Supplemental information

In planta processing of the SpCas9/gRNA complex

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Supplemental Figure Legends

Figure S1.
Targeted mutagenesis using SpCas9-rz-gRNA or SpCas9-gRNA systems at different target loci in rice calli.
(A) Mutation variations on *DL* gene locus (gDL-1) in the SpCas9-rz-gRNA vector transformed callus line #4. The wild type sequence is shown at the top with the PAM sequence in green, and the 20-nt target sequence in red. The blue arrowhead indicates the expected cleavage site. Dashes: deleted bases. The net changes in length are shown to the right of each sequence (+, insertion; -, deletion). The number of clones representing each mutant allele is shown in brackets. (B) CAPS analysis of three genes in each of SpCas9-rz-gRNA vector transformed calli and SpCas9-gRNA vector transformed calli. Mutation frequency of calli shown by red was calculated from ratio of sequenced clones with mutation. –: Non-digested PCR products, +: *Pst* I-digested PCR products. Blue arrowhead indicated the position of undigested PCR products. An undigested band indicates mutations in the target loci.

Figure S2.
Targeted mutagenesis using SpCas9-rz-gRNA or SpCas9-gRNA systems in *Arabidopsis*.
(A) Schematic representation of the constructs used in this study. The *SpCas9* gene was optimized for *Arabidopsis* codon usage, and controlled by ubiquitin 4-2 promoter from *Petroselinum crispum* (PcUbi; Fauser et al. 2012). (B) CAPS analysis of *BRI1* gene in each of SpCas9-rz-gRNA vector transformed T1 plants and SpCas9-gRNA vector transformed T1 plants. Mutation frequency of calli shown by red was calculated from ratio of sequenced clones with mutation. –: Non-digested PCR products, +: *Pst* I-digested PCR products. Blue arrowhead indicated the position of undigested PCR products. An undigested band indicates mutations in the target loci. (C) Mutation variations on *BRI1* gene locus in the SpCas9-rz-gRNA vector transformed T1 plant line #6. The wild type sequence is shown at the top with the PAM sequence in green, and the 20-nt target sequence in red. The blue arrowhead indicates the expected cleavage site. Dashes: deleted bases. The net changes in length are shown to the right of each sequence (+, insertion; -, deletion).

Figure S3.
Schematics of expected gRNA sequences by northern blot analysis.
(A) Schematics of gRNA sequence driven by OsU6 (pol III) or 2x35S (pol II) promoters. (B) Schematics of gRNA sequence connected to *HPT* gene. (C) Schematics of sequence of SpCas9-rz-gRNA. Blue character; target sequence, red character: gRNA scaffold sequence, orange character: poly (T) sequence, green character: 35S terminator sequence, purple character: pea3A terminator sequence gray character: poly (A) addition signal sequence, pink character: hammerhead ribozyme sequence, ‘G’ italic character: predicted transcription start site, under lined character: *HPT* or *SpCas9* gene sequence .

Supplemental Figure Legends

Figure S4.
Northern blot analysis of gRNA expression by prolonged exposure.
This figure shown prolonged exposure of Fig. 3B.

Figure S5.
Comparison of mutation frequency among three promoters using SpCas9-rz-gRNA vector in rice calli.
Mutation frequency of calli shown by red was calculated from ratio of sequenced clones with mutation. –: Non-digested PCR products, +: *Pst* I-digested PCR products. Blue arrowhead indicated the position of undigested PCR products. An undigested band indicates mutations in the target loci.

Figure S6.
Structure of in-vitro-transcribed gRNAs
Schematic representation of the expected size (nt) of in-vitro-transcribed gRNAs in this study. *: OsU6gRNA_T7 was synthesized using Guide-it sgRNA *In Vitro* Transcription Kit (TAKARA).

Figure S7.
Analysis of five in-vitro-transcribed gRNAs in a SpCas9 protein dependent manner.
(A) Northern blot analysis of five in-vitro-transcribed gRNAs in the presence or absence of the SpCas9 protein. Target gene was *DL* gene (gDL-1 locus). In-vitro-transcribed gRNAs No. 1: OsU6gRNA_T7, No. 2: 2x35SgRNA_T7, No. 3: SpCas9:fra-rz-gRNA, No. 4: SpCas9:fra-gRNA, No. 5: HPT-rz-gRNA_T7. *h* band: gRNA processed by the hammerhead ribozyme sequence from SpCas9:fra-gRNA. *i* band: gRNA processed by the hammerhead ribozyme sequence from HPT-rz-gRNA. (B) Cleavage efficiency of target DNA using five in-vitro-transcribed gRNAs in a SpCas9 protein dependent manner**.** The reaction solutions did not add RNase A after in vitro cleavage reaction. Target gene was *DL* gene (gDL-1 locus). Arrowheads indicated the position of cleaved PCR products. Cleavage efficiency was determined by the formula, 100*(1-sqrt(1-(b+c)/(a+b+c))), where a is the integrated intensity of the PCR product digested only with gRNA, and b and c are the integrated intensities of each cleavage product.

Supplemental Figure Legends

Figure S8.
SpCas9 protein- and RNases-mediated other target DNA cleavage in vitro.
Cleavage of the liner target DNA of the gDL-2 locus in a SpCas9 protein- and RNase III- or RNase T1-dependent manners. Yellow arrowheads indicated the position of cleaved PCR products. Cleavage efficiency was determined by the formula, 100*(1-sqrt(1-(b+c)/(a+b+c))), where a is the integrated intensity of the PCR product digested only with gRNA, and b and c are the integrated intensities of each cleavage product. +* means that the SpCas9 protein, RNase III or RNase T1 are added after 20 min of the in vitro cleavage reaction.

