Supplementary Material

Hexamer phasing governs transcription initiation in the 3'-leader of Ebola virus

Simone Bach[1], Nadine Biedenkopf[2], Arnold Grünweller[1], Stephan Becker[2], Roland K. Hartmann[1,#]

[1]Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, 35037 Marburg, Germany
[2]Institut für Virologie, Philipps-Universität Marburg, Hans-Meerwein-Str. 2, 35043 Marburg

Table S1: Primers used in this study for the construction of mutant minigenomes

no.	name	Cloning strategy 1: site-directed mutagenesis / plasmid template	primer fwd name	primer forward (5‘ to 3‘)	primer rev name	primer reverse (5‘ to 3‘)
		Overhang/inside-out primer insertion/substitution mutagenesis				
1	p3E5E_VP40	mutagenesis of p3E5E_Rluc_RC	pS_0006	**CGAGGTAGGTTTTTC**TTAATCTTC**A**TCATAGTTATTCGCACACAAAAGATCC	pS_0007	**GCTGAGAGAGTGTTTTTTCATTAACCTTCA**TCTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
2	p3E5E_GP	mutagenesis of p3E5E_ Rluc_RC	pS_0016	**CACTGTCGGC**TTAATCTTC**A**TCATAGTTATTCGCACACAAAAGATCC	pS_0017	**AGCGTAATCTTCA**TCTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
3	p3E5E_VP35	mutagenesis of p3E5E_ Rluc_RC	pS_0002	TAATCTTC**A**TCATAGTTATTCGCACACAAAAGATCC	pS_0003	**AAACCTTCA**TCTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
4	p3E5E_VP30	mutagenesis of p3E5E_ Rluc_RC	pS_0008	**AGATTACCTTTTTC**TTAATCTTC**A**TCATAGTTATTCGCACACAAAAGATCC	pS_0009	**TTCGATTATCTTTAATCTTCA**TCTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
5	p3E5E_L	mutagenesis of p3E5E_ Rluc_RC	pS_0012	**CCAATAAGCAGTTTTTC**TTAATCTTCCTC**A**TAGTTATTCGCACACAAAAGATCC	pS_0013	**GTCTTTCCGTGTTTTAGATGAAGCAGTTGAAATTC**TTCCTCTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
6	p3E5E_VP24	mutagenesis of p3E5E_ Rluc_RC	pS_0010	**GTTTATTCTTATCAGACCTCCGC**ATTAATCTTC**A**TCATAGTTATTCGCACACAAAAGATCC	pS_0011	**CTTATTATTCAGATTAGGCCCCAAGAGGCATTCTTCA**TCTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
7	p3E5E_VP35 +1 nt (stem)	mutagenesis of p3E5E_ Rluc_RC	pS_0002	TAATCTTC**A**TCATAGTTATTCGCACACAAAAGATCC	pS_0037	**AAACCTTCATC**CTCATTGAAATTTATATCGGAATTTAAATTGAAATTG
		Complementary primer mutagenesis				
8	p3E5E GP -2 (loop)	deletion mutagenesis of p3E5E_GP	pS_0098	GAGATGAAGATTACGCTC–TGTCGGCTTAATCTTCATCATAG	pS_0099	CTATGATGAAGATTAAGCCGACA–GAGCGTAATCTTCATCTC
