

Supplement Information

Rapid Detection of Avian Leukosis Virus Subgroup J by Cross-priming Amplification

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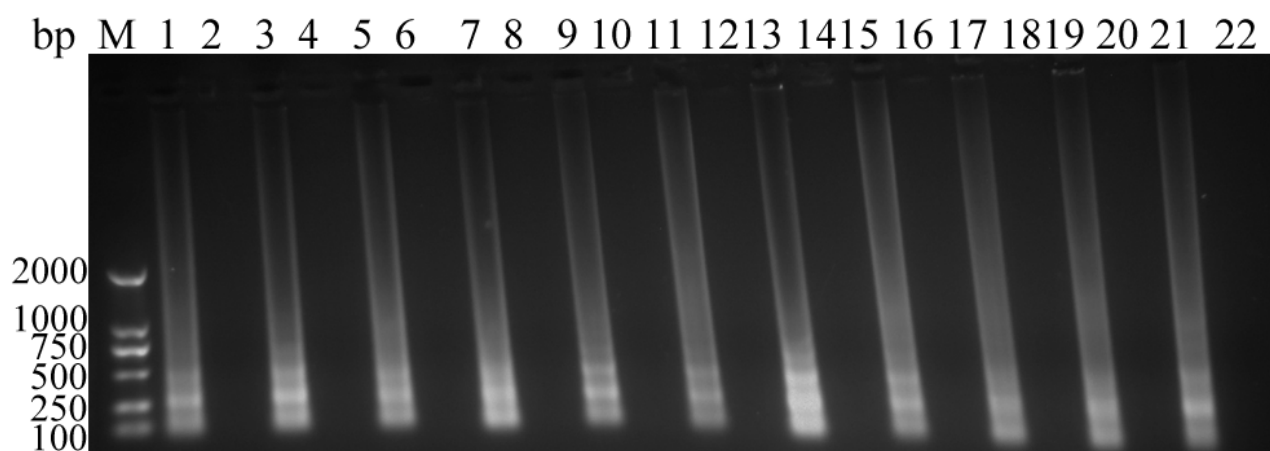
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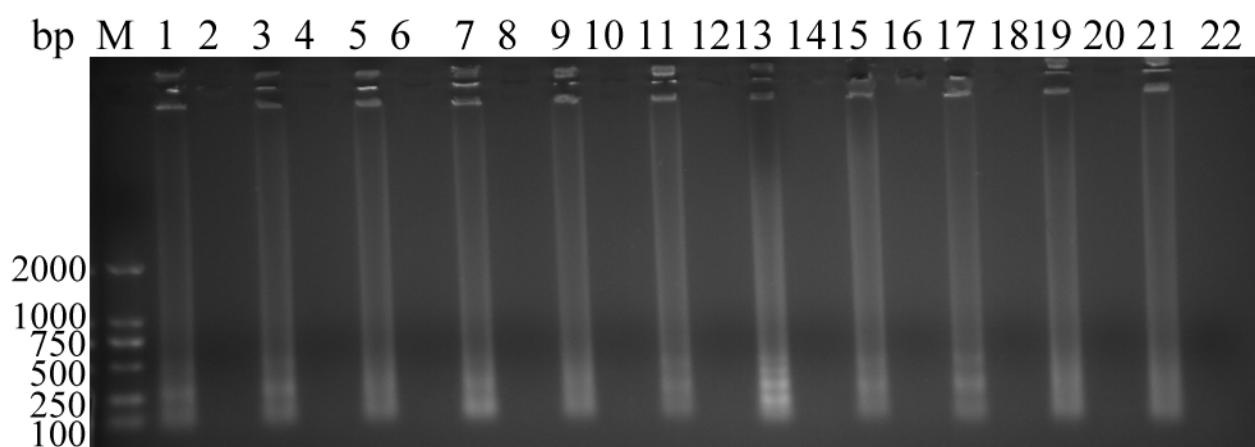
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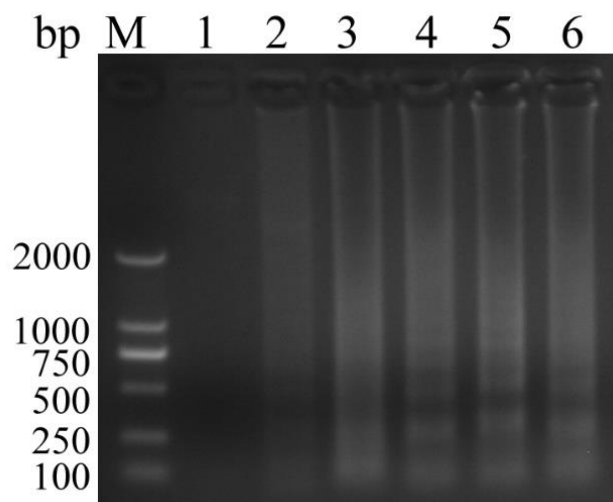
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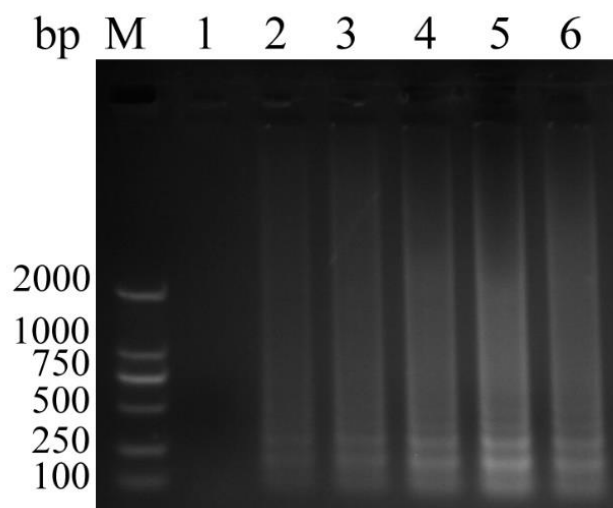
Supplementary Figure S1 Analysis of ALV-J CPA at different concentration of primers (Table 1) by agarose gel electrophoresis (repeated analyze). Lane M, DNA marker; 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, were primers groups 1-11 (Table 1) respectively; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 were negative controls for the corresponding primers groups. The full-length gel is presented in Supplementary Figure S26.



