

A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants

Xu Tang, Levi G. Lowder, Tao Zhang, Aimee A. Malzahn, Xuelian Zheng, Daniel F. Voytas, Zhaohui Zhong, Yiyi Chen, Qiurong Ren, Qian Li, Elida R. Kirkland, Yong Zhang and Yiping Qi

Nature Plants **3**, 17018 (2017); published 17 February 2017; corrected 19 June 2017.

In the Supplementary Information file originally published, the bottom oligo sequence in Supplementary Fig. 1b was incorrect. The sequence should have been 3'-ANNNNNNNNNNNNNNNNNNNNNNNNCCGG-5'. This has now been corrected.

In the format provided by the authors and unedited.

A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants

Xu Tang^{1†}, Levi G. Lowder^{2†}, Tao Zhang³, Aimee A. Malzahn⁴, Xuelian Zheng¹, Daniel F. Voytas⁵, Zhaohui Zhong¹, Yiyi Chen¹, Qiurong Ren¹, Qian Li¹, Elida R. Kirkland², Yong Zhang^{1*} and Yiping Qi^{4,6*}

¹Department of Biotechnology, School of Life Science and Technology, Centre for Informational Biology, University of Electronic Science and Technology of China, Chengdu 610054, China. ²Department of Biology, East Carolina University, Greenville, North Carolina 27834, USA. ³Jiangsu Key Laboratory of Crop Genetics and Physiology, Co-Innovation Centre for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, China. ⁴Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland 20742, USA. ⁵Department of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Minneapolis, Minnesota 55455, USA. ⁶Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, Maryland 20850, USA.

[†]These authors contributed equally to this work. *e-mail: yiping@umd.edu; zhangyong916@uestc.edu.cn

SUPPLEMENTARY METHODS

Gateway compatible attR1-attR2 destination vector. To accommodate Cpf1 expression in rice, a Gateway destination vector (pYPQ203) was generated by replacing the 2X 35 promoter of pMDC32¹ with the ZmUbi promoter from pUNos_C1 (Plamid # 33297 at Addgene) at HindIII-Ascl sites. Similarly, another Gateway destination vector (pYPQ202) was generated with the Arabidopsis ubiquitin 10 (AtUbi10) promoter. The resulting vectors were confirmed by Sanger sequencing.

Gateway compatible attL1-attR5 Cpf1 vectors. A short linker (AsLinker, **Supplementary Table 1**) was used to replace Cas9p sequence at NcoI and BamHI sites of pYPQ167² (Plasmid #69309 at Addgene) to generate pYPQ167-AsLinker. Meanwhile, a rice codon-optimized AsCpf1 coding sequence was generated with the IDT website and three gBlocks (AsCpf1-gBlock1, AsCpf1-gBlock2 and AsCpf1-gBlock3; see **Supplementary Table 1**) covering this ~4kb sequence were ordered. These gBlocks were sequentially cloned into pYPQ167-AsLinker at NcoI-HindIII, HindIII-MfeI, and MfeI-BamHI sites to make AsCpf1 entry clone, pYPQ220. Similarly, a rice codon-optimized LbCpf1 coding sequence was also designed at IDT. Three gBlocks (LbCpf1-gBlock1, LbCpf1-gBlock2 and LbCpf1-gBlock3) were sequentially cloned into pYPQ167-AsLinker at NcoI-HindIII, HindIII-NheI, and NheI-AatII sites to generate LbCpf1 vector, pYPQ230. Both Cpf1 vectors were verified by Sanger sequencing using the sequencing primers listed in **Supplementary Table 1**.

To generate AsCpf1 based transcriptional repressor pYPQ223 (dAsCpf1-SRDX), two PCR fragments amplified with primer pair one (Cpf1-D908A-F1 and pYPQ221-R1) and two (pYPQ221-F2 and Cpf1-D908A-R2) were recombined in *E. coli*, resulting in pYPQ221 (containing dAsCpf1). The sequence containing 3xSRDX was cut from pYPQ153² at BsmBI and AatII sites and inserted into pYPQ221 at the same sites to generate pYPQ223 (dLbCpf1-SRDX). LbCpf1 based transcriptional repressor pYPQ233 was generated in a similar fashion with PCR primers listed in **Supplementary Table 1**.

Gateway compatible attL5-attL2 crRNA expression vectors. The ZmUbi promoter was PCR amplified from pYPQ203 with primers 14N-ZmUbi-F-SpeI and 14N-ZmUbi-R-BamHI

(**Supplementary Table 1**) using NEB Q5 polymerase. The amplicon was cloned into pYPQ141A (Plasmid #69090 at Addgene) at SpeI and BamHI sites to generate pYPQ141A-ZmUbi. Two gBlocks (RZ-As-GBK and RZ-Lb-GBK) allowing for Golden gate cloning of crRNAs for AsCpf1 and LbCpf1 were ordered and cloned into BamHI and EcoRI sites of pYPQ141A-ZmUbi. The resulting crRNA cloning vectors, pYPQ141-ZmUbi-RZ-As and pYPQ141-ZmUbi-RZ-Lb, were confirmed by sequencing. To generate individual crRNA expression plasmid for each target site, a pair of oligos were phosphorylated and annealed, and then ligated into BsmBI sites of pYPQ141-ZmUbi-RZ-As or pYPQ141-ZmUbi-RZ-Lb. Refer to **Supplementary Table 1** and **Supplementary Methods** for more details.

Gateway® assembly of a CRISPR/Cpf1 T-DNA vector

Step1. Cloning guide RNA (gRNA) into expression vectors

I. Linearize guide RNA expression plasmids (pYPQ141-ZmUbi-RZ-As or pYPQ141-ZmUbi-RZ-Lb)

1. Digestion with Esp3I (BsmBI)

pYPQ141-ZmUbi-RZ-As or pYPQ141-ZmUbi-RZ-Lb	32 µl
10X OPTIZYME buffer 4	4 µl
DTT (20 mM)	2 µl
EPS3I (BsmBI) (10 u/µl; Thermo Scientific)	2 µl
Total	40 µl

Incubate at 37°C overnight; Inactivate enzymes at 80°C denature for 20 min, purify the vector using Qiagen PCR purification kit, and quantify DNA concentration using Nanodrop.

II. Cloning Oligos into linearized gRNA expression vector

2. Oligo phosphorylation and annealing

gRNA oligo forward (100 µM)	1 µl
gRNA oligo reverse (100 µM)	1 µl
10X T4 Polynucleotide Kinase buffer	1 µl
T4 Polynucleotide Kinase (10 u/µl; NEB)	0.5 µl
ddH ₂ O	6.5 µl
Total	10 µl

Phosphorylate and anneal the oligos using 37 °C for 30 min; 95 °C for 5 min; ramp down to 25 °C at 5 °C min⁻¹ (i.e., 0.08 °C/second) using a thermocycler (alternatively: cool down in boiled water) .