Figure S9.
Multiplex targeted mutagenesis with or without different positions of ribozyme sequence and gRNA in rice calli.
(A) Schematic representation of the SpCas9-gRNAs vectors and CAPS analysis of each of DL-1 and DL-2 locus on *DL* gene. –: Non-digested PCR products, +: *Pst* I-digested PCR products. (B) Schematic representation of the 1145 bp region with two gRNA-targeted sites. F1, F2, R1 and R2: PCR primers. Blue arrowheads : target loci (gDL-1 and gDL-2 loci). (C) Deletion and sequence of 1145 bp induced by using each of SpCas9-gRNA vectors in rice calli. Blue arrowhead indicated the position of 1145 bp deleted PCR products. WT band: 1937 bp, expected deletion band: 792 bp. (D) Mutation variations of large deleted PCR products on *DL* gene locus in line #2. The wild type sequence is shown at the top with the PAM sequence in green, and the 20-nt target sequence in red. The blue arrowhead indicates the expected cleavage site. Dashes: deleted bases.

(A)

Target gene : *DL* (gDL-1 locus)
SpCas9-rz-gRNA transformed callus : line #4

```
TTTTTCGTCTTTTGGGTAGCTGCAGGTTGGAGTCCCATG     WT      (x0)
TTTTTCGTCTTTTGGGTAGCTGC-GGTTGGAGTCCCATG     -1      (x2)
TTTTTCGTCTTTTGGGTAGC---AGGTTGGAGTCCCATG     -3      (x2)
TTTTTCGTCTTTTGGGTAGC----GGTTGGAGTCCCATG     -4      (x1)
TTTTTCGTCTTTTGGGTAG----------GAGTCCCATG     -10     (x1)
TTTTTCGTCTTTTGG----------TTGGAGTCCCATG      -11     (x1)
TTTTTCGTCTTTTGGGTAGCTGCA--ACAACCTCTC---     -22+10  (x1)
TTTTTCGTCTTTTGGGTAGCTGCACGGTTGGAGTCCCATG    +1(C)   (x2)
TTTTTCGTCTTTTGGGTAGCTGCAAGGTTGGAGTCCCATG    +1(A)   (x11)
TTTTTCGTCTTTTGGGTAGCTGCATGGTTGGAGTCCCATG    +1(T)   (x2)
TTTTTCGTCTTTTGGGTAGCTGCAGGGTTGGAGTCCCATG    +1(G)   (x1)
```

(B)

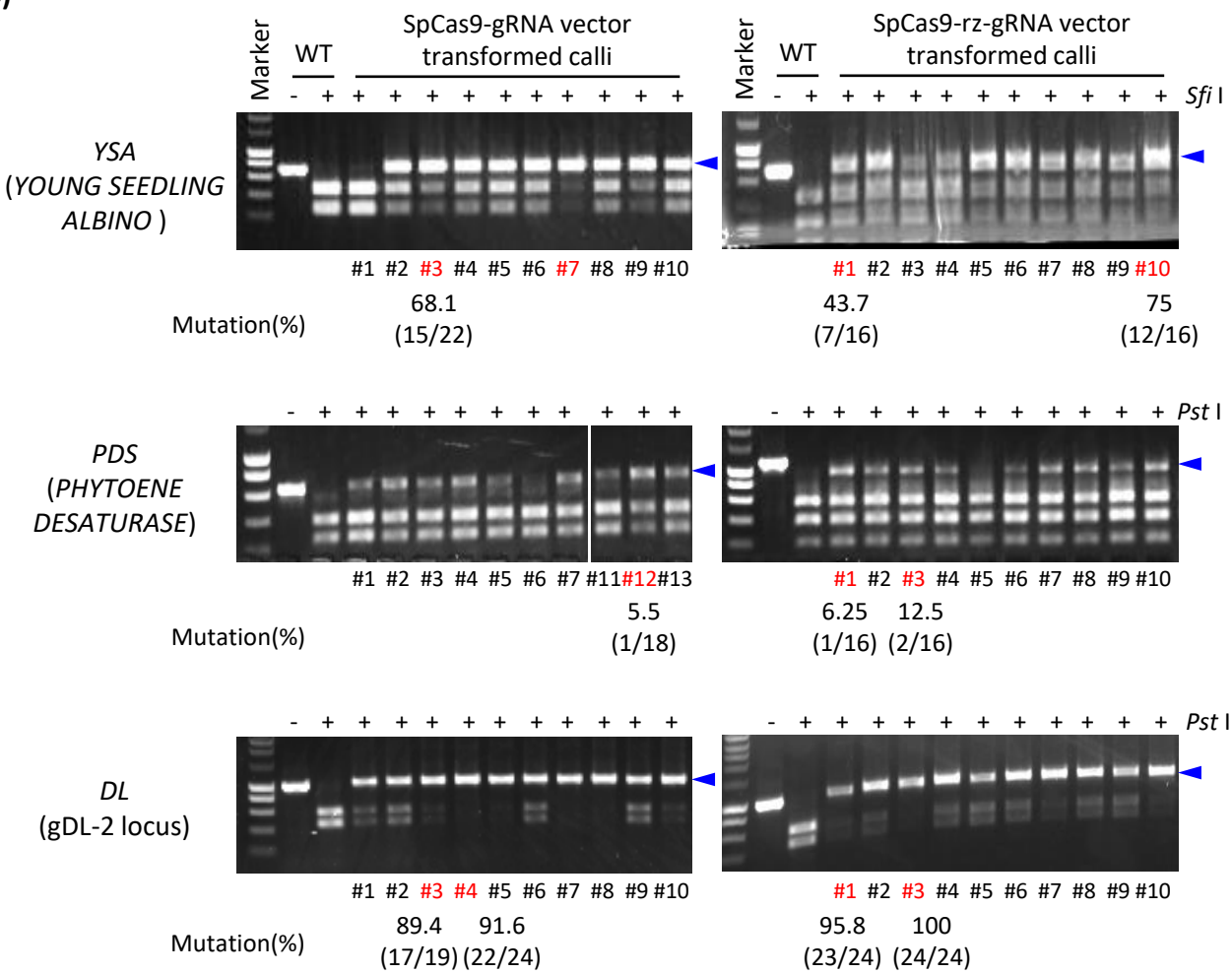
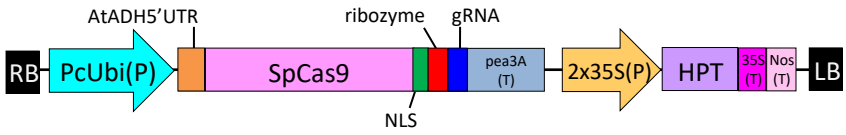