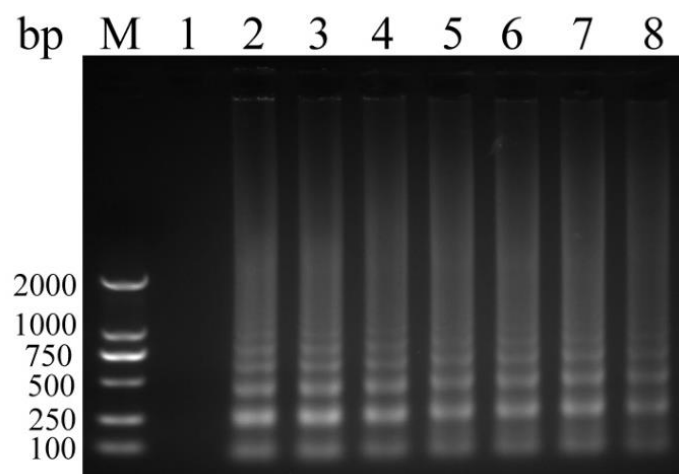
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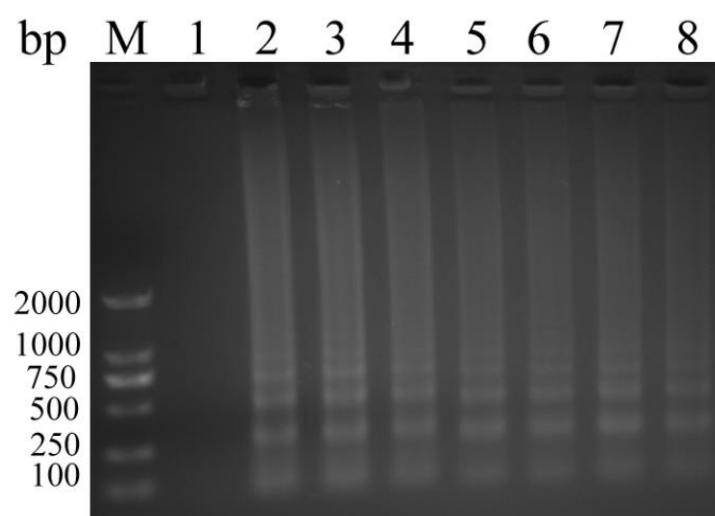
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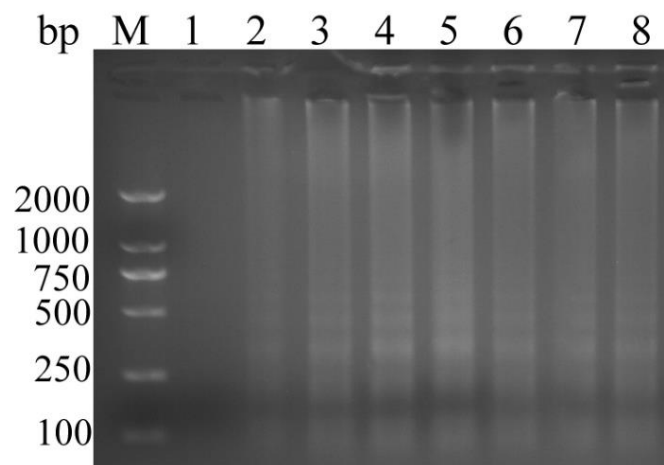
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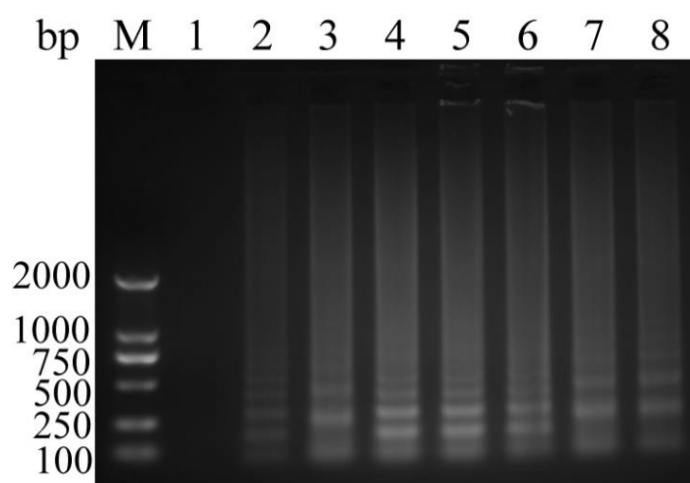
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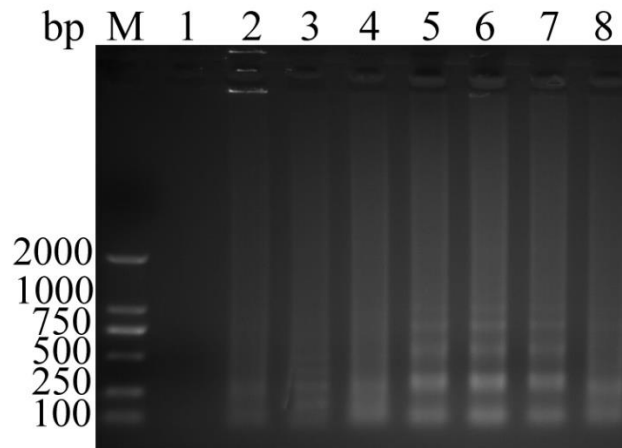
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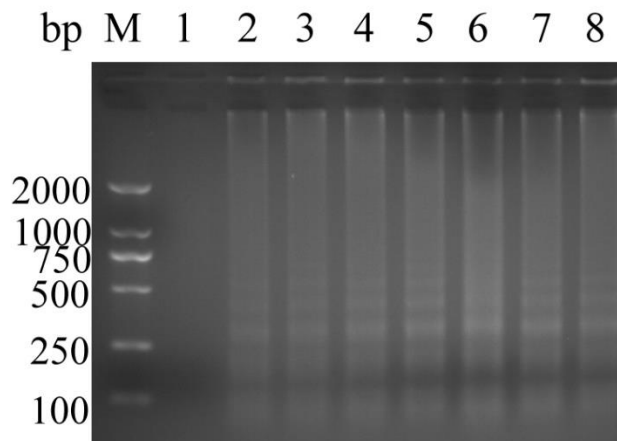
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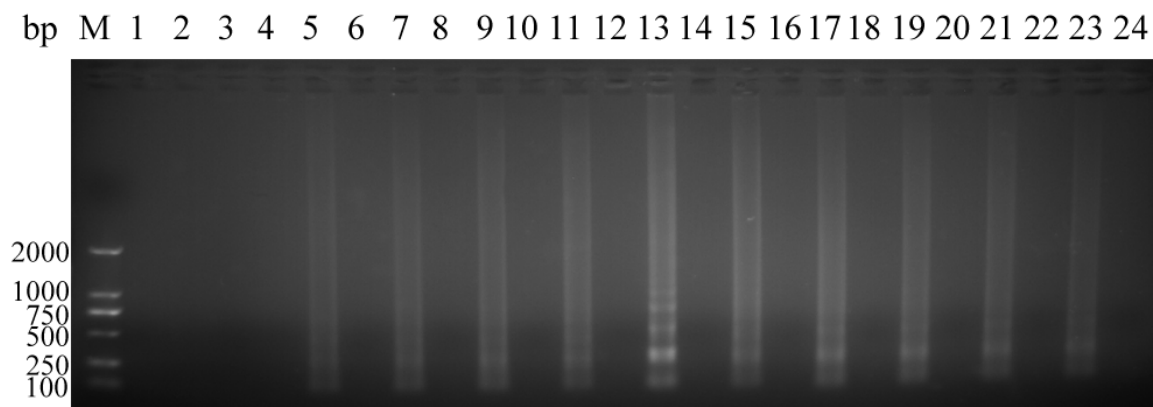
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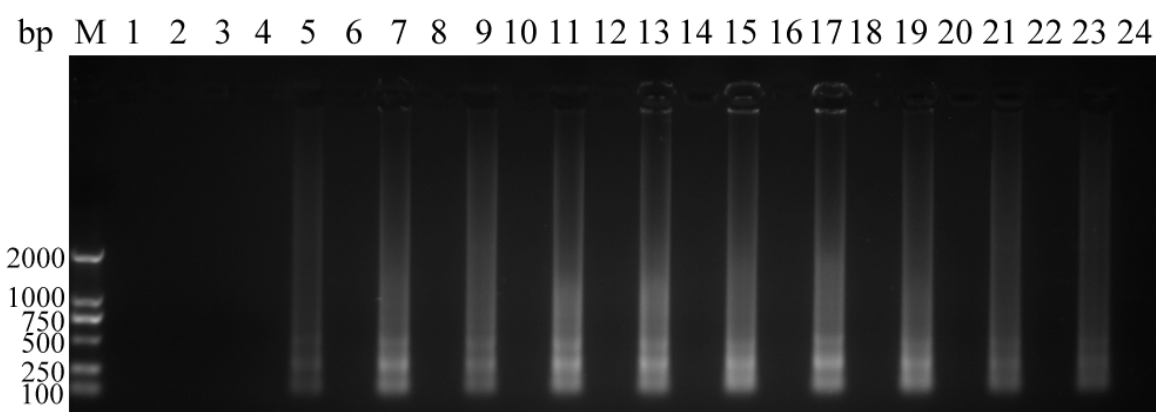
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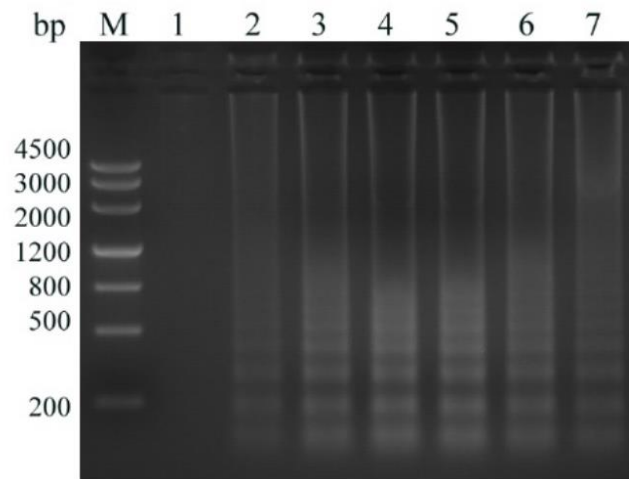
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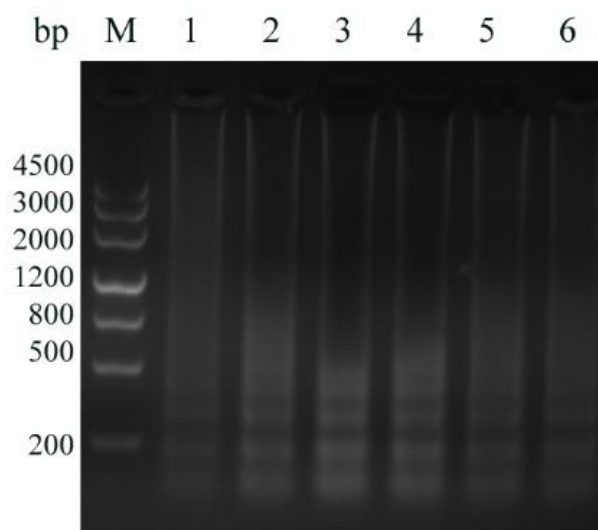
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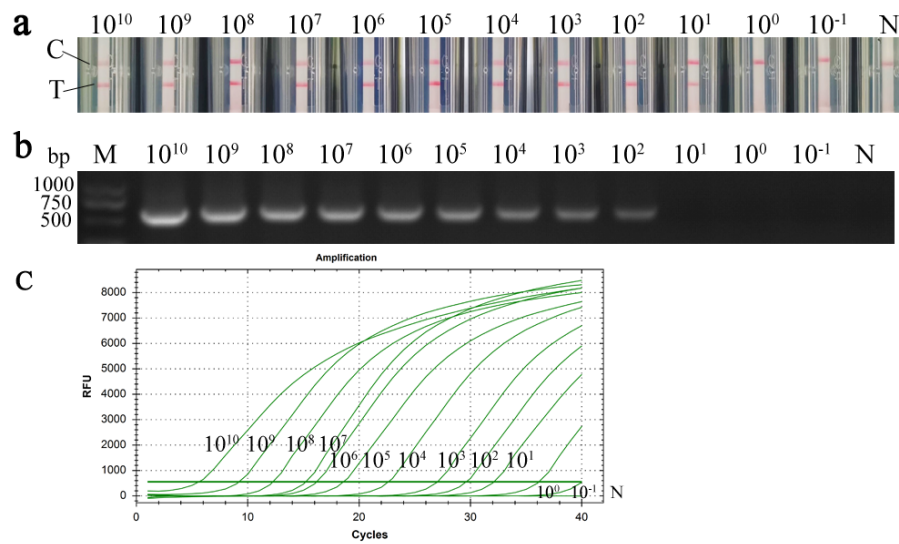
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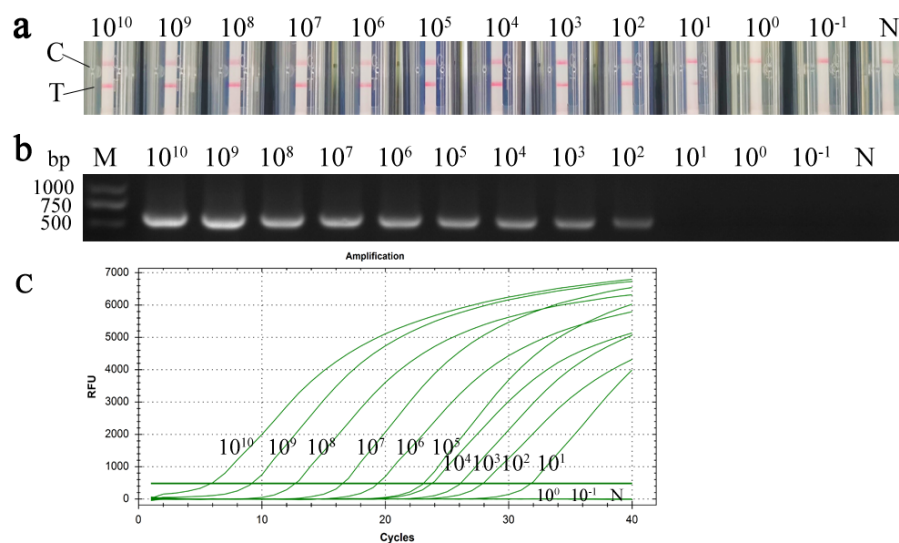
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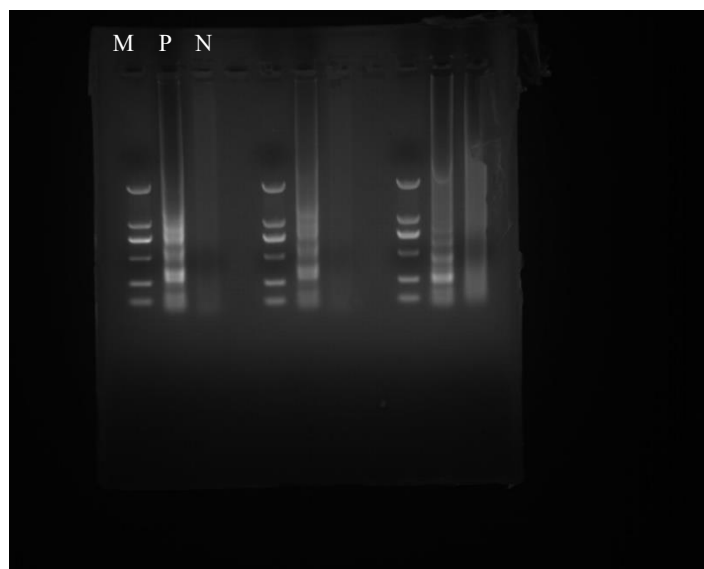
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Supplementary Figure S16 Comparison of sensitivity between the CPA, PCR and RT-PCR of ALV-J detect methods based on recombinant plasmid standards at different copy numbers ($1.86 \times 10^{-1} \sim 1.86 \times 10^{10}$ copies μL^{-1}) (repeated analyze). **(a)** Sensitivity of CPA based on a nucleic acid detection device; **(b)** Sensitivity of conventional PCR; The full-length gel is presented in Supplementary Figure S41. **(c)** Sensitivity of RT-PCR for ALV-J. C, control-line; T, test-line; N, negative control.