3. Ligate oligos into linearized gRNA expression vector and transformation of *E.coli* DH5α cells

ddH ₂ O	6.5 µl
10X NEB T4 ligase buffer	1 µl
Linearized gRNA plasmid	1 µl
Diluted annealed Oligos (1:200 dilution)	1 µl
T4 ligase	0.5 µl
Total	10 µl

Incubate at room temperature for 1 hour.

4. Transform *E. coli* DH5α cells and plate transformed cells on a Tet⁺ (5ng/µl) LB plate; 37 °C over night
5. Mini-prep two independent clones and verify gRNAs by Sanger sequencing with primer M13-R.

Step 2. Gateway® Assembly of CRISPR/Cpf1 components into a binary vector

1. Set up Gateway LR reaction as following:

Cpf1 entry vector (100 ng/ µl)	1 µl
Guide RNA entry vector (100ng/ µl)	1 µl
Destination vector (100 ng/ µl)	2 µl
LR Clonase II	1 µl
Total	5 µl

Incubate at room temperature for 1 hour or overnight (recommended)

2. Transform *E. coli* DH5α cells and plate transformed cells on a Kan⁺ (50 µg/ml) LB plate
3. Mini-prep two independent clones and verify by restriction digestion

Note 1: We have found regular LR Clonase II enzyme is efficient for the Gateway reactions. There is no need to use MultiSite Gateway recombination kit, which is much more expensive.

Note 2: If EcoRI digestion is used to confirm final T-DNA vector, there will be an extra ~3 kb band which likely results from an additional unannotated EcoRI site in the pYPQ203 vector backbone. Such an extra EcoRI site doesn't impact the functionality of the vector.

References

1. Curtis, M.D. & Grossniklaus, U. A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* **133**, 462-469 (2003).
2. Lowder, L.G. et al. A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. *Plant Physiol* **169**, 971-985 (2015).

Supplementary Figure 1. A double ribozyme system for precise processing of mature crRNAs

Top oligo: 5'-TAGATNNNNNNNNNNNNNNNNNNNNNNNNNN-3'
3'-ANNNNNNNNNNNNNNNNNNNNNNNNNNCCGG-5' : Bottom oligo

PstII **Bam**HI

CACCCCTGTTGTTGGTGTTACTTCTCGAGgGATCcaaaattactGATGAGTCCGTGAGGACGAACGAGTA

GTGGGACAAACCAACATGAAGACGTCCtCAggttaattGACTACTCAGGCACTCTGCTTTGCTCAT

UbiI (maize ubiquitin promoter 1) 1st... Hammerhead ribozyme

BsmBI **Eco**53kI **Sac**I **Sac**II **Bsm**BI

AGCTCGTCTAATTCTACTCTGTGATAGTgagacggagctcagcttgaccgcggcgctctctGGCCGGCATG

TCGAGCAGATTAAAGATGAGAACATCTActctgcctcgagtcagagctggcgcgcgagagaCCGGCCGTAC

Ham... AsCpfI crRNA repeat Guide RNA cloning site HDV ribozyme

KasI **Nar**I **Sfo**I **Plu**TI **Acc**65I **Kpn**II **Eag**II

GTCCACAGCCTCTCGCTGGCGGCGGCTGGGCAACATGCTTCGGCATGGCGAATGGGACggtaccgcggcg

CAGGGTCGGAGGAGCGACCGCGCGCGACCGCTTTGTACGAAGCCGTACCGCTTACCTTgcatgggcggcg

HDV ribozyme

EcoRI **Bsr**GI

gaattcgaccacgctttcttgtaacaagtggccattataaaaaataattgctcatcaattttgttgcacg

cttaagctgggtgcaagaagacattgttaaccgtaaatttttataacgagtagttaaacacggtgc

att 2

PstI **BamHI**

CACCCTGTTGTTGGTGTACTTCTGCAgGATCCaaattacGATGAGTCCGTGAGGACGAAACGAGTA

2170

GTGGGACAAACCAACCAATGAAGACGTCCtAGgtttaatGACTACTCAGGCACTCCTGCTTTGCTCAT

Ubi1 (maize ubiquitin promoter 1) 1st... Hammerhead ribozyme

BsmBI **Eco53kI** **SacI** **SacII** **BsmBI**

AGCTCGTCTAATTTCTACTAagTGtAGATgagacggagctcagctcgaccgcggcgtctctGGCCGGCAT

2240

TCGAGCAGATTAAAGATGattcACATCTActctgcctcgagtcagactggccgcgagagaCGGCGGTA

Hamm... LbCpf1 crRNA repeat Guide RNA cloning site HDV ribozyme

KasI **NarI** **SfoI** **PluTI** **Acc65I** **KpnI** **EagI**

GGTCCACGCTCCTCGCTGCGCGCGCGCTGGGCAACATGCTTCGGCATGGCGAATGGAGCggtaccggcc

2310

CCAGGGTCGAGGAGCGACCGCGCGCGACCGTGTGTACGAGCGGTACCGCTTACCTGccatgggcccgg

HDV ribozyme

EcoRI **BsrGI**

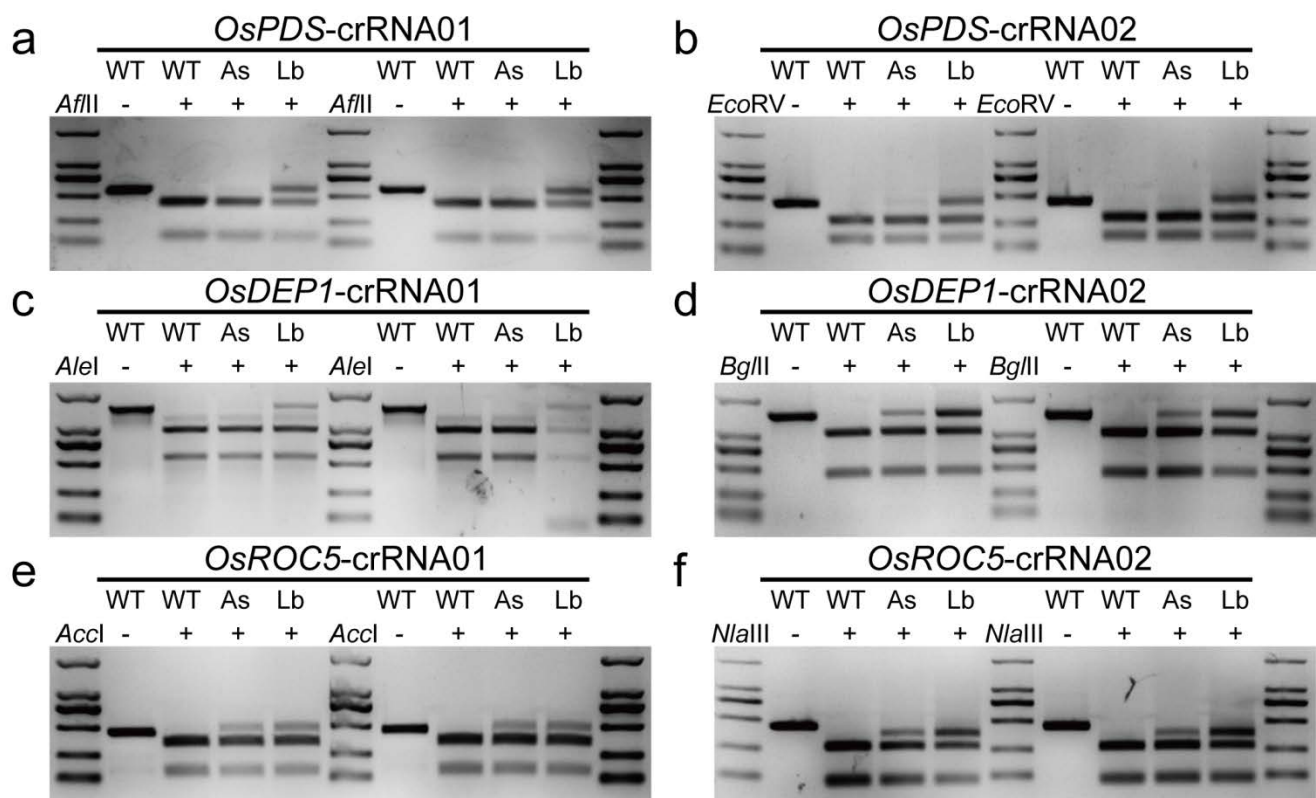
ggaattcgaccgagctttctgtacaaagtggcattataaaaaaattgtcatcaattttgtgaac