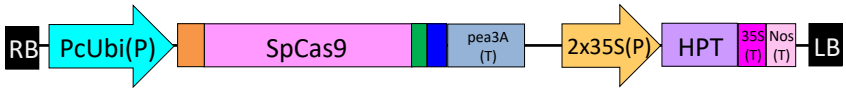
Figure. S1

(A)

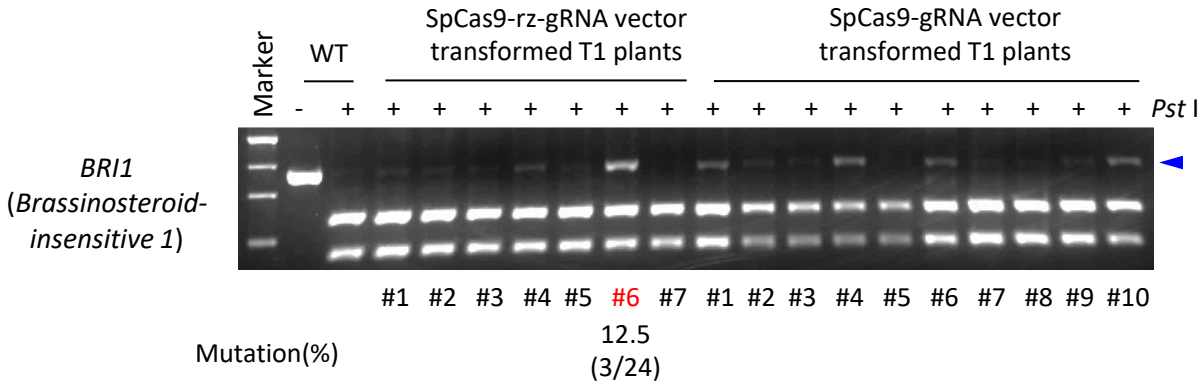
SpCas9-rz-gRNA vector



SpCas9-gRNA vector



(B)



(C)

SpCas9-rz-gRNA transformed plant : line #6

```
TTCTCAATTTGGGTCATAACGATATCTCTGGTTCGATTCCT   WT   (x21)
TTCTCAATTTGGGTCATAACGATA-CTCTGGTTCGATTCCT   -1   (x2)
TTCTCAATTTGGGTCATAA-----TCTCTGGTTCGATTCCT   -5   (x1)
```

Figure. S2

(A) SpCas9/OsU6gRNA and SpCas9/2x35SgRNA

OsU6-pro::gRNA::polyT-ter
CGCTGTCGCTTGTGTTGNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT

2x35S-pro::gRNA::35S-ter
TTTCATTTGGAGAGGNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA
GTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGATCTGTCGATCGACAAGCTCGAGTTTCT
CCATAATAATGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCCTATAGGGTTTCGCTCATGTGTTGAGCATA
TAAGAAACCCTTAGTATGTATTTGTATTTGTAAAATACTTCTATCAATAAAATTTCTAATTCCTAAAACCAAAATCC
AGTACTAAAATCCAGATCCCCC

(B) SpCas9-rz-gRNA and SpCas9-gRNA

SpCas9-hammerhead ribozyme::gRNA::pea3A-ter
GAATAGATCTTAGCCAGCTCGGCGGTGATTGAAAGCCATGGGATATCNNNNNNCTGATGAGTCCGTGAGGACG
AAACGAGTAAGCTCGTCNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTACTAGTCGCAGGCCTCCCAGCTTTCGTC
CGTATCATCGGTTTCGACAACGTTCGTCAAGTTCAATGCATCAGTTTCATTGCCCACACACCAGAATCCTACTAAG
TTTGAGTATTATGGCATTGGAAAAGCTGTTTTCTTCTATCATTTGTTCTGCTTGTAATTTACTGTGTTCTTTCAGTTT
TTGTTTTCGGACATCAAAATGCAAATGGATGGATAAGAGTTAATAAA

SpCas9::gRNA::pea3A-ter
GAATAGATCTTAGCCAGCTCGGCGGTGATTGAAAGCCATGGGATATCNNNNNNNNNNNNNNNNNNNGTTTT
AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT
TTTTACTAGTCGCAGGCCTCCCAGCTTTCGTCCGTATCATCGGTTTCGACAACGTTCGTCAAGTTCAATGCATCAG
TTTCATTGCCCACACACCAGAATCCTACTAAGTTTGAGTATTATGGCATTGGAAAAGCTGTTTTCTTCTATCATTT
GTTCTGCTTGTAATTTACTGTGTTCTTTCAGTTTTTGTTTTCGGACATCAAAATGCAAATGGATGGATAAGAGTTA
ATAAA

