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Electronic Supplementary Material

Generation and Application of a Luciferase Reporter Virus Based on Yellow Fever Virus 17D

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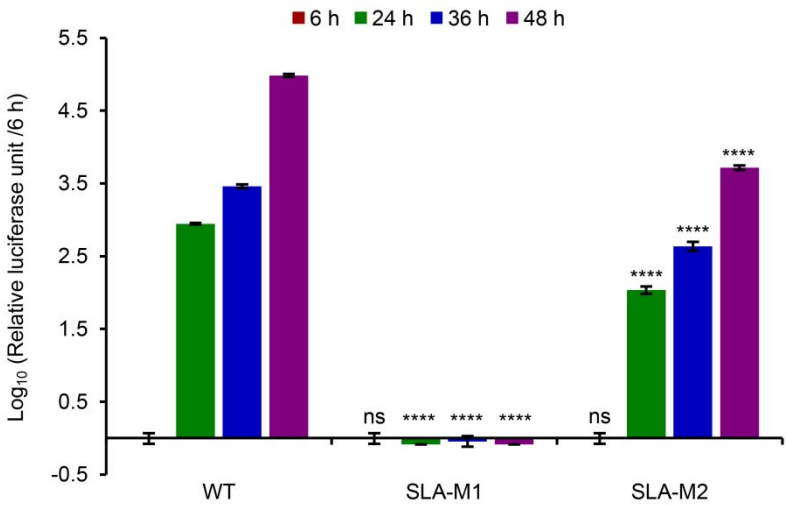

Fig. S1 The effect of 5'-SLA on yellow fever virus replication analyzed by 17D-Rluc.2A-based infection assay. Culture supernatants from BHK-21 cells transfected with WT or 5'-SLA mutant 17D-Rluc.2A RNA were collected at 48 h post-transfection. 10-μL of the supernatants were used to incubate with 1×10^5 of BHK-21 cells in a 48 well-plate. The reporter activities in the blind-infected BHK-21 cells were measured at different hours post-infection. Relative luciferase unit (the ratio of the reporter activity measured at later time points to the value measured at 6 h post-infection) was shown in the logarithmic form. The experiment was performed in triplicates. Two-way ANOVA and Tukey's multiple comparisons test were used to analyze statistical significance. *P* values referred to the comparisons of the logarithmic relative luciferase units between the SLA-M1 or SLA-M2 group and the WT group at the same hours post-infection. ns: $P > 0.05$; ****: $P \leq 0.0001$.

Table S1. Primers for 17D sequencing

Primers	Sequences（5'-3'）	Binding regions in 17D genome	Application
YFV-F1	AGTAAATCCTGTGTGCTAAT	1-20	Amplification and sequencing for S1
YFV-SLA-M2-F1*	TACACATACGATTTAGGTGACACTATAGAGTAAATCCTG	1-11	Amplification and sequencing for S1
YFV-R0	AGGTCCAGGTCTGTTTCCA	220-238	Sequencing for S1
P-YF-A-R	TCTTGGGTCCAGCATTTTCCACAACCTCTTTAG	319-343	Sequencing for S1
YF-Rluc-R	TCTTATCTTGATGCTCATAG	*Rluc* gene	Sequencing for S1
YF-Rluc-F	ACGCTGAAAGTGTAGTAGATGTGAT	*Rluc* gene	Sequencing for S1
YFV-R1	CCCTCAATGAAATCCCTGTC	995-1014	Amplification and sequencing for S1
YFV-F2	GACCATTGCCTACCTTGTG	889-907	Amplification and sequencing for S2
YFV-R2	CTTTCACCTGCATCACAACAGTG	1921-1943	Sequencing for S2
YFV-F3	AAGGACACAAATGACAAC	1769-1786	Sequencing for S2
YFV-R3	CCAAGTCTTCCAACCATAC	2788-2806	Amplification for S2
YFV-F4	GGAGAAGCAGGGCAGATGAG	2655-2674	Amplification and sequencing for S3
YFV-R4	AGCAGCCCTGGTCTGATTG	3750-3768	Sequencing for S3
YFV-R5	TCGATGATCTTAGGAGTGGGAAT	4592-4614	Amplification and sequencing for S3
YFV-F6	TGGGACCAGGTTGTGATGAC	4460-4479	Amplification and sequencing for S4
YFV-R6	GGTGTGGCTGTCATCAAG	5521-5538	Sequencing for S4
YFV-F7	GCCATGCCACCCTAACTT	5367-5384	Sequencing for S4
YFV-R7	ACACCCTTTCATCACACC	6369-6386	Amplification for S4
YFV-F8	GGCTGGTTTGAAGACGAATG	6238-6257	Amplification and sequencing for S5
YFV-R8	GATTCCCATCAACCACAGG	7325-7343	Sequencing for S5
YFV-F9	AGTGATGCCTCTGCTCTGT	7201-7219	Sequencing for S5
YFV-R9	TCACTGTTCCGCCAAACCTC	8236-8255	Amplification and sequencing for S5
YFV-F10	AGGACCGTGAGAGTTCTTG	8114-8132	Amplification and sequencing for S6
YFV-F11	CAGGTGTCGGACTTGTGTG	8974-8992	Sequencing for S6
YFV-R11	ACCATGTTGTGCGTCCTTGTG	10014-10034	Amplification and sequencing for S6
YFV-F12	GCTGGATGATCAAGGAAACAG	9882-9902	Amplification and sequencing for S7
YFV-R12	AGTGGTTTTGTGTTTGTCATCC	10841-10862	Amplification for S7
YFV-F14	ACAGAAGAAGTTGTCAGCCCAGAACCC	10525-10551	Sequencing for S7

*This primer is designed for the confirmation of the SLA-M2 mutation in the corresponding mutant virus. Note that the underlined sequence is not complementary to 17D genome, but is aimed to create an extension in the end of amplicons for a better sequencing readout.