2380

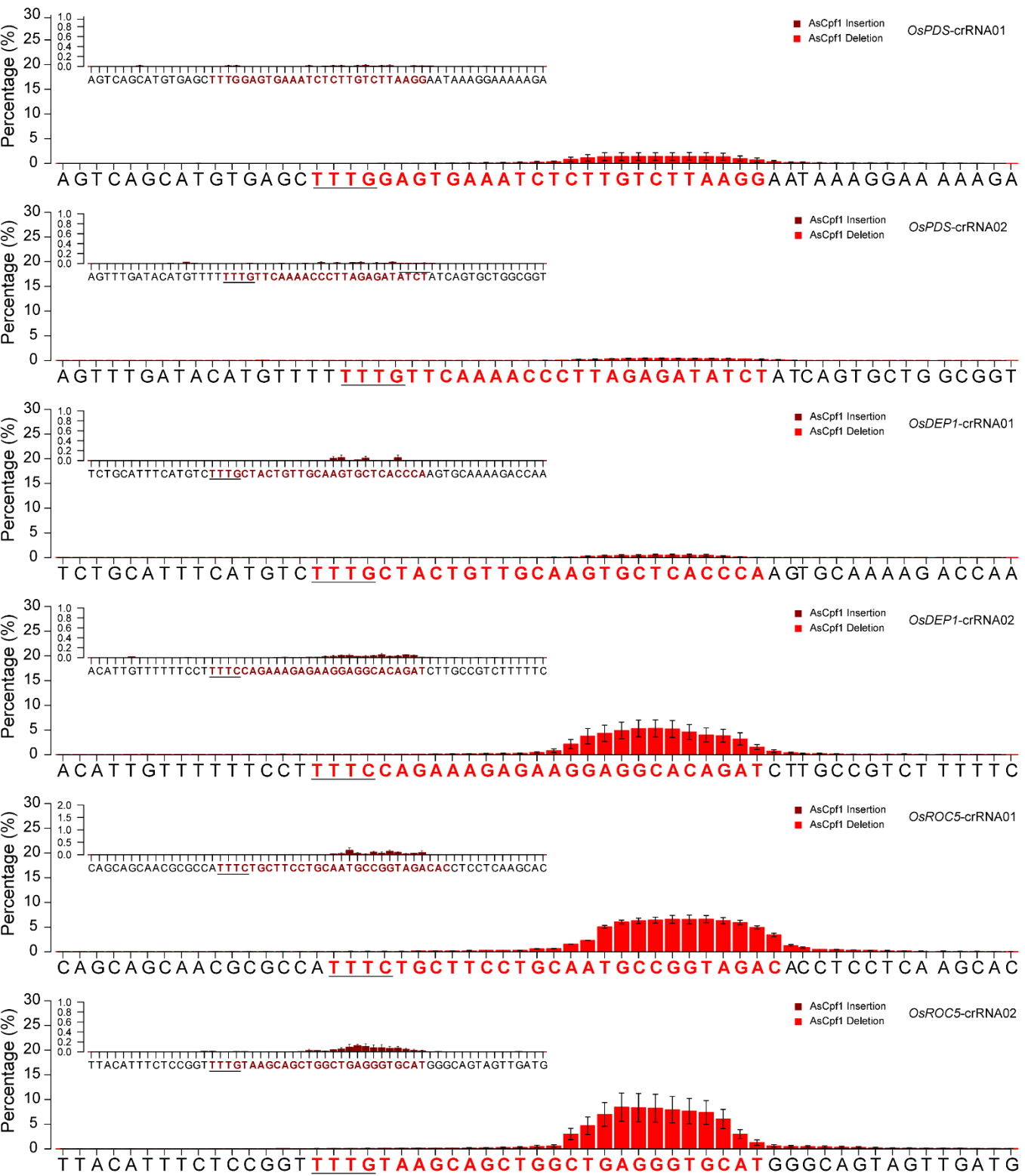
ccttaagctgggtcgaaagaacatgtttcaacgctaatttttaataacgtagttaaacacgttg

