Cell Host & Microbe, Volume *1*

Supplemental Data

A Plasmid-Based Reverse Genetics System for Animal Double-Stranded RNA Viruses

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Table S1. Plasmid-based reverse genetics systems for representative RNA viruses

Genome	Virus family	Reference
Plus-sense RNA	Picornaviruses	(Racaniello and Baltimore, 1981)
	Flaviviruses	(Rice et al., 1989; Gritsun and Gould, 1995; Kinney et al., 1997; Yun et al., 2003)
	Coronaviruses	(Almazan et al., 2000; Yount et al., 2003; Coley et al., 2005)
Minus-sense RNA	Bornaviruses	(Schneider et al., 2005)
	Rhabdoviruses	(Schnell et al., 1994; Lawson et al., 1995; Whelan et al., 1995)
	Paramyxoviruses	(Collins et al., 1995; Garcin et al., 1995; Yoneda et al., 2006)
	Bunyaviruses	(Bridgen and Elliott, 1996)
	Orthomyxoviruses	(Fodor et al., 1999; Neumann et al., 1999)

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Table S2. Reovirus Mutants Generated from Cloned cDNA

Mutant	Nucleotide change	Amino acid change
rsT3D-σ1T249I	ACT → ATT (757-759)	Thr → Ile (249)
rsT3D-σ3Y354H	TAT → CAC (1092-1094)	Tyr → His (354)
rsT3D/S4-GFP	S4 (150-768) → GFP ORF* (1-720)	σ3 (40-365) → GFP (1-239)

*Open reading frame, including termination codon.

Table S3. GenBank Accession Numbers for Reovirus cDNA Sequences

Gene segment cDNA	GenBank accession number
T3D L1	EF494435
T3D L2	EF494436
T3D L3	EF494437
T3D M1	EF494438
T3D M2	EF494439
T3D M3	EF494440
T3D S1	EF494441
T3D S2	EF494442
T3D S3	EF494443
T3D S4	EF494444
T1L S1	EF494445

Table S4. Oligodeoxynucleotide primers used for plasmid construction

Plasmid DNA		Nucleotide sequence (nucleotide positions)[a]
pT7-L1T3D	Forward	5'-AAACCCGGGTAATACGACTCACTATAGCTACACGTTCCACTTCCACGACAATGT-3' (1-28)
	Reverse	5'-GGTCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGATGAGTTGACGCACCACGACCCAT-3' (3836-3860)
pT7-L2T3D	Forward	5'-TTAGAATTCTAATACGACTCACTATAGCTAAAAGGCGCGATGGCGAACGTTTG-3' (1-27)
	Reverse	5'-GGTCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGATGAATTAGGCGCGCTCACGAGGG-3' (3892-3916)
pT7-L3T3D	Forward	5'-AAACCCGGGAATTCTAATACGACTCACTATAGCTAATCGTCAGGATGAAGCGGATTC-3' (1-26)
	Reverse	5'-GGTCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGATGAATCGGCCCAACTAGCATTGA-3' (3877-3901)
pT7-M1T3D	Forward	5'-AAACCCGGGTAATACGACTCACTATAGCTATTCGCGGTCATGGCTTACATCG-3' (1-26)
	Reverse	5'-TTTCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGATGAAGCGCGTACGTAGTC-3' (2285-2304)
pT7-M2T3D	Forward	5'-AAAGAGGAGGTGGAGATGCTCTAGATAATACGACTCACTATAGCTAATCTGCTGACCGT-3' (1-17)
	Reverse	5'-TTTGAGGAGGTGGAGATGCCATGCCGACCCGATGATTTGCCTGCATCCCT-3' (2184-2203)
pT7-M3T3D	Forward	5'-CCTCGCGGTCCGACCGGTTAATACGACTCACTATAGCTAAAGTGACCG-3' (1-13)
	Reverse	5'-GGTCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGATGAATGGGGGTCGGGAAGGCTTAAGGGA-3' (2212-2241)
pBacT7-S1T3D	Forward	5'-AAAGAGGAGGTGGAGATGCTCTAGATAATACGACTCACTATAGCTATTGGTCGGATGGATCC-3' (1-20)
	Reverse	5'-AAAGAGGAGGTGGAGATGCCATGCCGACCCGATGAAATGCCCCAGTGCCG-3' (1397-1416)
pBacT7-S1T1L	Forward	5'-AAAGAGGAGGTGGAGATGCTCTAGATAATACGACTCACTATAGCTATTCGCGCCTATGGAT-3' (1-19)
	Reverse	5'-AAAGAGGAGGTGGAGATGCCATGCCGACCCGATGATTGACCCCTTGTGCC-3' (1443-1462)
pT7-S2T3D	Forward	5'-AAAGAGGAGGTGGAGATGCTCTAGATAATACGACTCACTATAGCTATTCGCTGGTCAGT-3' (1-17)
	Reverse	5'-AAAGAGGAGGTGGAGATGCCATGCCGACCCGATGAATGTGTGGTCAGTCG-3' (1312-1331)
pT7-S3T3D	Forward	5'-CTCGCGGTCCGGTTAATACGACTCACTATAGCTAAAGTCACGCCTGTCGTCGTCACTATGGCTTCC-3' (1-36)
	Reverse	5'-AGGTCGGACCGCGAGGAGGTGGAGATGCCATGCCGACCCGATGATTAGGCGTC-3' (1185-1198)
pT7-S4T3D	Forward	5'-GGCATGGCATCTCCACCTCCTCGCGGGTTAATACGACTCACTATAGCTATTTTTGCCTCTTCC-3' (1-18)
	Reverse	5'-GAGGAGGTGGAGATGCCATGCCGACCCGATGAATGAAGCCTGTCCC-3' (1178-1196)

