VIROLOGICA SINICA

Electronic Supplementary Material

Development and Characterization of SYBR Green I Based RT-PCR Assay for Detection of Omsk Hemorrhagic Fever Virus

Ya-Nan Zhang[1] • Si-Qing Liu[2] • Cheng-Lin Deng[1] • Zhi-Ming Yuan[1] • Bo Zhang[1] • Xiao-Dan Li[3]✉ • Han-Qing Ye[1]✉

1. Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan 430071, China;
2. Department of Medical Genetics, Oslo University Hospital, Oslo 0450, Norway;
3. Hunan Normal University, School of Medicine, Changsha 410081, China

Supporting information to DOI: 10.1007/s12250-021-00389-5

Supplementary Materials

Cells

BHK-21 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Germany) containing 10% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37 °C with 5% CO_2.

Designing of Primers

In order to design efficient primer pairs, we identified the conserved regions of viral genome from *NS4A* to *NS4B* among strains of OHFV and other related tick-borne flaviviruses including TBEV, POWV, LGTV, and LIV as well as KFDV. Maximum care has been taken to reduce the cross-reactivity due to sequence similarities. All above genome sequences for analysis were retrieved from GenBank database and aligned using the sequence alignment software Clustal X. And the primers were designed by Primer Premier 5 software in this study.

Synthesis of *In Vitro* RNA Transcripts

Genomic RNA transcripts derived from in vitro transcription of the full-length infectious clones were used as RNA standards. OHFV full-length infectious clone (OHF-IC) was kindly provided by Professor Kentaro Yoshii. Other genomic RNAs such as JEV (SA14 strain), Dengue virus serotype 2 (DENV2, TSV01 strain), West

Nile virus (WNV, 3356 strain), tick-borne encephalitis virus (TBEV, WH2012 strain), Chikungunya virus (CHIKV) and YFV (17D strain), were also obtained through *in vitro* transcription from the corresponding full-length cDNA plasmids. OHFV-Rluc replicon was constructed by inserting the reporter gene of Rluc to replace the natural structural genes as described previously. Above all RNA transcripts were produced by using T7 mMESSAGE mMACHINE kit (Thermo, USA).

SYBR Green-I Based Real-Time RT-PCR

A real-time reverse transcription-polymerase chain reaction (RT-PCR) assay was performed in a reaction mixture with a total volume of 20 μL, containing 2 μL of an RNA template and 0.4 μL of each CHIKV primer (final concentration, 0.2 μmol/L) using a StepOnePlus™ real-time PCRsystem (Applied Biosystems, Foster City, USA). The RT/amplification conditions consisted of an RT step at 42 °C for 5 min, reverse transcriptase inactivation at 95 °C for 10 s, followed by 40 cycles of PCR at 95 °C for 10 s (denaturation) and 60 °C for 34 s (annealing and extension)

Conventional RT-PCR

To compare the sensitivity of the real-time RT-PCR-based assay, the conventional RT-PCR assay was conducted with the same set of primers to target the 191 bp region between *NS4A* and *NS4B* gene of OHFV genome. The amplification was carried out in a 20 μL total reaction volume by using One-Step RT-PCR kit (TaKaRa) following the manufacturer' instructions. 10 μL of 2× One-Step buffer, 5 μL RNAase-free water, 0.5 μmol/L of each primer, 2 μL of RNA templates and 1 μL of RNA transcriptase were mixed for amplification system and thermal cycling conditions were same as those for the real-time assay described above.

OHFV-Rluc Replicon RNA Transfection

Transfection was performed in 35 mm dishes of 70% confluent BHK monolayers. 4 μLof DMRIE-C reagents (Invitrogen, USA) was diluted with 1 mL Opti-MEM Medium (Invitrogen) and then combined with about 1μg of RNA transcripts. The RNA-DMRIE-C complex solution was immediately added to the dishes and incubated at 37 °C with 5% CO_2 for 4 h. After replaced with fresh DMEM containing 10% FBS, the transfected cells were incubated at 37 °C with 5% CO_2.