att1.2

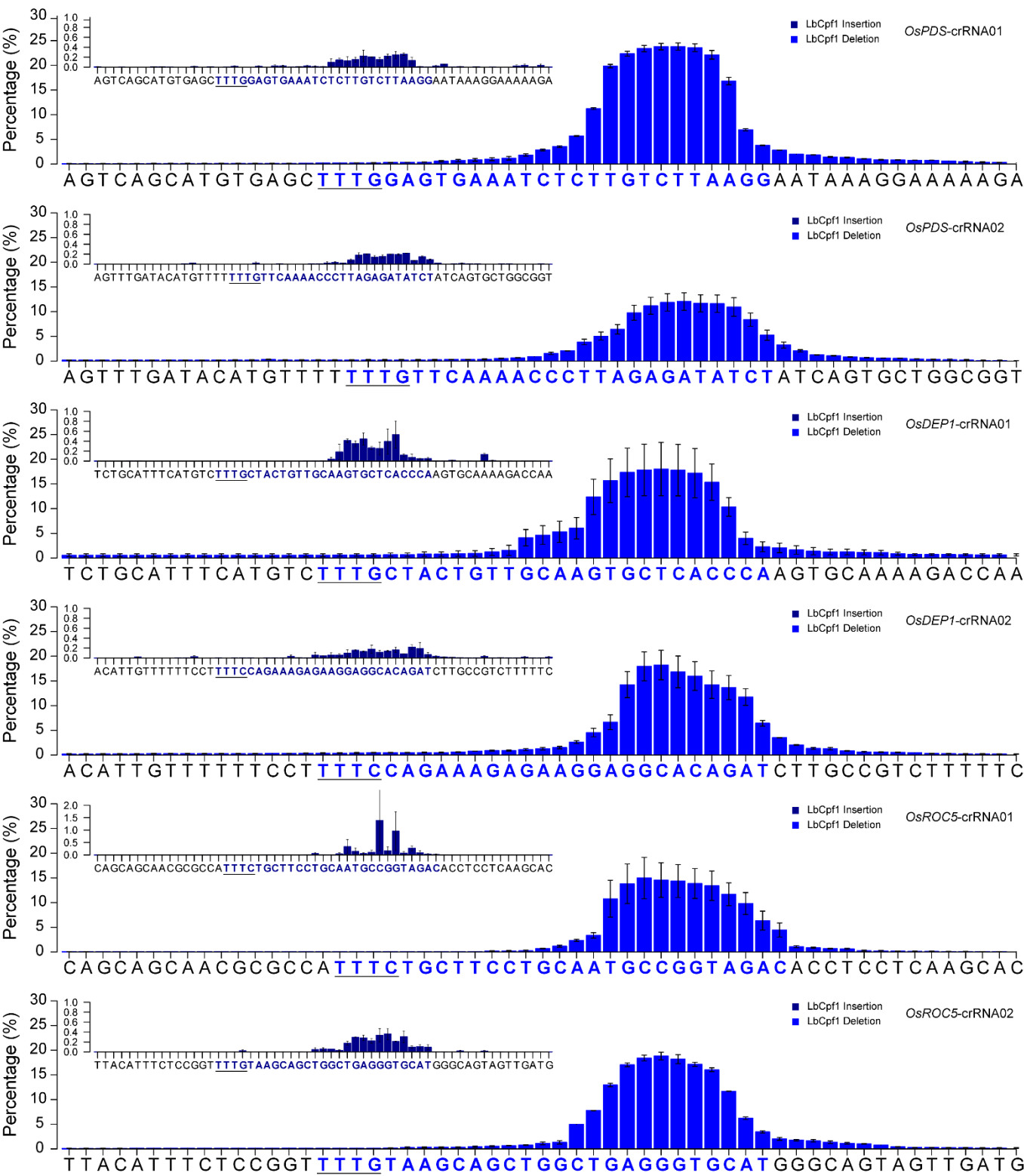
Supplementary Figure 2. Targeted mutagenesis at six endogenous sites in rice protoplasts with AsCpf1 and LbCpf1



Supplementary Figure 3. Positions of insertions and deletions at six rice target sites by AsCpf1



Supplementary Figure 4. Position of insertions and deletions at six rice target sites by LbCpf1



Supplementary Figure 5. Biallelic mutations confirmed by Sanger sequencing in additional T0 lines targeted by with LbCpf1 and *OsPDS*-crRNA01

WT (*OsPDS*): GTGAGC**TTTGGAGTGAAATCTCTTGTCTTAAGGA**AATAAAGGAA
#*OsPDS*-crRNA01-07
Locus-1: GTGAGC**TTTGGAGTGAAATCTCT**-----AATAAAGGAA -10bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCTT**-----AATAAAGGAA -9bp
#*OsPDS*-crRNA01-08
Locus-1: GTGAGC**TTTGGAGTGAAATCTCTTG**-----GAATAAAGGAA -7bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCTTG**-----GGAATAAAGGAA -6bp
#*OsPDS*-crRNA01-09
Locus-1: ----- -57bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCTTGT**-----GAATAAAGGAA -6bp
#*OsPDS*-crRNA01-10
Locus-1: GTGAGC**TTTGGAGTGAAATCTC**-----AGGAA -16bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCT**-----AATAAAGGAA -10bp
#*OsPDS*-crRNA01-11
Locus-1: GTGAGC**TTTGGAGTGAAA**-----AATAAAGGAA -15bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCTTG**-----GAATAAAGGAA -7bp
#*OsPDS*-crRNA01-12
Locus-1: GTGAGC**TTTGGAGTGAAATCTCTT**-----AATAAAGGAA -9bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCT**-----GGAATAAAGGAA -8bp
#*OsPDS*-crRNA01-13
Locus-1: GTGAGC**TTTGGAGTGAAATCTCT**-----GAATAAAGGAA -9bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCTT**-----GAATAAAGGAA -8bp
#*OsPDS*-crRNA01-14
Locus-1: GTGAGC**TTTGGAGTGAAATCT**+----- -238/+8bp
(+8bp: AGGATTTG)
Locus-2: GTGAGC**TTTGGAGTGAAATCT**+----- -238/+8bp
(+8bp: AGGATTTG)
#*OsPDS*-crRNA01-15
Locus-1: GTGAGC**TTTGGAGTGAAATCTCTTG**----- AATAAAGGAA -8bp
Locus-2: GTGAGC**TTTGGAGTGAAATCTCTTGTCTTAAGG**gAATAAAGGAA +1bp

Supplementary Figure 6. Biallelic mutations confirmed by Sanger sequencing in additional T0 lines targeted by with LbCpf1 and *OsROC5-crRNA02*

WT (*OsROC5*): TCCGGT**TTTG**TAAGCAGCTGGCTGAGGGTGCATGGGCAGTAGT

#*OsROC5-crRNA02-07*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTGA**a**----- -17/+1bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCT-----ATGGGCAGTAGT -8bp

#*OsROC5-crRNA02-08*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCT-----GGGCAGTAGT -10bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGC-----ATGGGCAGTAGT -9bp

#*OsROC5-crRNA02-09*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTG-----ATGGGCAGTAGT -7bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG-----GCAGTAGT -11bp

#*OsROC5-crRNA02-10*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCT----- -20bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG--GGTGCATGGGCAGTAGT -2bp

#*OsROC5-crRNA02-11*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTG-----GCAGTAGT -11bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG----TGCATGGGCAGTAGT -4bp

#*OsROC5-crRNA02-12*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTG-----GCAGTAGT -11bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG-----CATGGGCAGTAGT -6bp

#*OsROC5-crRNA02-13*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTGA-----GGCAGTAGT -9bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTGAG-----TGGGCAGTAGT -6bp

#*OsROC5-crRNA02-14*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTG-----GGCAGTAGT -10bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTGA-----ATGGGCAGTAGT -6bp

#*OsROC5-crRNA02-15*

Locus-1: -----GCATGGGCAGTAGT -30bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG-----T -19bp

#*OsROC5-crRNA02-16*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTGA-----GCAGTAGT -10bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTGA-----TGGGCAGTAGT -7bp

#*OsROC5-crRNA02-17*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTGA-----GTAGT -13bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTGAG-----TGGGCAGTAGT -6bp

