

CRISPR/Cas9-mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations

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Supplementary Information

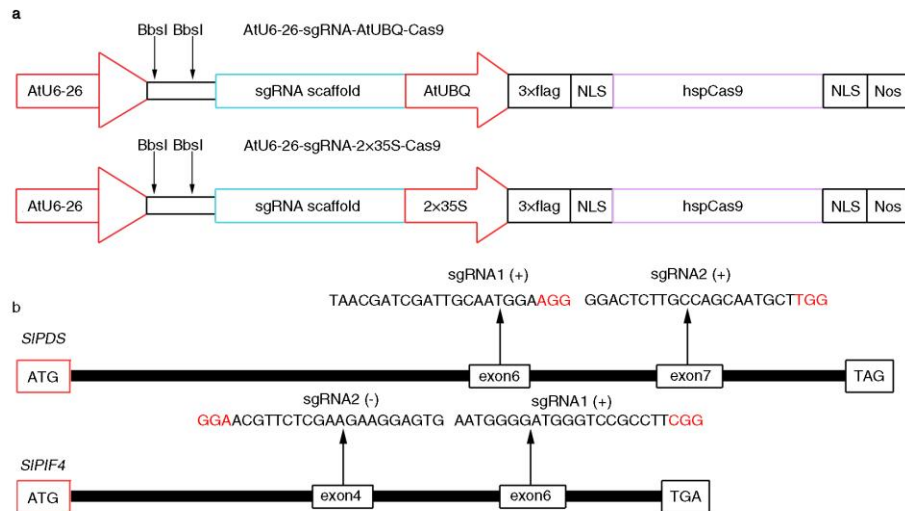


Figure S1. Schematic diagrams for binary vectors used in this study and selected target sites in *SIPDS* and *SIPIF4*. (a) Schematic view of the expression cassettes. Cas9 fused with a flag tag (3 ×flag) and two nuclear localization signal (NLS) is expressed by either *Cauliflower mosaic virus* 35S promoter or *Arabidopsis* UBQ promoter. The scaffold sequence of sgRNA is derived using U6 promoters of *Arabidopsis*. Two *Bbs* I sites are located among the U6 promoter and sgRNA scaffold. (b) Schematic illustrating the target sites of *SIPDS* and *SIPIF4*. All the 20-bp sequence are followed by a PAM sequence (NGG; red). +: plus strand; -: minus strand. ATG: initiation codon; TAG or TGA: termination codon.

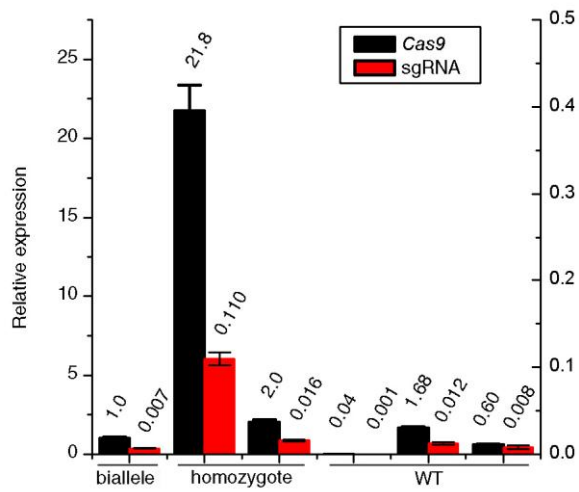


Figure S2. Expression analysis of *Cas9* and sgRNA by RT-qPCR. #WT, wild-type sequence with no mutation detected. \$The zygosity of homozygote, biallele and WT in T0 lines are putative. The *SIUbi3* was used as the internal control. The RT-qPCR products were verified by sequencing.

Supplementary Table S1. Genotyping of T0 independent transgenic lines of sgRNA1-*SIPDS*

Target	Lines [#]	Zygoty [§]	Genotypes	Total	Ratio
sgRNA1- <i>SIPDS</i>	T0-1	Chimera	1d1,6i1,2WT	15	71.4%
	T0-2	Chimera	8d1,1d8,1d28,1i1,2WT		
	T0-4	Chimera	1d28,1c14,3WT		
	T0-5	Chimera	1d2,6i1,3WT		
	T0-6	Chimera	1d6,1i1,1c8,7WT		
	T0-10	Chimera	2d1,1d65,3i1,4WT		
	T0-11	Chimera	1d1,1d1a,2i1,3WT		
	T0-12	Chimera	1d2,3d72,3WT		
	T0-14	Chimera	1d55,2i1,2i1b,3WT		
	T0-15	Chimera	1d1,1i1,4WT		
	T0-16	Chimera	3d1,6i1,1i1a,		
	T0-19	Chimera	5d5,2d11,1i1,3WT		
	T0-20	Chimera	2d5,1d12,1c102,6WT		
	T0-21	Chimera	1d2,1d4,2i1		
	T0-22	Chimera	1d2,7i1,2WT		
	T0-3	WT*		6	28.6%
	T0-7	WT			
	T0-8	WT			
	T0-9	WT			
	T0-13	WT			
	T0-18	WT			

[#]Line name is in the format of T0-#. [§]The zygoty of T0 lines is putative. *WT, no mutation detected at target sites. d#, # of bp deleted at the target sites; i#, # of bases insertion at target sites; i#a, the same number of insertions at one site; i#b, the same number of insertions at other sites; c#, combined mutation.

