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Supplemental information

Establishment of a reverse genetics system for SARS-CoV-2 using circular polymerase extension reaction

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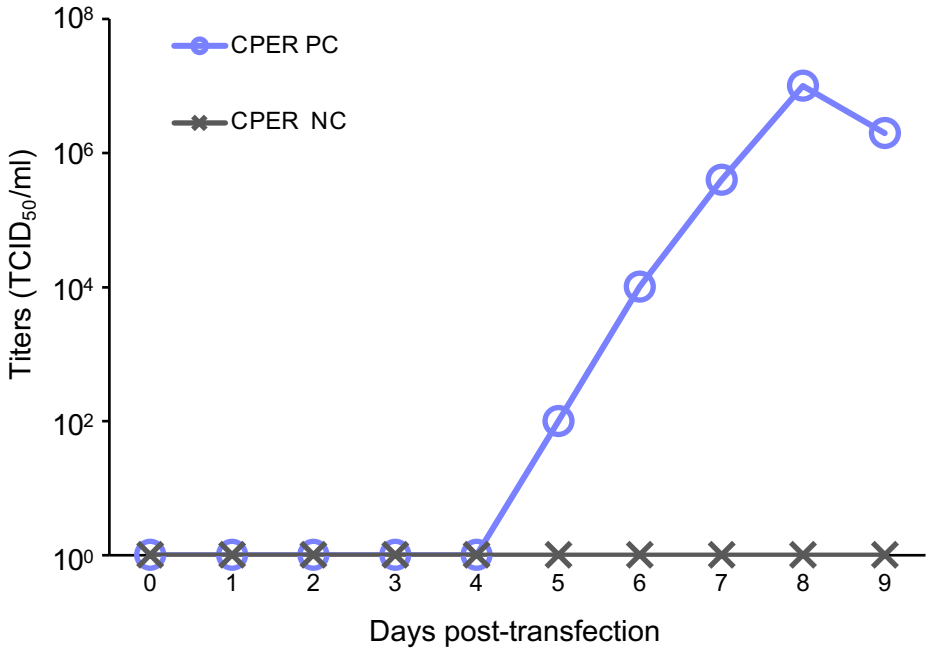

Figure S1. Production of recombinant SARS-CoV-2. Figure S1 is related to Figure 1A. CPER products were transfected into HEK293-3P6C33 cells (CPER PC) and infectious titers in the culture supernatants were measured at the indicated time points. As a negative control, the CPER product obtained without fragment F9/10 was transfected into cells (CPER NC).

Fig. S1 Torii et al.