(C) SpCas9 or GFP/HPT-rz-gRNA

2x35S-pro::HPT::hammerhead ribozyme::gRNA::35S-ter
AGCACTCGTCCGAGGGCAAAGAAATAGAAGGCCTCGNNNNNNCTGATGAGTCCGTGAGGACGAAACGAGTAA
GCTCGTCNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTAT
CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGATCTGTCGATCGACAAGCTCGAGTTTCTCCATAATAA
TGTGTGAGTAGTTCCCAGATAAGGGAATTAGGGTTCCTATAGGGTTTCGCTCATGTGTTGAGCATATAAGAAAC
CCTTAGTATGTATTTGTATTTGTAAAATACTTCTATCAATAAAATTTCTAATTCCTAAAACCAAAATCCAGTACTAA
AATCCAGATCCCCC

Figure. S3

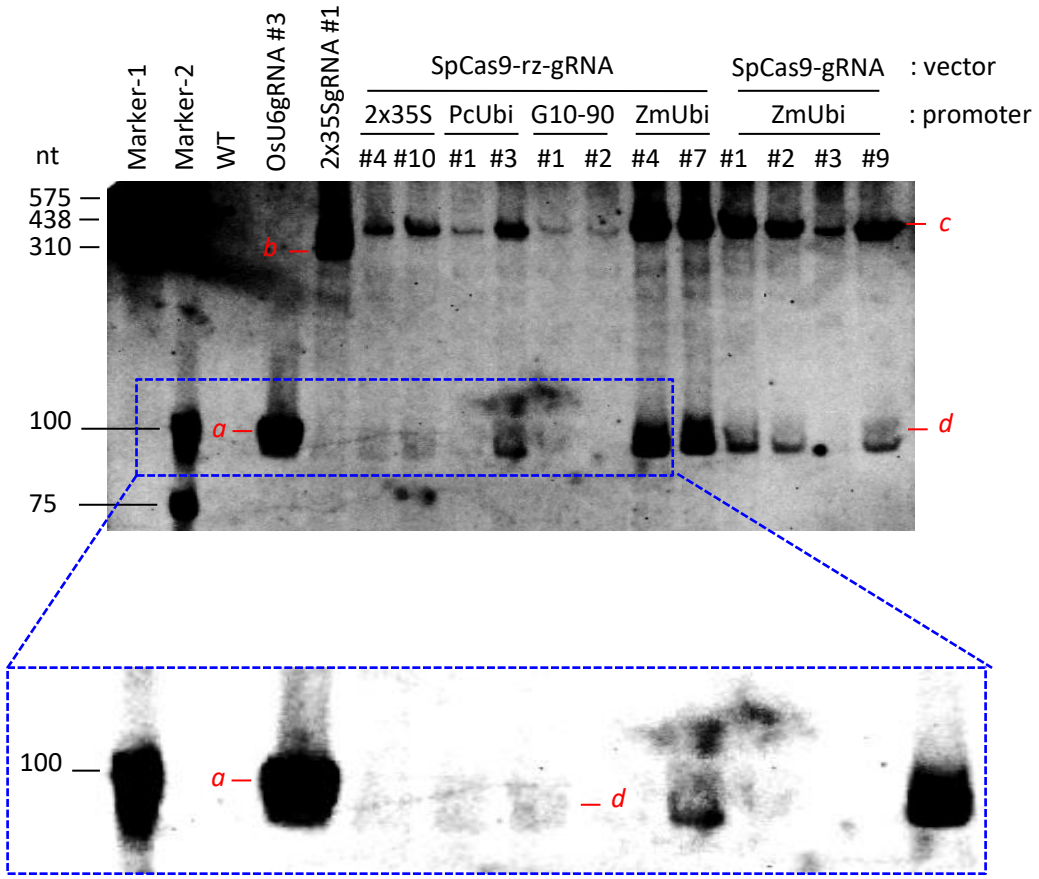

Marker-1
Marker-2
WT
OsU6gRNA #3
2x35SgRNA #1
SpCas9-rz-gRNA
SpCas9-gRNA
: vector
2x35S
PcUbi
G10-90
ZmUbi
ZmUbi
: promoter
nt
#4 #10 #1 #3 #1 #2 #4 #7 #1 #2 #3 #9
575
438
310
100
75
a
b
c
d
100
a
d

