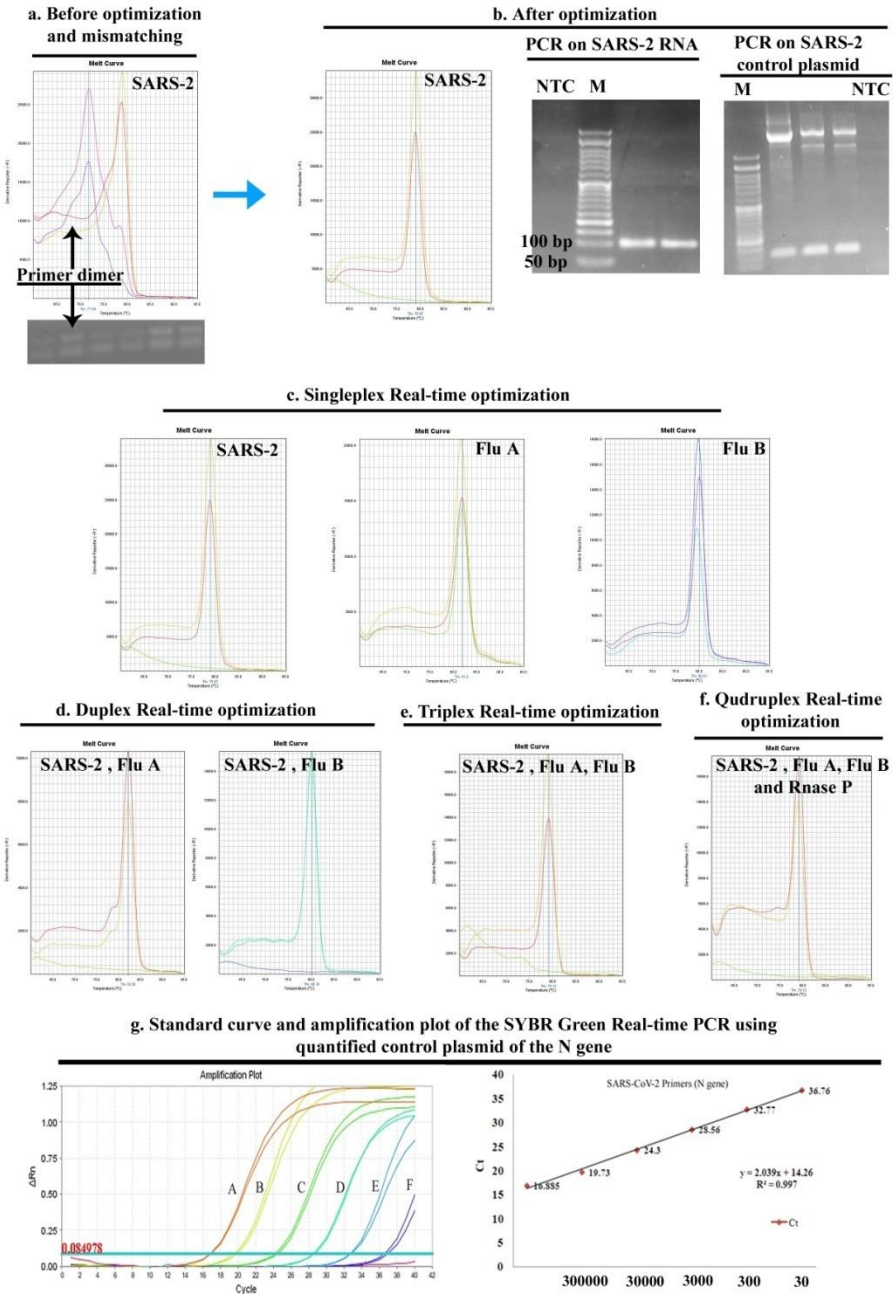
Supplemental Information

Supplementary file 1. Optimization of primer sets using SYBR Green Real-time PCR. The electrophoresis and melting curve analysis of amplified *N* gene using conventional RT-PCR and SYBR Green Real-time PCR before creating mismatches and optimization (a). The obtained melting curves using SYBR Green Real-time PCR in simplex, duplex, triplex and qudruplex conditions with *SARS-CoV-2* and *Flu A/B* primers (b-f). The obtained standard curve and amplification plots using SYBR Green Real-time PCR with quantified *SARS-CoV-2* positive control plasmid (g). * The full length gels are shown below.

