Supplementary Data

Synthesis-dependent repair of Cpf1-induced double-strand DNA breaks enables targeted gene replacement in rice

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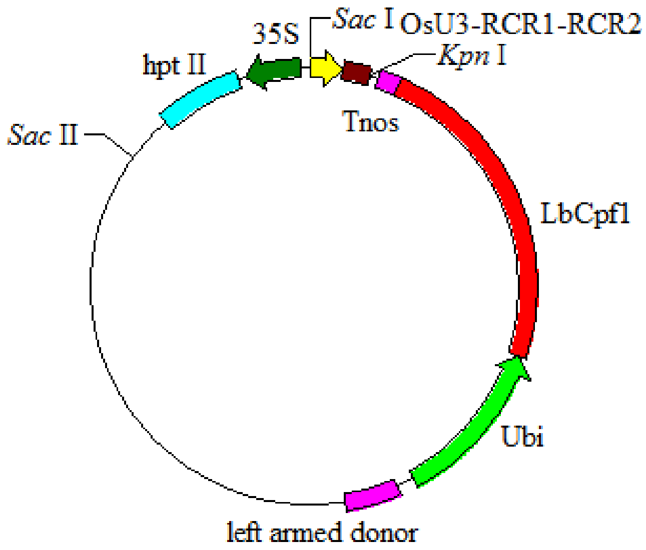
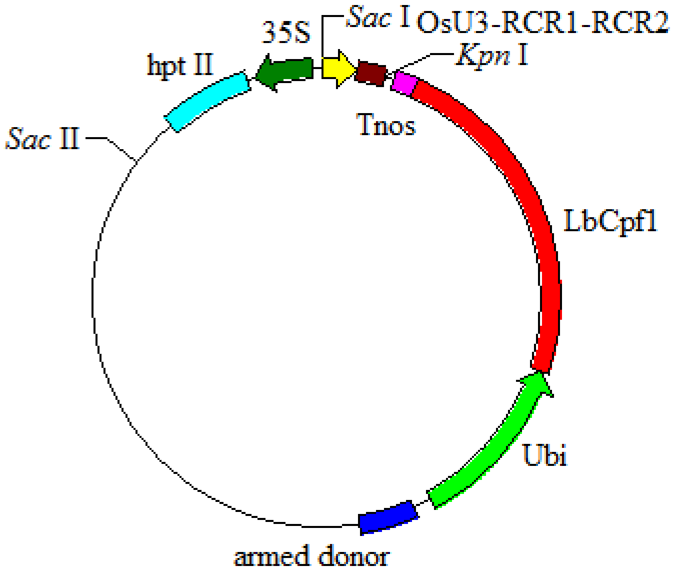
Contents

A



pCXUN-LbCpf1-OsU3-RGR1-RGR2-left-armed-DRT

B



pCXUN-LbCpf1-OsU3-RGR1-RGR2-armed-DRT

Fig. S1. A schematic show of the vectors used in this study

(A) pCXUN-LbCpf1-OsU3-RGR1-RGR2-left-armed-DRT

(B) The map of pCXUN-LbCpf1-OsU3-RGR1-RGR2-armed-DRT

Fig. S2. Sequences of the DRT with only left homology arm and the DRT with two homology arms

(**A**) DRT with only left homology arm; (**B**) DRT with two homology arms. The sequences shadowed in yellow are derived from the wild-type *ALS* gene sequence, sequences of two targets are underlined, the PAM and mutated PAM sequences are boxed, the left and right arms are shadowed in blue, the mutated *Eco*RV target site are shadowed in gray, the mutated amino acids are shadowed in green, and the mutated synonymous changes in the donor are in red lower case.

Table S1. The primer sets used in this study

Primer	Sequence（5′ to 3′）	Use of PCR products	Annealing (℃)	Fragment size (bp)
RGR1F2	TAATTTCTACTAAGTGTAGATGGTATGGTGGTGCAATGGGAGGAGGCCGGCATGGTCCCAGCCT	Construct vector for RGR1	58	112
RGR-common-R	GTCCCATTCGCCATGCCGAAGC			
RGRF1	AAATTACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCTAATTTCTACTAAGTGTAGAT	Construct vector for RGR1and RGR2	58	155
RGR-common-R	GTCCCATTCGCCATGCCGAAGC			
RGR2-F2	TAATTTCTACTAAGTGTAGATACCTGAATGACCCATAAAGAGTGGGCCGGCATGGTCCCAGCCT	Construct vector for RGR2	58	112
RGR-common-R	GTCCCATTCGCCATGCCGAAGC			
OsU3F	AAGGAATCTTTAAACATACGAACAGATCA	Construct vector for OsU3-RGR1-RGR2	56	404
OsU3R	TGCCACGGATCATCTGCAC			
OsU3-RGR1R	CACGGACTCATCAGTAATTTTGCCACGGATCATCTGCAC			
RGR-Common-F	AAATTACTGATGAGTCCGTGAGGAC	Construct vector for OsU3-RGR1-RGR2	58	172
RGR1-10 random-R	GTAATTTGGTCGTATTCGTCCCATTCGCCATGCCG			
RGR2-10 random-F	CGAATGGGACGAATACGACCAAATTACTGATGAGTCCGTGAGGAC	Construct vector for OsU3-RGR1-RGR2	58	195
Sac-RGR2R	CCCTTTCGCCAGGGGTACCGGTCCCATTCGCCATGCCGAAGC			
Sac-OsU3F	GATTACGAATTCGAGCTCAAGGAATCTTTAAACATACGAACAGATCA	Construct vector for OsU3-RGR1-RGR2	58	739
Sac-RGR2R	CCCTTTCGCCAGGGGTACCGGTCCCATTCGCCATGCCGAAGC			
Pme-donorF	GTTTCCCGCCTTCAGTTTAAACGTAAAACGACGGCCAGT	Construct vector for donor	56	873
Pme-donorR	GTCAAACACTGATAGTTTAAACCAGGAAACAGCTATGAC			