#*OsROC5-crRNA02-18*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCT-----AGTAGT -14bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG-----ATGGGCAGTAGT -7bp

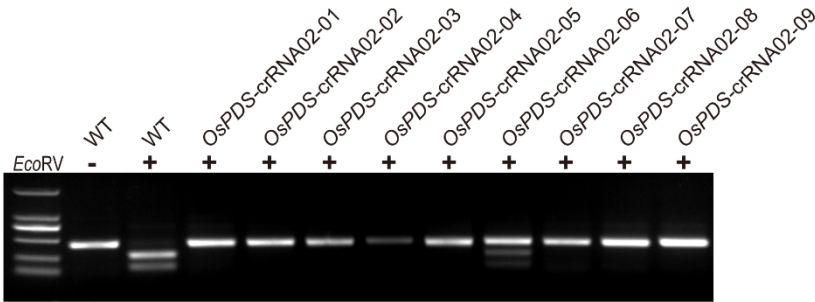
#*OsROC5-crRNA02-19*

Locus-1: TCCGGT**TTTG**TAAGCAGCTGGCTG-----AGT -16bp

Locus-2: TCCGGT**TTTG**TAAGCAGCTGGCTG-----GGCAGTAGT -10bp

Supplementary Figure 7. Generation of T0 rice mutants with LbCpf1 and *OsPDS*-crRNA02

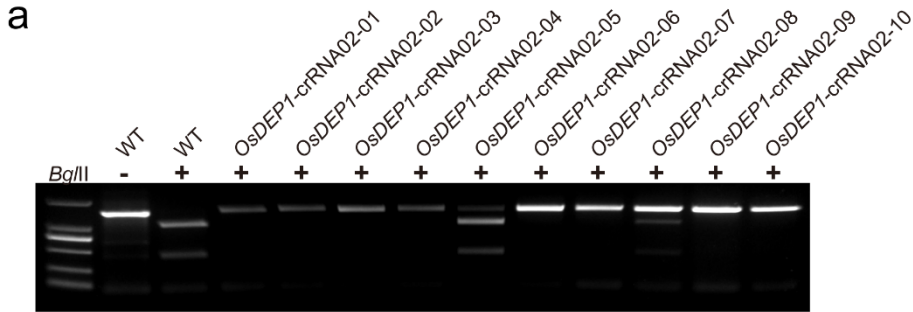
a



b

WT (*OsPDS*): CCAGCACTGATAGATATCTCTAAGGGTTTTGAACAAAAACA
 #*OsPDS*-crRNA02-01
 Locus-1: CCAGCACTGA-----TCTAAGGGTTTTGAACAAAAACA -8bp
 Locus-2: CCAGCACTGA-----TCTAAGGGTTTTGAACAAAAACA -8bp
 #*OsPDS*-crRNA02-02
 Locus-1: CCAGCACT-----TAAGGGTTTTGAACAAAAACA -12bp
 Locus-2: CCAGCACT-----GTAAGGGTTTTGAACAAAAACA -11bp
 #*OsPDS*-crRNA02-03
 Locus-1: CCAG-----AACAAAAACA -27bp
 Locus-2: CCAGCACT-----TAAGGGTTTTGAACAAAAACA -12bp
 #*OsPDS*-crRNA02-04
 Locus-1: CCAGCA-----GGGTTTTGAACAAAAACA -17bp
 Locus-2: CCAGCACT-----GGGTTTTGAACAAAAACA -15bp
 #*OsPDS*-crRNA02-05
 Locus-1: CCAGCACT-----TAAGGGTTTTGAACAAAAACA -12bp
 Locus-2: -----AAAAACA -48bp
 #*OsPDS*-crRNA02-06
 Locus-1: CCA-----CTAAGGGTTTTGAACAAAAACA -16bp
 Locus-2: CCAGCACTGATAGATA--TCTAAGGGTTTTGAACAAAAACA -2bp
 #*OsPDS*-crRNA02-07
 Locus-1: CCAGCACTG-----TCTAAGGGTTTTGAACAAAAACA -9bp
 Locus-2: CCAGCACTG-----TCTAAGGGTTTTGAACAAAAACA -9bp
 #*OsPDS*-crRNA02-08
 Locus-1: CCAGCACT-----GGTTTTGAACAAAAACA -16bp
 Locus-2: CCAGCACTGATAGATA----AAGGGTTTTGAACAAAAACA -5bp
 #*OsPDS*-crRNA02-09
 Locus-1: CCAGCACT-----GGGTTTTGAACAAAAACA -15bp
 Locus-2: CCAGCACTGATA-----CTAAGGGTTTTGAACAAAAACA -7bp
 #*OsPDS*-crRNA02-10
 Locus-1: CCAGCA-----GGGTTTTGAACAAAAACA -17bp
 Locus-2: CCAGCACTGA-----TCTAAGGGTTTTGAACAAAAACA -8bp
 #*OsDEP1*-crRNA02-11
 Locus-1: CCAGCACT-----TAAGGGTTTTGAACAAAAACA -12bp
 Locus-2: CCAGCACT-----GGGTTTTGAACAAAAACA -15bp
 #*OsDEP1*-crRNA02-12
 Locus-1: CCAGCACTGAT-----TAAGGGTTTTGAACAAAAACA -9bp
 Locus-2: CCAGCACTGATAG-----CTAAGGGTTTTGAACAAAAACA -6bp
 #*OsDEP1*-crRNA02-13
 Locus-1: CCAGCACT-----AAAAACA -54bp
 Locus-2: CCAGCACTG-----AAGGGTTTTGAACAAAAACA -12bp

Supplementary Figure 8. Generation of T0 rice mutants with LbCpf1 and *OsDEP1*-crRNA02



b

WT (*OsDEP1*): TTTCCCTTTTCAGAAAGAGAAGGAGGCACAGATCTTGCCGTCT

#*OsDEP1*-crRNA02-01

Locus-1: TTTCCCTTTTC----- -38bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----CTTGCCGTCT -8bp

#*OsDEP1*-crRNA02-02

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----CTTGCCGTCT -7bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----ATCTTGCCGTCT -5bp

#*OsDEP1*-crRNA02-03

Locus-1: TTTCCCTTTT----- -37bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----ATCTTGCCGTCT -5bp

#*OsDEP1*-crRNA02-04

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----GATCTTGCCGTCT -5bp

Locus-2: TTTCCCTTTTC-----+TTGCCGTCT -24/+14bp
(+14bp: ACTTTTAACATACA)

#*OsDEP1*-crRNA02-05

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----ATCTTGCCGTCT -5bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----ATCTTGCCGTCT -6bp

#*OsDEP1*-crRNA02-06

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----TCT -15bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----TTGCCGTCT -8bp

#*OsDEP1*-crRNA02-07

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGA-----TCTTGCCGTCT -8bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----cCTTGCCGTCT -8/+1bp

#*OsDEP1*-crRNA02-08

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----TGCCGTCT -10bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAGGCACAGATCTTGCCGTCT WT

