Supplementary Data

Genome Editing of Golden SNP-Carrying Lycopene Epsilon-Cyclase (*LcyE*)/HDR Gene using the CRSPR-Cas9 and Geminiviral Replicon System in Rice

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Figure S3. Confirmation of T-DNA insertion from calli obtained through HDR experiment in rice. M: 1kb DNA ladder; WT: wild-type; P: pGemBos::*LcyE* plasmid vector.

Table S2. The primers list used in this study.

**Supplementary Table S1.** Design of sgRNAs for HDR by CRISPR-Cas9 system in rice. using the Cas-designer (http://www.rgenome.net/cas-designer/). Under line is PAM sequence.

sgRNA	RGEN Target (5' to 3')	Direction	GC content (%, w/o PAM)	Out-of frame score	Mismatches			
					0	1	2	3
sgRNA1	CAGAGAGGAGATGTCTGACA<u>AGG</u>	-	50.0	67.8	1	0	0	0
sgRNA2	TTGAGCCGGTCGGATCAGAG<u>AGG</u>	-	60.0	77.9	1	0	0	0

LcyE (Os01g0581300) gene

→ donor and primers region, 1116 bp

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atCCCGGGCCTGGAACCTCTCAAAGTTCCAGTCCATCAATGCTTGGTAAGCATTCTTCTGAT
-> : Fw1(J169)
ATTTATTCAATTTTATTGACAAGCACACCAGTATTACAAATTGGAGTACATTGCTTCAGA
TGAGGGCAGTATTTTAGTATAATTTTGGAAATGTCAGGAACATTTTCTGGTACTAATTGC
TATGCGGTGTTGCAGCATGGAGAACATTATGGCCCCAAGAACGGAAACGTCAACGATCAT
TCTTCCTTTTTGGGCTGGCTTTGATAATCCAACTGAATAACGAAGGCATTCAGACATTCT
TTGAAACCTTTTTCCGGTTGCCCAAATGGTAATTCTACTCTTGATTGCATTTGCCTCTGT
TTTCAGTCTATTTACAAATACCATTATGTATGACCTGAAGATTGCCACCCAACAAAGTGT
TCATCACTGTTCTTTGTTACTATCAGGATGTGGCGAGGATTCCTTGGTTCGACGCTTTCT
TCAGTGGATCTCATACTCTTTGCATTCTACATGTTCACAATTGCGCCGAACCAAATGCGA

ATGAACCTTGTCAGACTTCTGCTCTCGGATCCGACCGGCTCAACGATGATCAAGACCTAC
         <- : Rv1(J171)            -> : Fw2(J173)
CTGACCTTGTAAAACCATTTCAGCAGTCTACAAGAATATTAGGAAATGTACAGTTTTGTA
<- : Rv2(J172)
GTTTGTACATAACATAGTGAGAGCCAGAGGATATGGGGGGTTGGGGGGTTACTTATCATGC
TAGAACAACAAAACACTGCAAGAATTTTATGCATGAATTTGGCAAATGGAATAGATTATG
CAGAATGGGAACACGTGAATACGTGATGCGTGTGCTGGAACAACAAGTAGGAGGAATAAA
ACCCATGGATTATGGATCTTGTAGTGAGTTGCATTATAGCCATTCTTAGGTCTTATCATC
TCTCTCCCCTTTCCATTTTTACCTCTATCCGGTTTTCGACATGCCGGATTACACACCCTA
TTTTAGATAGCTCGGCCAAAATGTTCCACAAACATTTTAGAAAAGGAACAAAATGGAAAA
GAGGAGAGAAAAAAAGAGATGGTGCAAGAATAGTCTGAGGCTATCCTTGCTCCATTGCC
ATGGCATTTAGGTCCCAGAAGAAGGGTCTAGAga
                        <- : Rv3(J170)
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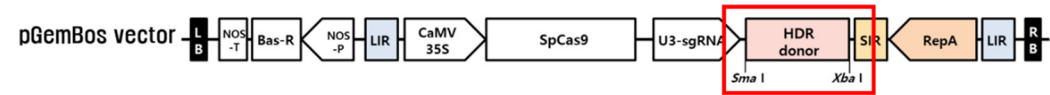
○ **primer SET 1 : 555bp** Section①

