

**Table S1 Primer sequences used in this study**

Primer	Sequence
Template 1-F	TAATACGACTCACTATATTGTGCAAATAGCATTCGTGGTTTTAGAGCTAGAAATAGC
Template 2-F	TAATACGACTCACTATAGTTGTGCAAATAGCATTCGTGGTTTTAGAGCTAGAAATAGC
Template 3-F	TAATACGACTCACTATAGGTTGTGCAAATAGCATTCGTGGTTTTAGAGCTAGAAATAGC
sgRNA-R	AAAAAAAGCACCGACTCGGTGCCAC
Target DNA-F	ATGCCACACTGGGTAACTGCTG
Target DNA-R	GAAGAAACCTTGCGTCCGTGT
KO-Test-F	ACCTCACCGAAGTTAGTTACG
KO-Test-R	TTTGGACAGGTGTCAAATGGG

**Table S2 The reagents used in this study**

Reagent	Company	Code
10×PCR Buffer (Mg <sup>2+</sup> free)	TAKARA	9151AM
TAKARA Taq <sup>TM</sup>	TAKARA	R001B
dNTP	TAKARA	4030Q
3M Sodium acetate	Promega	P135A
Acryl Carrier	Solarbio	SA1020
Guide-it <sup>TM</sup> Recombinant Cas9 (10 µg/µL)	TAKARA	632679
E.Z.N.A® Gel Extraction Kit	Omega	D2500-03
T7 RiboMAX <sup>TM</sup> Express Large Scale RNA Production System	Promega	P1320
DNAiso Regent	TAKARA	9770A
NE Buffer3	New England BioLabs	B7003S
T-Vector PMD <sup>TM</sup> 19 (Simple)	TAKARA	3271

**Figure S1 The profile of the T<sub>PMD19</sub>-sgRNA plasmid.**

$\xrightarrow{\text{Lac Z}}$   $\xrightarrow{\text{M13 F}}$   
 CGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTAC  
 GCATACAACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTTTGTCGATACTGGTACTAATG  
  
 GCCAAGTTTGCACGCCTGCCGTTTCGACGATTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC  
 CGGTTCAAACGTGCGGACGGCAAGCTGCTAACAAAATCTCGATCTTTATCGTTCAATTTTATCCG  
 crRNA-tracrRNA (83bp)  
 TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTAATCTCTGGAAGATCCGC  
 ATCAGGCAATAGTTGAACTTTTTCACCGTGGCTCAGCCACGAAAAAATTAGAGACCTTCTAGGCG  
  
 GCGTACCGAGTTCTAATTCAGTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTA  
 CGCATGGCTCAAGATTAAGTGACCGGCAGCAAAATGTTGCAGCACTGACCCTTTTGGGACCGCAAT  
 $\xleftarrow{\text{M13 R}}$

