VIROLOGICA SINICA

Electronic Supplementary Material

A Convenient and Biosafe Replicon with Accessory Genes of SARS-CoV-2 and Its Potential Application in Antiviral Drug Discovery

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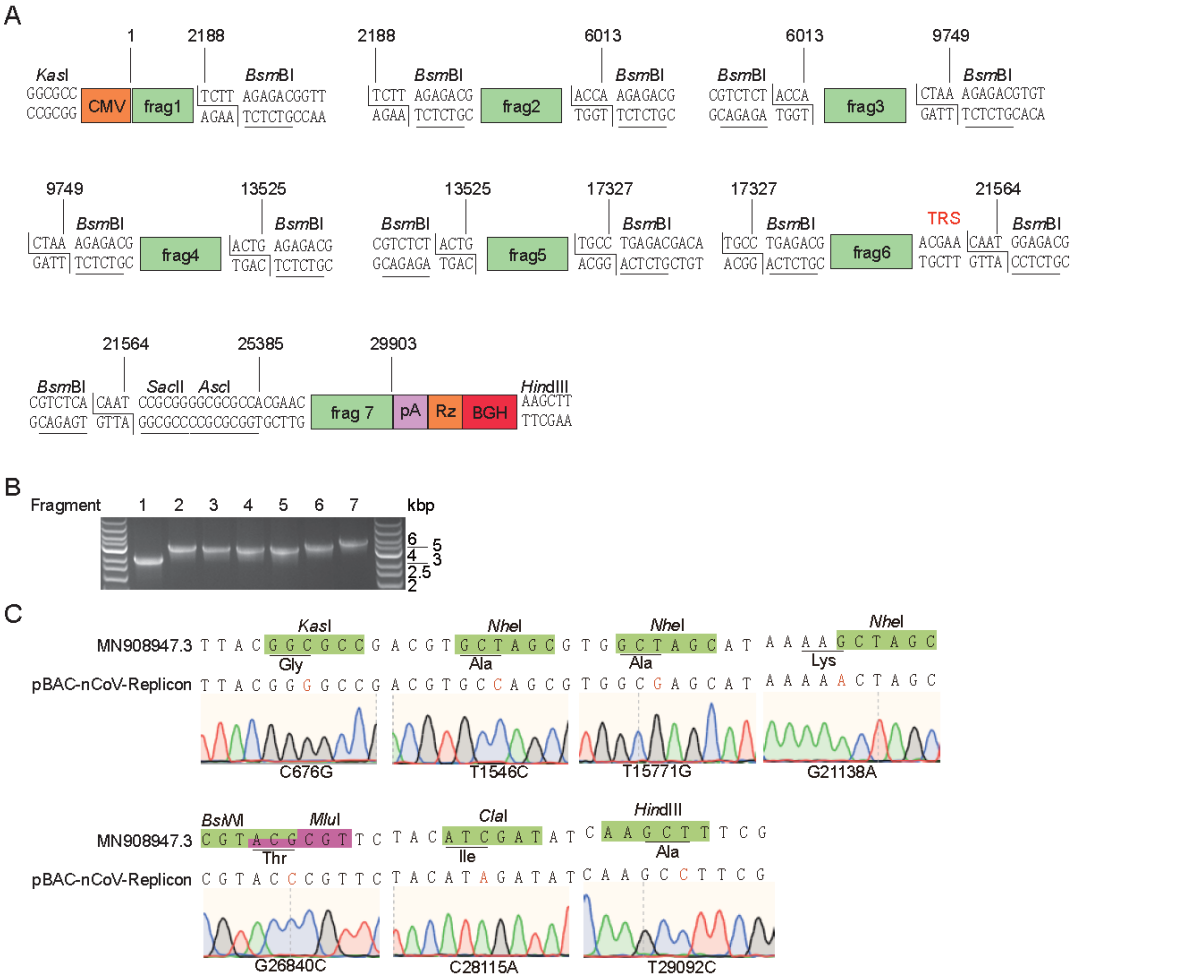

1. **Fig. S1** The strategy for the assembly of the SARS-CoV-2 replicon cDNA. A Schematic of the fragments for the assembly of cDNA of the SARS-CoV-2 replicon with indicated restriction sites, elements for transcription, and nucleotide positions in the viral genome. **B** The fragments were examined in DNA agarose gel electrophoresis. **C** The sequencing results of cloning markers.

