

Supplementary Materials: Development of High Yielding Glutinous Cytoplasmic Male Sterile Rice (*Oryza sativa* L.) Lines through CRISPR/Cas9 Based Mutagenesis of *Wx* and *TGW6* and Proteomic Analysis of Anther

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Table S1. Efficiency score and positions of four targets.

Target Name	Position	Strand	Off Target Score	GC (%)	Region	Pairing with SgRNA
WxT1	1522 - 1541	+	0.292	75.0	CDS	None
WxT2	2011 - 2030	+	0.075	55.0	CDS	None
TGW6T1	184 - 203	+	0.196	70.0	CDS	None
TGW6T2	751 - 770	+	0.288	65.0	CDS	None

Table S2. List of primers used in the study.

Primer name	Primer sequence (5'-3')
WxT1	GTCGTGTACGCCACCGGCGC
WxT2	CATCGACGGGTATGACACGC
TGW6T1	GCGTTCGACGGCAAAGGCCG
TGW6T2	TGGATCCGAGGCCCGAAGAC
OsWaxyT1	F: ATGTCGGCTCTCACCACG R: CGGATGGTCGATGAACACAC
OsWaxyT2	F: GTGTGTTTCATCGACCATCCG

	R: TCGTACTTGGCGGTGATGTA
Tgw6-T1	F: CGCTCATTGTCTTCCTCGTG R: GGACCAACTCGCATCAATCC
Tgw6-T2	F: GGATTGATGCGAGTTGGTCC R: CTGTTGAACCAGCTTCCCAC
U-F	CTCCGTTTTACCTGTGGAATCG
gR-R	CGGAGGAAAATTCCATCCAC
WxgRT1:	TCGTGTACGCCACCGGCGCGTTTTAGAGCTAGAAAT
OsU6aWxT1:	GCGCCGGTGGCGTACACGACGGCAGCCAAGCCAGCA
WxgRT2:	CATCGACGGGTATGAC ACGCGT TTTAGAGCTAGAAAT
WxOsU6bT2:	GCGTGTTCATACCCGTCGATGCAACACAAGCGGCAGC
TGgRT1	CGTTCGACGGCAAAGGCCGGTTTTAGAGCTAGAAAT
OsU6cTGT1	CGGCCTTTGCCGTCGAACGCTGAGCCTCAGCGCAG
TGgRT2:	TGGATCCGAGGCCCGAAGACGTTTTAGAGCTAGAAAT
OsU3TGT2	GTCTTCGGGCCCTCGGATCCATGCCACGGATCATCTGC
Pps-R	TTCAGAGGTCTCTACCG ACTAGT ATGGAATCGGCAGCAAAGG
Pgs-2	AGCGTGGGTCTCGT CAG GGTCCATCCACTCCAAGCTC
Pps-2	TTCAGAGGTCTCT CTG ACTGGAATCGGCAGCAAAGG
Pgs-3	AGCGTGGGTCTCGTCTTCACTCCATCCACTCCAAGCTC
Pps-3	TTCAGAGGTCTCTAAGACTTTGGAATCGGCAGCAAAGG
Pgs-4	AGCGTGGGTCTCG AGT CCTTTCCATCCACTCCAAGCTC
Pps-4	TTCAGAGGTCTCT ACT ACATGGAATCGGCAGCAAAGG
Pgs-L	AGCGTGGGTCTCGCTCG ACGCGT ATCCATCCACTCCAAGCTC
PB-L	GCGCGCGGTCTCGCTCG ACTAGT ATGG
Actin-F	GAGTATGATGAGTCGGGTCCAG
Actin-R	ACACCAACAATCCCAAACAGAG
W-F	GGCTGAGATCAAGGTTGCAG
W-R	TCTTCTCACCGGTCTTTCCC
TG-F	CCGGGCCATGTAAGTTGATG
TG-R	GCGATGAAGCGCTATCCAAT
Cas9-F	CTGACGCTAACCTCGACAAG