The full-length gels used in the main article which were cropped:

Supplementary Figure S17



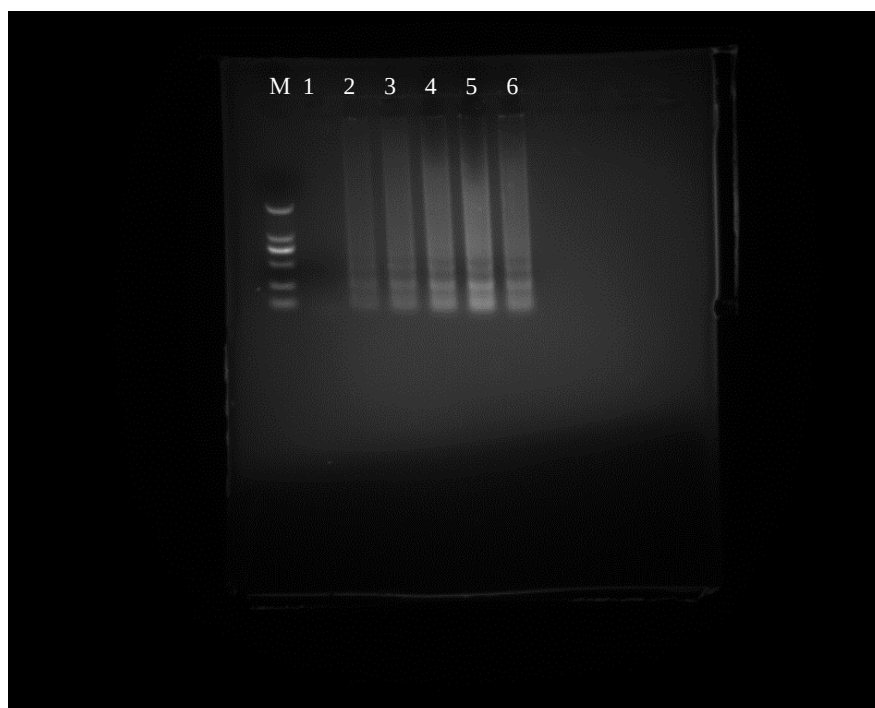
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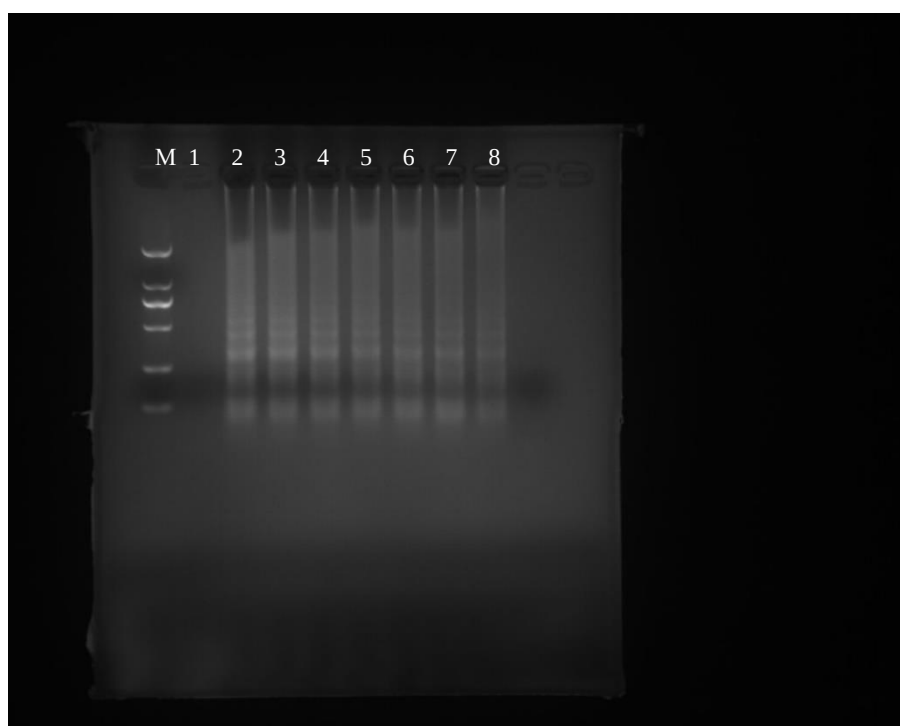
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Supplementary Figure S19



