

Supplemental Materials: A Melting Curve-Based Multiplex RT-qPCR Assay for Simultaneous Detection of Four Human Coronaviruses

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Supplemental Results: Optimization of the Primer Concentration

In the conventional PCR, primer concentration was 0.2 μM . When 0.2 μM each set of primers were used to amplify a mixed template containing 30,000 copies of each HCoV RNA, only low or no melting peaks were observed for HCoV-229E, -NL63, and -HKU1, suggesting a competitive amplification of HCoV-OC43 with other three HCoVs. To obtain well performance of the melting curve-based multiplex RT-qPCR, we optimized the primer inputs for HCoV-OC43 and other three HCoVs by orthogonal experiments with primer concentration from 0.2 to 0.6 μM . When GC-HCoV-OC43-F/R was remained at lower concentration of 0.2 μM , the melting peaks of HCoV-229E, -NL63, and -HKU1 were detectable although they were obviously lower than the melting peak of HCoV-OC43 (Figure S2A). In particular, the peaks of HCoV-229E, -NL63, and -HKU1 increased along with the increase of primer concentration. When the concentrations of primers for HCoV-229E, -NL63, and -HKU1 were increased to 0.8 μM , the melting peaks for four HCoVs appeared to have similar peak heights (Figure S2B). Therefore, 0.8, 0.8, 0.8 and 0.2 μM of HCoV-229E, -NL63, -HKU1, and -OC43 specific primers were selected for the assay, respectively.

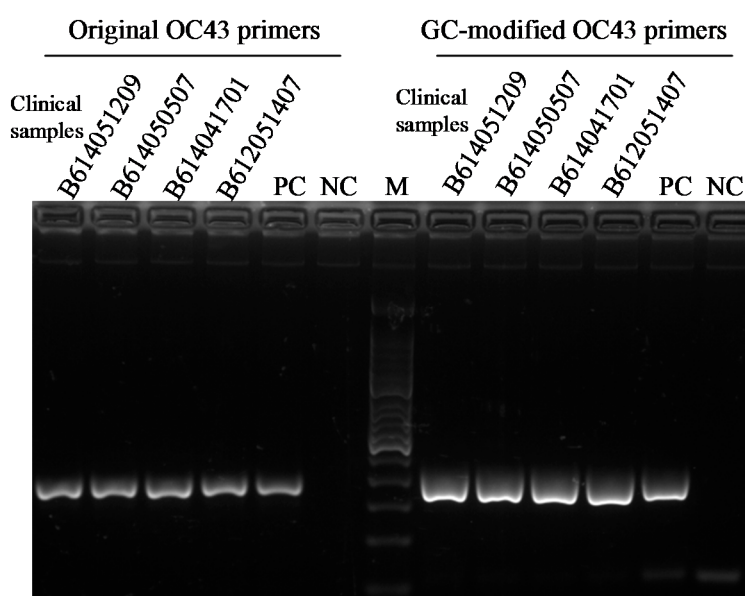


Figure S1. Comparison of the amplification effects between the original OC43 primers and the GC-modified OC43 primers. PC: positive control (OC43 standard strain VR-1558); NC: negative control; M: Marker.

