

Supplementary Table 1: Initial screening of the ϕ 6 RdRP mutants

Plasmid ^o	Mutation	Mutation site	Initial selection [†]			
			Clone obtained	Expression level	Purification	Polymerase activity
-	E172Q	plough	-			
-	R291S	specificity pocket	-			
-	K451A	specificity pocket	-			
-	K627L	rim of the template tunnel	-			
-	H642K	specificity pocket	-			
pNL7#4	R146L	rim of the template tunnel	x	-		
pNL6#5	K147L	rim of the template tunnel	x	-		
pNL2#3	E165Q	plough	x	-		
pNL16#1	K451L	specificity pocket	x	-		
pNL11#2	K627A	rim of the template tunnel	x	-		
pNL10#2	E652Q	plough	x	-		
pNL15#1	L628S	specificity pocket	x	+	-	
pNL1#1	M646V	specificity pocket	x	+	-	
pNL12#5	E139Q	plough	x	+	+++	+
pNL3#3	E165A	plough	x	++	+++	+
pNL4#7	E168Q	plough	x	+	+++	+
pNL8#7	K541A	rim of the template tunnel	x	+	+++	+
pNL9#4	K541L	rim of the template tunnel	x	++	+++	+
pNL19#1	R30A	rim of the template tunnel	x	++	+++	++
pNL20#1	R291K	specificity pocket	x	++	+++	+
pNL14#1	T633A	specificity pocket	x	+	+++	+
pNL13#1	E634Q	specificity pocket	x	+	+++	++

[†] Legend: - = no, x = yes, + = poor, ++ = moderate, +++ = good

^o ϕ 6 RdRP mutants shaded grey were selected for further biochemical characterisation