OLIGO   start   length   tm   gc%   any   3' seq

LEFT PRIMER   5   20   60.63   55.00   4.00   1.00 GGCCTGGAACCTCTCAAAGT

RIGHT PRIMER   553   20   59.13   40.00   4.00   2.00 TGACAAGGTTCATTCGCATT

○ **primer SET 2 : 84bp** Section②

OLIGO   start   length   tm   gc%   any   3' seq

LEFT PRIMER   2   18   62.92   50.00   4.00   2.00 ATTGCGCCGAACCAAATG

RIGHT PRIMER   85   22   58.20   45.45   6.00   2.00 CAGGTAGGTCTTGATCATCGTT

○ Section① + Section② = Section③

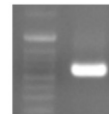○ **primer SET 3 : 539bp** Section④

OLIGO   start   length   tm   gc%   any   3' seq

LEFT PRIMER   7   19   60.36   52.63   6.00   2.00 CGGCTCAACGATGATCAAG
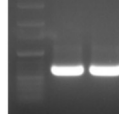
RIGHT PRIMER   543   23   61.79   52.17   6.00   1.00 TCTAGACCCTTCTTCTGGGACCT

pGemBos vector — LB — NOS-T — Bas-R — NOS-P — LIR — CaMV 35S — SpCas9 — U3-sgRNA — HDR donor — SIR — RepA — LIR — RB
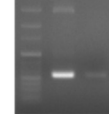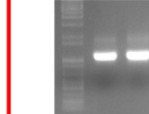
Sma I   Xba I

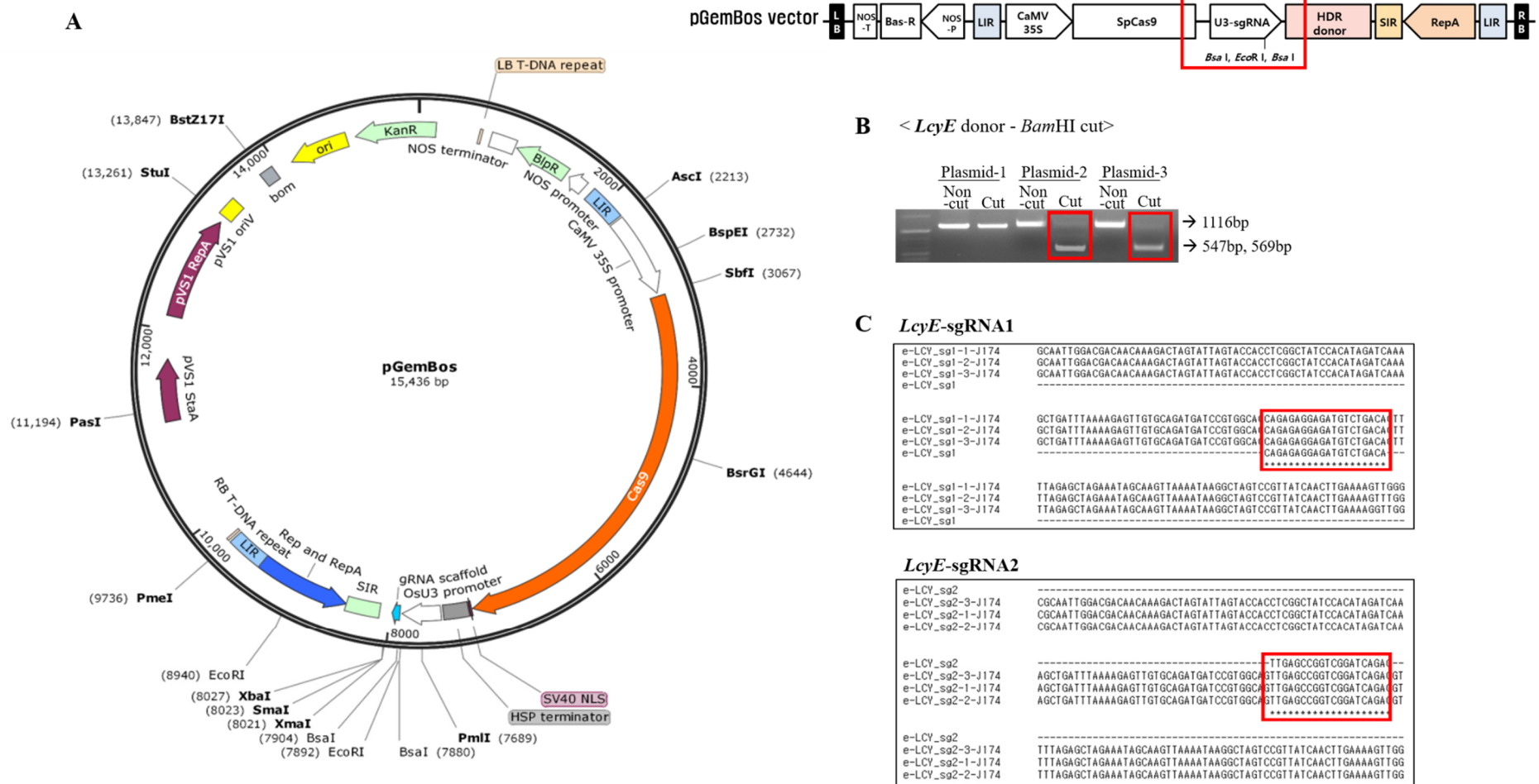Section① (555bp)   Section③ (605bp)   Section④ (539bp)   Completed Donor (1116bp)

Supplementary Figure S1. Design of donor template using the CRSPR-Cas9 and geminiviral replicon system.