Figure. S4

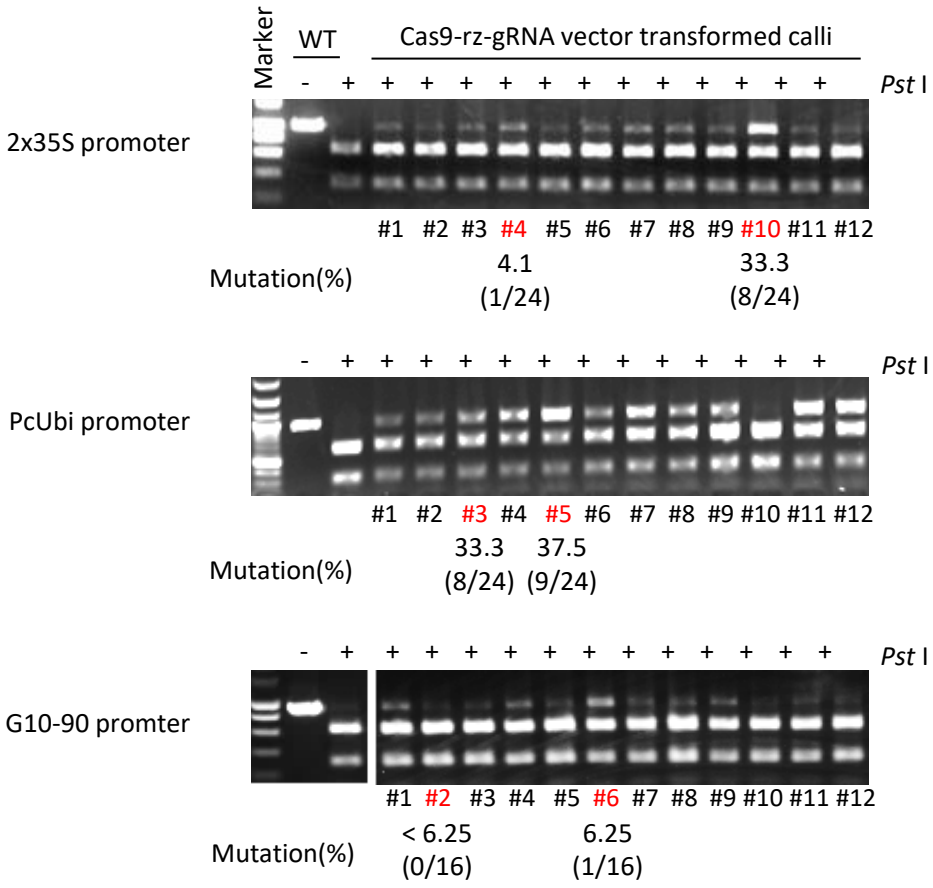
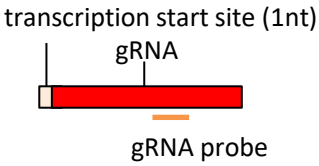
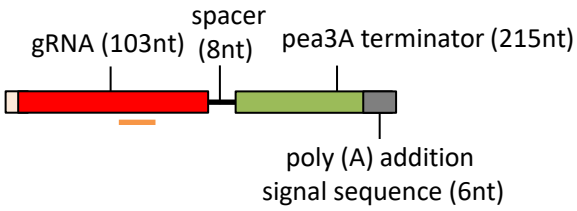

Marker
WT
Cas9-rz-gRNA vector transformed calli
- + + + + + + + + + + + + + Pst I
2x35S promoter
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12
Mutation(%)
4.1
(1/24)
33.3
(8/24)
- + + + + + + + + + + + + + Pst I
PcUbi promoter
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12
Mutation(%)
33.3
(8/24)
37.5
(9/24)
- + + + + + + + + + + + + + Pst I
G10-90 promter
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12
Mutation(%)
< 6.25
(0/16)
6.25
(1/16)

Figure. S5

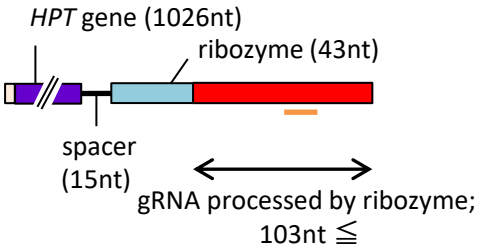
1. OsU6gRNA_T7*
Full length RNA; approximately 125nt ≦



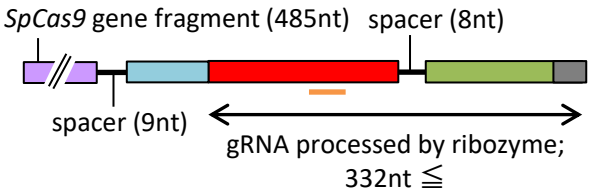
2. 2x35SgRNA_T7
Full length RNA; 333nt ≦