9	p3E5E GP-2 (Δ2 nt PE1)	deletion mutagenesis of p3E5E_GP	pS_0096	CTGTCGGCTTAATCTTCATCA–GTTATTCGCACACAAAAGATCC	pS_0097	GGATCTTTTGTGTGCGAATAAC–TGATGAAGATTAAGCCGACAG
10	p3E5E VP24 +2 (stem)	insertion mutagenesis of p3E5E_VP24	pS_0092	CCGATATAAATTTCAATGA**TA**GATGAAGAATGCCTCTTGGGGCC	PS_0093	GGCCCCAAGAGGCATTCTTCATC**TA**TCATTGAAATTTATATCGG
11	p3E5E VP24 +2 (loop)	insertion mutagenesis of p3E5E_VP24	pS_0094	GGGGCCTAATCTGAATAATAA**GG**GGTTTATTCTTATCAGACCTCCGC	pS_0095	GCGGAGGTCTGATAAGAATAAACC**CC**TTATTATTCAGATTAGGCCCC
12	p3E5E_VP35 +1 (loop)	mutagenesis of p3E5E_VP35	pS_0110	CAATGAGATGAAGGT**C**TTTAATCTTCATCATAGTTATTCGC	pS_0111	GCGAATAACTATGATGAAGATTAAA**G**ACCTTCATCTCATTG
13	p3E5E_VP30 +4 (stem)	mutagenesis of p3E5E_VP30	pS_0108	CCGATATAAATTTCAATGA**AACC**GATGAAGATTAAAGATAATCG	pS_0109	CGATTATCTTTAATCTTCATC**GGTT**TCATTGAAATTTATATCGG
14	p3E5E_L+1 (stem)	mutagenesis of p3E5E_L	PS_0102	CCGATATAAATTTCAATGA**C**GAGGAAGAATTTCAACTGCTTCATC	PS_0103	GATGAAGCAGTTGAAATTCTTCCTC**G**TCATTGAAATTTATATCGG
15	p3E5E_L+1 (loop)	mutagenesis of p3E5E_L	PS_0104	CAACTGCTTCATCTAAAA**C**CACGGAAAGACCCAATAAGCAG	PS_0105	CTGCTTATTGGGTCTTTCCGTG**G**TTTTAGATGAAGCAGTTG
16	p3E5E_ NP G_{-72}	substitution mutagenesis of p3E5E_Rluc_RC	pS_229	CAATGAGAGGAA**G**ATTATTAATCTTCCTCATAGTTATTCGC	pS_230	GCGAATAACTATGAGGAAGATTAATAAT**C**TTCCTCTCATTG
17	p3E5E_NP-1 (stem)	deletion mutagenesis of p3E5E_Rluc_RC	pS_231	CCGATATAAATTTCAATG–GAGGAAAATTATTAATCTTCCTC	pS_232	GAGGAAGATTAATAATTTTCCTC–CATTGAAATTTATATCGG
18	p3E5E_NP-1 (loop)	deletion mutagenesis of p3E5E_Rluc_RC	pS_233	CAATGAGAGGAAAAT–ATTAATCTTCCTCATAGTTATTCGCAC	pS_234	GTGCGAATAACTATGAGGAAGATTAAT–ATTTTCCTCTCATTG
19	p3E5E_NP+1 (stem)	insertion mutagenesis of p3E5E_Rluc_RC	pS_235	CCGATATAAATTTCAATGA**A**GAGGAAAATTATTAATCTTCCTC	pS_236	GAGGAAGATTAATAATTTTCCTC**T**TCATTGAAATTTATATCGG
20	p3E5E_NP+1 (loop)	insertion mutagenesis of p3E5E_Rluc_RC	pS_237	CAATGAGAGGAAAAT**A**TATTAATCTTCCTCATAGTTATTCGCAC	pS_238	GTGCGAATAACTATGAGGAAGATTAATA**T**ATTTTCCTCTCATTG
21	RD_p3E5E_ NP-1 (stem)	deletion mutagenesis of p3E5E_Rluc_RD	pS_231	CCGATATAAATTTCAATG–GAGGAAAATTATTAATCTTCCTC	pS_232	GAGGAAGATTAATAATTTTCCTC–CATTGAAATTTATATCGG
