SARS Coronavirus, Tor2, Complete Gateway® Clone Set, Recombinant in Escherichia coli

Catalog No. NR-19270

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Contributor:
Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

Product Description:
SARS Coronavirus, Tor2, Gateway® clones were designed for features based on the annotation from the GenBank entry AY274419 and the corresponding RefSeq entry for NC_004718. The sequences from both entries are identical but there are annotation differences. The clones were designed from the annotated ORFs from AY274419 and from the annotated protein coding regions of NC_004718. The clone set consists of twenty-seven clones that were constructed in vector pDONR 221 (Invitrogen). ATG start codons were added to the forward primer sequences when required and stop codon sequence was trimmed from the reverse primer sequences. Each clone has been sequenced using a combination of end sequencing and primer walking to determine each base at an average of 2-fold coverage.

Detailed information about each clone is shown in Table 1. Information related to the use of Gateway® Clones can be obtained from Invitrogen.

Material Provided:
Each well of the 96-well plate contains approximately 40 µL of Escherichia coli culture [strain DH10B-T1, or strain Stbl for clones NC_828851, NC_828869(a) and NC_828869(b)] in Luria Bertani (LB) Broth containing 50 µg/mL kanamycin supplemented with 15% glycerol.

Note: Production in the 96-well format has increased risk of cross-contamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources cannot confirm or validate any clone not identified on the plate information table.

Packaging/Storage:
NR-19270 was packaged asexptically in 96-well plates. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:
Media:
LB Broth or Agar containing 25 µg/mL kanamycin

Incubation:
Temperature: Clones should be grown at 37°C except for clones NC_828851, NC_828869(a) and NC_828869(b), which should be grown at 30°C.
Atmosphere: Aerobic
Propagation:
1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plates at the temperatures indicated above. All clones should be grown for 18 to 24 hours, except for clones NC_828851, NC_828869 and NC_828869b, which should be incubated for 16 to 18 hours.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: SARS Coronavirus, Tor2, Complete Gateway® Clone Set, Recombinant in Escherichia coli, NR-19270."

Biosafety Level: 1

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<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Well Position</th>
<th>Coordinates</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A</td>
<td>100% identity to target with &gt;= 2X coverage over the full length of the insert. These clones should be considered completely error free.</td>
<td>A01</td>
<td>265...801</td>
<td>A</td>
</tr>
<tr>
<td>Class B</td>
<td>100% identity to target with &lt; 2X coverage over the full length of the insert. These clones have no mismatches relative to the intended insert sequence, however there are regions where we have only been able to verify the sequence using one read.</td>
<td>A02</td>
<td>802...2718</td>
<td>C</td>
</tr>
<tr>
<td>Class C</td>
<td>Less than 100% identity to intended target sequence. These clones either have not been completely verified, or have sequence that differs from the Genbank Accession.</td>
<td>A03</td>
<td>9985...10902</td>
<td>B</td>
</tr>
<tr>
<td>Class D</td>
<td>These clones have no mismatches relative to the intended insert sequence, however there are regions where we have only been able to verify the sequence using one read.</td>
<td>A04</td>
<td>10903...11772</td>
<td>A</td>
</tr>
</tbody>
</table>

Table 1: SARS Coronavirus, Tor2, Gateway® Clones

NP_828860 A01 265...801 Leader protein nsp1-pp1/pp1ab A
NP_828861 A02 802...2718 Counterpart of MHV p65 protein nsp2-pp1a/pp1ab C
NP_828863 A03 9985...10902 3C-like protease nsp3-pp1a/pp1ab (3CL-PRO) B
NP_828864 A04 10903...11772 Transmembrane protein nsp6-pp1a/pp1ab B
NP_828865 A05 11773...12021 Protein nsp7-pp1a/pp1ab B
NP_828866 A06 12022...12615 Protein nsp8-pp1a/pp1ab A
NP_828867 A07 12616...12954 RNA-binding protein nsp9-pp1a/pp1ab A
NP_828868 A08 12955...13371 Protein nsp10-pp1a/pp1ab A
NP_828869(a) A09 13372...16166 RNA-dependent RNA polymerase nsp12-pp1ab (RdRp) (with leader) C
NP_828869(b) A10 13398...16166 RNA-dependent RNA polymerase nsp12-pp1ab (RdRp) (without leader) C
NP_828870 A11 16167...17969 Zinc-binding NTPase/helicase nsp13-pp1ab (ZD NTPase/HEL) A
NP_828871 A12 17970...19550 nsp14-pp1ab (nuclease ExoN homolog) A
NP_828872 B01 19551...20388 Replicative endonuclease NendoU nsp15-pp1ab A
NP_828873 B02 20589...21482 Ribose 2'-O-methyltransferase nsp16-pp1ab B
NP_828874 B03 21492...25259 E2 glycoprotein precursor; putative spike glycoprotein C
NP_828875 B04 25268...26092 Protein 3a (sars3a) B
NP_828876 B05 25689...26153 Putative protein 3a (sars3b) B
NP_828877 B06 26117...26347 Small envelope protein E B
NP_828878 B07 26398...27063 Matrix protein M B
NP_828879 B08 27074...27265 Putative protein (sars6) B
NP_828880 B09 27273...27641 Putative protein (sars7a) A
NP_849175 B10 27638...27772 Putative protein (sars7b) C
NP_849176 B11 27779...27986 Putative protein (sars8a) B
NP_849177 B12 27964...28118 Putative protein (sars8b) B
NP_828878 C01 28120...29388 Nucleocapsid protein N A
NP_828879 C02 28130...28426 Putative protein (sars9b) B
AAP41049 C03 28583...28795 Orf14 (SARS coronavirus, Tor2) B

Notes:

- Class A: 100% identity to target with >= 2X coverage over the full length of the insert. These clones should be considered completely error free.
- Class B: 100% identity to target with < 2X coverage over the full length of the insert. These clones have no mismatches relative to the intended insert sequence, however there are regions where we have only been able to verify the sequence using one read.
- Class C: Less than 100% identity to intended target sequence. These clones either have not been completely verified, or have sequence that differs from the Genbank Accession.
- Class D: These clones have not yet been completely verified, due to sequencing gaps.