Cas9-R	CCGATCTAGTAACATAGATGACACC
hptF	AAGCTG CATCATCGAAATTGC
hptR	AAGAATCTC GTGCTTTCAGCTTCG
SP-L1	GCGGTGTCATCTATGTTACTAG
SP-R	GCCTATACCAAGTTATTGCA
Primers used to check the off-target effects	
POT1	F: GCCGAGTTCCCATTTGATCAC R: CCATGGCTCCTCGTCGATC
POT2	F: CCAAACCTCAGTACGTGCCTG R: ACATATACCTGGGTCACGGAC
POT3	F: GCTACCACCTCTTTGTCAGC R: CCACTAGCCGCTACTCAAAT
POT4	F: AGCCATCCCATGTCCATTCT R: CGAAGCACTACACCGAAGTT
POT5	F: CAAAGGTCAAGGGAAGTGGG R: TTCACCTTTGTCTTCCGGGT
POT6	F: CCTGTGGCTGAAAACGAACA R: TGGCTGCTATGTGGTGAAGA
POT7	F: AACACCAACAGCCAACGATC R: TTGAACATTCTCGCTGTGGC
POT8	F: CACTTTGGTCGTCGTCTGTC R: GGTGTCTGGCATCATCATCG
POT9	F: GAAGTTCCCGATGAGACGC R: TTTGGAAGAATGATGGCGGC
POT10	F: ATGGCCGAGGTGCTACTATC R: AGGTAGATGCTTCGCTGGTT
POT11	F: CAACCTGCTGCTCAACTACA R: TGTGTGGTACCATGCATGC
POT12	F: CGCTAAGGTCGAGAAAAGGC R: CGGTGTCGTCCTCTGATGTA

Note: **ACTAGT** and **ACGCGT**: *SpeI* and *MluI* restriction enzyme cutting sites.-

Table S3: Detection of mutations on the putative off-target sites.

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
<i>WxT1</i>	OT1	Chr3: 5219388-2519410	GTCGTCTCCGCCACCAGCGC CGG	3	25	0
	OT2	Chr9: 19024204-19024226	GTCGTGTGCGTCACCGGCGC CGG	2	25	0
	OT3	Chr2: 19954891-19954913	GTCGTGAACGGCACCGGCGC CGG	2	25	0
<i>WxT2</i>	OT4	Chr12: 21533939-21533961	TATCGAACATAGTTCATTCC TGG	5	25	0
	OT5	Chr9: 8873383-8873405	CATCGATCATCAGTAATTCA AGG	4	25	0
	OT6	Chr1: 36331821-36331843	CACCGACCATCCTTCATGCA AGG	3	25	0
<i>TGW6T1</i>	OT7	Chr6: 25085711-25085733	GCGTTCGACGGCAAGGGACA CGG	3	25	0
	OT8	Chr12: 6170-6192	GCGTGCGACGGTGAAGGCCA TGG	4	25	0
	OT9	Chr2: 27118550-27118572	GCCTGCGACGACGAAGGCCG CGG	4	25	0
<i>TGW6T2</i>	OT10	Chr6: 8590755-8590777	TGAACCAGAGGCCCGAAGAC CCGG	3	25	0
	OT11	Chr3: 16250669-16250719	TGGATGCGGGACCCGAAGAC GGG	3	25	0
	OT12	Chr8: 18484973-18484995	AGGATCCGTAGCCCGAAGGC TGG	4	25	0

*The PAM motif (NGG) is shown in red.

Table S4. Pollen fertility status of F1 lines.

Serial No.	Lines	Pollen Fertility (PF)	Spikelet's fertility (SF)
1	4-1A	28.89 ± 1.25	21.33 ± 2.23
2	4-2A	0.00 ± 0.00	0.00 ± 0.00
3	4-3A	88.56 ± 3.15	86.57 ± 1.36
4	4-7A	73.20 ± 2.31	71.65 ± 3.47
5	4-8A	8.13 ± 1.02	6.36 ± 1.25
6	4-4B	23.65 ± 2.16	19.25 ± 2.14
7	4-5C	72.26 ± 2.35	76.26 ± 1.59
8	7-3A	5.23 ± 1.21	6.44 ± 1.16
9	7-5B	5.12 ± 1.32	6.32 ± 2.19
10	14-4A	98.23 ± 2.35	97.13 ± 3.23
11	14-4C	81.56 ± 3.19	89.68 ± 3.73
12	19-5A	0.00 ± 0.00	0.0 ± 0.00
13	19-3B	0.00 ± 0.00	0.0 ± 0.00
14	19-3C	97.56 ± 2.10	94.65 ± 3.28
15	23-7A	59.53 ± 2.12	63.25 ± 3.15
16	23-5B	53.25 ± 4.13	49.56 ± 2.82