Table S1. Oligonucleotide primers used in SYBR Green-I based real-time RT-PCR

Primer name	Sequences	Tm value	Region	Product length (bp)	GC%
OHFV-4A-F1	aggcagaggagtagcgatgac	58.6	NS4A	191	57.1
OHFV-4B-R1	gcgggttggatgtctatgtt	57.3	NS4B		50
OHFV-4A-F1	aggcagaggagtagcgatgac	58.6	NS4A	199	57.1
OHFV-4B-R2	aagacttggcgggttggaT	59.2	NS4B		52.6
OHFV-4A-F1	aggcagaggagtagcgatgac	58.6	NS4A	197	57.1
OHFV-4B-R3	gacttggcgggttggatg	58.9	NS4B		61.1
OHFV-4A-F2	gctggcaggcagaggagtag	59.9	NS4A	197	65
OHFV-4B-R1	gcgggttggatgtctatgtt	57.3	NS4B		50

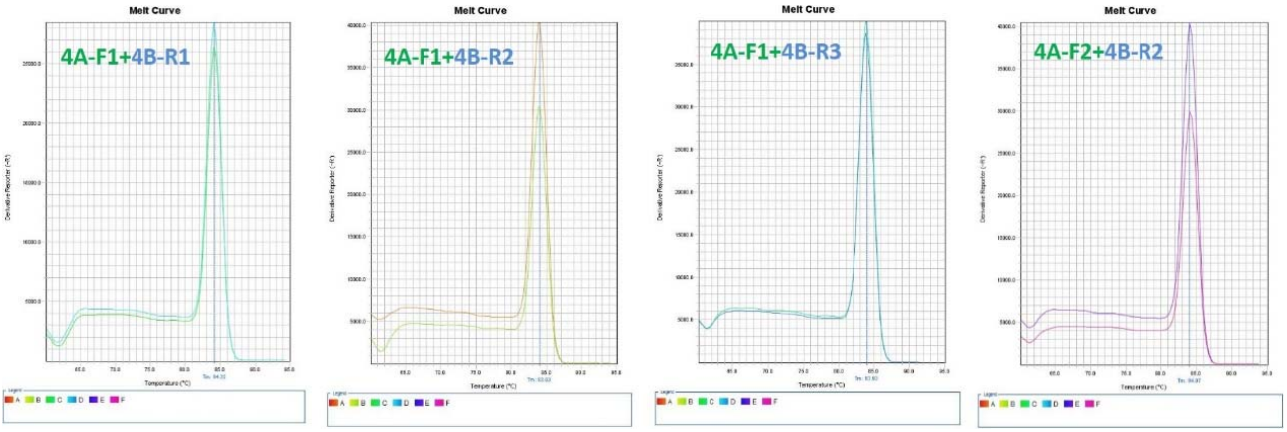
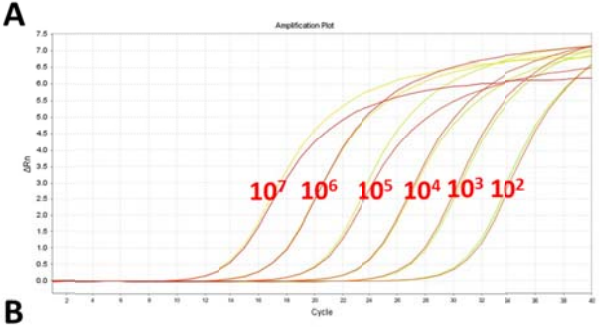


Fig. S1 The melting curves of the real-time PCR reactions using the OHFV RNA as the template with the four designed primer pairs.

A



B

Copies/reaction	10^7	10^6	10^5	10^4	10^3	10^2
Mean C_T	12.87	16.01	19.40	22.75	26.02	29.62

C

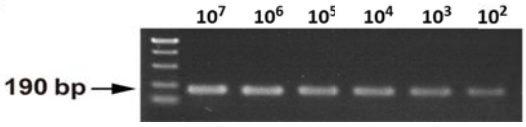


Fig. S2 Amplification analysis of 4A-F1+4B-R1 primers. **A** Amplification plot of the real-time RT-PCR using 4A-F1+4B-R1 primers with 10^2 to 10^7 RNA copies per reaction. **B** C_T values corresponding to 10^2 to 10^7 copies per reaction with real-time RT-PCR. **C** Agarose gel analysis of the real-time RT-PCR amplicon with 10^2 to 10^7copies reaction.