#*OsDEP1*-crRNA02-09

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGA-----CTTGCCGTCT -9bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----TCTTGCCGTCT -6bp

#*OsDEP1*-crRNA02-10

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGA----- -30bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----ATCTTGCCGTCT -5bp

#*OsDEP1*-crRNA02-11

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----CTTGCCGTCT -8bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----ATCTTGCCGTCT -5bp

#*OsDEP1*-crRNA02-12

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----ACAGATCTTGCCGTCT -2bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----CAGATCTTGCCGTCT -2bp

#*OsDEP1*-crRNA02-13

Locus-1: TTTCCCTTTTCAGAAAGAGAA-----TCTTGCCGTCT -11bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----TTGCCGTCT -9bp

#*OsDEP1*-crRNA02-14

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGA-----TTGCCGTCT -10bp

Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAGC-----TTGCCGTCT -7bp

#*OsDEP1*-crRNA02-15

Locus-1: TTTCCCTTTTCAGAAAGAGAAGGAG-----TGCCGTCT -10bp

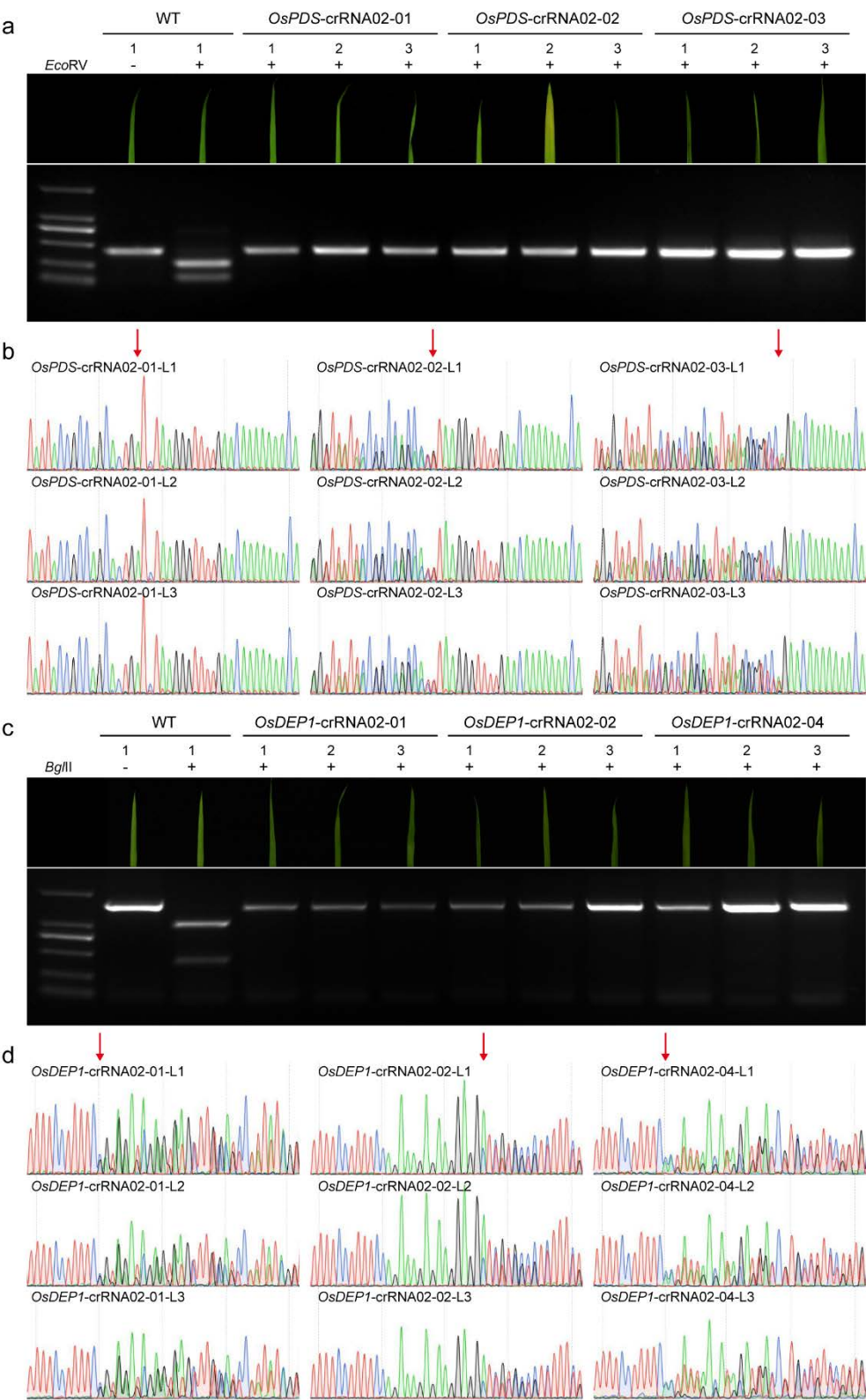
Locus-2: TTTCCCTTTTCAGAAAGAGAAGGAG-----GATCTTGCCGTCT -5bp

#*OsDEP1*-crRNA02-16

Locus-1: TTTCCCTTTTCAGAAAGAGAA-----TGCCGTCT -14bp

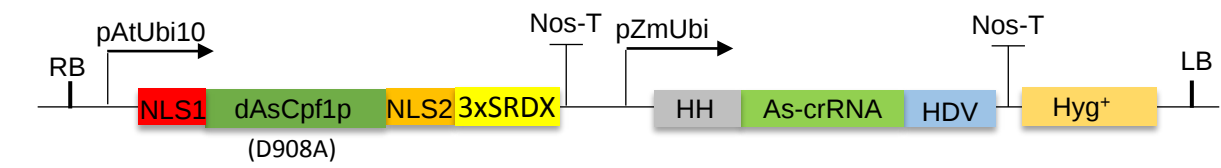
Locus-2: TTTCCCTTTTCAGAAAGAGAAGG-----ATCTTGCCGTCT -8bp