3. HPT-rz-gRNA_T7
Full length RNA; 1188nt ≦



4. SpCas9:fra-rz-gRNA
Full length RNA; 878nt ≦



5. SpCas9:fra-gRNA
Full length RNA; 835nt ≦

Figure. S6

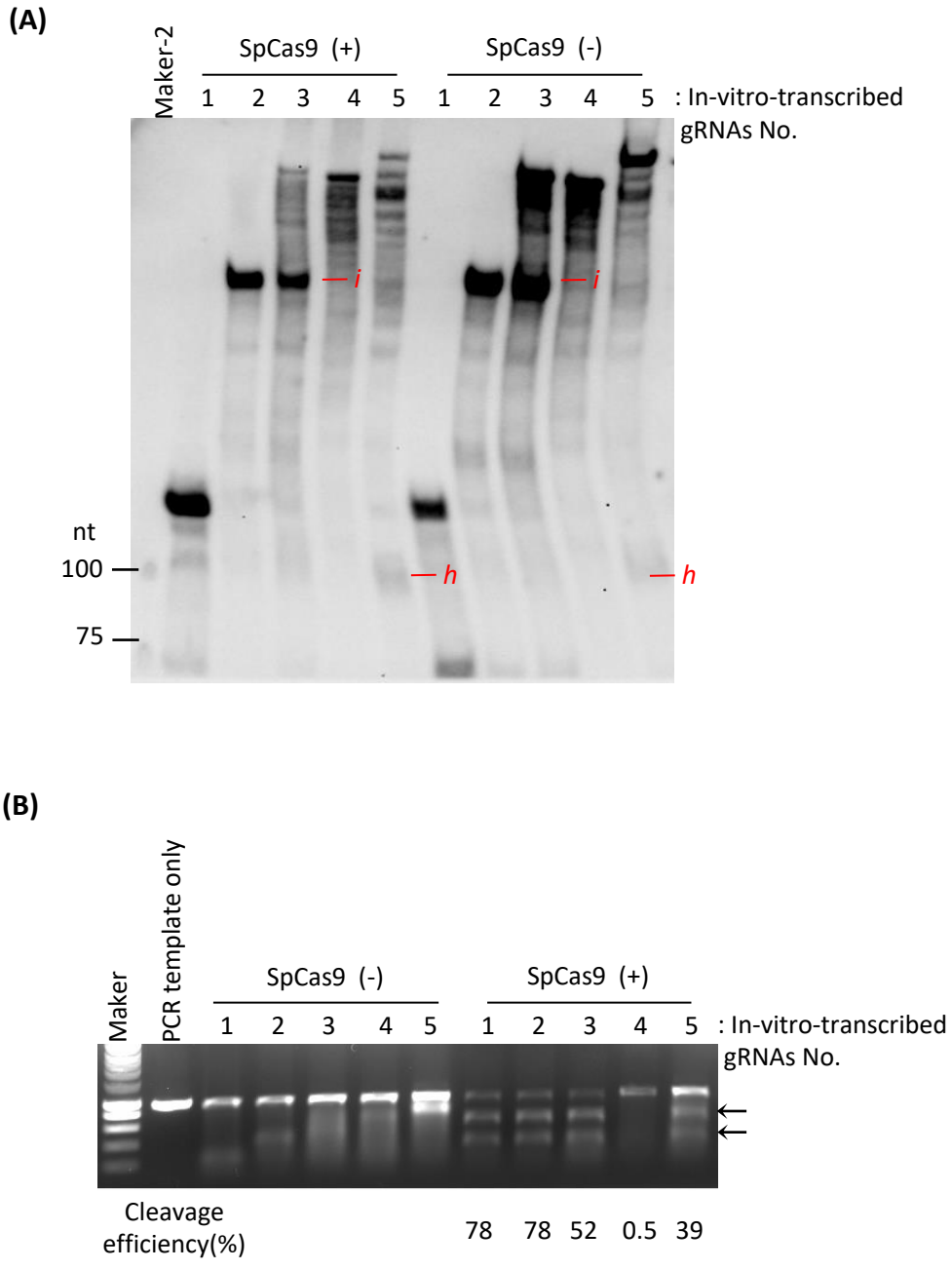

(A)
Maker-2
SpCas9 (+)
SpCas9 (-)
1 2 3 4 5 1 2 3 4 5 : In-vitro-transcribed gRNAs No.
i
i
nt
100
75
h
h
(B)
Maker
PCR template only
SpCas9 (-)
SpCas9 (+)
1 2 3 4 5 1 2 3 4 5 : In-vitro-transcribed gRNAs No.
Cleavage efficiency(%)
78 78 52 0.5 39

Figure. S7

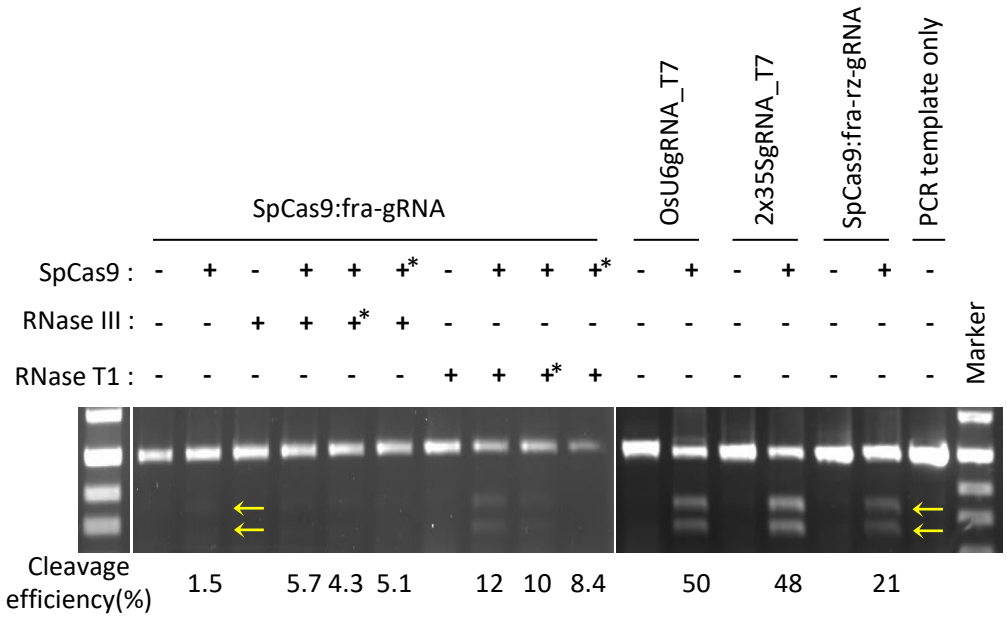
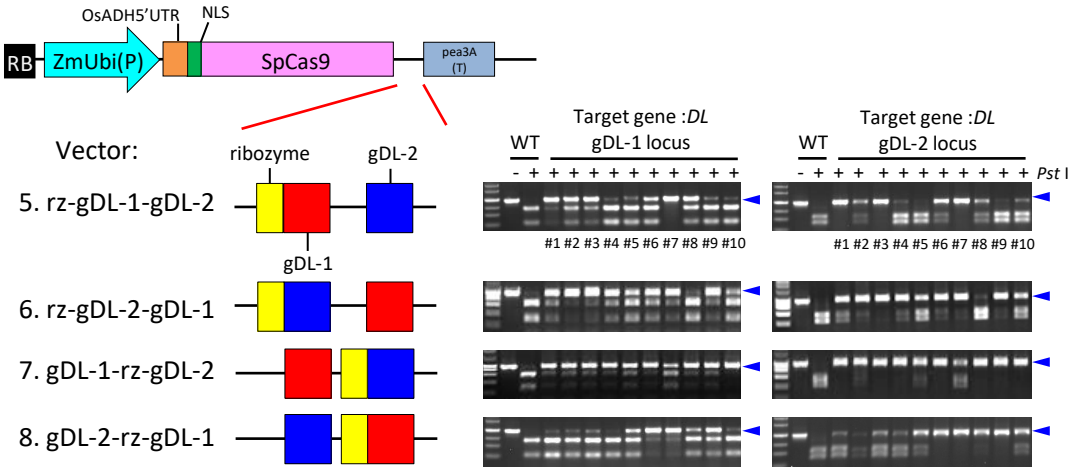
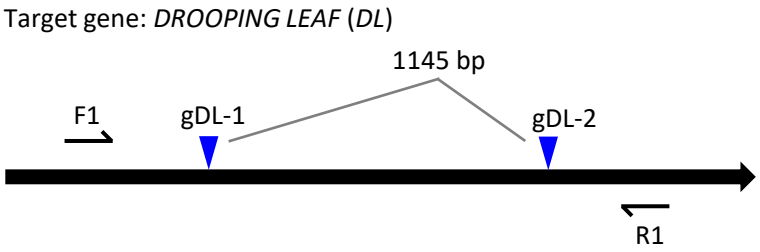