22	RD_p3E5E_ NP-1 (loop)	deletion mutagenesis of p3E5E_Rluc_RD	pS_233	CAATGAGAGGAAAAT–ATTAATCTTCCTCATAGTTATTCGCAC	pS_234	GTGCGAATAACTATGAGGAAGATTAAT–ATTTTCCTCTCATTG
23	RD_p3E5E_ NP+1 (stem)	insertion mutagenesis of p3E5E_Rluc_RD	pS_235	CCGATATAAATTTCAATGA**A**GAGGAAAATTATTAATCTTCCTC	pS_236	GAGGAAGATTAATAATTTTCCTC**T**TCATTGAAATTTATATCGG
24	RD_p3E5E_ NP+1 (loop)	insertion mutagenesis of p3E5E_Rluc_RD	pS_237	CAATGAGAGGAAAAT**A**TATTAATCTTCCTCATAGTTATTCGCAC	pS_238	GTGCGAATAACTATGAGGAAGATTAATA**T**ATTTTCCTCTCATTG
25	p3E5E_ NP U_{-75}	substitution mutagenesis of p3E5E_Rluc_RC	pS_252	CCGATATAAATTTCAATGAGAG**T**AAAATTATTAATCTTCCTCATAG	pS_253	CTATGAGGAAGATTAATAATTTT**A**CTCTCATTGAAATTTATATCGG
26	p3E5E_ NP U_{-75}/G_{-72}	substitution mutagenesis of p3E5E_NP stab.	pS_250	CCGATATAAATTTCAATGAGAG**T**AAGATTATTAATCTTCCTCATAG	pS_251	CTATGAGGAAGATTAATAATCTT**A**CTCTCATTGAAATTTATATCGG
27	RD_p3E5E_ NP U_{-75}	substitution mutagenesis of p3E5E_Rluc_RD	pS_252	CCGATATAAATTTCAATGAGAG**T**AAAATTATTAATCTTCCTCATAG	pS_253	CTATGAGGAAGATTAATAATTTT**A**CTCTCATTGAAATTTATATCGG
28	RD_p3E5E_ NP U_{-75}/G_{-72}	substitution mutagenesis of p3E5E_Rluc_RD	pS_250	CCGATATAAATTTCAATGAGAG**T**AA**G**ATTATTAATCTTCCTCATAG	pS_251	CTATGAGGAAGATTAATAAT**C**TT**A**CTCTCATTGAAATTTATATCGG
		Inside-out primer deletion mutagenesis				
29	RD_p3E5E_ VP30	mutagenesis of p3E5E_VP30	pS_0029	TTTCCAGGAATCCTTTTTGCAACG	pS_0030	ACTATAGTGAGTCGTATTAACCCGGGATCG
30	RD_p3E5E_ VP40	mutagenesis of p3E5E_VP40	pS_0029	TTTCCAGGAATCCTTTTTGCAACG	pS_0030	ACTATAGTGAGTCGTATTAACCCGGGATCG
31	RD_p3E5E_ VP35	mutagenesis of p3E5E_VP35	pS_0029	TTTCCAGGAATCCTTTTTGCAACG	pS_0030	ACTATAGTGAGTCGTATTAACCCGGGATCG
32	RD_p3E5E_ GP	mutagenesis of p3E5E_GP	pS_0029	TTTCCAGGAATCCTTTTTGCAACG	pS_0030	ACTATAGTGAGTCGTATTAACCCGGGATCG
33	RD_p3E5E_ VP35+1 (stem)	mutagenesis of p3E5E_VP35+1 (stem)	pS_0029	TTTCCAGGAATCCTTTTTGCAACG	pS_0030	ACTATAGTGAGTCGTATTAACCCGGGATCG
no.	**name**	**Cloning strategy 2: restriction cloning** 1) PCR with respective replication-competent plasmid (no. 8, 9, 12, 13) as template 2) restriction cloning with BamHI and BstBI into vector p3E5E_Rluc_RD	**primer fwd name**	**primer fwd (5‘ to 3‘)**	**primer rev name**	**primer rev (5‘ to 3‘)**