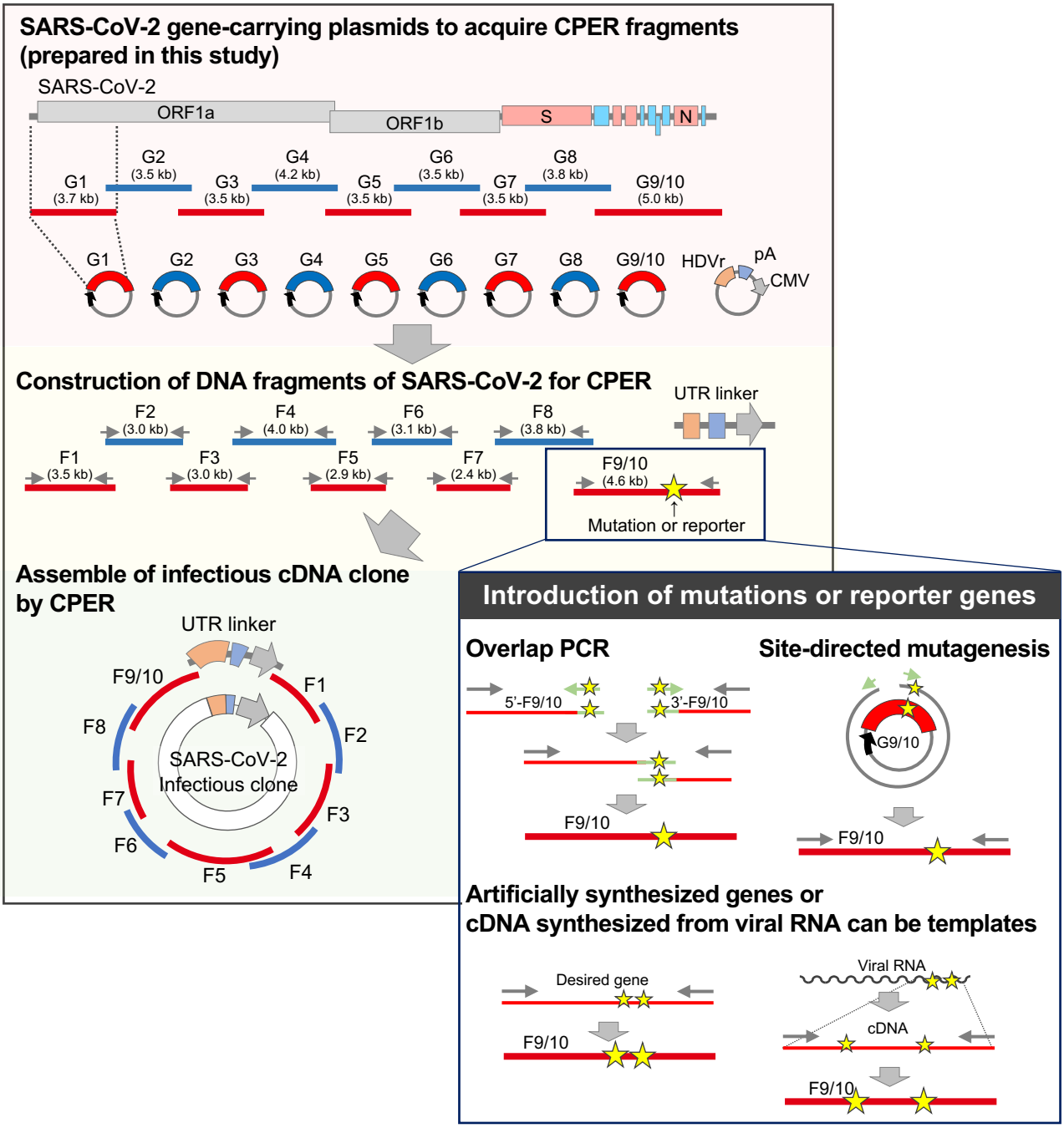

Figure S2. Workflow to generate infectious cDNA clone for SARS-CoV-2 with desirable mutations or reporters. Figure S2 is related to Figure 2.
By using DNA fragments and overlapping primers, which possess mutations or reporter genes, mutation can be introduced. By site-directed mutagenesis using SARS-CoV-2 gene-encoding plasmids, mutations can also be introduced. Using artificially synthesized gene fragments with mutations, multiple mutations can be inserted more quickly. In addition, reverse-transcribed cDNA from viral RNA can be used as templates for amplification of DNA fragments of SARS-CoV-2.

Fig. S2 Torii et al.

