

**SARS-Related Coronavirus 2,  
USA-WA1/2020 Recombinant Infectious  
Molecular Clone Plasmid Kit**

**Catalog No. NR-53762**

**For research use only. Not for use in humans.**

**Contributor:**

Ralph S. Baric, Ph.D., Department of Epidemiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

**Manufacturer:**

BEI Resources

**Product Description:**

Note: The vial labels for NR-53752 to NR-53758 indicate these products are a molecular clone in vector pU57. The correct vector is pUC57 and each plasmid produces a viral fragment that must be combined with additional fragments to produce the molecular clone.<sup>1,2</sup> The NR-53755 label also lists this clone as wildtype (WT); however, NR-53755 is not WT and includes a T15102A silent mutation.<sup>2</sup>

The vectors for the recombinant infectious molecular clone from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), USA-WA1/2020 (GenBank: [MT461669](#)) was designed by RT-PCR amplification of SARS-CoV-2 virus (GenBank: [MT020880](#)) with restriction sites and four-nucleotide cohesive ends at the 5' and 3' insert termini and subcloned into the [pUC57](#) expression vector.<sup>1,2</sup>

NR-53762 can be used to assemble recombinant infectious SARS-CoV-2, USA-WA1/2020 and consists of the eight plasmids listed in Table 1. Descriptions of each component are included below.

**Table 1: SARS-CoV-2 Molecular Clone Plasmid Kit**

Plasmid Type	Insert	BEI Resources Catalog Number
Virus fragment	cDNA fragment A	NR-53752
Virus fragment	cDNA fragment B	NR-53753
Virus fragment	cDNA fragment C	NR-53754
Virus fragment	cDNA fragment D	NR-53755
Virus fragment	cDNA fragment E	NR-53756
Virus fragment	cDNA fragment F	NR-53757
Virus fragment	cDNA fragment G	NR-53758
Helper Plasmid	sgRNA-N	NR-53761

NR-53752 to NR-53755 and NR-53758 were designed with *Bsa*I restriction sites for plasmid excision.<sup>1,2</sup> The cDNA fragment A contains nucleotides 1 to 5415 as well as an upstream T7 promoter sequence, fragment B contains

nucleotides 5406 to 10456, fragment C contains nucleotides 10450 to 14498, fragment D contains nucleotides 14493 to 17848 as well as a T15102A silent mutation and fragment G contains nucleotides 25412 to 29895 as well as a 25 nucleotide poly-A tail downstream.

NR-53756 and NR-53757 were designed with *Bsm*BI restriction sites for plasmid excision.<sup>1,2</sup> The cDNA fragment E contains nucleotides 17842 to 21497 and fragment F contains nucleotides 21492 to 25417.

NR-53761 is an sgRNA-N plasmid designed to enhance the efficiency of recovering SARS-CoV-2 virus in the cell culture. This plasmid includes a 75 base pair leader sequence, SARS-CoV-2 virus nucleocapsid (N) gene, 3' untranslated region (UTR) and a 25-nucleotide poly-A tail under control of a T7 promoter.

All plasmids contain the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli* (*E. coli*).

The complete plasmid sequences and maps are provided on the BEI Resources webpage. The plasmids were produced in *E. coli* and extracted.

**Material Provided:**

Each kit contains one vial of each plasmid DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. The DNA concentrations and volumes provided are shown on the Certificate of Analysis. The vials should be centrifuged prior to opening. Note: The contents of each vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression.

**Packaging/Storage:**

NR-53762 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

**Citation:**

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, USA-WA1/2020 Recombinant Infectious Molecular Clone Plasmid Kit, NR-53762."

**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. [Biosafety in Microbiological and Biomedical Laboratories](#). 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see [www.cdc.gov/biosafety/publications/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

Note: Infectious viral particles produced by use of this kit are a BSL3 organism. Virus production should be performed with appropriate biosafety controls.

**Disclaimers:**

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**References:**

1. Baric, R. S., Personal Communication.
2. Hou, Y. J., et al. "SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract." *Cell* 182 (2020): 429-446. PubMed: 32526206.

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