Supplementary Table S2. Genotyping of T0 independent transgenic lines of sgRNA2-*SIPDS*

Target	Lines	Zygoty	Genotypes	Total	Ratio
sgRNA2- <i>SIPDS</i>	T0-5	Biallele	14d3,5d5	1	14.3%
	T0-1	Chimera	1d2,1d4,1d23,1i2,1WT	6	85.8%
	T0-2	Chimera	1d2,3d5,1i1		
	T0-3	Chimera	3d1,1d5,1WT		
	T0-4	Chimera	2d1,1d3,1d4,1d102		
	T0-6	Chimera	1d1,2d3,3WT		
	T0-7	Chimera	6d3,1d5,4d14		

See Supplementary Table S1 for lines naming and genotype denotation.

Supplementary Table S3. Genotyping of T0 independent transgenic lines of sgRNA1-*SIP1F4*.

Target	Lines	Zygosity	Genotypes	Total	Ratio
sgRNA1- <i>SIP1F4</i>	T0-22	Homozygote	d1	2	8.0%
	T0-23	Homozygote	d1		
	T0-7	Biallele	7c2,7c14	3	18.8%
	T0-15	Biallele	8d1,4d3		
	T0-19	Biallele	9d1,1d19		
	T0-1	Chimera	1d1,1d6,2WT	16	64.0%
	T0-2	Chimera	2d4,2d18,5WT		
	T0-3	Chimera	3d3,1d4,5i1		
	T0-4	Chimera	2d1,1d20,1d61		
	T0-5	Chimera	1d3,1d30,2WT		
	T0-8	Chimera	1d1,1d3,1d7,1WT		
	T0-9	Chimera	2d1,2d18,1c5		
	T0-10	Chimera	2c51,1c126,1WT		
	T0-11	Chimera	4d8,1c51,1c52,3WT		
	T0-13	Chimera	2d1,1d4,1d7		
	T0-14	Chimera	2d1,2d4,1WT		
	T0-16	Chimera	2d1,5d3,1d5,1i1		
	T0-20	Chimera	1d1,1d5,2WT		
	T0-21	Chimera	4d1,1d4,1d50,3i1		
	T0-24	Chimera	2d1,1d4,1WT		
	T0-25	Chimera	5d1,1d4,1d5		
	T0-6	WT		4	16.0%
	T0-12	WT			
	T0-17	WT			
	T0-18	WT			

See Supplementary Table S1 for lines naming and genotype denotation.

Supplementary Table S4. Genotyping of T0 independent transgenic lines of sgRNA2-*SIP1F4*.

Target	Lines	Zygoty	Genotypes	Total	Ratio
sgRNA2- <i>SIP1F4</i>	T0-13	Homozygote	d1	3	15.8%
	T0-17	Homozygote	d1		
	T0-19	Homozygote	d1		
	T0-2	Biallele	13d1,2d3	5	26.3%
	T0-7	Biallele	9d2,4d9		
	T0-8	Biallele	6d2,6d9		
	T0-10	Biallele	6d3,7i1		
	T0-18	Biallele	7i1,6c43		
	T0-1	Chimera	1d127,5i1,1WT	9	47.4%
	T0-4	Chimera	1d1,4d6,3d17		
	T0-6	Chimera	3d2,2d2a,2d3,1d4,2d9		
	T0-9	Chimera	3d1,5d3,2d6		
	T0-11	Chimera	3d2,1d3,2i1		
	T0-12	Chimera	3d2,1d6,1d17,4d6a		
	T0-14	Chimera	2d1,3d6,1d17,2i1		
	T0-15	Chimera	5d1,4d2,1d2a		
	T0-16	Chimera	3d1,1d2,1d3		
	T0-3	WT		2	10.5%
	T0-5	WT			

See Supplementary Table S1 for lines naming and genotype denotation. d#a, the same number bases of deletion at one site.

Supplementary Table S5. Compare the genotypes of leaves and mixed tissues.