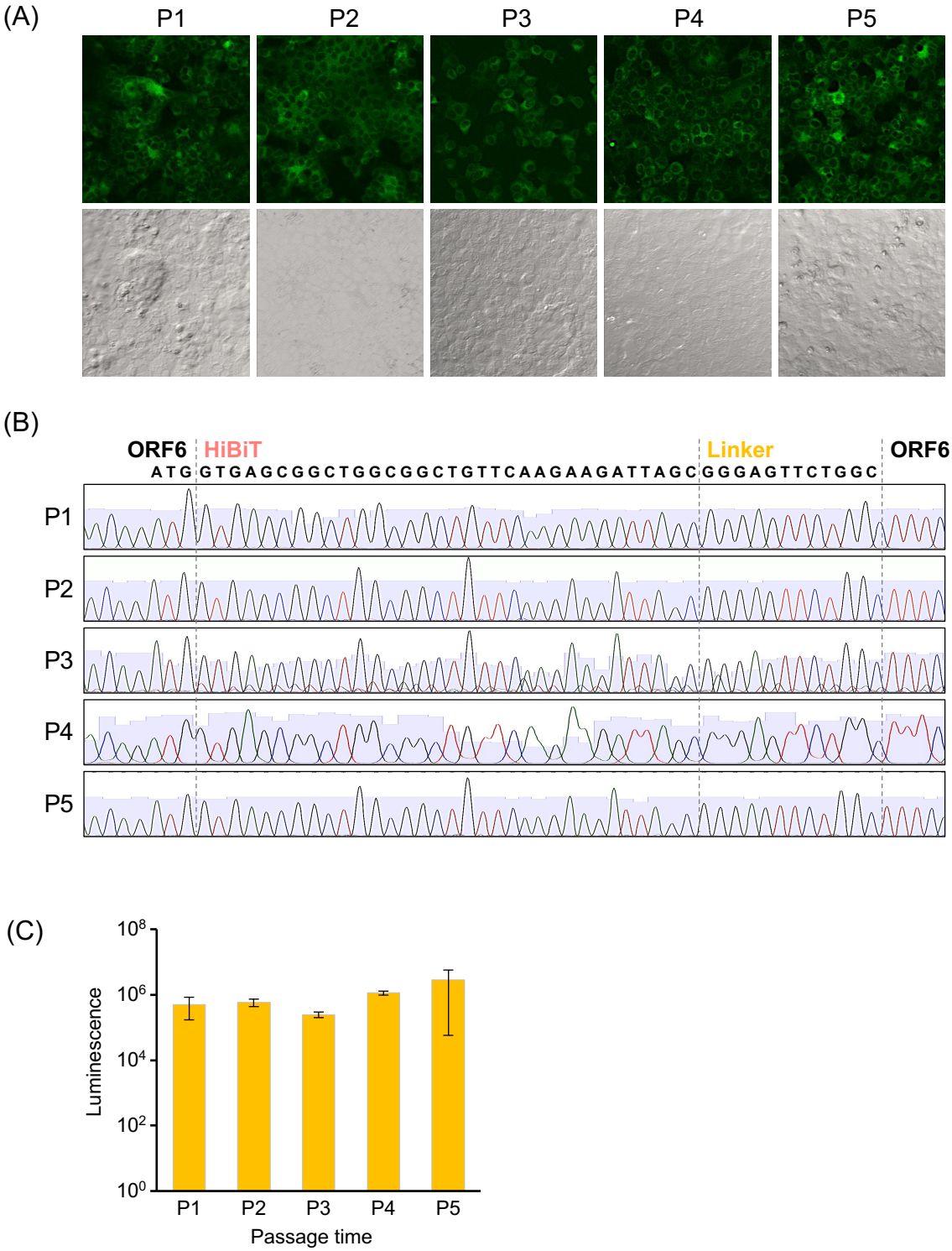

Figure S3. Stability of reporter genes in recombinant SARS-CoV-2 engineered by CPER. Figure S3 is related to Figure 2A-E.
(A) GFP viruses serially passaged for five times (P1-P5) were infected with VeroE6/TMPRSS2 cells at an MOI of 0.01 and the fluorescent signals in cells were observed at 24 hpi. (B) HiBiT viruses were serially passaged for five rounds (P1-P5), and the sequence of HiBiT and a linker inserted into the ORF6 of each virus was determined. (C) HiBiT viruses (P1-P5) were inoculated into VeroE6/TMPRSS2 cells at an MOI of 0.01 and luciferase activities were determined at 24 hpi.

Fig. S3 Torii et al.