Table S1 Primers for the amplification of the viral genome

Names	Primer sequences (5′–3′)
373r	CTCCACGGAGTCTCCAAAGCC
1379f	GCATGTCACAATTCAGAAGTAGG
1412f	AGTCTTGCCGAATACCATAATGAATC
1463f	GGTGGTCGCACTATTGCCTTTGG
2072f	GGTGGTGTTGTTCAGTTGACTTCGC
2356r	TCCACCAATAATGATAGAGTCAGCACAC
2900r	CAGCATCTGCCACAACACAG
4125f	TGGGTGATGTTGTTCAAGAGGG
4295r	TCTTTGCCTCCTCTACAGTGTAACC
5230f	GTGGAAATACCCACAAGTTAATGG
5370f	CAAGGGCTGGTGAAGCTGCTAAC
5831f	TCCTCAGAATACAAAGGTCCTATTACGG
6331r	TGATGTTTCAACTGGTTTTGTGCTCC
6563r	CATAAGCAGCCATTAGATCTGTGTG
6618f	GTTTGAAAACCCTTGCTACTCATG
6852r	GTCGGCATAGATGCTTTAATTCTAG
7385f	ATGGCCCCGATTTCAGCTATGG
7994f	GTGTCTGATGTTGGTGATAGTGCGG
8320f	CCGTGACCTTGGTGCTTGTATTG
8718r	CGAGTGACACCACCATCAATAGCC
8975f	GAGTACACTGACTTTGCAACATC
9093f	TTGCTTATGAAAGTTTACGCCCTGAC
9370f	TCAACCTATTGGTGCTTTGGACATATC
10041r	GAGGTGATAGAGGTTTGTGGTGGTTG
10436f	TGTGCTATGAGGCCCAATTTCAC
10560r	GTTGGTAATTCCATATGGTGCATG
10578r	GTGCCAGCATGAACTCCAGTTGG
13179f	GTCAGGCAATAACAGTTACACCGGA
13237f	ATCGTGTTGTCTGTACTGCCGTTGC
13633r	AATTGTCATCTTCGTCCTTTTCTTGG
13811f	TGGCAGACCTCGTCTATGCTTTAAG
13835f	GGCATTTTGATGAAGGTAATTGTGAC
14036r	GCATTTCGCATGGCATCACAG
14786r	GCAGCATTACCATCCTGAGCAAAG
16386f	CAATGCTCCAGGTTGTGATGTC
16573f	GACTGGACAAATGCTGGTGATTAC
17061f	AAAGTATTCTACACTCCAGGGACCACCT
17556f	TTGTCGGCGTTGTCCTGCTG
18179r	TTAGGTATGCCAGGTATGTCAACAC
18322r	GACACCCCTCGACATCGAAGCC
18904r	GCTTAACAAAGCACTCGTGGACAG
21219f	CGCATGGTGGACAGCCTTTGTTAC
22203r	CGCACTAAATTAATAGGCGTGTGC
21224f	GGTGGACAGCCTTTGTTACTAATGTG
21566r	GGCCCGCGGATTGTTCGTTTAGTTGTTAACAAGAACATCACTAG
21923r	AAACAATAAGTAGGGACTGGGTCTTCG
22218r	CCCTGAGGGAGATCACGCAC
25264f	TTGCTGTATGACCAGTTGCTGTAGTTG
25513r	CAACGATACCGATACAAGCCTCAC
25546r	GTGCAACGCCAACAATAAGCCATC
28632r	CCAGCTTCTGGCCCAGTTCC
28812r	CTGCCGCCTCTGCTCCCTTC
2890r	CACAACACAGGCGAACTCATTTAC
29870r	GTCATTCTCCTAAGAAGCTATTAAAATCACATGG

