7.6 (-11.17) 69 -5.4 (-9.23) 728 -5.4 (-10.03) 147 -5.3 (-10.03) 178	69C 147U 178A 728G	-4.4 (-9.45) <b>534/535</b>
3p	150 (171, 409, 577, 714)	GdDgDg	0.6299 (S)	0.2180 (M)	-6.9 (-11.76) 714 -5.9 (-9.74) 171 -5.9 (-9.74) 150 -5.9 (-9.74) 577 -5.4 (-9.74) 409	150U 171U 409U 577U 714C	-4.2 (-8.53) <b>52</b> -4.2 (-8.53) <b>59</b> -4.1 (-5.47) <b>69/70</b>
4p	148 (179, 407, 410, 729)	DgDdGg	0.4967 (S)	0.4877 (S)	-6.4 (-12.19) 407 -5.2 (-9.61) 179 -5.2 (-9.61) 729 -5.1 (-9.61) 410 -4.6 (-9.61) 148	148A 179A 407C 410U 729A	-4.7 (-10.08) <b>536/537</b> -4.6 (-4.77) <b>69/70</b> -4.5 (-4.77) <b>171/172</b> -4.5 (-4.77) <b>577/578</b>
5p	142	dDgDcg	0.4913 (S)	0.1412 (M)	-5.4 (-9.58) 142	142U	
6p	52 (59, 713, 719)	dDgDgg	0.4837 (S)	0.8894 (S)	-7.0 (-12.96) 719 -6.5 (-12.96) 713 -5.1 (-10.03) 59 -5.0 (-10.03) 52	52G 59U 713C 719C	-4.6 (-10.03) <b>267/268</b> -4.2 (-8.52) <b>729</b> -4.2 (-8.52) <b>179</b> 4.1 (-10.55) <b>148/149</b>
7p	106 (123, 269, 412)	dCdGdg	0.4772 (S)	0.3867 (S)	-7.1 (-11.96) 269 -5.4 (-9.83) 123 -5.4 (-9.83) 412 -4.6 (-9.83) 106	106A 123U 269C 412U	-4.1 (-5.96) <b>538/539</b> -4.1 (-5.96) <b>180/181</b> -4.1 (-5.96) <b>730/731</b> -4.1 (-5.96) <b>211/212</b>
8p	163	dDgGug	0.4470 (S)	0.1264 (M)	-7.0 (-12.07) 163	163C	
9p	68 (534)	dDgUgg	0.4449 (S)	0.787 (S)	-6.3 (-12.37) 534 -6.2 (-12.37) 68	68C 534C	

10p	107 (117, 250, 683)	dDcDgg	0.2661 (M)	0.1429 (M)	-4.4 (-9.15) 250 -4.4 (-9.15) 107 -4.2 (-9.15) 683 -4.2 (-9.15) 117	107U 117G 250A 683G	-4.3 (-5.62) <b>412/413</b> -4.2 (-5.62) <b>269/270</b> -4.2 (-5.62) <b>123/124</b>
11p	194 (254, 390)	GdGdDg	0.2659 (M)	-	-5.7 (-9.5) 194 -5.0 (-9.5) 390 -5.0 (-9.5) 254	194G 254U 390U	-4.8 (-9.8) <b>148/149</b> -4.6 (-8.29) <b>729</b> -4.6 (-8.29) <b>179</b> -4.6 (-9.8) <b>407/408</b> -4.3(-6.39) <b>57/58</b> -4.3 (-6.39) <b>266/267</b>
12p	58 (267)	dGdGdg	0.2393 (M)	0.3886 (S)	-5.4 (-10.71) 267 -4.8 (-10.71) 58	58A 267A	-5.1 (-10.38) <b>536/537</b> -5.0 (-6.39) <b>194/195</b> -4.8 (-8.3) <b>719</b> -4.3 (-7.89) <b>390/391</b> -4.4 (-6.39) <b>254/255</b>
13p	17 (118, 251, 684, 854)	dDdcDg	0.2387 (M)	0.1989 (M)	-4.2 (-10.49) 251 -3.9 (-10.49) 684 -3.9 (-10.49) 118 -1.8 (-8.47) 17 -1.8 (-8.47) 854	17G 118C 251C 684C 854A	-3.5 (-8.46) <b>107</b>
14p	121 (169, 210, 253, 389, 537, 575)	dGdDdg	0.2302 (M)	0.2805 (M)	-5.0 (-11.13) 537 -2.9 (-9.00) 121 -2.7 (-9.00) 210 -2.1 (-9.00) 253 -2.1 (-9.00) 169 -2.1 (-9.00) 389 -2.1 (-9.00) 575	121U 169A 210A 253G 389A 537C 575A	
15p	80 (275)	dDcUag	0.2241 (M)	-	-2.9 (-7.89) 80 -2.3 (-7.89) 275	80G 275A	
16p	141	dGdCdg	0.2072 (M)	0.1543 (M)	-4.6 (-9.83) 141	141A	-4.3 (-9.50) <b>250/251</b> -4.0 (-9.50) <b>117/118</b> -4.0 (-9.50) <b>683/684</b>
17p	60 (775)	cDdGdg	0.1966 (M)	0.5904 (S)	-6.0 (-11.62) 60 -5.2 (-9.49) 775	60C 775U	-4.0 (-9.10) <b>719</b>
18p	13 (436)	AcCcUg	0.1905 (M)	-	-7.6 (-10.18) 13 -7.1 (-10.18) 436	13A 436U	

