

A plasmid-launched reverse genetics system for the human coronavirus HCoV-OC43.

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Overview

Coronaviruses, including the etiological agent of the COVID-19 pandemic (SARS-CoV-2), are positive-sense single-stranded RNA viruses which infect a wide variety of vertebrate species. Human coronavirus OC43 (HCoV-OC43) generally causes cold-like disease in humans and is a good model to safely study the replication of HCoVs. Reverse genetics systems enable the engineering and recovery of viruses in order to better understand the biological function(s) of viral proteins. We designed a reverse genetics system utilizing yeast-based transformation-associated recombination (TAR) for HCoV-OC43. Following verification and full-genome sequencing, we successfully recovered HCoV-OC43 transfection of the ~41.4 kbp plasmid into HEK-293T cells and inoculation of the supernatant onto MRC-5 cells.

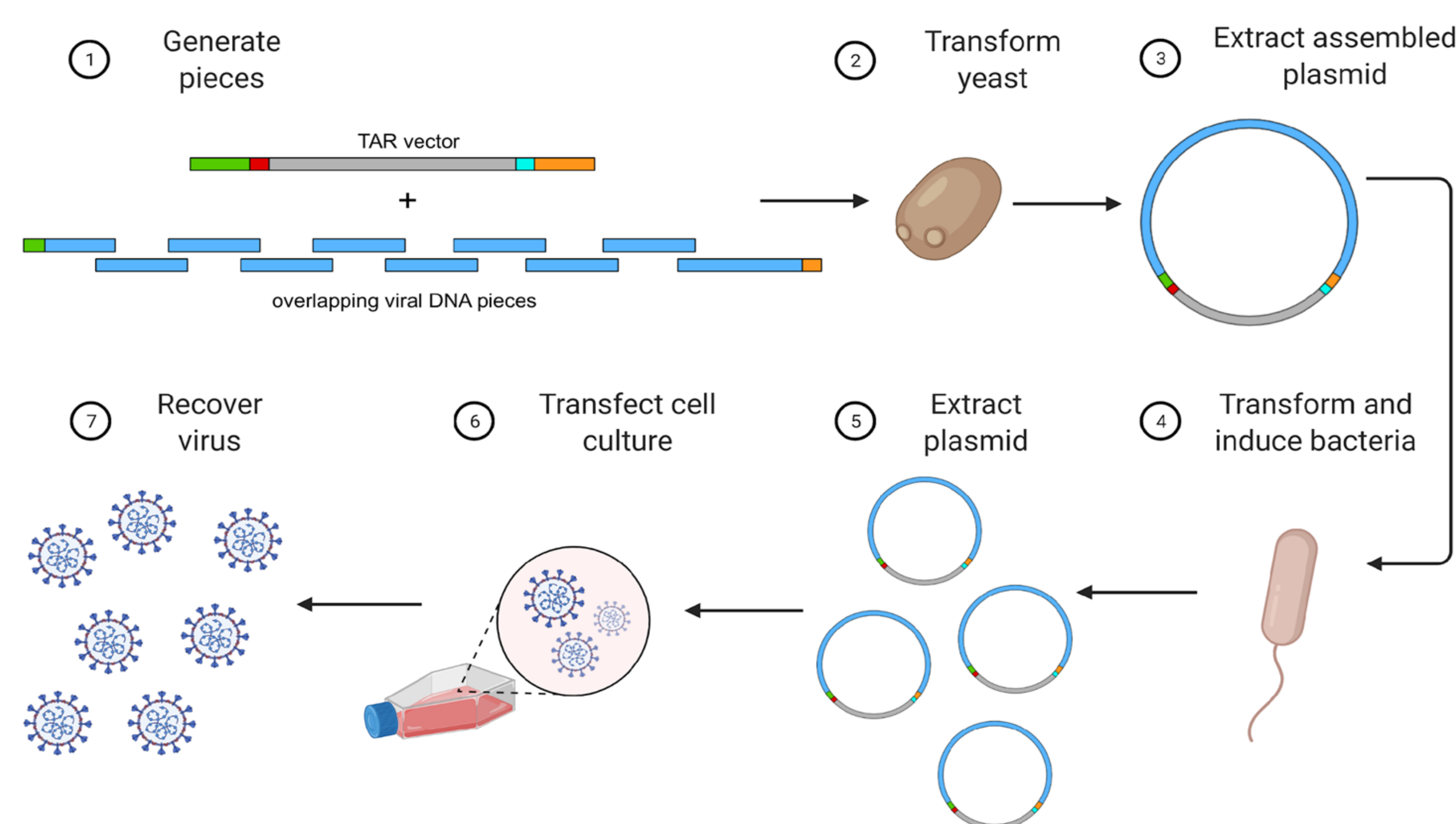


FIG. 1. Schematic of TAR assembly, subsequent transfection, and viral recovery. Yeast-based TAR facilitates the recombination of DNA viral genome pieces and the TAR vector due to the presence of overlapping DNA sequences. The assembled plasmid is then replicated in bacteria, purified, and transfected into appropriate host cells. Cells are monitored for cytopathic effect during recovery.

Results

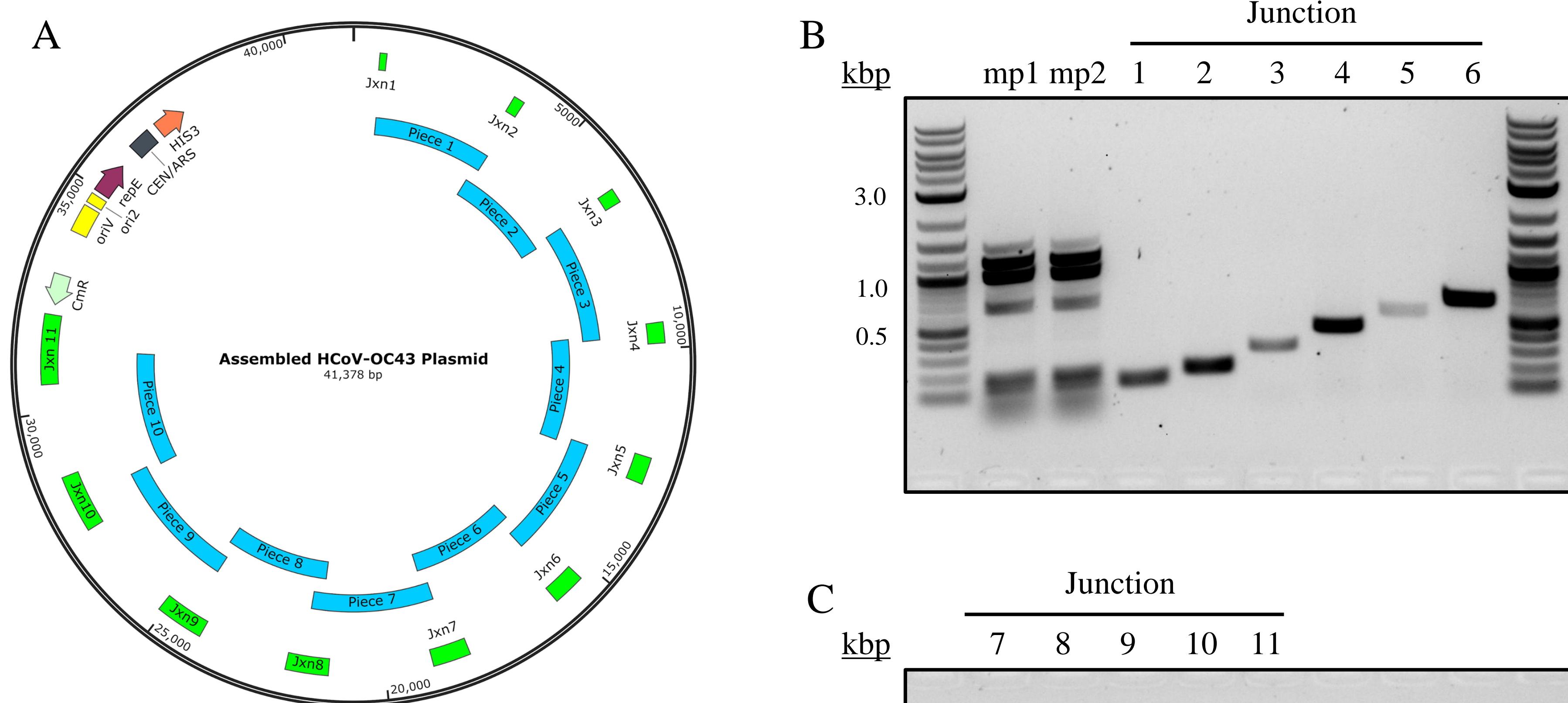


FIG. 2. Junction analysis of the TAR-assembled HCoV-OC43 genome. (A) Schematic of assembled vector showing HCoV-OC43 genome pieces (cyan), junctions to confirm assembly (green), and replication/selection features (others). (B and C) Agarose gels showing individual junctions (Jxn 1-11; green in Figure 1A) and multiplex PCR attempts to assess all junctions (mp1 and mp2).

