

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

Catalog No. NR-53762

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. The NR-53755 label also lists this clone as wildtype (WT); however, NR-53755 is not WT and includes a T15102A silent mutation. 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.

The deposited plasmids were transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani or Terrific broth with ampicillin (100 µg per mL) for 1 day at 37°C in an aerobic atmosphere, extracted using a Plasmid Plus Maxi Kit (QIAGEN® 12963) and vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

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

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Table 1: Molecular Clone Plasmid Kit Components

COMPONENT NUMBER	DESCRIPTION	LOT NUMBER	DATE OF MANUFACTURE
NR-53752	cDNA fragment A	70038955	16SEP2020
NR-53753	cDNA fragment B	70038946	16SEP2020
NR-53754	cDNA fragment C	70041812	19FEB2021
NR-53755	cDNA fragment D	70038948	16SEP2020
NR-53756	cDNA fragment E	70038949	16SEP2020
NR-53757	cDNA fragment F	70038950	16SEP2020
NR-53758	cDNA fragment G	70038951	16SEP2020
NR-53761	sgRNA-N	70038954	16SEP2020

Table 2: Virus Fragment Plasmids (NR-53752 to NR-53758)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	Consistent with depositor reported size ¹
Genotypic Analysis Sequencing of insertion	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence ^{2,3}
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ⁴	<i>bla</i> sequence present	<i>bla</i> sequence present
Agarose Gel Electrophoresis NR-53752 Digestion with <i>Sall</i> (pre-vial) Digestion with <i>Bsal</i> + <i>NotI</i> (pre-vial) NR-53753 Digestion with <i>Bsal</i> (pre-vial) Digestion with <i>BamHI</i> + <i>Sall</i> (pre-vial) NR-53756 Digestion with <i>BsmBI</i> (pre-vial) NR-53758 Digestion with <i>KpnI</i> and <i>Sall</i>	~ 5.5 kb and ~ 3 kb ~ 5.5 kb, ~ 1.3 kb and ~ 1.3 kb ~ 5 kb, ~ 1.5 kb and ~ 1.5 kb ~ 5 kb and ~ 3 kb ~ 4 kb, ~ 2 kb and ~ 0.4 kb ~ 4.5 kb and ~ 2.5 kb	~ 5.5 kb and ~ 3 kb (Figure 1) ~ 5.5 kb, ~ 1.3 kb and ~ 1.3 kb (Figure 1) ~ 5 kb, ~ 1.5 kb and ~ 1.5 kb (Figure 2) ~ 5 kb and ~ 3 kb (Figure 2) ~ 4 kb, ~ 2 kb and ~ 0.4 kb (Figure 3) ~ 4.5 kb and ~ 2.5 kb (Figure 4)
Concentration by PicoGreen® Measurement NR-53752 NR-53753 NR-53754 NR-53755 NR-53756 NR-53757 NR-53758	≥ 2 µg/mL ≥ 2 µg/mL ≥ 2 µg/mL ≥ 2 µg/mL ≥ 2 µg/mL ≥ 2 µg/mL ≥ 2 µg/mL	0.8 µg in 30 µL per vial (27 µg/mL) 0.4 µg in 20 µL per vial (20 µg/mL) 0.3 µg in 20 µL per vial (14 µg/mL) 1.7 µg in 30 µL per vial (56 µg/mL) 0.7 µg in 30 µL per vial (24 µg/mL) 0.4 µg in 20 µL per vial (22 µg/mL) 0.4 µg in 20 µL per vial (18 µg/mL)
Amount per Vial NR-53752 NR-53753 NR-53754 NR-53755 NR-53756 NR-53757 NR-53758	Report results Report results Report results Report results Report results Report results Report results	0.8 µg 0.4 µg 0.3 µg 1.7 µg 0.7 µg 0.4 µg 0.4 µg
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9 to 2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	≥ 50 colonies per ng

¹The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

²The NR-53753 insert has two point mutations compared to the deposited GenBank sequence: T5409C (missense mutation resulting in ORF1ab mutation L1715S) and T8782C (silent mutation).

³The sequence MT461669 includes a silent mutation T15102A compared to the SARS-CoV-2, USA-WA1/2020 sequence (GenBank: MT020880).

⁴The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.