donor-armLF	AACCAACATTTGGGTATGGTG	Construct vector for donor (with only left arm)	60	486
donor-armLR1	CATAGGCCGGGCTGGT			
donor-armLF	AACCAACATTTGGGTATGGTG	Construct vector for donor (with only left arm)	58	522
donor-armLR2	CACTCTTTATGGGTCATTCAGGTCAAACATAGGCCGGGCTGGT			
pme-donor-armLF	GTTTCCCGCCTTCAGTTTAAACAACCAACATTTGGGTATGGTG	Construct vector for donor (with only left arm)	58	566
pme-donor-armLR	GTCAAACACTGATAGTTTAAACCACTCTTTATGGGTCATTCAGGT			
ALSTestF	GGCAATCATCGCTACTGGTG	Detect edited *ALS* gene in calli	58	648
T2MR	CACTCTTTATGAGTCATTCACGTCAG			
ALSTestF	GGCAATCATCGCTACTGGTG	Detect edited *ALS* gene in plants	58	803
ALSTestR	ATGCCTACAGAAAACAACACACTACA			
hptF	GAGGGCGTGGATATGTCCTG	Detect *hptII* gene in plants	58	306
hptR	ATTGACCGATTCCTTGCGGT			
U3F	AAGGAATCTTTAAACATACGAACAGATC	Detect crRNA sequence in plants	57	509
U3R	TTTGGATCCTCTAGAGATTATGTGG			
LbCpf1F	GCCTGTTGCACAAGGATTAGACTTC	Detect LbCpf1 transgene in plants	58	684
LbCpf1R	GGAGCAGAGCTGTTCATGAGG			
ALS-OFF1-F	GCTGCTCTGTTGTCTGGGTC	Detect mutations on putative off-target site of *ALS* target 1	59	800
ALS-OFF1-R	ACGAGCAACAGCCAGAATCA			

ALS-OFF2-F	GGTTGCAAAGTCACATGCGTG	Detect mutations on putative off-target site of *ALS* target 1	59	818
ALS-OFF2-R	TCCGGAGATTCCTGACGTGAG			
ALS-OFF3-F	GGTTTACCTTTCTAGCGCACTTC	Detect mutations on putative off-target site of *ALS* target 1	59	658
ALS-OFF3-R	CGATGTTCTTAGGCGCACC			
ALS-OFF4-F	CACCCATAAGGAATGGGTCTTC	Detect mutations on putative off-target site of *ALS* target 2	57	999
ALS-OFF4-R	GCTCTTGATTGGGCCGGC			
ALS-OFF5-F	CTACCTAGGGCAGCTGCTG	Detect mutations on putative off-target site of *ALS* target 2	59	524
ALS-OFF5-R	CAGAGCCATCCCAGACTTTAC			

Table S2.Characterization and genotyping of regenerated plants in T_0 generation

Vector	NO. of calli for transform	NO. of resistant calli	NO. of individual rice plants recovered	Line	Genotype
pCXUN-LbCpf1-OsU3-RGR1-RGR2-left-armed-DRT	152	15	71	171-2	Heterozygous HR
				171-9	chimeric HR
				170-1 172-1	Partial HR
pCXUN-LbCpf1-OsU3-RGR1-RGR2-armed-DRT	164	20	94	169-2	chimeric HR
				169-3	
				168-2	Partial HR
				168-4	
				168-5	
				169-5	
				169-6	
				169-1	Random indels

Table S3. Analysis of potential off-target effects

Target	Name of putative off-target site	Putative off-target locus	Sequence of the putative off-target site	No. of mismatching bases	No. of plants sequenced	No. of plants with mutations
Target 1	ALS1-OFF1	chr02:4712511-4712537	TTTAGAAATTGTTGTGCAAGGGGAGGA	5	12	12
	ALS1-OFF2	chr05:26016305-26016331	TTTGGGTAAGATCATGCAAAGGGAGAA	6	12	12
	ALS1-OFF3	chr03:33738572-33738598	TTTGATTTTGGTGGTGGAATGGAAGAA	6	12	12
Target 2	ALS2-OFF1	chr08:4284947-4284973	TTTCACCTAAATAACCCATTAGGAGTA	5	12	12
	ALS2-OFF2	chr07:584165-584191	TTTGCCCTGGATGGCCCTTTAAGAGGG	6	12	12

Note: The PAM motif is underlined; mismatching bases are shown in red.