VIROLOGICA SINICA

Electronic Supplementary Material

Construction of a One-vector Multiplex CRISPR/Cas9 Editing System Used to Inhibit BmNPV Replication in Silkworms

Zhanqi Dong[1#], Qi Qin[1#], Zhigang Hu[1], Peng Chen[1], Liang Huang[1], Xinling Zhang[1], Ting Tian[1], Cheng Lu[1,2]✉, Minhui Pan[1,2]✉

1. State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400716, China
2. Key Laboratory of Sericultural Biology and Genetic Breeding, Ministry of Agriculture, Southwest University, Chongqing 400716, China

Supporting information to DOI: 10.1007/s12250-019-00121-4

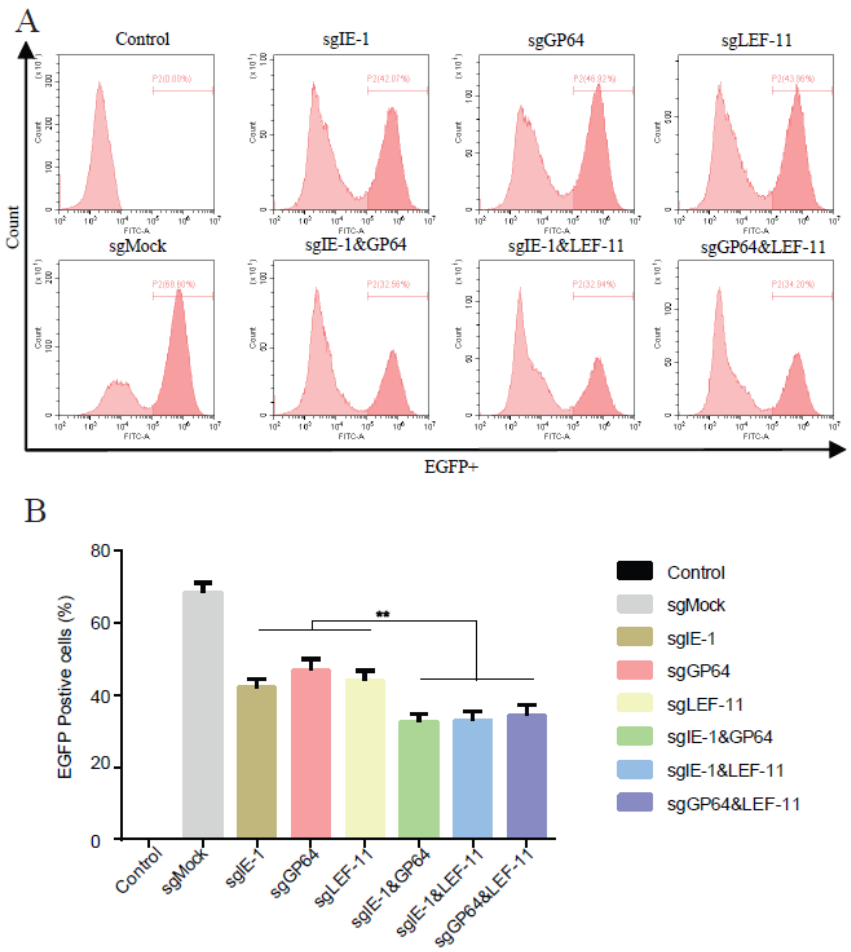

Figure S1. Comparison of CRISPR/Cas9 editing efficiency with single and double sgRNAs. (A) BmN-SWU1 cells transfected with the indicted sgMock, single sgRNAs (sgIE-1, sgGP64, or sgLEF-11), or double sgRNAs (sgLEF-11 & IE-1, sgLEF-11 & GP64, or sgIE-1 & GP64) and infected with BmNPV at MOI of 10. The sgRNA knockout cells inhibited the expression of BmNPV. Flow cytometric analysis were taken 72 h post infection after co-culturing. (B) Statistical analysis of BmN-SWU1 EGFP-positive cells transfected with Cas9 and the indicted sgRNA.