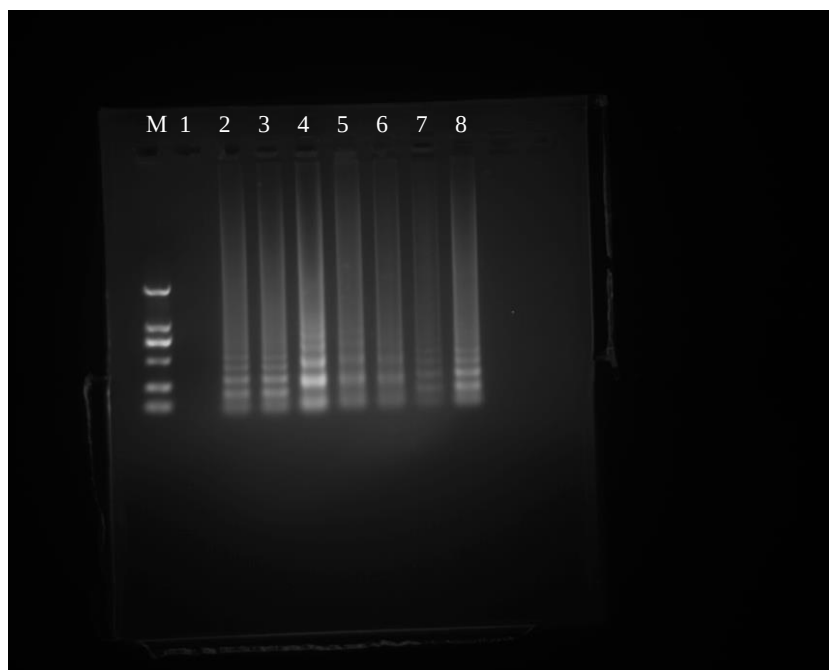
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Supplementary Figure S20



Supplementary Figure S20 The full-length gel of Fig. 4 in the main article. Analysis of ALV-J CPA at different concentration of Betaine by agarose gel electrophoresis. Lane M, DNA marker; 1, 0 mol L^{-1} ; 2, 0.2 mol L^{-1} ; 3, 0.4 mol L^{-1} ; 4, 0.6 mol L^{-1} ; 5, 0.8 mol L^{-1} ; 6, 1.0 mol L^{-1} ; 7, 1.2 mol L^{-1} ; 8, 1.4 mol L^{-1} .

Supplementary Figure S21



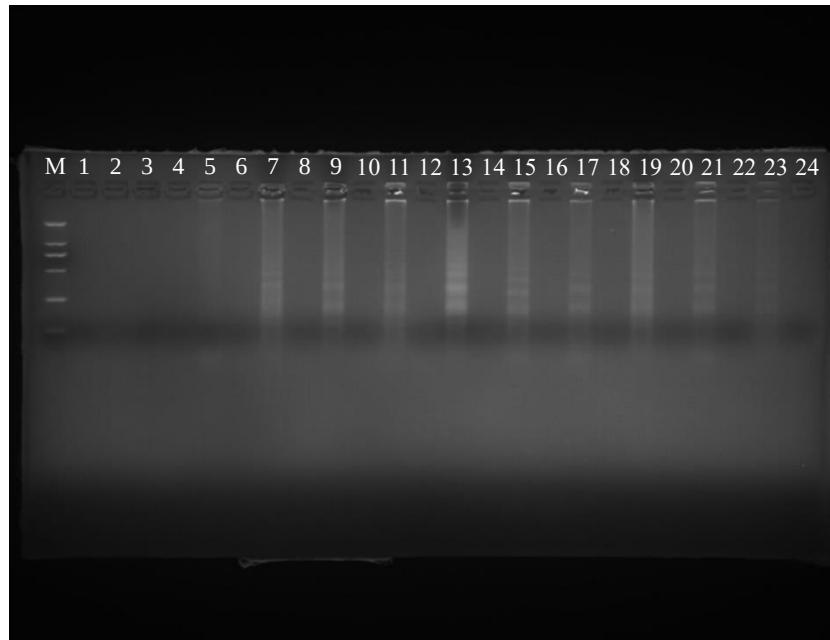
Supplementary Figure S21 The full-length gel of Fig. 5 in the main article. Analysis of ALV-J CPA at different concentration of dNTPs by agarose gel electrophoresis. Lane M, DNA marker; 1, 0 mmol L⁻¹; 2, 0.2 mmol L⁻¹; 3, 0.4 mmol L⁻¹; 4, 0.6 mmol L⁻¹; 5, 0.8 mmol L⁻¹; 6, 1.0 mmol L⁻¹; 7, 1.2 mmol L⁻¹; 8, 1.4 mmol L⁻¹.

Supplementary Figure S22



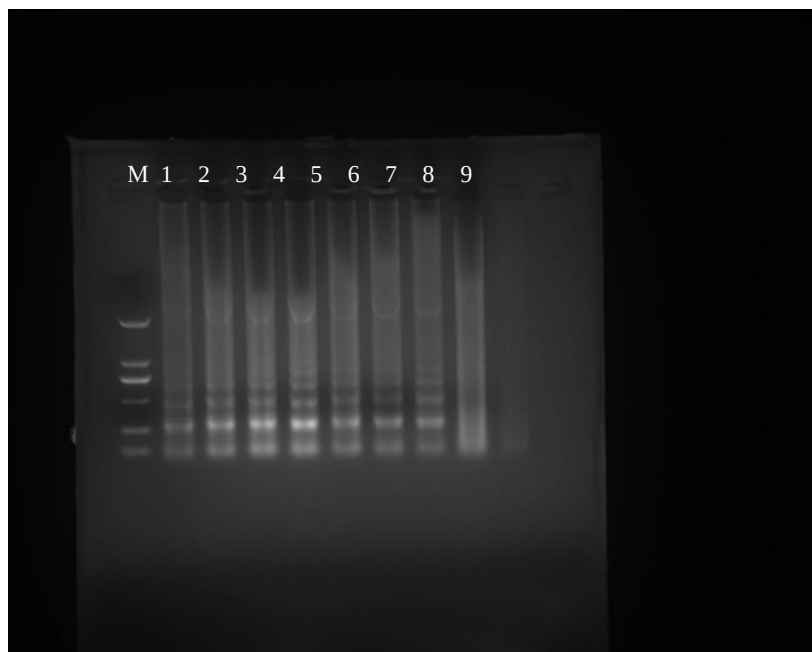
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Supplementary Figure S23



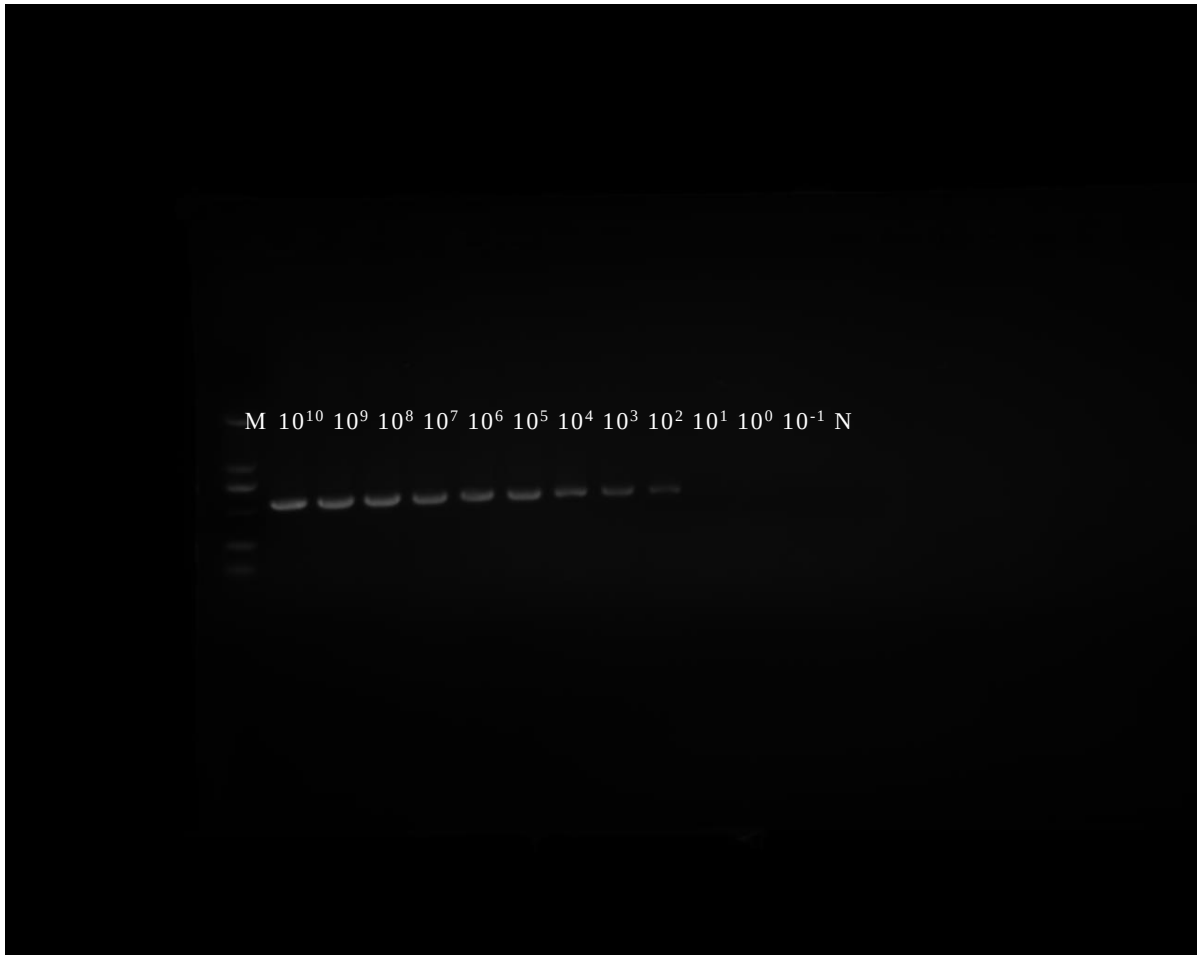
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Supplementary Figure S24



Supplementary Figure S24 The full-length gel of Fig. 8 in the main article. Analysis of ALV-J CPA with different reaction times by agarose gel electrophoresis. Lane M, DNA marker; 1, 15 min; 2, 30 min; 3, 45 min; 4, 60 min; 5, 75 min; 6, 90 min; 7, 105 min; 8, 120 min; 9, Negative control amplification for 120 min.