SpCas9:fra-gRNA
OsU6gRNA_T7
2x35SgRNA_T7
SpCas9:fra-rz-gRNA
PCR template only
SpCas9 : - + - + + +* - + + +* - + - + - + -
RNase III : - - + + +* + - - - - - - - - - - -
RNase T1 : - - - - - - + + +* + - - - - - - -
Marker
Cleavage efficiency(%)
1.5
5.7
4.3
5.1
12
10
8.4
50
48
21

Figure. S8

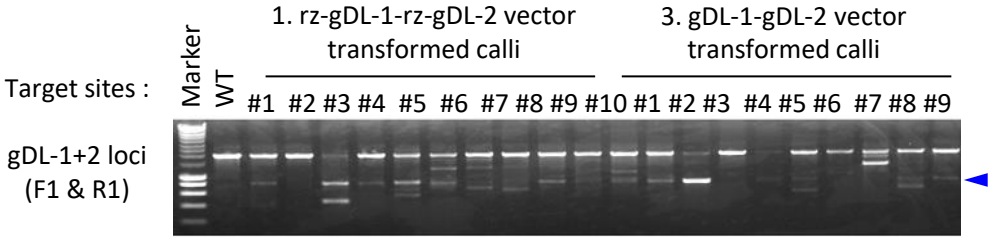
(A)



(B)



(C)



(D)

3. gDL-1-gDL-2 vector transformed callus : line #2

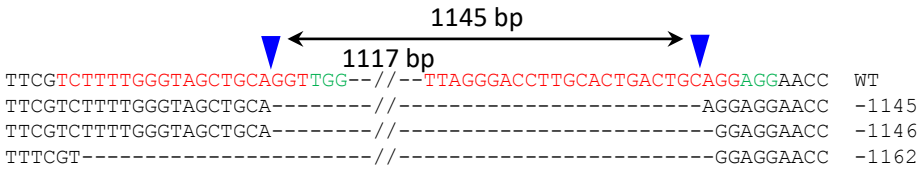


Figure. S9

Table. S1

Mutation variations of transgenic plants regenerated from SpCas9-gRNAs vetor transformed calli.

	1. rz-gDL-1-rz-gDL-2 vector transformed regenerated plants																									
callus No.	#2						#4										#6					#8				
Regenerated plants	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	1	2	3	4	5	6
gDL-1 locus	M	B	B	B	B	M	B	M	M	M	M	WT	B	WT	WT	WT	M	M	B	M	M	M	WT	WT	WT	WT	M
gDL-2 locus	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	M	M	M	M	M
gDL-1+2 loci	×	×	×	×	○	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	○	×	×
phenotype	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	WT	WT	Dr	WT	Dr

	2. rz-gDL-2-z-rgDL-1 vector transformed regenerated plants																				
callus No.	#1							#2					#4								
Regenerated plants	1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	5	6	7	8	9
gDL-1 locus	B	B	B	B	M	M	B	M	WT	M	B	WT	B	B	B	B	B	B	B	B	B
gDL-2 locus	B	B	B	B	B	B	B	B	M	B	M	B	B	B	B	B	B	B	B	B	B
gDL-1+2 loci	×	×	×	×	×	×	×	×	○	×	×	×	×	×	×	×	×	×	×	×	×
phenotype	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr

	3. gDL-1-gDL-2 vector transformed regenerated plants																				
callus No.	#1								#2								#5				
Regenerated plants	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5
gDL-1 locus	WT	M	B	M	B	B	B	B	B	B	B	B	B	B	B	B	M	WT	WT	M	WT
gDL-2 locus	M	B	B	B	B	B	M	B	B	B	B	B	B	B	B	B	M	M	M	M	B
gDL-1+2 loci	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	○	×	×	×	×
phenotype	WT	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	WT	WT	Dr