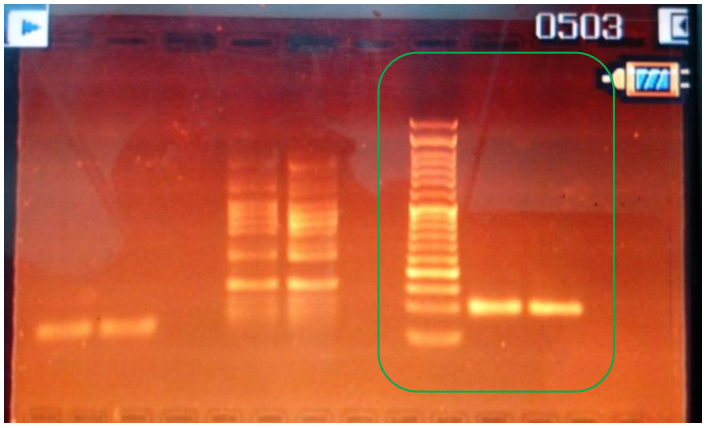


*Full length gels

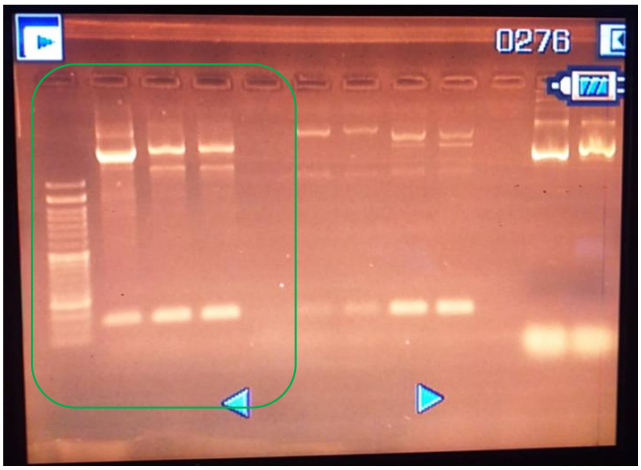
Before optimization of PCR reaction conditions and insertion of mismatches in primer-probe binding regions.

After optimization of PCR reaction conditions and insertion of mismatches in primer-probe binding regions.