34	RD_p3E5E GP-2 (loop)		pS_1	CTTTCGAAGTCATGGTGGTATGAGA C	pS_2	GTTAGCAGCCGGATCCTTTTTTTTG
35	RD_p3E5E GP-2 (Δ2 nt PE1)		pS_1	CTTTCGAAGTCATGGTGGTATGAGA C	pS_2	GTTAGCAGCCGGATCCTTTTTTTTG
36	RD_VP35+1 (loop)		pS_1	CTTTCGAAGTCATGGTGGTATGAGA C	pS_2	GTTAGCAGCCGGATCCTTTTTTTTG
37	RD_VP30+4 (stem)		pS_1	CTTTCGAAGTCATGGTGGTATGAGA C	pS_2	GTTAGCAGCCGGATCCTTTTTTTTG

The different PCR-based strategies for site-directed mutagenesis are illustrated in Fig. S2 (except for the classical restriction cloning). The EBOV wt minigenome pANDY 3E5E is termed p3E5E_Rluc_RC; RC indicates the replication-competent and RD replication-deficient minigenomes. Hyphens in primer sequences mark nucleotide deletions, boldface underlined nucleotides substitutions or insertions.

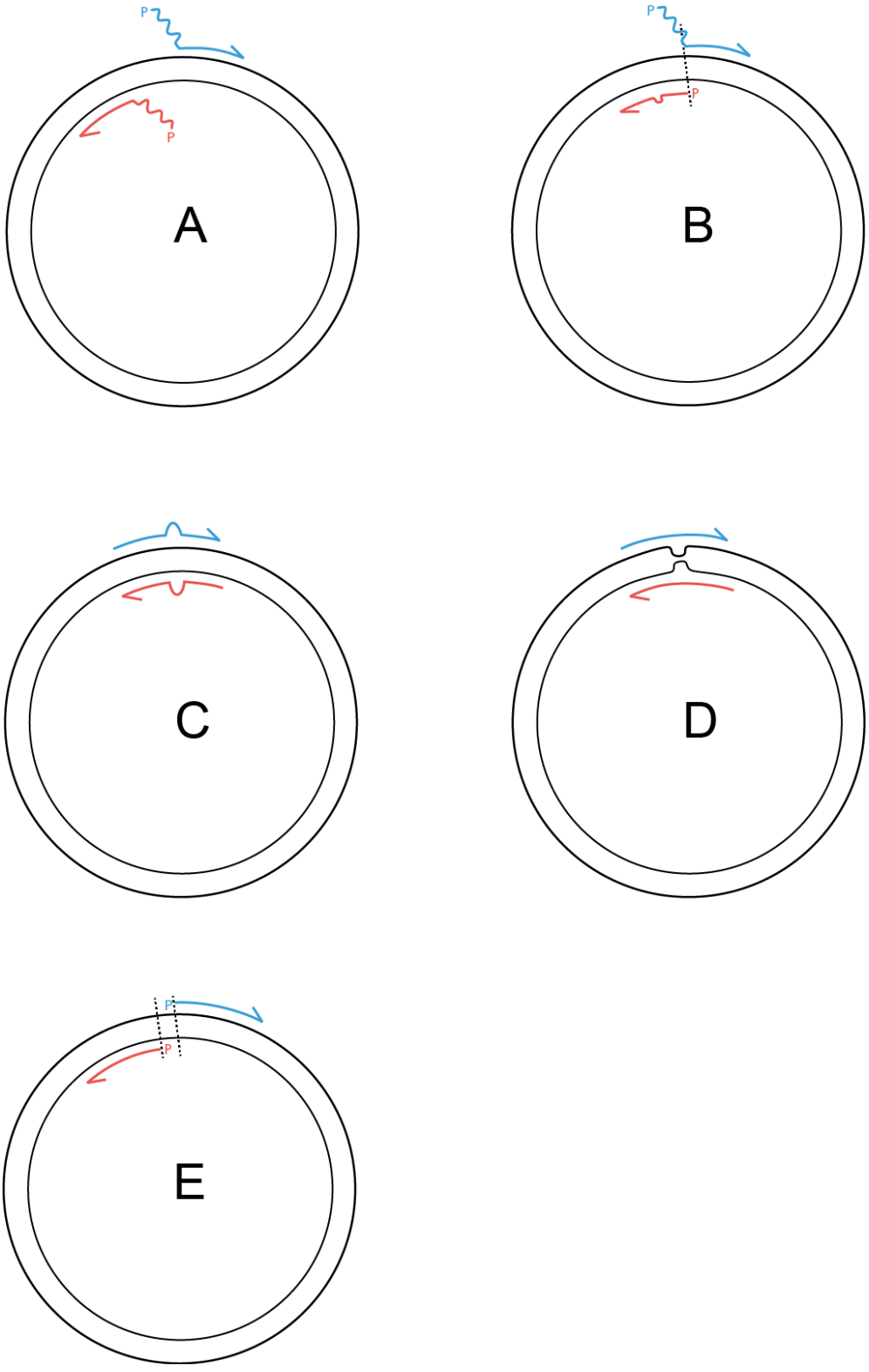

Fig. S1: PCR-based strategies (essentially performed as previously described, Li et al., 2009) that were used for the construction of mutant minigenomes. (**A, B**) Overhang/inside-out primer insertion/substitution mutagenesis. (**C, D**) Complementary primer mutagenesis; insertions and substitutions were