# A novel, quick, and easy system for genetic analysis of SARS-CoV-2

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## Abstract

Our group has established a novel PCR-based, bacterium-free reverse genetics system for SARS-CoV-2 using the CPER method. This method is simpler and faster than previous methods. Large numbers of recombinant SARS-CoV-2 can be produced in about two weeks. This method can be used to modify the viral genome, allowing detailed study of the functions of mutations and the pathogenesis of COVID-19, and could speed the development of preventative measures and therapeutic strategies for the disease.

## Background & Results

SARS-CoV-2 is the virus responsible for the COVID-19 pandemic. We know that mutations in the genome of SARS-CoV-2 have occurred and spread, but what effect do those mutations have? Current methods for studying mutations in the SARS-CoV-2 genome are very complicated and time-consuming because coronaviruses have large genomes, but now a team from Osaka University and Hokkaido University have developed a quick, PCR-based reverse genetics system for analyzing SARS-CoV-2 mutations.

This system uses the polymerase chain reaction (PCR) and a circular polymerase extension reaction (CPER) to reconstruct the full-length cDNA of viral genome. This process does not involve the use of bacteria, which can introduce further unwanted mutations, and takes only two weeks using simple steps to generate infectious virus particles. Previous methods took a couple of months and were very complicated procedures.

## Significance of the research and Future perspective

This method allows us to quickly examine the biological features of mutations in the SARS-CoV-2. The CPER technique can be used to create recombinant viruses with each mutation and examine their biological features in comparison with the parental virus and contribute to understand the mechanisms underlying propagation and pathogenesis of SARS-CoV-2, as well as help determine the biological significance of emerging mutations. This method could could even allow a recombinant virus that is unable to cause disease to be generated, which could be used as a safe and effective vaccine for SARS-CoV-2.

Mutations are arising in the SARS-CoV-2 population all the time, as well as questions as to what those mutations do and whether they could affect the efficacy of vaccines. This simple and rapid method allows scientists around the globe to characterize the mutants, which is a vital step forward in our fight against the SARS-CoV-2.

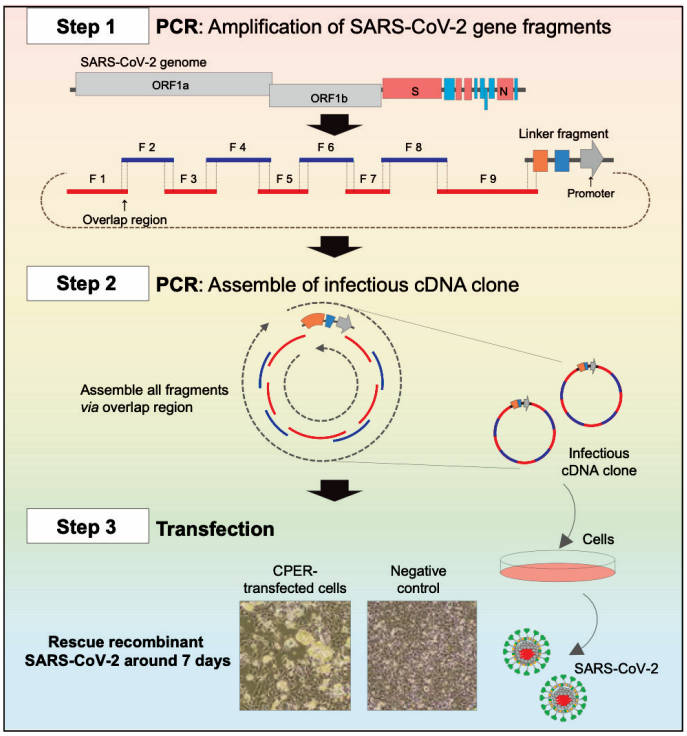


Figure. Reverse genetics for SARS-CoV-2 by CPER method In total of 9 gene fragments covering the full-length SARS-Cov-2 genome and a linker fragment were amplified. Since all fragments were designed to include overlapping ends with adjacent fragments, they can be assembled as a circular viral genome by additional PCR. By transfection of the circular viral genome into the susceptible cells, recombinant SARS-CoV-2 were rescued. Cytopathic effects were observed only in the cells transfected with CPER products.

Patent

Treatise Torii, Shiho; Ono, Chikako; Suzuki, Rigel et al. Establishment of a reverse genetics system for SARS-CoV-2 using circular polymerase extension reaction. Cell Rep. 2021 Apr; 35(3): 109014. doi: 10.1016/j.celrep.2021.109014. Epub 2021 Apr 1.

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