Supplementary Figure 9. Non-mosaic editing by the LbCpf1 system in rice

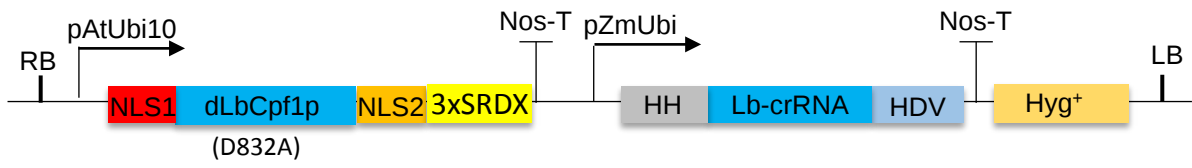


Supplementary Figure 10. A dicot expression system for dCpf1 based transcriptional repression

a



b



Name	Sequence (5'-3')	Experiment
AsLinker-F	catggcgcacAAGCTTtaggtCAATTGgcggccgcG	Constructing Cpf1 entry clone
AsLinker-R	GATCCGcgggccgcCAATTGacctAAGCTTgtgcgc	Constructing Cpf1 entry clone
AsCpf1-gBlock1	cttcaccatggctcctaagaagaagcggaaggttggtattcacggggtgcctg	Constructing Cpf1 entry clone
AsCpf1-gBlock2	CTCACCGGGATCAAGCTTGAGATGGAACCTAGCTTGAGCTT	Constructing Cpf1 entry clone
AsCpf1-gBlock3	GCTTATCGACAAGCTCAATTGCCTGGTGCTCAAGGACTATC	Constructing Cpf1 entry clone
AsCpf1-seq1	GGCACCGTCACGACCACAGAGC	Constructing Cpf1 entry clone
AsCpf1-seq2	TCCGCAACGAGAATGTGCTC	Constructing Cpf1 entry clone
AsCpf1-seq3	GGGTGGGACGTTAACAAAGAGAAG	Constructing Cpf1 entry clone
AsCpf1-seq4	GAACTCAACCCACTCCTGTATCAC	Constructing Cpf1 entry clone
AsCpf1-seq5	AGCGGAACCTCATCTACATCAC	Constructing Cpf1 entry clone
AsCpf1-seq6	GGTTTCGACTTCCTGCACTATGAT	Constructing Cpf1 entry clone
LbCpf1-gBlock1	cttcaccATGgctcctaagaagaagcggaaggttggtattcacggggtgcctg	Constructing Cpf1 entry clone
LbCpf1-gBlock2	CAGGAAAAGCTTCAAAAAGATCGGAAGTTTCAGCCTGGAA	Constructing Cpf1 entry clone
LbCpf1-gBlock3	TGCGGCGCGCTAGCCTTAAGAAGGAGGAGCTTGTAGTCCAI	Constructing Cpf1 entry clone
LbCpf1-seq1	TCAACGGATTACACAACAGCATTCA	Constructing Cpf1 entry clone
LbCpf1-seq2	GAATGGAACGTGATCAGAGACAAA	Constructing Cpf1 entry clone
LbCpf1-seq3	ACGATCCTGAGGTATGGTTC	Constructing Cpf1 entry clone
LbCpf1-seq4	CTTGTAGTCCACCCTGCGAATAGT	Constructing Cpf1 entry clone
LbCpf1-seq5	CGGCGGCGCACTCAAAGGTTAC	Constructing Cpf1 entry clone
Cpf1-D908A-F1	GCACCCAGAGACACCCATAATCGGGATTGcCCGGGGGGGAG	Constructing Cpf1 entry clone
pYPQ221-R1	gagcggtatcagctcactcaaagg	Constructing Cpf1 entry clone
pYPQ221-F2	cttttgctggccttttgctcacat	Constructing Cpf1 entry clone
Cpf1-D908A-R2	GATGTAGATGAGGTTCCGCTCCCCCGGgCAATCCCGATTA	Constructing Cpf1 entry clone
Lb-D832A-F1	ACAATCCTTACGTCAATTGGGATTGcTCGGGGCGAGAGGAAC	Constructing Cpf1 entry clone
Lb-D832A-R2	TATAGAGGAGGTTCTCTCGCCCCGAgCAATCCCAATGACG	Constructing Cpf1 entry clone
14N-ZmUbi-F-Spel	CCTTactagtGTGCTGCCCCCTCTCTAGAGATAATGAGCAT	Constructing crRNA cloning vector
14N-ZmUbi-R-BamHI	CGCAggatccCTGCAGAAGTAACACCAAACAACAGGGTGA	Constructing crRNA cloning vector
RZ-As-GBK	CTTCTGCAGgGATCCaaattaCTGATGAGTCCGTGAGGACGA	Constructing crRNA cloning vector
RZ-Lb-GBK	CTTCTGCAGgGATCCaaattaCTGATGAGTCCGTGAGGACGA	Constructing crRNA cloning vector
Cpf1-OsPDS-gR1-F	TAGATGAGTGAAATCTCTTGCTTAAAGG	Constructing crRNA expression entry clone
Cpf1-OsPDS-gR1-R	GGCCCCCTTAAGACAAGAGATTTCACTCA	Constructing crRNA expression entry clone
Cpf1-OsPDS-gR2-F	TAGATttcaaaacccttagagatatcta	Constructing crRNA expression entry clone
Cpf1-OsPDS-gR2-R	GGCCtagatatctctaagggttttgaaA	Constructing crRNA expression entry clone
Cpf1-OsDEP1-gR1-F	TAGATctactgttgcaagtgtcaccca	Constructing crRNA expression entry clone
Cpf1-OsDEP1-gR1-R	GGCCtgggtagcacttgcaacagtagA	Constructing crRNA expression entry clone
Cpf1-OsDEP1-gR2-F	TAGATcagAAAGAGAAGGAGGCACAGAT	Constructing crRNA expression entry clone
Cpf1-OsDEP1-gR2-R	GGCCATCTGTGCCTCCTTCTCTTTctgA	Constructing crRNA expression entry clone
Cpf1-OsROC5-gR1-F	TAGATTGCTTCCTGCAATGCCGGTAGAC	Constructing crRNA expression entry clone
Cpf1-OsROC5-gR1-R	GGCCGTCTACCGGCATTGCAGGAAGCAA	Constructing crRNA expression entry clone
Cpf1-OsROC5-gR2-F	TAGATTAAGCAGCTGGCTGAGGGTGCAT	Constructing crRNA expression entry clone
Cpf1-OsROC5-gR2-R	GGCCATGCACCCTCAGCCAGCTGCTTAA	Constructing crRNA expression entry clone
OsPDS-MS1-F	TAGATctGTGAAATCTCTTGCTTAAAGG	Constructing crRNA expression entry clone
OsPDS-MS1-R	GGCCCCCTTAAGACAAGAGATTTCAcagA	Constructing crRNA expression entry clone
OsPDS-MS2-F	TAGATGAGTctAATCTCTTGCTTAAAGG	Constructing crRNA expression entry clone
OsPDS-MS2-R	GGCCCCCTTAAGACAAGAGATTagACTCA	Constructing crRNA expression entry clone
OsPDS-MS3-F	TAGATGAGTGAAAActTCTTGCTTAAAGG	Constructing crRNA expression entry clone
OsPDS-MS3-R	GGCCCCCTTAAGACAAGAagTTTCACTCA	Constructing crRNA expression entry clone
OsPDS-MS4-F	TAGATGAGTGAAATCTCaaGTCTTAAAGG	Constructing crRNA expression entry clone
OsPDS-MS4-R	GGCCCCCTTAAGACTtgAGATTTCACTCA	Constructing crRNA expression entry clone
OsPDS-MS5-F	TAGATGAGTGAAATCTCTTGtgTAAGG	Constructing crRNA expression entry clone
OsPDS-MS5-R	GGCCCCCTTAtcACAAGAGATTTCACTCA	Constructing crRNA expression entry clone
OsPDS-MS6-F	TAGATGAGTGAAATCTCTTGCTTActG	Constructing crRNA expression entry clone
OsPDS-MS6-R	GGCCCagTAAGACAAGAGATTTCACTCA	Constructing crRNA expression entry clone
Cpf1-m159b-gR1-F	TAGATattgtatgaatatatgagttagt	Constructing crRNA expression entry clone
Cpf1-m159b-gR1-R	GGCCactaactcatatattcatacaatA	Constructing crRNA expression entry clone
M13-F	TTCCAGTCACGACGTTGTAAAC	Sequencing Cpf1 and crRNA entry clones
M13-R	TTTGAGACACGGGCCAGAGCTGC	Sequencing Cpf1 and crRNA entry clones
Cpf-OsPDS-F1	CTGGCTGCCTGTCATCTATGAA	Amplifying the OsPDS-crRNA01 target site
Cpf-OsPDS-R1	CCAAAACATCCCTTGCCCTCA	Amplifying the OsPDS-crRNA01 target site
Cpf-OsPDS-F2	GGAAATGCCTTGAACAGATAGCT	Amplifying the OsPDS-crRNA02 target site
Cpf-OsPDS-R2	TTGGAAGGGAATAGTAGGTTGA	Amplifying the OsPDS-crRNA02 target site
Cpf-OsDEP1-F	TCACCGATTCTTTCCATGCG	Amplifying the OsDEP1-crRNA01/OsDEP1-crRNA02 target site
Cpf-OsDEP1-R	GCCACAATCGGGTTTGCAAT	Amplifying the OsDEP1-crRNA01/OsDEP1-crRNA02 target site
Cpf-OsROC5-F	CTTATGTTCCGTTCCAATCCT	Amplifying the OsROC5-crRNA01/OsDEP1-crRNA02 target site
Cpf-OsROC5-R	CCTACACTTCACATTTCCACCT	Amplifying the OsROC5-crRNA01/OsDEP1-crRNA02 target site
HTS-1F	ATCACGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-1R	CGATGTggaggtcttgaaagtctctgg	High-throughput sequencing
HTS-2F	TTAGGCctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-2R	TGACCAggaggtcttgaaagtctctgg	High-throughput sequencing
HTS-3F	ACAGTGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-3R	GCCAATggaggtcttgaaagtctctgg	High-throughput sequencing
HTS-4F	CAGATCctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-4R	ACTTGAggaggtcttgaaagtctctgg	High-throughput sequencing