	4. gDL-2-gDL-1 vector transformed regenerated plants																									
callus No.	#1						#2								#3								#4			
Regenerated plants	1	2	3	4	5	6	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5
gDL-1 locus	WT	WT	WT	WT	WT	WT	B	B	B	B	B	B	B	B	WT	M	B	B	B	WT	B	WT	WT	WT	WT	M	M
gDL-2 locus	WT	WT	WT	WT	WT	WT	B	B	B	B	B	B	B	B	B	B	B	B	B	WT	B	WT	B	B	B	B	B
gDL-1+2 loci	◎	◎	◎	◎	◎	◎	×	×	×	×	×	×	×	×	×	×	×	×	×	◎	×	◎	×	×	×	×	×
phenotype	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr	Dr

B, bi-allelic mutation
M, mono-allelic mutation
○, deletion between gDL-1+2 loci on mono-allelic
◎, deletion between gDL-1+2 loci on bi-allelic
Dr, drooping leaves phenotype
WT, wild type

Table S2. List of primers used in this study.

Usage	Primer name	Sequence (5'→3')
gRNA-oligo for rice	rz-gDL-1-F	TATCAAAAGACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCTCTTTTGGGTAGCTGCAGGT
	rz-gDL-1-R	AAACACCTGCAGCTACCCAAAAGAGACGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGTCTTTT
	rz-gDL-2-F	TATCAAGGTCCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCGACCTTGCACTGACTGCAGG
	rz-gDL-2-R	AAACCCTGCAGTCAGTGCAAGGTCGACGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGGACCTT
	rz-gYSA-F	TATCGCGCGCCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCGCGCGCCACCTCGGCCGAAG
	rz-gYSA-R	AAACCTTCGGCCGAGGTGGCGCGCGACGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGGCGCGC
	rz-gPDS-F	TATCTGGACGCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCCGTCCAACCCATTCCTCTGC
	rz-gPDS-R	AAACGCAGAGGAATGGGTTGGACGGACGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGCGTCCA
	gDL-1-F	TATCTCTTTTGGGTAGCTGCAGGT
	gDL-1-F for 2x35S	GAGGTCTTTTGGGTAGCTGCAGGT
	gDL-1-R	AAACACCTGCAGCTACCCAAAAGA
	gDL-2-F	TATCGACCTTGCACTGACTGCAGG
	gDL-2-R	AAACCCTGCAGTCAGTGCAAGGTC
	gYSA-F	TATCGCGCGCCACCTCGGCCGAAG
	gYSA-R	AAACCTTCGGCCGAGGTGGCGCGC
	gPDS-F	TATCCGTCCAACCCATTCCTCTGC
	gPDS-R	AAACGCAGAGGAATGGGTTGGACG
	gDMC1A-F for 2x35S	GAGGTGGAGATGTGAAGAAGCTGC
	gDMC1A-R	AAACGCAGCTTCTTCACATCTCCA
gRNA-oligo for *Arabidopsis*	rz-gBRI1-F	TATCACCCAACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCTTGGGTCATAACGATATCTC
	rz-gBRI1-R	AAACGAGATATCGTTATGACCCAAGACGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGTTGGGT
	gBRI1-F	TATCTTGGGTCATAACGATATCTC
	gBRI1-R	AAACGAGATATCGTTATGACCCAA
Primers for Guide-it sgRNA *In Vitro* Transcription Kit	In vitro_T7-gDL-1	GCGGCCTCTAATACGACTCACTATAGGGTCTTTTGGGTAGCTGCAGGTGTTTTAGAGCTAGAAATAGCA
	In vitro_T7-gDL-2	GCGGCCTCTAATACGACTCACTATAGGGGACCTTGCACTGACTGCAGGGTTTTAGAGCTAGAAATAGCA
Primers for *in Vitro* Transcription T7 Kit	T7-gDL-1-F	AATACGACTCACTATAGGTCTTTTGGGTAGCTGCAGGTGTTTTAGAGC
	T7-gDL-2-F	AATACGACTCACTATAGGGACCTTGCACTGACTGCAGGGTTTTAGAGC
	T7-SpCas9-F	AATACGACTCACTATAGGACGGGAGAAAGCGTATGCTGGCGTCGGCGG
	T7-HPT-F	AATACGACTCACTATAGGATGAAAAAGCCTGAACTCACCGCGACGTCT
	PolyT-gRNA-Scaffold-R	AAAAAAAGCACCGACTCGGTGCCACTTTTT
	pea3A-polyA-signal-R	TTTATTAACTCTTATCCATCCATTTGCATTTTGATGTCCG
PCR	OsDL-1F	CAGTGTCATGTTCCATCTTTCGCTTCCATT
	OsDL-1R	ATGGGCAAGAGAGAAATCTTTTGCAATCCA
	OsDL-2F	TGCAAAAGATTTCTCTCTTGCCCATCTGTG
	OsDL-2R	TTTCTCACCTCATGAAGCGGTTGTAAGCAG
	OsYSA-F	CATGCGCTCTCTTCCCCACCTGTACTTTAC
	OsYSA-R	CCCTAGCACCCATCTCCGAGTACACTGATT
	OsPDS-F	TGCAAGGTACTAACTAGGAGACATTA
	OsPDS-R	TTGTAAACAGATCTGTAACAGTGA
	AtBRI1-F	GATGGGATGAAGAAGAGTG
	AtBRI1-R	CTCATCTCTCTACCAACAAG