Target	Lines	Zygoty	Genotypes of leaf	Genotypes of mixed tissues*
sgRNA1- <i>SIPIF4</i>	T0-22	Homozygote	d1	d1
sgRNA1- <i>SIPIF4</i>	T0-23	Homozygote	d1	d1
sgRNA2- <i>SIPIF4</i>	T0-13	Homozygote	d1	d1
sgRNA2- <i>SIPIF4</i>	T0-17	Homozygote	d1	d1
sgRNA2- <i>SIPIF4</i>	T0-19	Homozygote	d1	d1
sgRNA1- <i>SIPIF4</i>	T0-7	Biallele	7c2,7c14	4c2,6c14
sgRNA1- <i>SIPIF4</i>	T0-15	Biallele	8d1,4d3	5d1,4d3
sgRNA2- <i>SIPIF4</i>	T0-7	Biallele	9d2,4d9	5d2,2d9
sgRNA2- <i>SIPIF4</i>	T0-8	Biallele	6d2,6d9	6d2,2d9
sgRNA2- <i>SIPIF4</i>	T0-9	Chimera	3d1,5d3,2d6	2d1,5d3,3d6
sgRNA1- <i>SIPDS</i>	T0-22	Chimera	1d2,7i1,2WT	<u>1d6,1d13,2i1,6WT</u>
sgRNA2- <i>SIPIF4</i>	T0-15	Chimera	5d1,4d2,1c3	8d1,8d2
sgRNA1- <i>SIPIF4</i>	T0-6	WT	N [#]	N
sgRNA2- <i>SIPIF4</i>	T0-5	WT	N	N
sgRNA1- <i>SIPDS</i>	T0-13	WT	N	N
sgRNA1- <i>SIPDS</i>	T0-18	WT	N	N

See Supplementary Table S1 for lines naming and genotype denotation. *Mixed tissues included leaves, shoots and flowers; [#]N, no mutation detected. Underline means new mutation were detected.

Supplementary Table S6. Genotyping the regeneration plantlets in T0 generation.

Target	Lines [#]	Zygoty	Genotypes
sgRNA2- <i>SIP4</i>	T0-13	Homozygote	d1
sgRNA2- <i>SIP4</i>	T0-13(1)	Homozygote	d1
sgRNA2- <i>SIP4</i>	T0-13(2)	Homozygote	d1
sgRNA2- <i>SIP4</i>	T0-17	Homozygote	d1
sgRNA2- <i>SIP4</i>	T0-17(1)	Homozygote	d1
sgRNA2- <i>SIP4</i>	T0-17(2)	Homozygote	d1
sgRNA1- <i>SIP4</i>	T0-22	Homozygote	d1
sgRNA1- <i>SIP4</i>	T0-22(1)	Homozygote	d1
sgRNA1- <i>SIP4</i>	T0-22(2)	Homozygote	d1
sgRNA1- <i>SIP4</i>	T0-7	Biallele	7c2,5c14
sgRNA1- <i>SIP4</i>	T0-7(1)	Biallele	8c2,3c14
sgRNA2- <i>SIP4</i>	T0-7	Biallele	9d2,4d9
sgRNA2- <i>SIP4</i>	T0-7(1)	Biallele	6d2,3d9
sgRNA2- <i>SIP4</i>	T0-7(2)	Biallele	4d2,4d9
sgRNA1- <i>SIP4</i>	T0-1	Chimera	1d1,1d6,2WT
sgRNA1- <i>SIP4</i>	T0-1(1)	Chimera	2d1,4 <u>d10</u> ,3WT
sgRNA1- <i>SIP4</i>	T0-1(2)	Chimera	1d1,6 <u>d10</u> ,3WT
sgRNA2- <i>SIP4</i>	T0-9	Chimera	3d1,5d3,2d6
sgRNA2- <i>SIP4</i>	T0-9(1)	Chimera	1 <u>d2</u> ,2d3,5 <u>i1</u> ,2WT
sgRNA2- <i>SIP4</i>	T0-9(2)	Chimera	1d1,1 <u>i1</u> ,7WT
sgRNA1- <i>SIP4</i>	T0-6	WT	WT
sgRNA1- <i>SIP4</i>	T0-6(1)	WT	WT
sgRNA1- <i>SIP4</i>	T0-6(2)	WT	WT
sgRNA2- <i>SIP4</i>	T0-5	WT	WT
sgRNA2- <i>SIP4</i>	T0-5(1)	WT	WT
sgRNA2- <i>SIP4</i>	T0-5(2)	WT	WT

[#]Line name of regeneration plantlets is in the format of T0-M(N), with N designated the plantlets regenerated from the T0 plant T0-M. Underline means new mutation were detected.

Supplementary Table S7. Segregation patterns of CRISPR/Cas9-mediated targeted mutagenesis during the T1 to T2 generation in T-DNA-free plants.