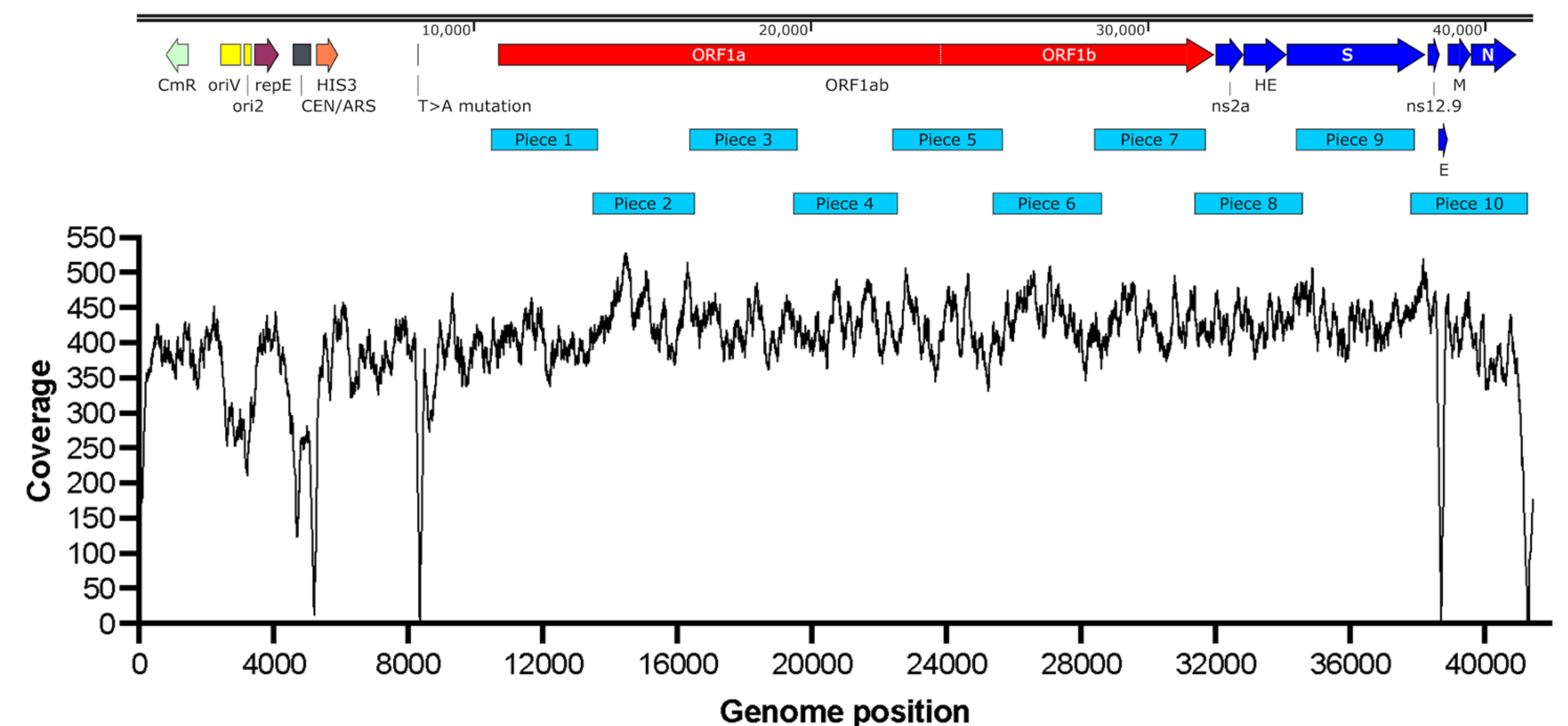


FIG. 3. Full-genome sequencing of the assembled HCoV-OC43 genome. The assembled plasmid was sequenced at the MGH CCIB DNA Core (Massachusetts General Hospital). Illumina MiSeq V2 chemistry generated paired-end reads (2 x 150 b), which were then assembled using *de novo* assembly [UltraCycler v1.0. (Brian Seed and Huajun Wang, unpublished)]. Only one mutation was detected (T>A at position 8351), but this is within a non-coding region of the plasmid and outside of the viral genome sequence.