19p	122 (180, 211, 411, 538, 730)	CdGdDg	0.1614 (M)	0.1508 (M)	-7.1 (-11.09) 180 -7.1 (-11.09) 730 -6.2 (-11.09) 411 -5.0 (-9.07) 211 -4.5 (-9.07) 122 -4.3 (-9.07) 538	122U 180C 211U 411C 538U 730C	-4.0 (-8.29) <b>148</b>
20p	405	dDgGdg	0.1302 (M)	-	-6.5 (-12.72) 405	405C	-4.0 (-8.47) <b>729</b> -4.0 (-8.47) <b>179</b>
21p	535	dDdGug	0.1254 (M)	-	-4.0 (-10.48) 535	535C	-3,7 (-8.45) <b>68</b>
22p	268	CdGdGg	0.1239 (M)	-	-6.7 (-9.86) 268	268U	-6.8 (-10.33) <b>179/180</b> -6.8 (-10.33) <b>729/730</b> -5.9 (-10.33) <b>410/411</b> -4.7 (-7.08) <b>211</b> -4.2 (-7.08) <b>122</b> -4.1 (-5.56) <b>58/59</b> -4.0 (-5.96) <b>538</b> -3.7 (-7.53) <b>148</b> -3.6 (-5.1) <b>163/164</b>
23p	108 (192)	GDdCdg	0.1126 (M)	-	-7.4 (-10.64) 108 -5.2 (-8.51) 192	108C 192U	-4.6 (-8.92) <b>250/251</b> -4.3 (-8.92) <b>683/684</b> -4.3 (-8.92) <b>117/118</b>
24p	53 (143, 720)	uDdGdg	-	1.000 (S)	-5.3 (-10.91) 720 -4.5 (-10.91) 53 -2.9 (-8.78) 143	53C 143G	-4.5 (-10.91) <b>59/60</b>
25p	61 (91)	GcDdGg	-	0.1904 (M)	-7.5 (-9.87) 91 -7.1 (-9.87) 61	61U 91G	
26p	62 (873)	DgCdDg	-	0.2114 (M)	-6.2 (-11.89) 62 -4.5 (-9.87) 873	62C 873U	
27p	63 (874)	GdGCdg	-	0.1555 (M)	-7.2 (-10.44) 63 -6.2 (-10.44) 874	63U 874U	-7.4 (-10.17) <b>107/108</b>
28p	66	GUgGdg	-	0.3200 (M)	-9.4 (-12.52) 66	66C	-5.0 (-7.27) <b>847</b>

29p	716	DgGdDg	-	0.1506 (M)	-6.4 (-12.19) 716	716C	-4.1 (-9.78) <b>148/149</b> -4.1 (-7.48) <b>729</b>
30p	736	CGdGdg	-	0.2762 (M)	-6.8 (-10.24) 736	736A	-5.7 (-12.37) <b>59/60</b> -4.4 (-9.10) <b>719</b> -4.3 (-6.17) <b>52</b>
31p	847	dUgGdg	-	0.3015 (M)	-4.7 (-10.46) 847	847A	
32p	868	dDgCdg	-	0.1824 (M)	-6.4 (-12.42) 868	868C	-4.2 (-9.11) <b>62</b> -4.2 (-6.76) 873/ <b>874</b> -3.9 (-7.59) <b>117/118</b> -3.5 (-7.69) <b>107</b> -3.2 (-5.39) <b>123/124</b>
33p	181*	CCdGdg	-	0.1133 (M)	-7.6 (-10.27) 181*	181U	-4.5 (-5.96) <b>730/731</b> -3.8 (-7.46) <b>122</b> -3.8 (-5.96) <b>105/106</b>