Target gene	sgRNA	Line#	T1			T2	
			Zygoty	Genotype	T-DNA	Mutation segregation	T-DNA
<i>SIPIF4</i>	1	T1-22(14)	Homozygote	d1d1	-	15d1d1	15-
<i>SIPIF4</i>	1	T1-8(10)	Homozygote	d1d1	-	17d1d1	17-
<i>SIPIF4</i>	2	T1-16(3)	Homozygote	d2d2	-	10d2d2	10-
<i>SIPIF4</i>	2	T1-12(7)	Homozygote	d2d2	-	12d2d2	12-

#Line name is in the format of T1-M(N), with N designated the progeny of T1 plant T1-M. -, T-DNA was not detected.

Supplementary Table S8. Sequencing results of sgRNA1/2-*SIPDS* and sgRNA1/2-*SIPIF4* in T0 plants.

Target	Genotypes	The sequences of target sites	Frequency
sgRNA1 <i>SIPDS</i> -	T0-1		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	2/9
	d1	GGGATTAACGATCGATTGCAA-GGAAGGAACATTC	1/9
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	6/9
	T0-2		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	2/13
	d1	GGGATTAACGATCGATTGCAA-GGAAGGAACATTC	8/13
	d8	GGGATTAACGATC-----TGGAAGGAACATTC	1/13
	d28	GATCGA-----TTGCAA	1/13
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	1/13
	T0-3		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC	
	T0-4		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/5
	d28	GGGATTAACGATCGATTGCAA-----	1/5
	c14	GGGATTAACGATTGGAAGGAA-----CATTC	1/5
	T0-5		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/10
	d2	GGGATTAACGATCGATTGC--TGGAAGGAACATTC	1/10
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	6/10
	T0-6		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	7/10
	d6	GGGATTAACGATCGAT-----GGAAGGAACATTC	1/10
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	1/10
	c5	GGGATTAACGATCGATTGCAATGAAA-----CATTC	1/10
	T0-7		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC	
	T0-8		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC	
	T0-9		
WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC		
T0-10			
WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	4/10	
d2	GGGATTAACGATCGATTGC--TGGAAGGAACATTC	2/10	
d65	ATTAGG-----AATTCA	1/10	
i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/10	

	T0-11			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/7	
	d1	GGGATTAACGATCGATTGCAA-GGAAGGAACATTC	1/7	
	d1b	GGGATTAACGATCGATTGCA-TGGAAGGAACATTC	1/7	
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	2/7	
	T0-12			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/7	
	d3	GGGATTAACGATCGATTGC---GGAAGGAACATTC	1/7	
	d72	GGAGAATT-----CAGCCG	3/7	
	T0-13			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC		
sgRNA1	T0-13	Mixed tissues		
SIPDS-	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC		
	T0-14			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/8	
	d55	TATTCA-----ATGATA	1/8	
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	2/8	
	i1b	GGGATTAACGATCGATTGCAAATGGAAGGAACATTC	2/8	
	T0-15			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	4/6	
	d1	GGGATTAACGATCGATTGCA-TGGAAGGAACATTC	1/6	
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	1/6	
	T0-16			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC		
	d1	GGGATTAACGATCGATTGCAA-GGAAGGAACATTC	3/10	
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	6/10	
	i1a	GGGATTAACGATCGATTGCAATAGGAAGGAACATTC	1/10	
	T0-18			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC		
	T0-18	Mixed tissues		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC GGGATTAACGATCGATTGCAATGGAAGGAACATTC		
	T0-19			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	3/11	
	i1	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	1/11	
	d5	GGGATTAACGATCGATTG-----GAAGGAACATTC	5/11	
	d12	GGGATTAACGATCG-----GGAACATTC	2/11	
	T0-20			
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	6/10	
	d5	GGGATTAACGATCGATT-----GGAAGGAACATTC	2/10	
	d12	GGGATTAACGATCG-----GGAACATTC	1/10	