HTS-5F	GATCAGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-5R	TAGCTTggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-6F	CATTTTggaaggatgaagatggagattg	High-throughput sequencing
HTS-6R	CCAACAgctcatgatatttatgtgacgttaa	High-throughput sequencing
HTS-7F	CGGAATggaaggatgaagatggagattg	High-throughput sequencing
HTS-7R	CTAGCTgctcatgatatttatgtgacgttaa	High-throughput sequencing
HTS-8F	CTATACggaaggatgaagatggagattg	High-throughput sequencing
HTS-8R	CTCAGAgctcatgatatttatgtgacgttaa	High-throughput sequencing
HTS-9F	GACGACggaaggatgaagatggagattg	High-throughput sequencing
HTS-9R	TAATCGgctcatgatatttatgtgacgttaa	High-throughput sequencing
HTS-10F	TACAGCggaaggatgaagatggagattg	High-throughput sequencing
HTS-10R	TATAATgctcatgatatttatgtgacgttaa	High-throughput sequencing
HTS-11F	TCATTGggcataataatctgtactactgcca	High-throughput sequencing
HTS-11R	ATCACGgaaggggtcttgcagcaact	High-throughput sequencing
HTS-12R	CGATGTgaaggggtcttgcagcaact	High-throughput sequencing
HTS-13R	TTAGGCgaaggggtcttgcagcaact	High-throughput sequencing
HTS-14R	TGACCAgaaggggtcttgcagcaact	High-throughput sequencing
HTS-15R	ACAGTGgaaggggtcttgcagcaact	High-throughput sequencing
HTS-16F	TCCCGAtggtacaaaacatgaccct	High-throughput sequencing
HTS-16R	TCCCGAgggactagaggcactttcaga	High-throughput sequencing
HTS-17R	CAGATCgggactagaggcactttcaga	High-throughput sequencing
HTS-18R	ACTTGAgggactagaggcactttcaga	High-throughput sequencing
HTS-19R	GATCAGgggactagaggcactttcaga	High-throughput sequencing
HTS-20R	TAGCTTgggactagaggcactttcaga	High-throughput sequencing
HTS-21F	TCGAAGaggtttgggctaattgcctcc	High-throughput sequencing
HTS-21R	GGCTACcacctgtagctcagccttcat	High-throughput sequencing
HTS-22R	CTTGTAcacctgtagctcagccttcat	High-throughput sequencing
HTS-23R	AGTCAAcacctgtagctcagccttcat	High-throughput sequencing
HTS-24R	AGTTCCacctgtagctcagccttcat	High-throughput sequencing
HTS-25R	ATGTCAcacctgtagctcagccttcat	High-throughput sequencing
HTS-26F	TCGGCAgctgctggtgagtgctgat	High-throughput sequencing
HTS-26R	CCGTCCaccattgggagtgcttgc	High-throughput sequencing
HTS-27R	GTAGAGaccattgggagtgcttgc	High-throughput sequencing
HTS-28R	GTCCGCaccattgggagtgcttgc	High-throughput sequencing
HTS-29R	GTGAAAaccattgggagtgcttgc	High-throughput sequencing
HTS-30R	GTGGCCaccattgggagtgcttgc	High-throughput sequencing
HTS-31F	GGCTACctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-31R	CTTGTAggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-32F	AGTCAActggctgcctgtcatctatgaa	High-throughput sequencing
HTS-32R	AGTTCCggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-33F	ATGTCActggctgcctgtcatctatgaa	High-throughput sequencing
HTS-33R	CCGTCCggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-34F	GTAGAGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-34R	GTCCGCggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-35F	GTGAAActggctgcctgtcatctatgaa	High-throughput sequencing
HTS-35R	GTGGCCggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-36F	GTTTCGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-36R	CGTACGggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-37F	GAGTGGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-37R	GGTAGCggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-38F	ACTGATctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-38R	ATGAGCggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-39F	ATTCTTctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-39R	CAAAAGggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-40F	CAACTActggctgcctgtcatctatgaa	High-throughput sequencing
HTS-40R	CACCGGggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-41F	CACGATctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-41R	CACTCAggaggtcttggaaagtctctgg	High-throughput sequencing
HTS-42F	CAGGCGctggctgcctgtcatctatgaa	High-throughput sequencing
HTS-42R	CATGGCggaggtcttggaaagtctctgg	High-throughput sequencing