Supplementary Figure S2. Ti-plasmid vector construction using the CRSPR-Cas9 and geminiviral replicon system (A) pGemBos vector construction (B) Confirmation of donor template into pGemBos::*LcyE* vector (C) Confirmation of sgRNA by sequencing analysis.
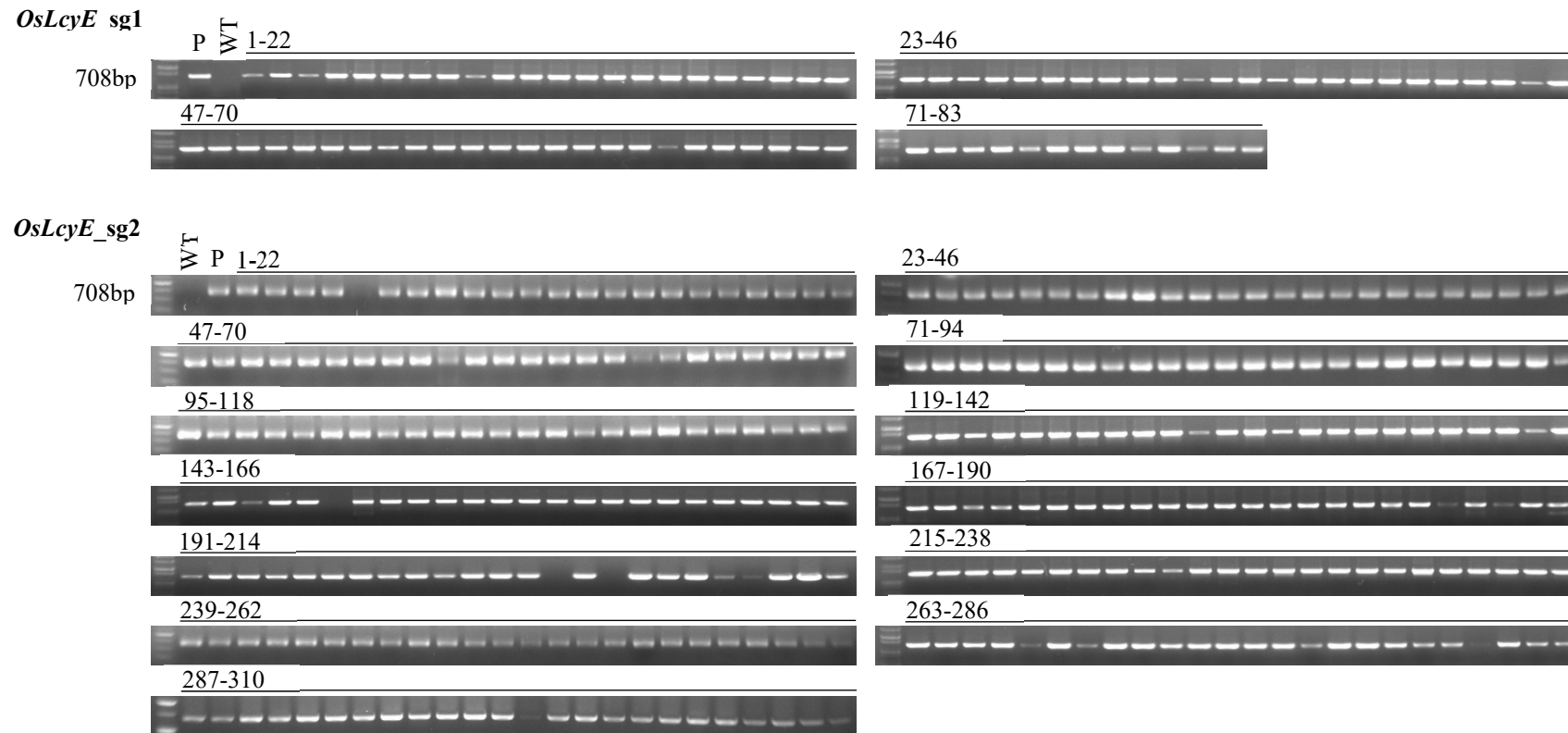
Supplementary Figure S3. Confirmation of T-DNA insertion from calli obtained through HDR experiment. M: 1kb DNA ladder; WT: wild-type; P: pGemBos::*LcyE* plasmid vector.