Table S2 Primers used for the mutagenesis of nsp12 and nsp14

Names	Primer sequences (5'-3')
nsp14 D90A/E92Af	TTGGCTTCGCTGTCGCGGGGTGTCATGCTACTAGAGAAGC
nsp14 D90A/E92Ar	TGACACCCCGCGACAGCGAAGCCAATCCATGCACGTAC
nsp14 D331Af	CAGTTCTTCACGCCATTGGTAACCCTAAAGCTATTAAGTG
nsp14 D331Ar	GGGTTACCAATGGCGTGAAGAACTGGGAATTTGTC
nsp12 SDD mt f	TGATACTCGCTGCCGCTGCTGTTGTGTGTTTCAATAGCAC
nsp12 SDD mt r	CAACAGCAGCGGCAGCGAGTATCATCATTGAGAAATG

Table S3 Primers used for deep sequencing on the subgenomic RNAs

Subgenomic RNA Species	Signature leader-body junction sequence / complementary sequence
S	TCTAAACGAACAATGTTTGTT / AACAAACATTGTTCGTTTAGA
ORF3	TCTAAACGAACTTATGGATTT / AAATCCATAAGTTCGTTTAGA
E	TCTAAACGAACTTATGTACTC / GAGTACATAAGTTCGTTTAGA
M	TCTAAACGAACTAAATATTAT / ATAATATTTAGTTCGTTTAGA
ORF6	TCTAAACGAACGCTTTCTTAT / ATAAGAAAGCGTTCGTTTAGA
ORF7	TCTAAACGAACATGAAAATTA / TAATTTTCATGTTCGTTTAGA
ORF8	TCTAAACGAACATGAAATTTC / GAAATTTCATGTTCGTTTAGA
N	TCTAAACGAACAAACTAAAAT / ATTTTAGTTTGTTCGTTTAGA

Table S4 Primers for sequencing of the viral genome

Names	Primer sequences (5′–3′)
203f	CGTCCGTGTTGCAGCCGATC
1835f	GGTGAACAGAAATCAATACTGAGTCCTC
1922r	CAAGAGTGCGGGAGAAAATTGATCG
3624r	GGGCCGACAACATGAAGACAG
3551f	CCACTTAAAGTGGGTGGTAGTTGTG
5361r	TAAGCATCTTGTAGAGCAGGTGG
6986f	GGTTCTTTAATCTACTCAACCGCTGC
7072r	GCCTTCTCTGTAACCAGTACAGTAAG
7751f	GTGAAGAATGGTTCCATCCATCTTTAC
9960f	GTCATCTCGCAAAGGCTCTCAATG
11679f	GACTGACTCTTGGTGTTTATGATTAC
11758r	GGGTGGGAGTAGTCCCTGTG
13423f	CCGCGAACCCATGCTTCAG
13487r	GCACTTACACCGCAAACCCG
15174f	AATAGCCGCCACTAGAGGAGC
15243r	CATGTTGTGCCAACCACCATAG
15546r	GGCCGTGACAGCTTGACA
16897f	GTTGGTGATTATTTTGTGCTGACATC
16971r	GTGCTCTTGTGGCACTAGTGTAGG
18020f	GGAATGTGGCAACTTTACAAGC
18828f	CCATGGTAATGCACATGTAGCTAG
19480f	GGTGCTGTCTGTAGACATCATGC
19804r	TGCGCTTAGCCCAAAGCTC
20476f	GGTTCATCTAAGTGTGTGTGTTCTG
20610f	GCTTTGGTGTAAAGATGGCCATG
20682r	ACCCGGTTGCCACGCTTGAC
25513r	CAACGATACCGATACAAGCCTCAC
28221f	GAGTATCATGACGTTCGTGTTG
29090f	GCTTTCGGCAGACGTGGTCC

Table S5 Primers for qRT-PCR

Names	Primer sequences (5′–3′)
15f	CCTTCCCAGGTAACAAACCAACC
273r	CTCTCCATCTTACCTTTCGGTCAC
25546r	GTGCAACGCCAACAATAAGCCATC
26395r	GACTCACGTTAACAATATTGCAGCAG
26641r	GCATAGGCAAATTGTAGAAGACAAATCC
27219r	GTCAACGAGATGAAACATCTGTTGTCAC
27577r	GAGTGCTAAAGCAAGTCAGTGC
28060r	GGTGCTGATTTTCTAGCTCCTACTC
28421r	CAGTATTATTGGGTAAACCTTGGGGC