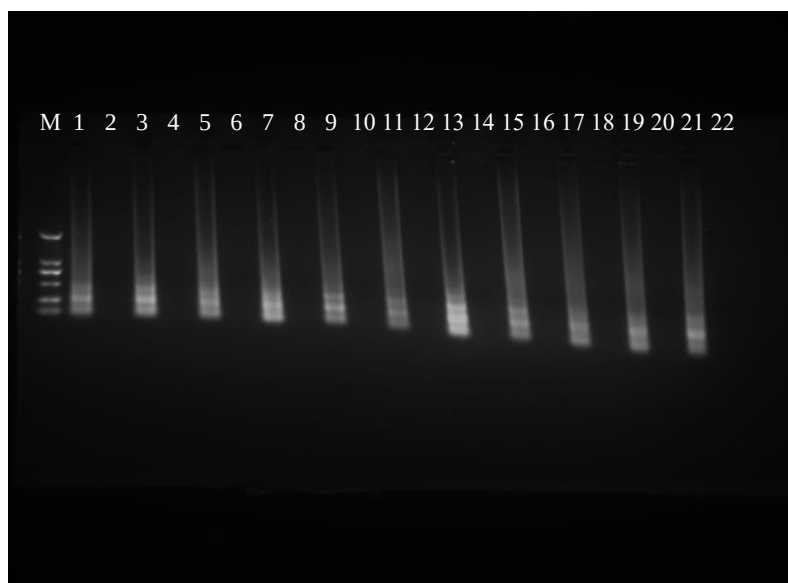
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Supplementary Figure S25 The full-length gel of Fig. 10b in the main article. Sensitivity of conventional PCR. Lane M, DNA markers; N, negative control.

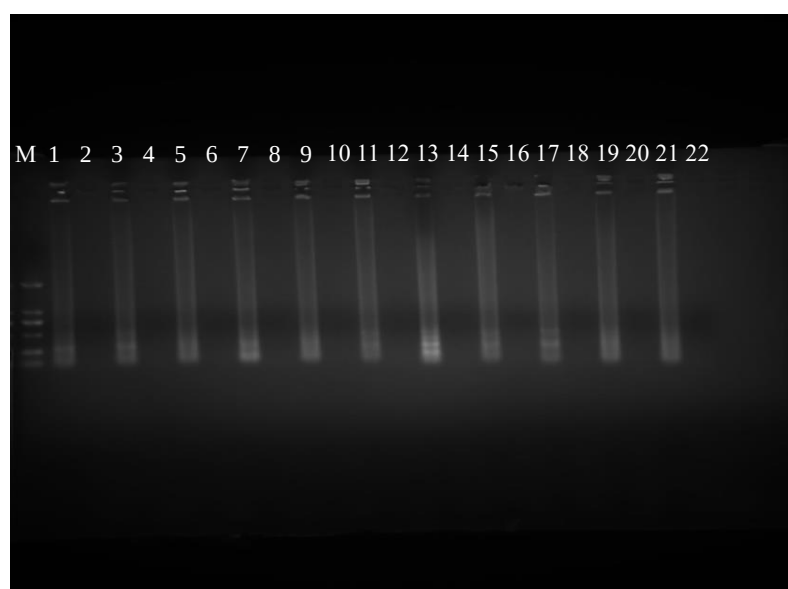
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Supplementary Figure S26



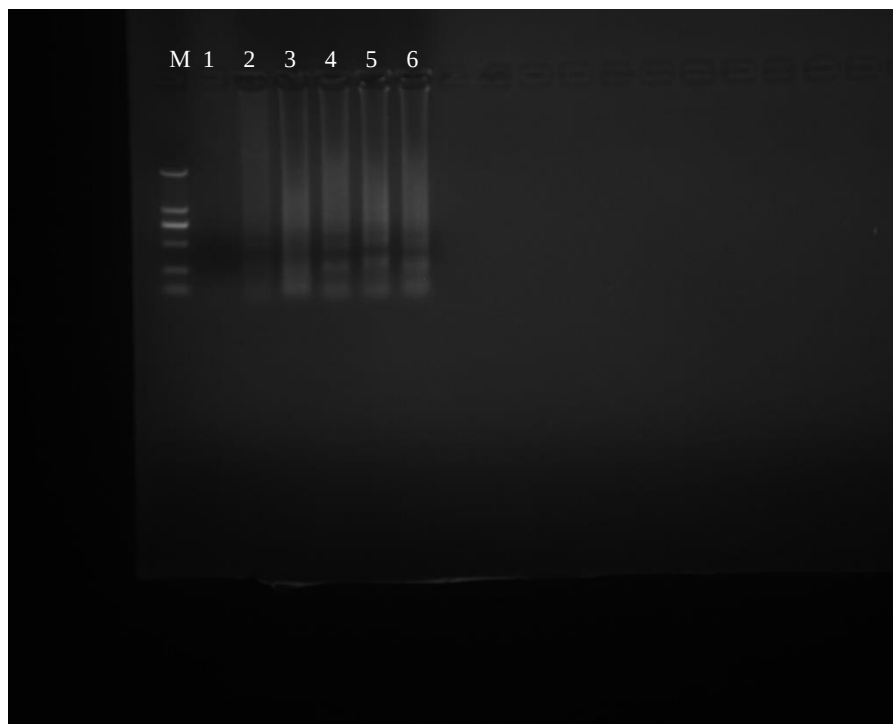
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Supplementary Figure S27



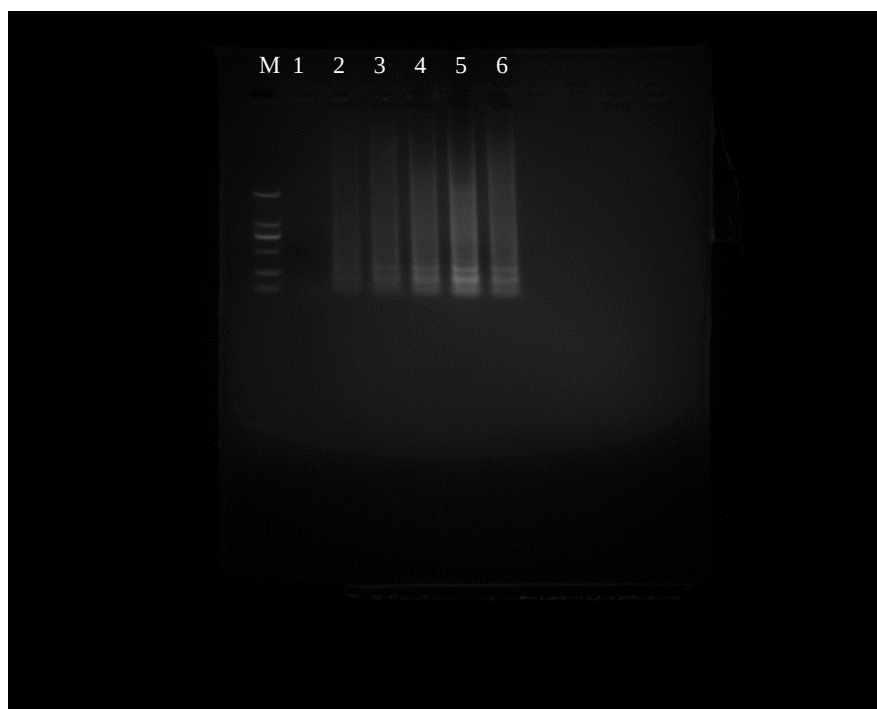
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Supplementary Figure S28



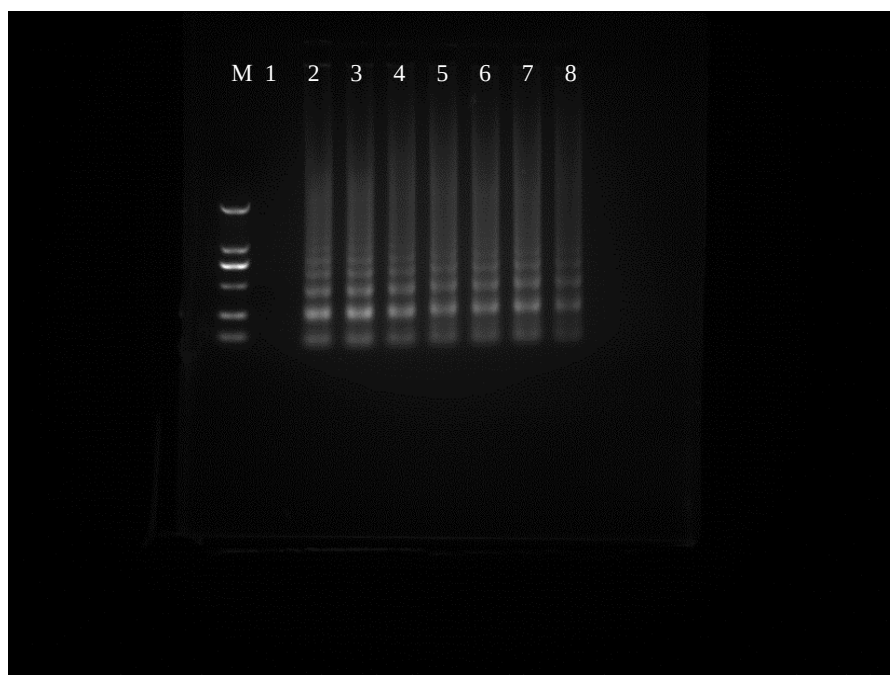
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Supplementary Figure S29