*a* – All sites mapped by microarray mapping are marked on Figure S3; binding sites of probes are denoted by the middle nucleotide of the complementary RNA region (or two nucleotides for probes with an even number of nucleotides); *b* – probes 1p-23p bind vRNA8 and part of them mini-vRNA8, probes 24p-33p bind only mini-vRNA8; *c* - in parenthesis are other fully complementary binding sites for the probe; sites in *italic* do not exist in mini-vRNA8; *d* - nucleotides in capital letter (A, C, G, U, D) are 2'-O-methyl-RNA nucleotides, in small letter (a, c, g, u, d) are LNA nucleotides, D and d are 2,6-diaminopurine (2'-O-methyl type or LNA, respectively); *e* - value:  $0.33 \leq$  strong (S),  $0.11 \leq$  medium (M)  $< 0.33$  and no binding (-)  $< 0.11$ . Condition: buffer A (300 mM NaCl, 5 mM MgCl<sub>2</sub>, 50 mM HEPES, pH 7.5), 37°C; *f* - calculated in RNAstructure program as RNA/RNA duplex and (in parenthesis) calculated considered modification of probe as modified probe/RNA duplex [1,2] the last number is the site of binding for which calculation was done; *g* - bolded are complementary sites of probe which binds strongly or moderately; \*- only mini-vRNA8 site.



**Table G. Deduced strong and medium binding sites in mini-vRNA8 for microarray probes.**

Probable binding sites <sup>a</sup>	Probe sequence <sup>b</sup>	Predicted $\Delta G^{\circ}_{37}$ of probe/vRNA8 duplex <sup>c</sup> (kcal/mol)	Sites of strong RNase H cleavage in vRNA8 <sup>d</sup>	Strength of binding <sup>e</sup>	Deduced sites	Comments
58	dGdGdg	-10.71	-	M	58	No alternative sites
68	dDgUgg	-12.37	70	S	68	No alternative sites
107 (117)	dDcDgg	-9.15/ -9.15	117	M	117	Strong RNase H cleavage at 117 in vRNA8, which is in the region of similar folding in mini-vRNA8 and vRNA8
141	dGdCdG	-9.83	143-144	M	141	Strong RNase H cleavage at 143-144 in vRNA8, which is in the region of similar folding in mini-vRNA8 and vRNA8
142	dDgDcg	-9.58	143-144	S	142	No alternative sites
163	dDgGug	-12.07	166-167	M	163	No alternative sites
847	dUgGdg	-10.46	-	M	847	No alternative sites

*a* - binding sites are denoted by the middle nucleotide of the complementary sequence of the target; *b* - nucleotides in capital letter (A, C, G, U, D) are 2'-O-methyl-RNA nucleotides, in small letter (a, c, g, u, d) - LNA nucleotides; D and d - 2,6-diaminopurine (2'-O-methyl type or LNA, respectively); *c*-  $\Delta G^{\circ}_{37}$  calculated as modified probe/RNA duplex [1,2]; *d* - vRNA8 nucleotide preceding RNase H cleavage. Cleavage within 3 nucleotides of probe site was considered confirmation of probe site, “-“ – not tested; *e* – symbols: S – strong binding, M – medium binding.

## References

1. Pasternak A, Kierzek E, Pasternak K, Fraczak A, Turner DH, and Kierzek R. The thermodynamics of 3'-terminal pyrene and guanosine for the design of isoenergetic 2'-O-methyl-RNA-LNA chimeric oligonucleotide probes of RNA structure. *Biochemistry* 2008;47: 1249-1258.
2. Kierzek E, Ciesielska A, Pasternak K, Mathews DH, Turner DH and Kierzek R. The influence of locked nucleic acid residues on the thermodynamic properties of 2'-O-methyl RNA/RNA heteroduplexes. *Nucleic Acids Res.* 2005; 33: 5082-5093.