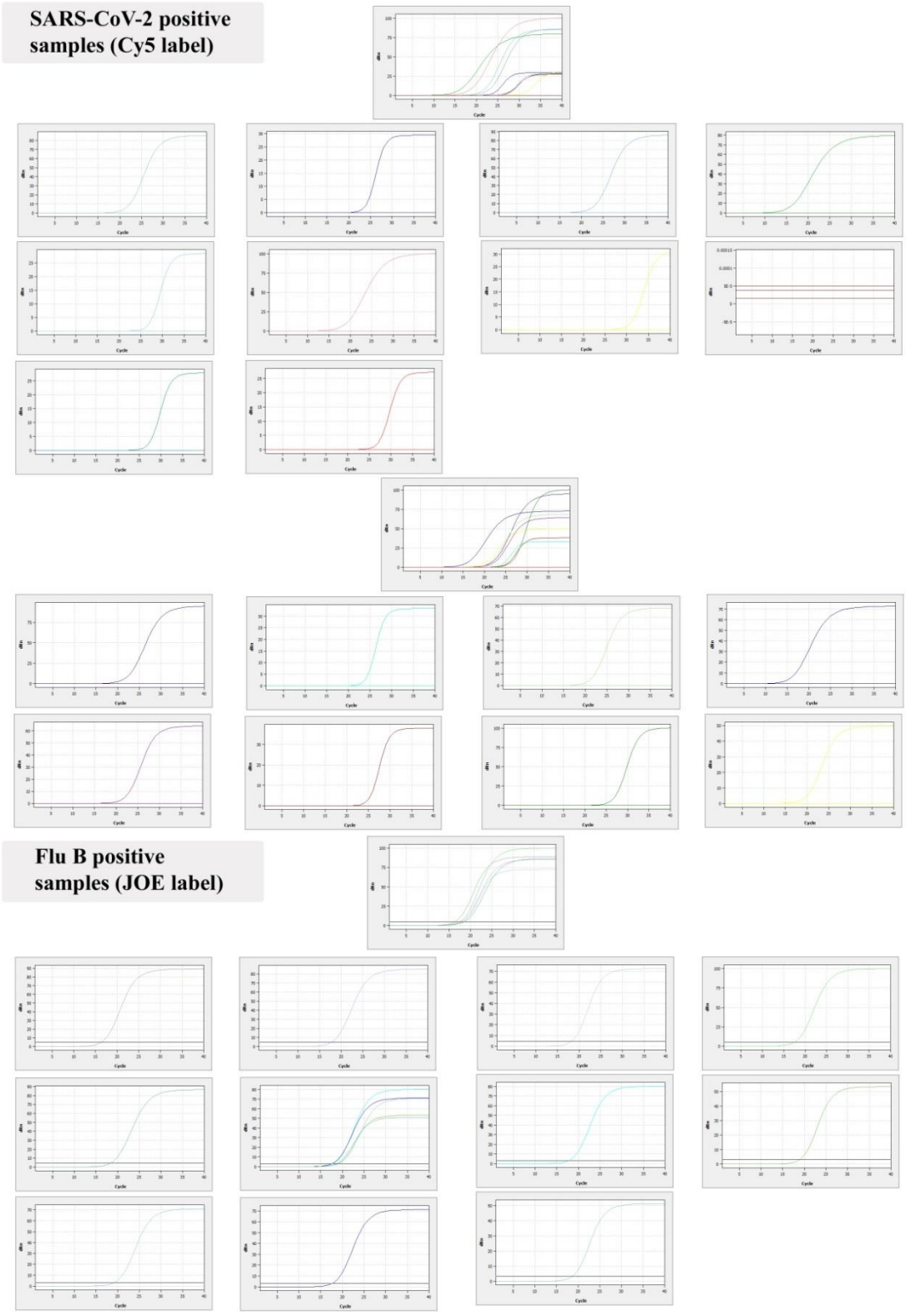
PCR on SARS-CoV-2 RNA



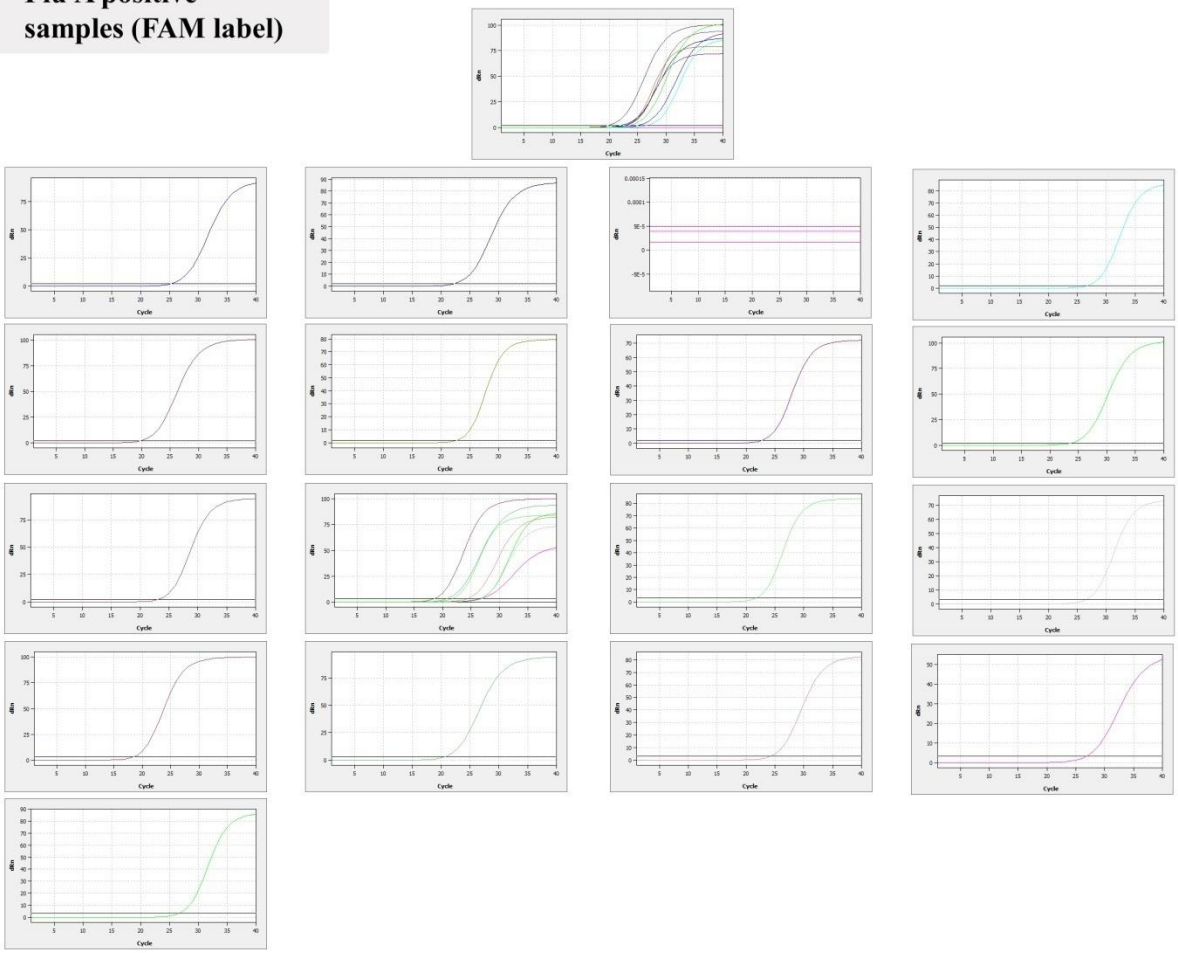
PCR on SARS-CoV-2 control plasmid



Supplementary file 2. The obtained amplification plots for clinical samples by our multiplex assay



Flu A positive samples (FAM label)



Supplementary file 3. The amplification plots of detected co-infected samples by our multiplex assay an