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Supplementary Figure S30



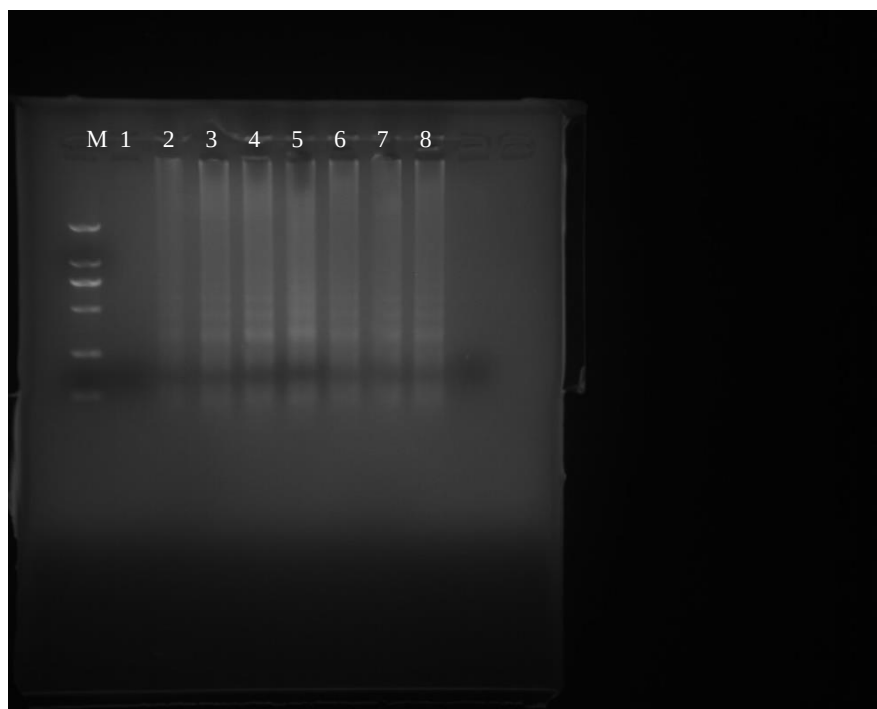
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Supplementary Figure S31



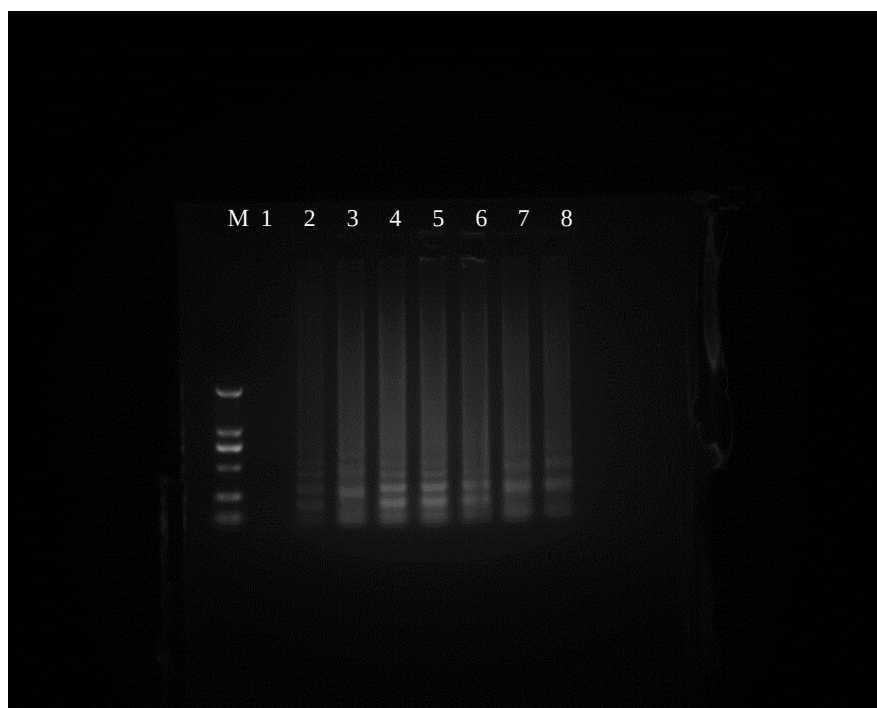
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Supplementary Figure S32



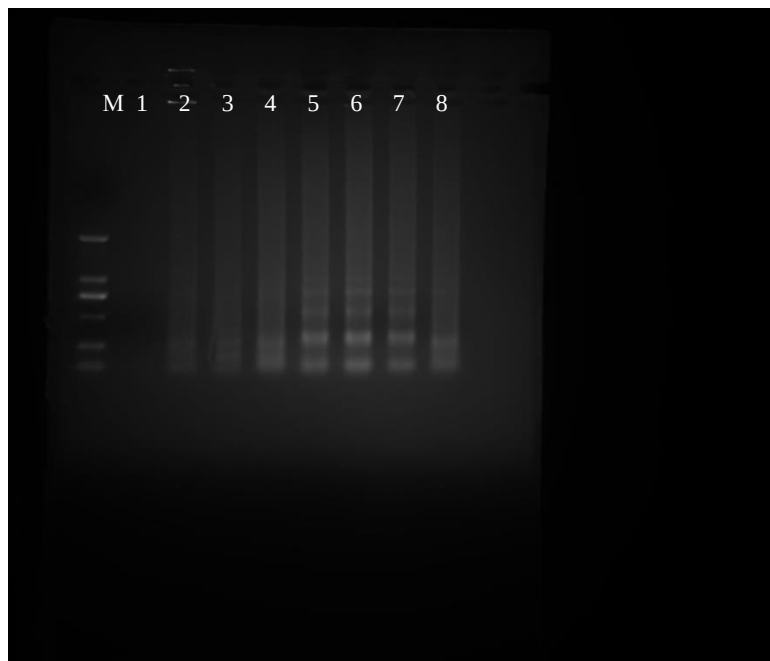
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Supplementary Figure S33



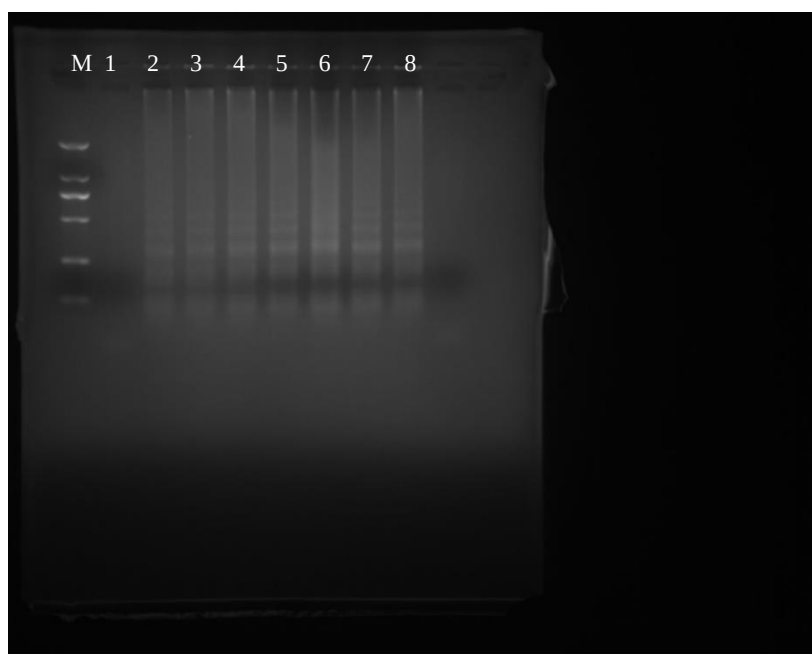
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Supplementary Figure S34



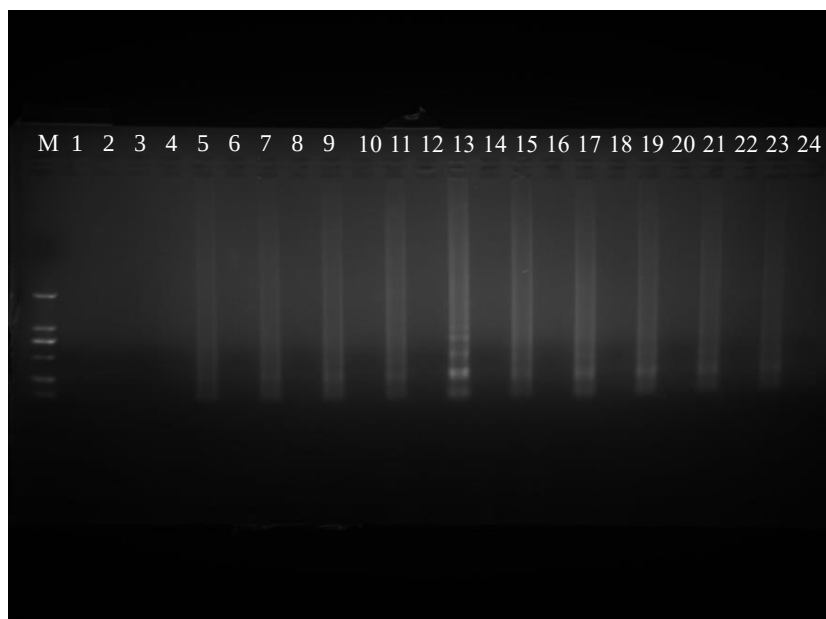
Supplementary Figure S34 The full-length gel of Supplementary Fig. S9 in the supplementary information. Analysis of ALV-J CPA at different units of *Bst* DNA polymerase (8 units μL^{-1}) by agarose gel electrophoresis (repeated analyze). Lane M, DNA markers; 1, 0 units μL^{-1} ; 2, 0.064 units μL^{-1} ; 3, 0.128 units μL^{-1} ; 4, 0.192 units μL^{-1} ; 5, 0.256 units μL^{-1} ; 6, 0.32 units μL^{-1} ; 7, 0.48 units μL^{-1} ; 8, 0.64 units μL^{-1} .