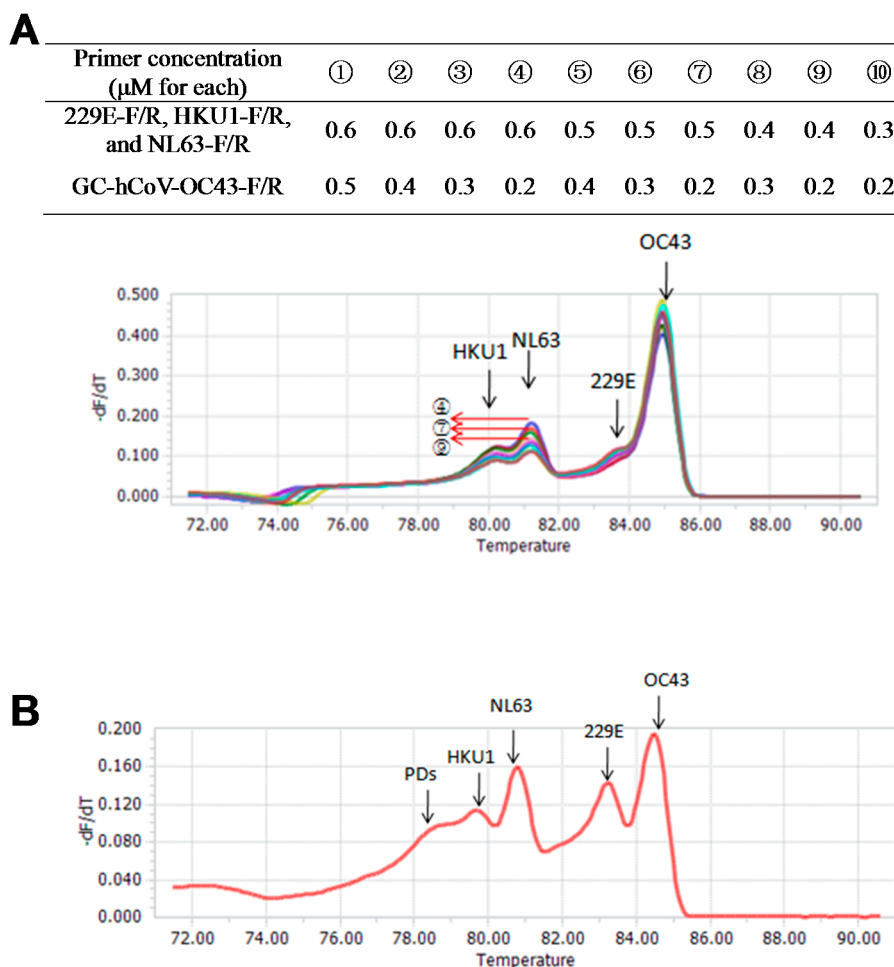


Figure S2. Optimization of the primer concentrations for the multiple RT-qPCR assay. (A) Optimization of the multiple RT-qPCR assay using various concentrations of GC-modified HCoV-OC43 and other three HCoVs' primers; (B) Melting peaks of four HCoVs using the multiple RT-qPCR assay under optimized primer concentrations. PDs: primer dimers.