Table 3: Helper Plasmid (NR-53761)

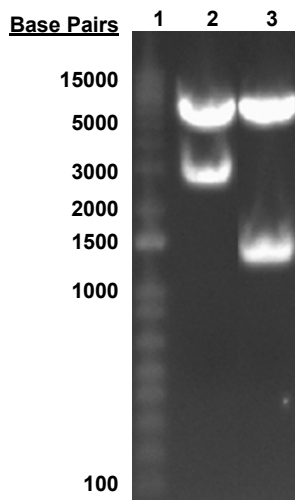
TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 4490 base pairs	4490 base pairs ¹
Genotypic Analysis Sequencing of insert (~ 1730 base pairs) N gene and 3' UTR T7 promoter sequence Leader sequence (75 base pairs) Poly-A tail sequence (25 base pairs)	≥ 99% sequence identity to SARS-CoV-2, USA-WA1/2020 (GenBank: MT020880.1) T7 promoter sequence confirmed Leader sequence confirmed Poly-A tail sequence confirmed	100% sequence identity to SARS-CoV-2, USA-WA1/2020 (GenBank: MT020880.1) T7 promoter sequence confirmed Leader sequence confirmed Poly-A tail sequence confirmed ²
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Agarose Gel Electrophoresis Digestion with <i>SalI</i> (pre-vial)	~ 2.7 kb and ~ 1.8 kb	~ 2.7 kb and ~ 1.8 kb (Figure 5)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.1 µg in 20 µL per vial (5 µg/mL)
Amount per Vial	Report results	0.1 µg per vial
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	> 500 colonies per ng

¹The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI website.

²The NR-53761 poly-A tail sequence has an additional 8 nucleotides, resulting in a 33-base pair poly-A tail sequence. The longer poly-A tail is within normal poly-A variation for viral mRNA transcripts. For more information, please see Kim, D., et al. "The Architecture of SARS-CoV-2 Transcriptome." *Cell* 181 (2020): 914-921. PubMed: 32330414.

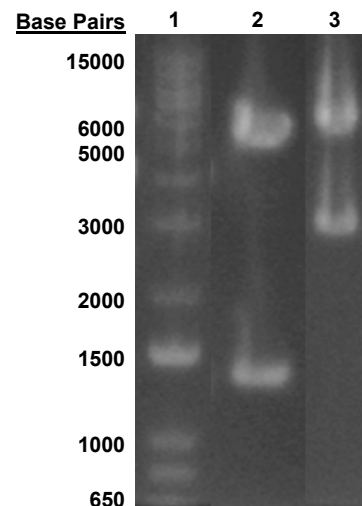
³The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid expansion to avoid plasmid loss and increased antibiotic concentrations may be necessary.

Figure 1: Agarose Gel of NR-53752



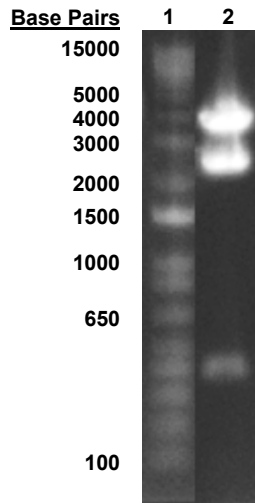
Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-53752 *SalI* digested
Lane 3: NR-53752 *Bsal/NotI* digested

Figure 2: Agarose Gel of NR-53753



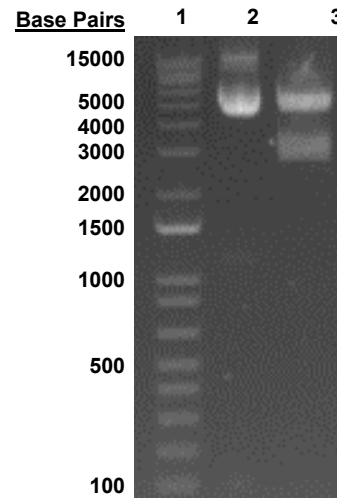
Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-53753 *Bsal* digested
Lane 3: NR-53753 *BamHI/SalI* digested

Figure 3: Agarose Gel of NR-53756



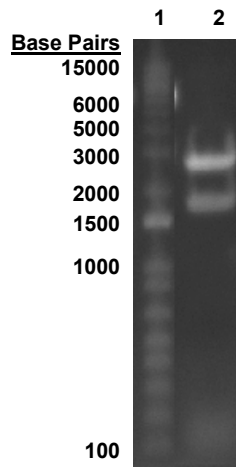
Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-53756 *BsmBI* digested

Figure 4: Agarose Gel of NR-53758



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-53758 undigested
Lane 3: NR-53758 *KpnI/SalI* digested

Figure 5: Agarose Gel of NR-53761



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-53761 *SalI* digested

/Heather Couch/
Heather Couch

Program Manager or designee, ATCC Federal Solutions

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