Supplementary Figure S35



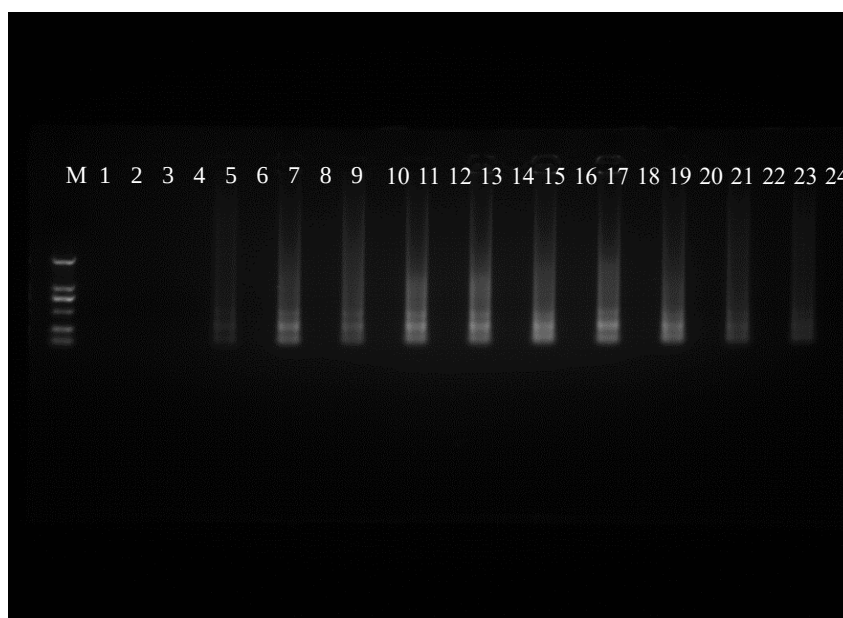
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Supplementary Figure S36



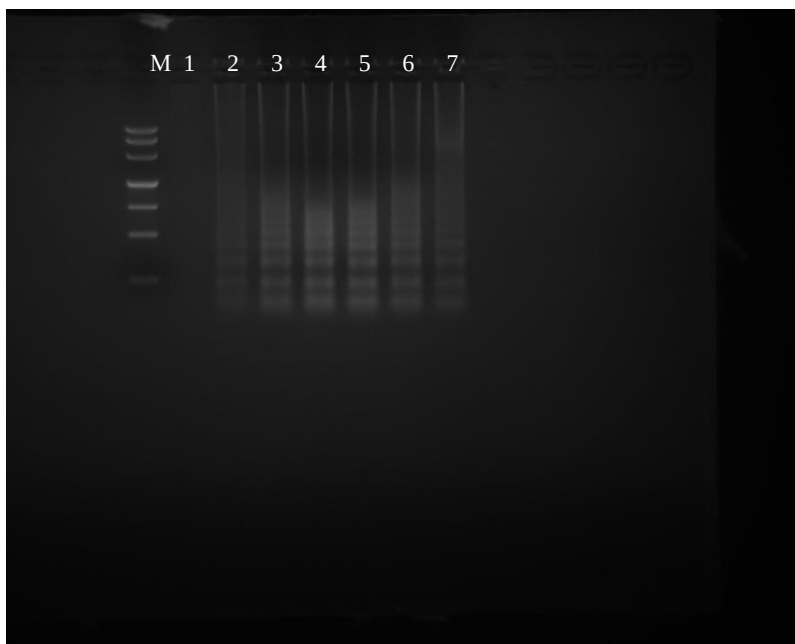
Supplementary Figure S36 The full-length gel of Supplementary Fig. S11 in the supplementary information. Analysis of ALV-J CPA at different temperatures by agarose gel electrophoresis (repeated analyze). Lane M, DNA marker; 1, 54°C; 3, 55°C; 5, 56°C; 7, 57°C; 9, 58°C; 11, 59°C; 13, 60°C; 15, 61°C; 17, 62°C; 19, 63°C; 21, 64°C; 23, 65°C; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 were negative controls for the corresponding temperatures.

Supplementary Figure S37



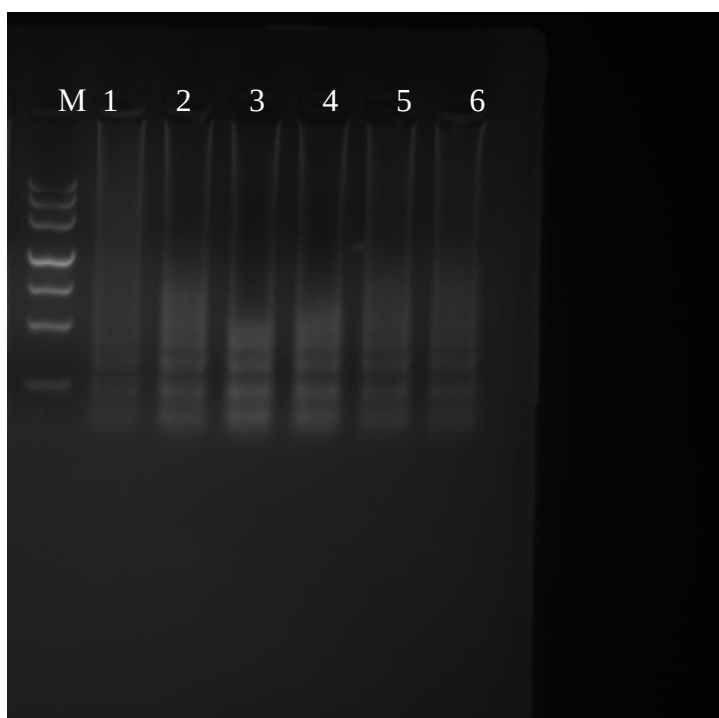
Supplementary Figure S37 The full-length gel of Supplementary Fig. S12 in the supplementary information. Analysis of ALV-J CPA at different temperatures by agarose gel electrophoresis (repeated analyze). Lane M, DNA marker; 1, 54°C; 3, 55°C; 5, 56°C; 7, 57°C; 9, 58°C; 11, 59°C; 13, 60°C; 15, 61°C; 17, 62°C; 19, 63°C; 21, 64°C; 23, 65°C; 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 were negative controls for the corresponding temperatures.

Supplementary Figure S38



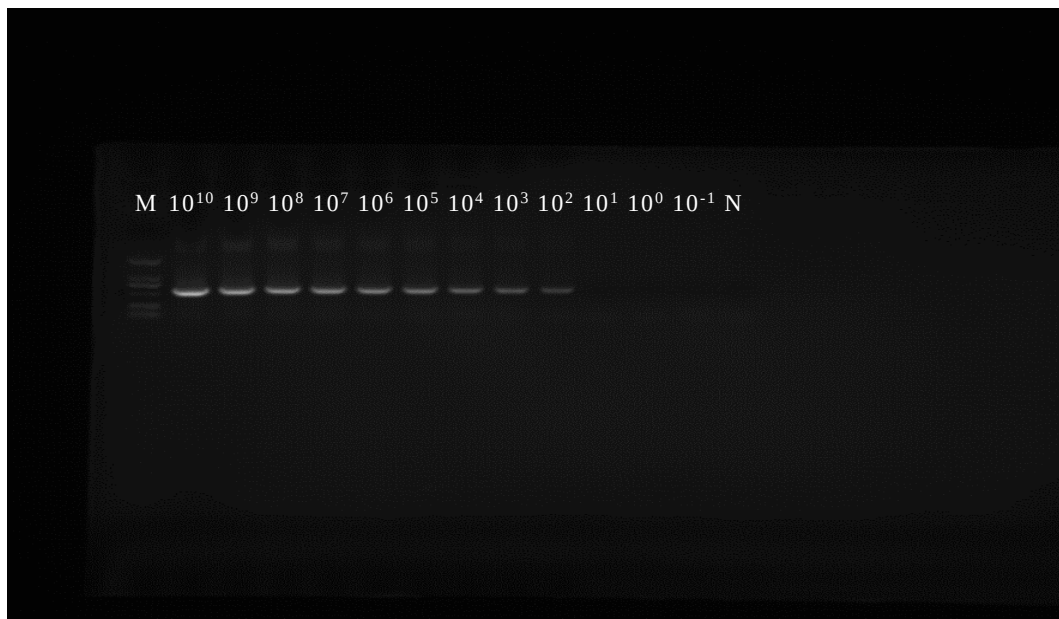
Supplementary Figure S38 The full-length gel of Supplementary Fig. S13 in the supplementary information. Analysis of ALV-J CPA with different reaction times (30min -75min) agarose gel electrophoresis. Lane M, DNA marker; 1, Negative control amplification for 75 min; 2, 30 min; 3, 45 min; 4, 60 min; 5, 65 min; 6, 70 min; 7, 75 min.