Supplementary Table 2: Oligonucleotides used in this study

Oligonucleotide	Sequence (5' – 3')	Description (ref.)
1. p2_E139Q_up	DNA CGGCTTATGTTTTCAGATCTACAGCCGGTACCCTGAAGATCCG	
2. p2_E139Q_down	DNA CCGATCTTCAGCGGTACCGGCTGTAGATCTGAAAACATAAGCCG	
3. p2_E165A_up	DNA GGAACGAAGATCGCGATCGCCGAGCGCTCTCGAGAAAAGCGG	
4. p2_E165A_down	DNA CCGCTTCTCGAGAGCGCGCTCGGCGATCGCGATCTTCGTTCC	
5. p2_E165Q_up	DNA GGAACGAAGATCCAGATCGCCGAGCGCGCTCTCGAGAAAAGCGG	
6. p2_E165Q_down	DNA CCGCTTCTCGAGAGCGCGCTCGGCGATCTGGATCTTCGTTCC	
7. p2_E168Q_up	DNA CGAAGATCGAGATCGCCGAGCGGCTCTCGAGAAAAGCGG	
8. p2_E168Q_down	DNA CCGCTTCTCGAGAGCGCGCTGGGCGATCTCGATCTTCG	
9. p2_E172Q_up	DNA CGCCGAGCGCGCTCTGCAGAAAAGCGGAAGAAGC	
10. p2_E172Q_down	DNA GCTTCTTCCGCTTCTGCAGAGCGCGCTCGGCG	
11. p2_E652Q_up	DNA GGTACTGATGCACGGCGTATCGGTCCAAAAGACTGAGCG	
12. p2_E652Q_down	DNA CGCTCAGTCTTTTGGACCGATACGCCGTGCATCAGTACC	
13. p2_R146L_up	DNA GTTTTCAGATCTCGAGCCTGTTCCGCTGAAGATCCTTAAAGGATCGTC	
14. p2_R146L_down	DNA GACGATCCTTTAAGGATCTTCAGCGGAACAGGCTCGAGATCTGAAAAC	
15. p2_K147L_up	DNA GTTTTCAGATCTCGAGCCTGTTCCGCTGAAGATCCGCTAGGATCGTC	
16. p2_K147L_down	DNA GACGATCCTAGACGGATCTTCAGCGGAACAGGCTCGAGATCTGAAAAC	
17. p2_K541A_up	DNA CCAATCGGGCGTACGCGACCGATCTGCGCGCAACCG	
18. p2_K541A_down	DNA CCGTTTGCAGCGATCGGTCGCGTACGCCCGATTGG	
19. p2_K541L_up	DNA CCAATCGGGCGTACGCGACCGATCTCTGCGCAACCG	
20. p2_K541L_down	DNA CCGTTTGCAGCGATCGGTCGCGTACGCCCGATTGG	
21. p2_K627A_up	DNA GTGCTTGCTGACCCGAACGCACTGCAGTATAAGTGGACCG	
22. p2_K627A_down	DNA CGGTCCACTTATACTGCAGTGCCTTCGGGTGAGCAAGCAC	
23. p2_K627L_up	DNA GTGCTTGCTGACCCGAACCTACTGCAGTATAAGTGGACCGAGG	
24. p2_K627L_down	DNA CCTCGGTCCACTTATACTGCAGTAGGTTCCGGTGCAGCAAGCAC	
25. p2_R30A_up	DNA GCCCAACAAGCCTCGAAGGCTAGCTTCAAAGAGGGGGCGATTGAAACG	
26. p2_R30A_down	DNA CGTTTCAATCGCCCCCTCTTTGAAGCTAGCCTTCGAGGCTTGTGGGC	
27. p2_R291K_up	DNA GCGCAACCTGTGAAAACAAAATTTATTCGAAGTACGCTTACACC	
28. p2_R291K_down	DNA GGTGTAAGCGTACTTCAATAAAATTTGTTTTTTCACAGGTTGCGC	
29. p2_R291S_up	DNA GCGCAACCTGTGTCAAACAAAATTTATTCGAAGTACGCTTACACC	
30. p2_R291S_down	DNA GGTGTAAGCGTACTTCAATAAAATTTGTTTTGACACAGGTTGCGC	
31. p2_K451A_up	DNA GCCAAGCATAGCATCATCAGATGCTGAGATCTGACGGATCTCCTCG	
32. p2_K451A_down	DNA GCCAAGCATAGCATCATCAGATGCTGAGATCTGACGGATCTCCTCG	
33. p2_K451L_up	DNA GCCAAGCATAGCATCATCAGATGCTGAGATCTGACGGATCTCCTCG	
34. p2_K451L_down	DNA CGAGGAGATCCGTCAGATCTCACTATCTGATGATGCTATGCTTGGC	
35. seq3_p2	DNA CCAGTTCAGCCCTGAGTACGGTGT	
36. p2_L628S_down	DNA GCCATGCATCAGTACCTCGTGGATATTCGCCGAGACGTCGGCCTCGGTCCACT-TATACTGGGATTTGTTCCGG	
37. p2_T633A_down	DNA GCCATGCATCAGTACCTCGTGGATATTCGCCGAGACGTCGGCCTCGGCCACT-TATAC	
38. p2_E634Q_down	DNA GCCATGCATCAGTACCTCGTGGATATTCGCCGAGACGTCGGCCTGAGTCCACTT	
39. p2_H642K_down	DNA GCCATGCATCAGTACTTCTTGATATTCGCCGAGA	
40. p2_M646V_down	DNA GGCCCTAGGCATTACAGAACGAGAAAACCGCTCAGTCTTTTCGACCGATACGC-CATGCACCAGTACCTCGT	
41. T7-1	DNA CGCGTAATACGACTCACTATAG	Upstream (1)
42. 3'end	DNA AGAGAGAGAGCCCCCGA	Downstream (2)
43. 3'end_1	DNA AAGAGAGAGAGCCCCCGA	Downstream (2)
44. 3'end_2	DNA CAGAGAGAGAGCCCCCGA	Downstream (2)
45. 3'end_3	DNA GAGAGAGAGAGCCCCCGA	Downstream (2)
46. 3'end_4	DNA TAGAGAGAGAGCCCCCGA	Downstream (2)
47. pT7_3'end	DNA TAAGCTTGGGCTGCAGGT	Downstream (2)
48. pT7_3'end_1	DNA ATAAGCTTGGGCTGCAGGT	Downstream (2)
49. pT7_3'end_2	DNA CTAAGCTTGGGCTGCAGGT	Downstream (2)
50. pT7_3'end_3	DNA GTAAGCTTGGGCTGCAGGT	Downstream (2)
51. pT7_3'end_4	DNA TTAAGCTTGGGCTGCAGGT	Downstream (2)
52. T7-Phi6_2628-2650_fwd	DNA CGCGTAATACGACTCACTATAGGGACACGGAAGTTATTGAAGCAGC	
53. anti-S_2929-2948	RNA AGAGAGAGAGCCCCGAAGG	
54. anti-S_2928-2947	RNA GAGAGAGAGCCCCGAAGGG	
55. anti-S_2927-2946	RNA AGAGAGAGCCCCGAAGGGG	
56. anti-S_2926-2945	RNA GAGAGAGCCCCGAAGGGGC	
57. anti-S_2925-2944	RNA AGAGAGCCCCGAAGGGGCC	
58. anti-S_2924-2943	RNA GAGAGCCCCGAAGGGGCCG	
59. anti-S_2923-2942	RNA AGAGCCCCGAAGGGGCCGU	
60. anti-S_2919-2938	RNA CCCCAGAGGGGCCGUCCUUAU	
61. anti-S_2909-2928	RNA GCCGUCCUUAUUGGACGCUCA	
62. anti-S_2889-2908	RNA ACAUAGUGAGUCUCGCCUUG	
63. anti-S_2729-2748	RNA UAGAUUUCGAUCAGAUCAAG	
64. anti-S_2529-2548	RNA GAGACUGGCAUUCACAUUC	

Oligonucleotide	Sequence (5' – 3')	Description (ref.)
65. anti-S_117-136	RNA CGACUCAUGGACCUUGGGAG	
66. anti-luc_1797-1816	RNA UAAGCUUGGGCUGCAGGUCG	
67. anti-luc_1792-1811	RNA UUGGGCUGCAGGUCGACCUC	
68. anti-luc_1787-1806	RNA CUGCAGGUCGACCUCGAGGC	
69. anti-luc_1782-1801	RNA GGUCGACCUCGAGGCCUCGG	
70. anti-luc_1777-1796	RNA ACCUCGAGGCCUCGGAGGAU	
71. anti-luc_1772-1791	RNA GAGGCCUCGGAGGAUUACAA	
72. anti-luc_1762-1781	RNA AGGAUUACAAUAGCUAAGAA	

1. Makeyev, E. V. and Bamford, D. H. (2000b) The polymerase subunit of a dsRNA virus plays a central role in the regulation of viral RNA metabolism. *EMBO J.*, **19**, 6275-84.
2. Makeyev, E. V. and Bamford, D. H. (2001) Primer-independent RNA sequencing with bacteriophage $\phi 6$ RNA polymerase and chain terminators. *RNA.*, **7**, 774-81.