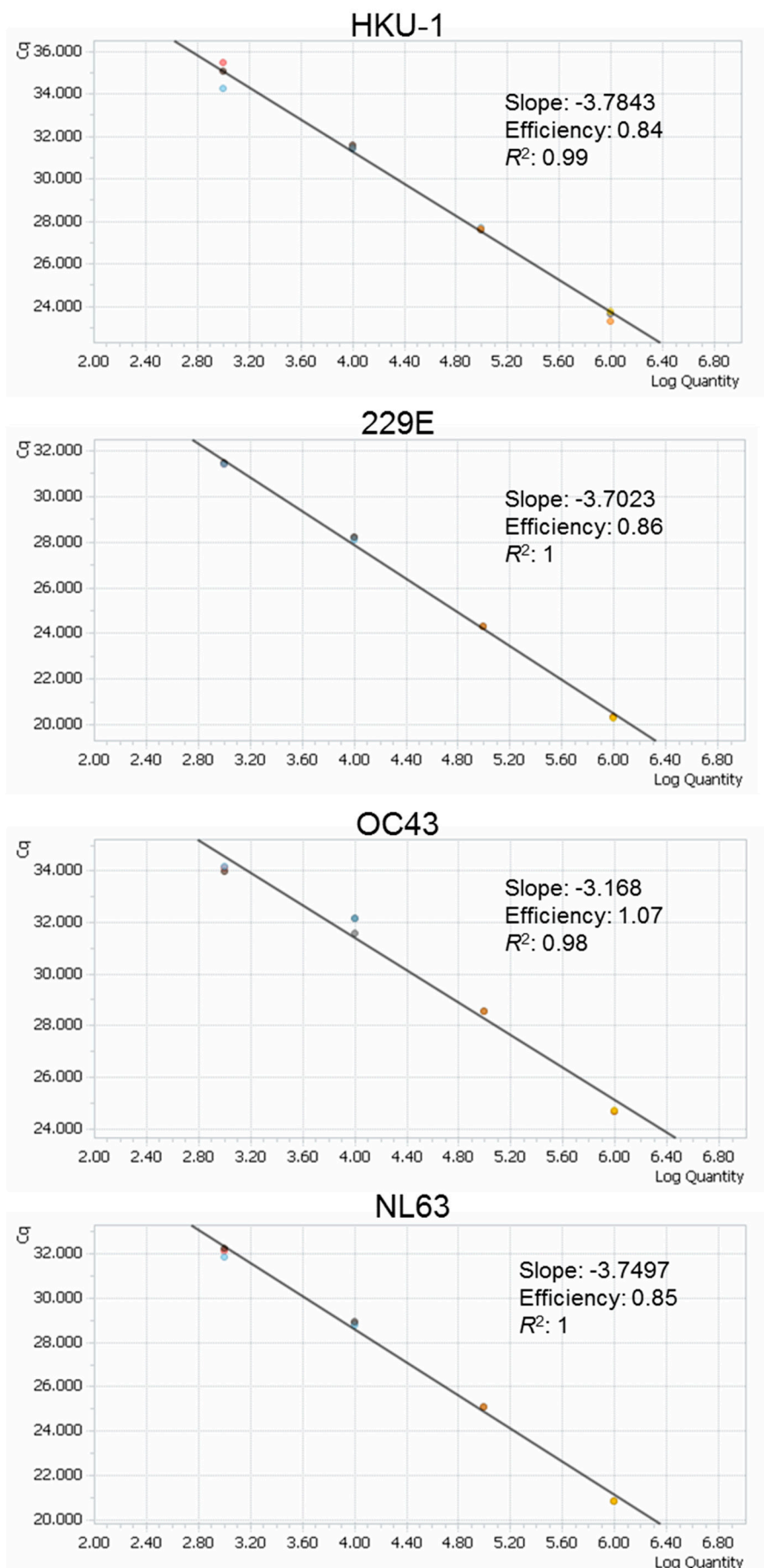


Figure S3. Standard curves of four HCoVs using the multiple RT-qPCR assay.

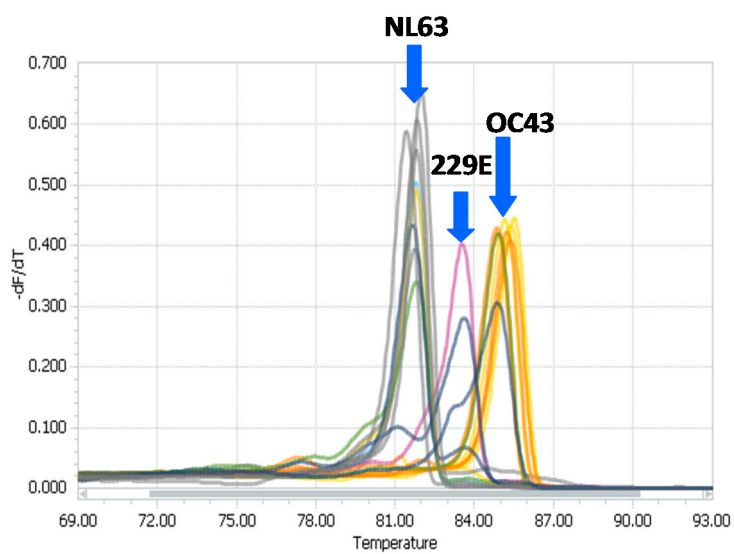


Figure S5. Melting curves of 9 HCoV-OC43, 2 HCoV-229E, and 9 HCoV-NL63 positive samples subjected to sequencing.