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Electronic Supplementary Material

Reverse Transcriptionrecombinase Polymerase Amplification Assays for Rapid Detection of Tick-Borne Encephalitis Virus Infection

Jia Jia[1,2#],YuchangLi[1#], Xiaoyan Wu[1], Sen Zhang[1], Yi Hu[1], Jing Li[1], Tao Jiang[1,3✉], Xiaoping Kang[1✉]

1. State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China
2. Center for Disease Control and Prevention of Beijing, Beijing 101300, China
3. Graduate school, Anhui Medical University, Hefei 230032, China

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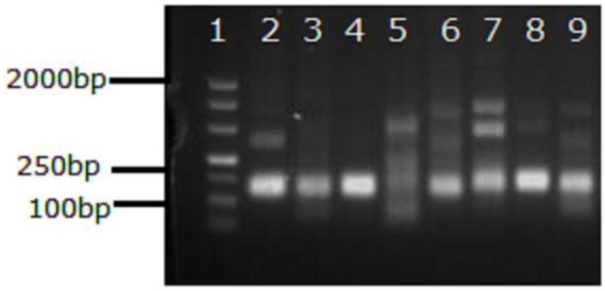

Figure S1. Results of the basic RPA assay performed using primer sets designed for TBEV detection. Lanes: 1, marker DL500bp; 2–9, assay performed using primer sets designed for TBEV detection (Sets 1–8, respectively). A Basic RPA Kit was used to amplify 1000 copies of TBEV VLP RNA. Different primer sets were added to the basic RPA assays. After incubation at 42°C for 20min, the basic RPA products were visualized by DNA gel electrophoresis. Sets 1–8: set 1, TBEV-F1-RPA and TBEV-R1-RPA; set 2, TBEV-F2-RPA and TBEV-R1-RPA; set 3,TBEV-F3-RPA and TBEV-R1-RPA; set 4, TBEV-F4-RPA and TBEV-R1-RPA; set 5, TBEV-F1-RPA and TBEV-R2-RPA; set 6, TBEV-F2-RPA and TBEV-R2-RPA; set 7,TBEV-F3-RPA and TBEV-R2-RPA; set 8, TBEV-F4-RPA and TBEV-R2-RPA.