	c102	GCTTAC-----A-----AGCCGC	1/10
	T0-21		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	
	i1	GGGATTAACGATCGATTGCAAT T GGAAGGAACATTC	2/4
	d2	GGGATTAACGATCGATTGCAAT--AAGGAACATTC	1/4
	d4	GGGATTAACGATCGATTG----GGAAGGAACATTC	1/4
	T0-22		
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	2/10
	i1	GGGATTAACGATCGATTGCAAT T GGAAGGAACATTC	7/10
sgRNA1	d2	GGGATTAACGATCGATTGC--TGGAAGGAACATTC	1/10
-SIPDS	T0-22	Mixed tissues	
	WT	GGGATTAACGATCGATTGCAATGGAAGGAACATTC	6/10
	i1	GGGATTAACGATCGATTGCAAT T GGAAGGAACATTC	2/10
	d6	GGGATTAACGATCGAT-----GGAAGGAACATTC	1/10
	d13	GGGATTAACGATC-----GGAACATTC	1/10
<hr/>			
	T0-1		
	WT	CAATTGGACTCTTGCCAGCAATGCT TGG AGGGCAA	1/5
	d4	CAATTGGACTCTTGCCA-----TGCT TGG AGGGCAA	1/5
	d23	C----- TGG AGGGCAA	1/5
	d2	CAATTGGACTCTTGCCAGC--TGCT TGG AGGGCAA	1/5
	i2	AATTGGACTCTTGCCAGCAAT T ATGCT TGG AGGGCAA	1/5
	T0-2		
	WT	CAATTGGACTCTTGCCAGCAATGCT TGG AGGGCAA	
	d5	CAATTGGACTCTTGCC-----TGCT TGG AGGGCAA	3/5
	d2	CAATTGGACTCTTGCCAGCA--GCT TGG AGGGCAA	1/5
	i1	CAATTGGACTCTTGCCAGCAAT T GCT TGG AGGGCAA	1/5
	T0-3		
	WT	CAATTGGACTCTTGCCAGCAATGCT TGG AGGGCAA	1/5
	d1	CAATTGGACTCTTGCCAGCA-TGCT TGG AGGGCAA	3/5
	d5	CAATTGGACTCTTGCC-----TGCT TGG AGGGCAA	1/5
	T0-4		
	WT	CAATTGGACTCTTGCCAGCAATGCT TGG AGGGCAA	
	d1	CAATTGGACTCTTGCCAGCA-TGCT TGG AGGGCAA	2/5
sgRNA2	d3	CAATTGGACTCTTGCCAG---TGCT TGG AGGGCAA	1/5
-SIPDS	d4	CAATTGGACTCTTGCCA-----TGCT TGG AGGGCAA	1/5
	d102	CTTA-----AAAG	1/5
	T0-5		
	WT	CAATTGGACTCTTGCCAGCAATGCT TGG AGGGCAA	
	d5	CAATTGGACTCTTGCCAGC----- TGG AGGGCAA	5/19
	d3	CAATTGGACTCTTGCCAGCAA--- TGG AGGGCAA	14/19
	T0-6		
	WT	CAATTGGACTCTTGCCAGCAATGCT TGG AGGGCAA	3/6
	d3	CAATTGGACTCTTGCCAGC---GCT TGG AGGGCAA	2/6
	d1	CAATTGGACTCTTGCCAGCAA-GCT TGG AGGGCAA	1/6

	T0-7			
	WT	CAATTGGACTCTTGCCAGCAATGCTTGGAGGGCAA		
	d14	CAATTGG-----TGCTTGGAGGGCAA	4/11	
	d5	CAATTGGACTCTTGCCA-----GCTTGGAGGGCAA	1/11	
	d3	CAATTGGACTCTTGCCAGC---GCTTGGAGGGCAA	6/11	
<hr/>				
	T0-1			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	2/4	
	d6	ATGGGAATGGGGATGG-----CTTCGGTGCCTTC	1/4	
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	1/4	
	T0-1(1)			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	3/9	
sgRNA1	d10	ATGGGAATGGGGAT-----TCGGTGCCTTC	4/9	
-SIP1F4	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	2/9	
	T0-1(2)			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	3/10	
	d10	ATGGGAATGGGGAT-----TCGGTGCCTTC	6/10	
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	1/10	
	T0-2			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	5/9	
	d4	ATGGGAATGGGGATGGGT---CTTCGGTGCCTTC	2/9	
	d18	ATGG-----CTTCGGTGCCTTC	2/9	
	T0-3			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
	d4	ATGGGAATGGGGATGGGT---CTTCGGTGCCTTC	1/9	
	i1	ATGGGAATGGGGATGGGTCCGCACCTTCGGTGCCTTC	5/9	
	d3	ATGGGAATGGGGATGGGTTC---CTTCGGTGCCTTC	3/9	
	T0-4			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
	d1	ATGGGAATGGGGATGGGTCC-CCTTCGGTGCCTTC	2/4	
	d20	ATGGGAA-----GTGCCTTC	1/4	
	d61	CATTT-----GCTA	1/4	
	T0-5			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	2/4	
	d3	ATGGGAATGGGGATGGGTCCGCC---GGTGCCTTC	1/4	
	d30	C-----GGTGCCTTC	1/4	
	T0-6			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
		ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
	T0-6	Mixed tissues		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
		ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
	T0-6(1)			
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		
		ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC		