Supplementary Table S2. The primers list used in this study.

Primer name	Sequence (primer direction 5'-3')
LcyE donor 1st Fw	TCCTGCAACCGGTACTAACA
LcyE donor 1st Rv	GTGGTGCAAGGAAGGAGAAG
LcyE donor 2nd Fw	ATCCCGGGCCTGGAACCTCTCAAAGTTCC
LcyE donor 2nd Rv	TCTCTAGACCCTTCTTCTGGGACCTAAA
LcyE donor OE-PCR Fw1	CGGCTCAACGATGATCAAG
LcyE donor OE-PCR Rv1	TGACAAGGTTCATTCGCATT
LcyE donor OE-PCR Rv2	CAGGTAGGTCTTGATCATCGTT
T-DNA confirm-Nos ter Fw	TTGCGCGCTATATTTTGTTTT
T-DNA confirm-Bar R Rv	CGTCAACCACTACATCGAGA
Replicon confirm-Rep Fw	TTCTCCCAGAGAAACTGGAA
Replicon confirm-35S Rv	CCATCTGTGGGTTAGCATTC
NGS (Next Generation Sequencing) primers	
LcyE 1st Fw	TTCAATTCTTGCAAATTTCTTCA
LcyE 1st Rv	TCATTTTTGGTCTAGTTACACATCA
LcyE 2nd Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTTTTTGGCGTATGAGCAG
LcyE 2nd Rv	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCATGTCCTCGGTGAACT
LcyE 3rd-1	AATGATACGGCGACCACCGAGATCTACACtatagcctACACTCTTTCCCTACACGAC
LcyE 3rd-2	AATGATACGGCGACCACCGAGATCTACACtagagggcACACTCTTTCCCTACACGAC

LcyE 3rd-3	AATGATACGGCGACCACCGAGATCTACACcctatcctACACTCTTTCCCTACACGAC
LcyE 3rd-4	AATGATACGGCGACCACCGAGATCTACACggctctgaACACTCTTTCCCTACACGAC
LcyE 3rd-5	AATGATACGGCGACCACCGAGATCTACACaggcgaagACACTCTTTCCCTACACGAC
LcyE 3rd-6	AATGATACGGCGACCACCGAGATCTACACtaatcttaACACTCTTTCCCTACACGAC
LcyE 3rd-7	AATGATACGGCGACCACCGAGATCTACACcaggacgtACACTCTTTCCCTACACGAC
LcyE 3rd-8	AATGATACGGCGACCACCGAGATCTACACgtactgacACACTCTTTCCCTACACGAC
LcyE 3rd-9	CAAGCAGAAGACGGCATACGAGATcgagtaatGTGACTGGAGTTCAGACGTGT
LcyE 3rd-10	CAAGCAGAAGACGGCATACGAGATtctccggaGTGACTGGAGTTCAGACGTGT
LcyE 3rd-11	CAAGCAGAAGACGGCATACGAGATaatgagcgGTGACTGGAGTTCAGACGTGT
LcyE 3rd-12	CAAGCAGAAGACGGCATACGAGATggaatctcGTGACTGGAGTTCAGACGTGT
LcyE 3rd-13	CAAGCAGAAGACGGCATACGAGATttctgaatGTGACTGGAGTTCAGACGTGT
LcyE 3rd-14	CAAGCAGAAGACGGCATACGAGATacgaattcGTGACTGGAGTTCAGACGTGT
LcyE 3rd-15	CAAGCAGAAGACGGCATACGAGATagcttcagGTGACTGGAGTTCAGACGTGT
LcyE 3rd-16	CAAGCAGAAGACGGCATACGAGATgcgcattaGTGACTGGAGTTCAGACGTGT
LcyE 3rd-17	CAAGCAGAAGACGGCATACGAGATcatagccgGTGACTGGAGTTCAGACGTGT
LcyE 3rd-18	CAAGCAGAAGACGGCATACGAGATttcgcggaGTGACTGGAGTTCAGACGTGT
LcyE 3rd-19	CAAGCAGAAGACGGCATACGAGATgcgcgagaGTGACTGGAGTTCAGACGTGT
LcyE 3rd-20	CAAGCAGAAGACGGCATACGAGATctatcgctGTGACTGGAGTTCAGACGTGT