introduced according to scheme C and deletions according to scheme D. (**E**) Inside-out primer deletion mutagenesis. For more information, see also the NEB web site for the Q5 Site-Directed Mutagenesis Kit (https://www.neb-online.de/en/pcr-and-dna-amplification/q5-site-directed-mutagenesis-kit/). In approaches A, B and E, the

entire plasmid is amplified with 5‘-phosphorylated primers that introduce the desired mutations/substitutions/insertions/deletions, followed by circularization of PCR products and template removal by Dpn I treatment before bacterial transformation. In approaches C and D, phosphorylation of 5'-ends and ligation are carried out by bacterial enzymes after DNA transformation.

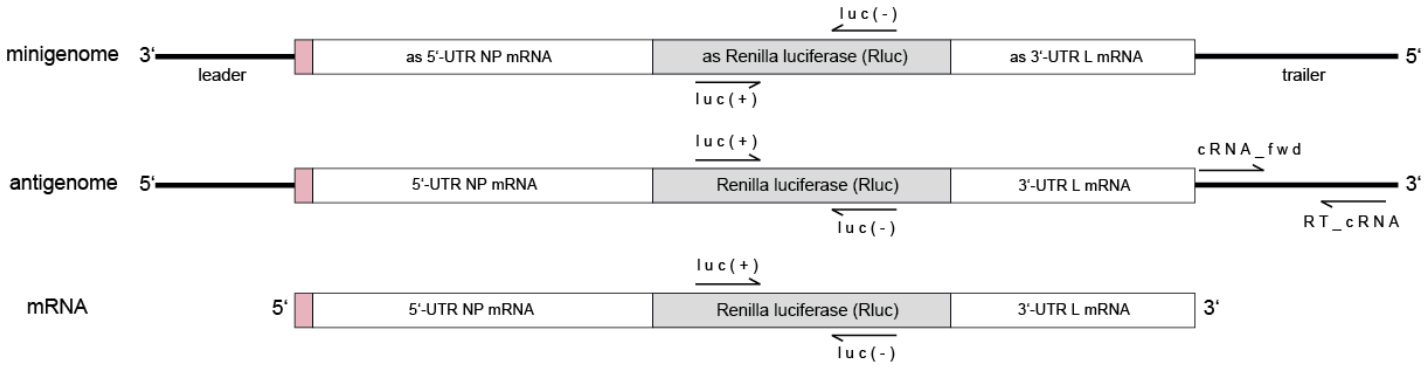

Fig. S2: Schematic representation of primers used for qRT-PCR of viral RNA species; as, antisense. See Fig. 2A of the main text for the color code.

Reference

Li D, Willkomm DK, Hartmann RK. 2009. Minor changes largely restore catalytic activity of archaeal RNase P RNA from *Methanothermobacter thermoautotrophicus*. *Nucleic Acids Res* **37**:231-242.