	T0-6(2)				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
		ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
	T0-7				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
	c2	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	7/12		
	c14	GGGAATGGGGATGGTGTCCAA-CACTACATT-TC	5/12		
	T0-7	Mixed tissues			
sgRNA1	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
- <i>SIP1F4</i>	c2	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	4/10		
	c14	GGGAATGGGGATGGTGTCCAA-CACTACATT-TC	6/10		
	T0-7(1)				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
	c2	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	8/11		
	c14	GGGAATGGGGATGGTGTCCAA-CACTACATT-TC	3/11		
	T0-8				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	1/4		
	d7	ATGGGAATGGGGATG-----CTTCGGTGCCTTC	1/4		
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	1/4		
	d3	ATGGGAATGGGGATGGGTG---CTTCGGTGCCTTC	1/4		
	T0-9				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	2/5		
	d18	ATGGGAATGGGGATGGGTCC-----GCAC	2/5		
	c62	GTTTCCAACACTACATGTCCA---AATATATGATTC	1/5		
	T0-10				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	1/4		
	c51	TGGTGATATTCAGATGATGTGGATGGGAAGTG--G	2/4		
	c126	ATATTCAGATGATGTGGATGGGAAGTG--GCATTT	1/4		
	T0-11				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	3/9		
	d8	ATGGGAATGGGGAT-----CTTCGGTGCCTTC	4/9		
	c52	TTCCA-----TGACAATGCTAT-----GCATTT	1/9		
	c51	TGATATTCAGATGATGTGGATGGGAAGTG-GCATT	1/9		
	T0-12				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
		ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
sgRNA1	T0-13				
- <i>SIP1F4</i>	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC			
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	2/4		
	d4	ATGGGAATGGGGATGGGT----CTTCGGTGCCTTC	1/4		
	d7	ATGGGAATGGGGATG-----CTTCGGTGCCTTC	1/4		
	T0-14				
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	1/5		

	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	2/5
	d4	ATGGGAATGGGGATGGGT-----CTTCGGTGCCTTC	2/5
	T0-15		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d3	ATGGGAATGGGGATGGGTCCGC---CGGTGCCTTC	4/12
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	8/12
	T0-15	Mixed tissues	
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d3	ATGGGAATGGGGATGGGTCCGC---CGGTGCCTTC	4/9
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	5/9
	T0-16		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d3	ATGGGAATGGGGATGGGTCCGC---CGGTGCCTTC	5/9
	d5	ATGGGAATGGGGATGGGTCT-----TTCGGTGCCTTC	1/9
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	2/9
	i1	ATGGGAATGGGGATGGGTCCGCACCTTCGGTGCCTTC	1/9
	T0-17		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	T0-18		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	T0-19	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	9/10
sgRNA1	d19	ATGG-----TTCGGTGCCTTC	1/10
-SIPIF4	T0-20		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	2/4
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	1/4
	d5	ATGGGAATGGGGATGGG-----CTTCGGTGCCTTC	1/4
	T0-21		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	i1	ATGGGAATGGGGATGGGTCCGCACCTTCGGTGCCTTC	3/9
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	4/9
	d4	ATGGGAATGGGGATGGGT-----CTTCGGTGCCTTC	1/9
	d50	GGTGTC-----TTCC	1/9
	T0-22		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	
	T0-22	Mixed tissues	
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	
	T0-22(1)		
	WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
	d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	

T0-22(2)		
WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	
T0-23		
WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	
T0-23 Mixed tissues		
WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	
T0-24		
WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	1/4
d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	2/4
d4	ATGGGAATGGGGATGGGT----CTTCGGTGCCTTC	1/4
T0-25		
WT	ATGGGAATGGGGATGGGTCCGCCTTCGGTGCCTTC	
d1	ATGGGAATGGGGATGGGTCCG-CTTCGGTGCCTTC	5/7
sgRNA1 -SIPIF4	d5 ATGGGAATGGGGATGGG-----CTTCGGTGCCTTC	1/7
	d4 ATGGGAATGGGGATGGGT----CTTCGGTGCCTTC	1/7

T0-1		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	3/9
i1	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	5/9
d128	CATGA-----TTAAGGTG	1/9
T0-2		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d3	GTAGAGTGAGGAAGAAGCT---GCAAGGCCTTCAT	2/15
sgRNA2 -SIPIF4	d1 GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	13/15
T0-3		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
T0-4		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d6	GTAGAGTGAGGAAGAA-----GCAAGGCCTTCAT	4/8
d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	1/8
d17	GTAGAGTGAGG-----CCTTCAT	3/8
T0-5		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
T0-5 Mixed tissues		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
T0-5(1)		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
T0-5(2)		

WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
T0-6		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d2	GTAGAGTGAGGAAGAAGCT--TGCAAGGCCTTCAT	3/10
d3	GTAGAGTGAGGAAGAAGCT---GCAAGGCCTTCAT	2/10
d9	GTAGAGTGAGGAA-----GCAAGGCCTTCAT	2/10
d2a	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	2/10
d4	GTAGAGTGAGGAAGAAGC---GCAAGGCCTTCAT	1/10
T0-7		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d9	GTAGAGTGAGGAA-----GCAAGGCCTTCAT	4/13
d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	9/13
T0-7	Mixed tissues	
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d9	GTAGAGTGAGGAA-----GCAAGGCCTTCAT	2/7
d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	5/7
T0-7(1)		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d9	GTAGAGTGAGGAA-----GCAAGGCCTTCAT	3/9
d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	6/9
T0-7(2)		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d9	GTAGAGTGAGGAA-----GCAAGGCCTTCAT	4/8
d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	4/8
T0-8		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	6/12
d9	GTAGAGTGAGGAA-----GCAAGGCCTTCAT	6/12
T0-9		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d6	GTAGAGTGAGGAAGA-----TGCAAGGCCTTCAT	2/10
d3	GTAGAGTGAGGAAGAAGC---TGCAAGGCCTTCAT	5/10
d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	3/10
T0-9	Mixed tissues	
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
d6	GTAGAGTGAGGAAGA-----TGCAAGGCCTTCAT	3/10
d3	GTAGAGTGAGGAAGAAGC---TGCAAGGCCTTCAT	5/10
d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	2/10
T0-9(1)		
WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	2/10
d3	GTAGAGTGAGGAAGAAG---TTGCAAGGCCTTCAT	2/10
d2	GTAGAGTGAGGAAGAAGC---TTGCAAGGCCTTCAT	1/10
i1	GTAGAGTGAGGAAGAAGCTCTTGTGCAAGGCCTTCAT	5/10

sgRNA2
-SIPIF4

	T0-9(2)			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		7/9
	i1	GTAGAGTGAGGAAGAAGCTCTTGTCAAGGCCTTCAT		1/9
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		1/9
	T0-10			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d3	GTAGAGTGAGGAAGAAGCT---GCAAGGCCTTCAT		6/13
	i1	GTAGAGTGAGGAAGAAGCTCTTGTCAAGGCCTTCAT		7/13
	T0-11			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d3	GTAGAGTGAGGAAGAAGCT---GCAAGGCCTTCAT		1/6
	i1	GTAGAGTGAGGAAGAAGCTCTTGTCAAGGCCTTCAT		2/6
	d2	GTAGAGTGAGGAAGAAGCT--TGCAAGGCCTTCAT		3/6
	T0-12			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d17	GTAGAGTGAGG-----CCTTCAT		1/9
	d6	GTAGAGTGAGGAAGAA-----GCAAGGCCTTCAT		1/9
	d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT		3/9
sgRNA2	d6a	GTAGAGTGAGGAAGA-----TGCAAGGCCTTCAT		4/9
-SIP1F4	T0-13			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		
	T0-13	Mixed tissues		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		
	T0-13(1)			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		
	T0-13(2)			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		
	T0-14			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d17	GTAGAGTGAGG-----CCTTCAT		1/8
	d6	GTAGAGTGAGGAAGAA-----GCAAGGCCTTCAT		3/8
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		2/8
	i1	GTAGAGTGAGGAAGAAGCTCTTGTCAAGGCCTTCAT		2/8
	T0-15			
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT		5/10
	d2	GTAGAGTGAGGAAGAAGCT--TGCAAGGCCTTCAT		4/10
	d2a	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT		1/10
	T0-15	Mixed tissues		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT		

	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	8/16
	d2	GTAGAGTGAGGAAGAAGCT--TGCAAGGCCTTCAT	8/16
	T0-16		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	3/5
	d3	GTAGAGTGAGGAAGAAGCT---GCAAGGCCTTCAT	1/5
	d2	GTAGAGTGAGGAAGAAGCTC--GCAAGGCCTTCAT	1/5
	T0-17		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	
	T0-17	Mixed tissues	
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	
	T0-17(1)		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	
	T0-17(2)		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	
sgRNA2	T0-18		
- <i>SIP1F4</i>	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	i1	GTAGAGTGAGGAAGAAGCTCTTTGCAAGGCCTTCAT	7/13
	c43	AA--GATCGTGTATACTTCCGTGCAAGGCCTTCAT	6/13
	T0-19		
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	
	T0-19	Mixed tissues	
	WT	GTAGAGTGAGGAAGAAGCTCTTGCAAGGCCTTCAT	
	d1	GTAGAGTGAGGAAGAAGCTCT-GCAAGGCCTTCAT	

DNA was extracted from the leaves or mixed tissues of T0 plants, then amplified by PCR and sequenced. On the first round, homozygote and WT were identified, with PCR product was one type. For others, DNA were cloned and a number of clones were sequenced. In X/Y, Y is the number clones sequenced, and X is the number of clones which showed a specific sequence. #WT, no mutations were detected at target sites. § Mixed tissues included leaves, shoots and flowers. d#, # of bp deleted at the target sites; d#a, same number base of deletion at one site; i#, # number of bases insertion at target sites; i#a, same number base of deletion at one site; i#b, same number base of deletion at other sites ; c#: combined mutation. PAM (NGG) is indicated in orange; The 20 bp target sequence is marked in green; the bases colored red and blue mean insertion and substitution respectively.