Supplementary Figure S39



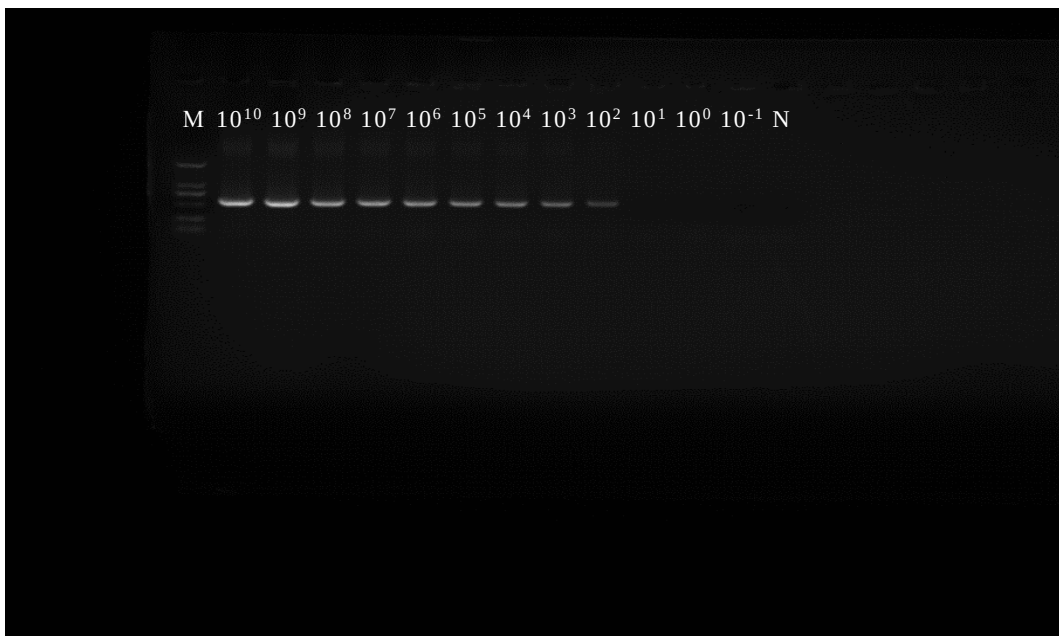
Supplementary Figure S39 The full-length gel of Supplementary Fig. S14 in the supplementary information. Analysis of ALV-J CPA with different reaction times (30min -75min) by agarose gel electrophoresis (repeated analyze). Lane M, DNA marker; 1, 30 min; 2, 45 min; 3, 60 min; 4, 65 min; 5, 70 min; 6, 75 min.

Supplementary Figure S40



Supplementary Figure S40 The full-length gel of Supplementary Fig. S15 in the supplementary information. Sensitivity of conventional PCR. Lane M, DNA markers; N, negative control.

Supplementary Figure S41



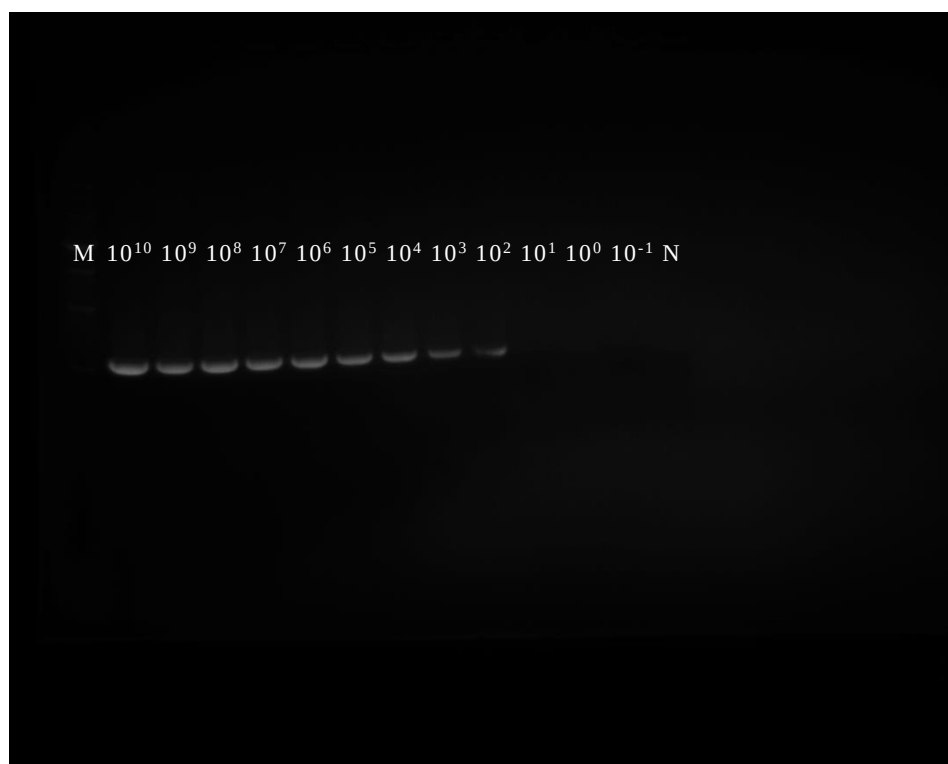
Supplementary Figure S41 The full-length gel of Supplementary Fig. S16 in the supplementary information. Sensitivity of conventional PCR; Lane M, DNA markers; N, negative control.

Supplementary Figure S42



Supplementary Figure S42 Sensitivity of conventional PCR to detect ALV-J (repeated analyze); Lane M, DNA markers; N, negative control.

Supplementary Figure S43



Supplementary Figure S43 Sensitivity of conventional PCR to detect ALV-J (repeated analyze); Lane M, DNA markers; N, negative control.

Supplementary Figure S44



Supplementary Figure S44 Sensitivity of conventional PCR to detect ALV-J (repeated analyze); Lane M, DNA markers; N, negative control.

Supplementary Tables:

Supplementary Table S1 Detection results for cell cultures inoculated with clinical plasma with S/P >0.2

Sample No.	Three methods of detection results								
	CPA			RT-PCR			PCR		
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+
10									
11	+	+	+	+	+	+	+	+	+
12									
13									
14									
15	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+
18									
19									
20	+	+	+	+	+	+	+	+	+
21									
22									
23									
24									
25	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+
27									
28									
29									
30									
31									
32									
33	+	+	+	+	+	+			
34	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+	+	+
37									
38	+	+	+	+	+	+	+	+	+
39									

40	+	+	+	+	+	+	+	+	+	+
41	+	+	+	+	+	+	+	+	+	+
42	+	+	+	+	+	+	+	+	+	+
43	+	+	+	+	+	+	+	+	+	+
44	+	+	+	+	+	+	+	+	+	+
45										
46										
47										
48										

“+” indicates that the ALV-J test result is positive

Supplementary Table S2 Detection results for cell cultures inoculated with clinical plasma with $0.1 \leq S/P \leq 0.2$

Sample		Three methods of detection results								
No.		CPA			qPCR			PCR		
1	+	+	+	+	+	+	+			
2										
3	+	+	+	+	+	+	+			
4	+	+	+	+	+	+	+			
5										
6	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
8										
9	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+
12										
13	+	+	+	+	+	+	+			
14	+	+	+	+	+	+	+			
15										
16					+	+	+			
17	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+			
19	+	+	+	+	+	+	+	+	+	+
20										
21	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+			
23	+	+	+	+	+	+	+			
24										
25										
26	+	+	+	+	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+	+
28										
29										
30										
31	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+
33					+	+	+			
34										
35	+	+	+	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+			
38	+	+	+	+	+	+	+			
39										
40	+	+	+	+	+	+	+	+	+	+
41										
42	+	+	+	+	+	+	+	+	+	+
43	+	+	+	+	+	+	+	+	+	+
44										
45	+	+	+	+	+	+	+	+	+	+
46	+	+	+	+	+	+	+	+	+	+

47									
48									
49	+	+	+	+	+	+	+	+	+
50									
51	+	+	+	+	+	+	+	+	+
52	+	+	+	+	+	+			
53	+	+	+	+	+	+			
54	+	+	+	+	+	+	+	+	+
55	+	+	+	+	+	+	+	+	+
56									
57	+	+	+	+	+	+	+	+	+
58									
59	+	+	+	+	+	+			
60									
61	+	+	+	+	+	+			
62									
63	+	+	+	+	+	+	+	+	+
64	+	+	+	+	+	+	+	+	+
65									
66	+	+	+	+	+	+	+	+	+
67	+	+	+	+	+	+	+	+	+
68	+	+	+	+	+	+	+	+	+
69									
70	+	+	+	+	+	+			
71									
72									
73									
74	+	+	+	+	+	+			
75									

“+” indicates that the ALV-J test result is positive