Table S1. Sequences of primers used in this study

Primer name	Primer sequence*
Bombyx mori U6 amplification primers	
U6- EcoR I /F	5' CGGAATTCAGGTTATGTAGTACACATT3'
U6- EcoR I /R	5' CGGAATTCAAAAAAAGCACCGACTCG 3'
CRISPR/Cas9 system primers	
IE-1^{prm}-EcoR I /F	5' AAGCTTTTGCAGTTCGGGAC 3'
IE-1^{prm}-Cla I /R	5' CCATCGATTAGATCCCTAGTCG 3'
Cas9- Cla I /F	5' CCATCGATATCGATATGGACTATAAGGA 3'
Cas9-Xba I/R	5' GC TCTAGATTACTTTTTCTTTTTTGCCTGG 3'
SV40-NcoI/F	5' CATGCCATGGGACTCTAGATCATAATC 3'
SV40-EcoR I/R	5' CGGAATTCTACATTGATGAGTTTGGACA 3'
GP41 primers	
GP41/F	5' CCTATTCTGTGCTGGTGGTGG 3'
GP41/R	5' ATGTTGATGTGCGGAAAGC 3'
Detection primers	
LEF-11/F	ATGCCCCCCAAAAATTGCA
LEF-11/R	TTACCATGTTTGATTTTTGTAAAC
IE-1/F	CGTGCCAGACTTTCATTACCA
IE-1/R	GGTCGGAGAACCTGTTGGAA
GP64/F	AACACACAAGCGAGATGGTAGG
GP64/R	TCAAACGCTCGTCCACCTT

* The restriction enzyme sites are marked in red.

Table S2. The sgRNA target sequences of primers used in this study

Primer name	Primer sequence*
SgLEF-11/F	5' AAGTGCACTTAGGCGGGTGTAATT 3'
SgLEF-11/R	5' AAACAATTACACCCGCCTAAGTGC 3'
sgMock/F	5' AAGTGGAGGATGCATTAGCACAAC 3'
sgMock/R	5' AAACGTTGTGCTAATGCATCCTCC 3'
SgIE-1/F	5' AAGTGAATCTTTTGAGCAGTCTGT 3'
SgIE-1/R	5' AAACACAGACTGCTCAAAAGATTC 3'
sgGP64/F	5' AAGTGGCGGCGCATTCTGCCTTTG 3'
sgGP64/R	5' AAACCAAAGGCAGAATGCGCCGCC 3'
sgVP39/F	5' AAGTGATATATAACAACGAAGAGG 3'
sgVP39/R	5' AAACCCTCTTCGTTGTTATATATC 3'
sgPOLY/F	5' AAGTGTTTGTGAACCGCGTCATAT 3'
sgPOLY/R	5' AAACATATGACGCGGTTCACAAAC 3'
SgLEF-1/F	5' AAGTGAAAATTTACATTGGCGCCA 3'
SgLEF-1/R	5' AAACTGGCGCCAATGTAAATTTTC 3'
SgLEF-3/F	5' AAGTGCAATCTTTCAAAGACATGG 3'
SgLEF-3/R	5' AAACCCATGTCTTTGAAAGATTGC 3'
sgP143/F	5' AAGTGCCCAACTGGCCCAAAGGGC 3'
sgP143/R	5' AAACGCCCTTTGGGCCAGTTGGG 3'
sgDNApol/F	5' AAGTGACATTGAGACGCATTCGGA 3'
sgDNApol/R	5' AAACTCCGAATGCGTCTCAATGTC 3'
sgP35/F	5' AAGTGTACGACGTCTTAGCTTACG 3'
sgP35/R	5' AAACCGTAAGCTAAGACGTCGTAC 3'
sgIE-2/F	5' AAGTGAAGACAACGTGCAGATTAT 3'
sgIE-2/R	5' AAACATAATCTGCACGTTGTCTTC 3'

* The restriction enzyme sites are marked in red.