Supplementary Table S9. Chromosomal locations of tomato U6 genes.

Gene	Chromosome	Strand	Location
<i>SIU6-1</i>	ch01	-	5813051-5813153
<i>SIU6-2</i>	ch01	+	92151263-92151365
<i>SIU6-3</i>	ch07	-	292992-293094
<i>SIU6-4</i>	ch07	+	327864-327966
<i>SIU6-5</i>	ch07	+	329337-329439
<i>SIU6-6</i>	ch07	-	2045653-2045755
<i>SIU6-7</i>	ch09	-	5169327-5169429

Supplementary Table S10. Primers used in the study.

Primer names	Sequence of primers	Aims
sgRNA1- <i>SIPDS</i> -F	5' TCTCCTATCCTCGTTTGTCGT 3'	Genotyping
sgRNA1- <i>SIPDS</i> -R	5' TTGCTTTCTCATCCAGTCCTT 3'	
sgRNA2- <i>SIPDS</i> -F	5' ATGGTGAGCTAATCACGAGTAA 3'	
sgRNA2- <i>SIPDS</i> -R	5' AAAGAGAGGCAGGTAGACAATC 3'	
sgRNA1- <i>SIPIF4</i> -F	5' ATACGGGGTGAGTTAGCGA 3'	
sgRNA1- <i>SIPIF4</i> -R	5' AGTGGCAGGTGCATTACTATTA 3'	
sgRNA2- <i>SIPIF4</i> -F	5' GAGGAAACAAGTCAGCCCA 3'	
sgRNA2- <i>SIPIF4</i> -R	5' TCGCTAACTACCCCGTAT 3'	
OFF1-F	5' ACGATTGATGGATTGTGAAG 3'	Off-target analysis
OFF1-R	5' TTCAGGGACGAATGTTTGTAG 3'	
OFF2-F	5' GTGCCTTGCCTATCTACTGG 3'	
OFF2-R	5' CATTGCGTCAACATCACCT 3'	
OFF3-F	5' CATGATTAGGCCAGGATTC 3'	
OFF3-R	5' GAAGTGTAACCCCTGAAT 3'	
OFF4-F	5' ACAATGGATTTACGACTCTC 3'	
OFF4-R	5' AAACCCGTTCCGTCTGTAG 3'	
OFF5-F	5' AGCTGAATATCATAGGTTTTCT 3'	
OFF5-R	5' CTCTATCACCGAAGTGGCTAG 3'	
OFF6-F	5' AAGAAATCTGTTGTTTCAGTATCAGT 3'	
OFF6-R	5' AGTTGTAACGGTTAAAGGGC 3'	
OFF7-F	5' CACCTCAGTTTTTCACATCAGC 3'	
OFF7-R	5' CTCGCTCTACGATAGCACATT 3'	
OFF8-F	5' ATGGGACGATAATGAACACTG 3'	
OFF8-R	5' GCATCTCTTTAGGTCAGCATC 3'	
OFF9-F	5' GCCTCCTTACATTACAAGAACTA 3'	
OFF9-R	5' AGACGCACAAATCGGACTAT 3'	
SIUbi-1F	5' TCCATCTCGTGCTCCGTCT 3'	
SIUbi-1R	5' CTGAACCTTTCCAGTGTCAT 3'	
Cas9-F	5' GACGCTAATCTGGACAAAGTG 3'	
Cas9-R	5' TCAGGGTAAACAGGTGGATG 3'	
sgRNA1- <i>SIPDS</i> -F	5' CGATTGCAATGGAGTTTTAGAG 3'	
sgRNA1- <i>SIPDS</i> -R	5' GTGCCACTTTTTCAAGTTGAT 3'	
sgRNA1- <i>SIPIF4</i> -F	5' GGGTCCGCCTTGTTTTAGA 3'	
sgRNA1- <i>SIPIF4</i> -R	5' CCGACTCGGTGCCACTT 3'	
sgRNA2- <i>SIPIF4</i> -R	5' GAAGCTCTTGCAAGTTTTAGAGC 3'	
sgRNA2- <i>SIPIF4</i> -R	5' CGGTGCCACTTTTTCAAGT 3'	
Cas9-pCAMBIA1301-F	5' ATAAGCCCATCAGAGAGCAG 3'	Detect the T-DNA region
Cas9-pCAMBIA1301-R	5' TAGCATTCGCCATTCAGG 3'	