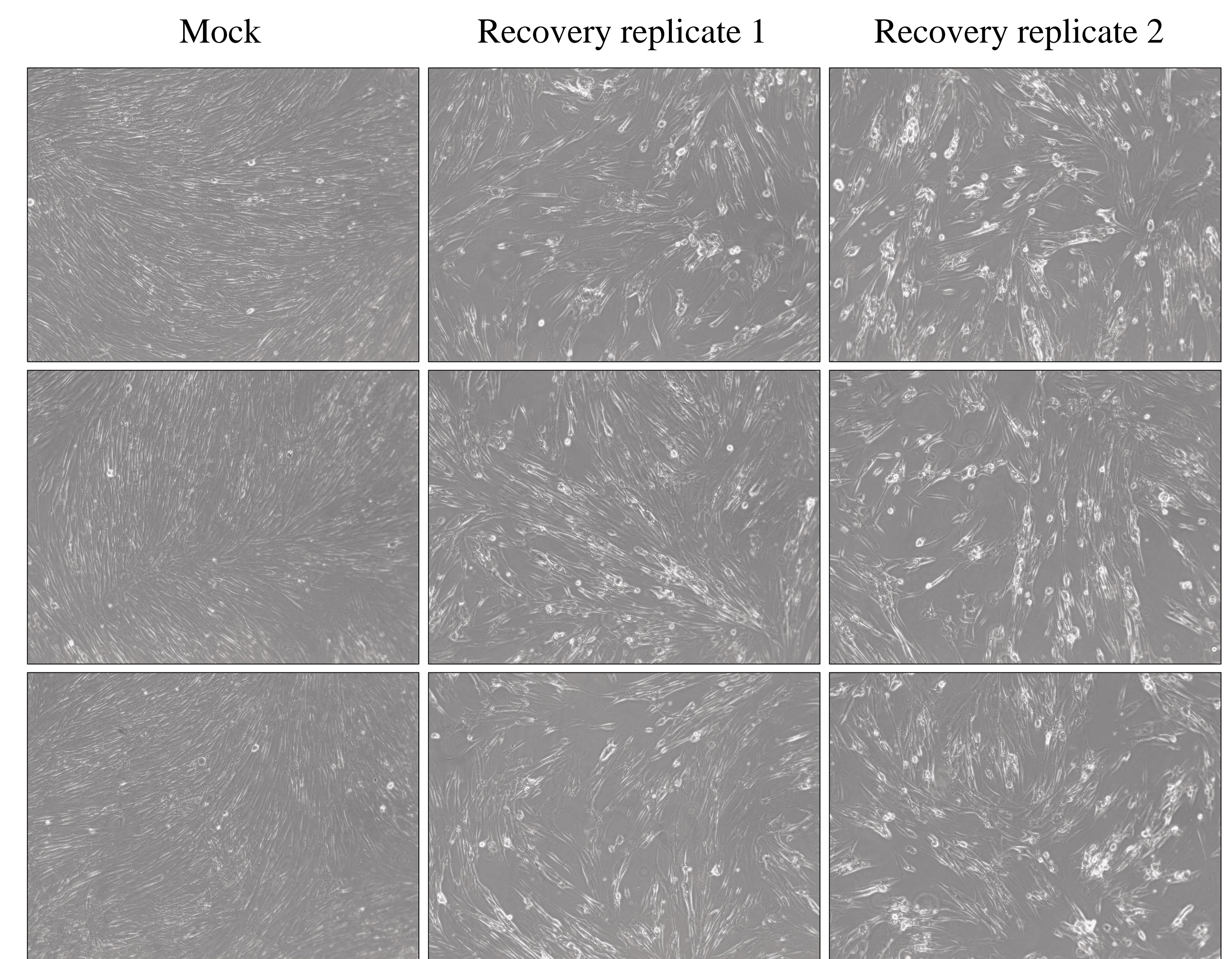


FIG. 4. Cytopathic effect (CPE) observed in human MRC-5 cells at day three post-infection. Recovered supernatant from transfected HEK-293T cells was transferred to human MRC-5 cells to observe potential cytopathic effect (CPE). Extensive CPE was observed at three days post-infection, as evidenced by cell rounding and lower cell density (recovery replicates 1 and 2) compared to mock samples. Images are representative of each condition and were taken at 100x total magnification.

Acknowledgements

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