pBacT7-S1T3DT249I	Forward	5'-CTCAAGGATAGGCGCAA**T**TGAGCAAAGTTACGTGG-3' (741-775)
	Reverse	5'-CCACGTAACTTTGCTCA**A**TTGCGCCTATCCTTGAG-3' (741-775)
pT7-S4T3DY354H	Forward	5'-GGGGATTTGAAT**C**A**C**CCAGTGATGATTGGCGATCCG-3' (1080-1115)
	Reverse	5'-CGGATCGCCAATCATCACTGG**G**T**G**ATTCAAATCCCC-3' (1080-1115)
pT7-S4GFP	Forward	5’-GACAAAACAATCTCAGCACAGgaattcCCAGATATGATGG-3’ (129-149, 150-162)
	Reverse	5’-CCATCATATCTGGgaattcCTGTGCTGAGATTGTTTTGTC-3’ (129-149, 150-162)

[a]The T7 promoter sequence is underlined. Nucleotide substitutions are indicated in bold. Nucleotide insertions are indicated in lowercase letters. Numbers in parentheses correspond to nucleotide positions of sequences contained in each gene-specific primer. Reverse primers are numbered according to the complementary nucleotide positions of the plus strand.

Supplemental Experimental Procedures

Plasmid construction

To construct T3D L1, L3, and M1 plasmid cDNAs for rescue of recombinant infectious reovirus, RT-PCR products generated using viral genomic RNA and gene-specific primer sets (Table S3) were cloned into the EcoRV-RsrII (L1 and M1) or SmaI-RsrII site (L3) of p3E5EGFP, resulting in pT7-L1T3D, pT7-L3T3D, and pT7-M1T3D. The T3D L2 gene cDNA, amplified from viral genomic RNA with specific primers, was inserted into the EcoRI-RsrII site of pT7-L3T3D, thereby replacing the L3 cDNA and generating pT7-L2T3D. pT7-M2T3D, containing the T3D M2 gene cDNA, was constructed by RT-PCR amplification of the M2 gene using viral genomic RNA and specific primers and insertion of the resultant cDNA into the EcoRV-BseRI site of p3E5EGFP. To construct pT7-M3T3D, pT7-S2T3D, and pT7-S4T3D, which contain the T3D M3, S2, and S4 genes, respectively, RT-PCR amplification products generated using viral genomic RNA and specific primers were inserted into RsrII (M3) and BseRI (S2 and S4) sites of p3E5EGFP. p3E5EGFP constructs containing cloned T3D M3, S2, and S4 genes were treated with the SmaI and AvrII (pT7-M3T3D) or EcoRV and AvrII (pT7-S2T3D and pT7-S4T3D) and self-ligated to remove GFP-encoding sequences and the Ebola virus leader and trailer. The T3D S3 cDNA was amplified by RT-PCR using viral genomic RNA and gene-specific primer sets and inserted into pCR2.1. The pCR2.1-based construct then was used as template for secondary PCR amplification of S3 sequences with the M13-reverse and gene-specific primers, and the amplification product was inserted into the EcoRV-RsrII site of p3E5EGFP to generate pT7-S3T3D. The T1L and T3D S1 cDNAs were amplified by RT-PCR using viral genomic RNA and gene-specific primer sets containing BseRI sites and inserted into pCR2.1. A BseRI fragment containing the S1 cDNA and T7 promoter was subcloned into p3E5EGFP to generate S1-p3E5EGFP. An XbaI fragment of S1-p3E5EGFP containing the S1 cDNA fused to the T7 promoter and HDV ribozyme was inserted into the XbaI site of pBacPak8 (Clontech) to generate pBacT7-S1T1L or pBacT7-S1T3D. To generate pT7-S4GFP, sequences corresponding to the open reading frame (ORF) of enhanced GFP (pEGFP-N1, Clontech) were substituted for S4 gene nucleotide sequences 150-768 in pT7-S4T3D. A unique *EcoR*I site first was introduced into the S4 gene of pT7-S4T3D using the QuickChange site-directed mutagenesis kit, yielding the construct, pT7-S4T3DEcoRI. Subsequently, a PCR product containing the GFP ORF was cloned into the *EcoR*I-*Xho*I site of pT7-S4T3DEcoRI to generate pT7-S4GFP. The construct, pCXN-S4T3D, which contains the entire T3D σ3 ORF, was created by subcloning T3D S4 sequences from pCMVS4wt (Becker et al., 2003) into the

*EcoR*I-*Kpn*I site of pCXNMCS, which was derived by modifying the multiple-cloning site of pCXN2 (Niwa et al., 1991).

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Figure S1. Kinetics of rsT3D/S4GFP Growth in L cells and L-σ3 cells

Parental L cells or those stably expressing σ3 protein (L-σ3) were infected with rsT3D/S4GFP at an MOI of 1 PFU/cell, and viral titers in cell lysates at the time points shown were determined by plaque assay. Results are presented as mean viral titers for triplicate experiments. Error bars indicate SD.