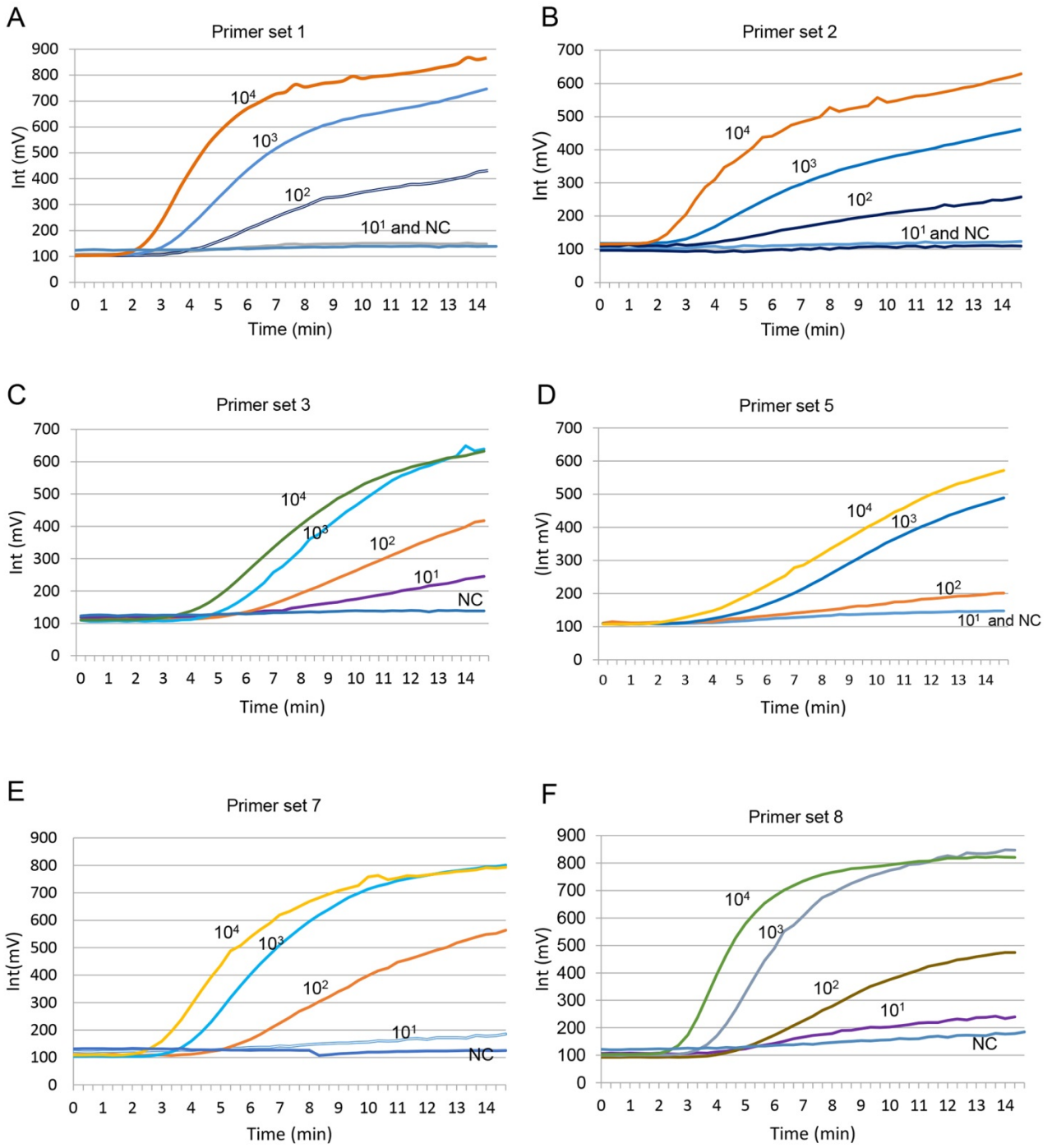

Figure S2. Primer sets and probe screened by RT-RPA. **(A)** Amplification curve by primer set 1 (TBEV-F1-RPA and TBEV-R1-RPA). (**B**) Amplification curve by primer set 2 (TBEV-F2-RPA and TBEV-R1-RPA). (**C**) Amplification curve by primer set 3 (TBEV-F3-RPA and TBEV-R1-RPA). (**D**) Amplification curve by primer set 5 (TBEV-F1-RPA and TBEV-R2-RPA). (**E**) Amplification curve by primer set 7 (TBEV-F3-RPA and TBEV-R2-RPA). (**F**) Amplification curve by primer set 8 (TBEV-F4-RPA and TBEV-R2-RPA).The RNA template from TBEV VLP was 10-fold serially diluted (10^0 copies/μL to 10^5 copies/μL) and applied to RT-RPA assay, at 5 μL/reaction; each reaction contained a different primer set and TBEV-RPA-probe and the enzyme complex pellet. After incubating at 39 °C for 20 min in the ESE Quant TS, the fluorescence intensity was documented every 20 s, and amplification curves were generated by Tube canner studio software.

Table S1. Sequences of primers and probe for the RPA assay

Name	Sequence (5′–3′)	Position
TBEV-F1-RPA	TCAGGATTTTTCCTCCTCCTATACAAAATTCC	10,649–10,679
TBEV-F2-RPA	AGGATTCTTCCTCCTCCTATACCAAATTCCC	10,651–10,680
TBEV-F3-RPA	GAAGCATGCTTCCGGGAGGAGGGAAGAGAG	10,602–10,631
TBEV-F4-RPA	AGGGAAGAGAGAAATTGGCAACTCTCTTCAGG	10,621–10,652
TBEVR1-RPA	TCCGAGTCACACGTCACCTCCTTGTCAGACT	10,742–10,771
TBEV-R2-RPA	TTCCGAGTCACACATCACCTCCTTGTCAG	10,743–10,770
TBEV-P-RPA	AGGGGGGGCGGTTCTTGTTCTCCCTGAGCCFCHAQCA CCCAGACACAGAT-[3′-block]	10,692–10,739

F: FAM-dT, thymidine nucleotide carrying fluorescein; H: THF, tetrahydrofuran spacer; Q: BHQ1-dT, thymidine nucleotide carrying Black-Hole Quencher 1; 3′-block: 3′-phosphate introduced to block elongation.