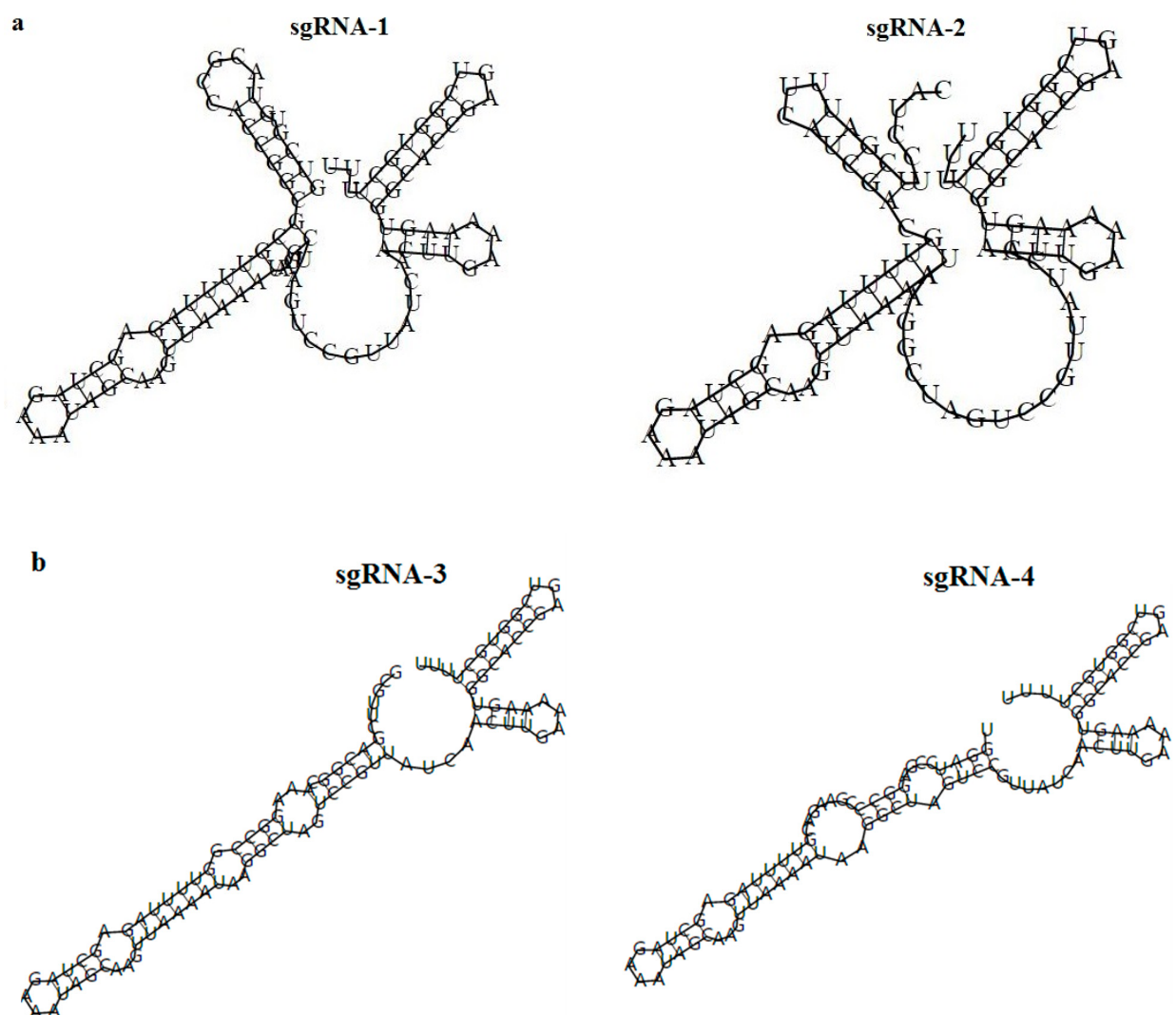


Figure S1. Schematic representation of secondary structures of both sgRNAs used in this experiment. **(a)** structure both sgRNA's for *Wx* targets and **(b)** structure of both sgRNA's for *TGW6* targets. The stem loop sgRNA secondary structure was predicted by online tool (<http://crispr.hzau.edu.cn/cgi-bin/CRISPR/CRISPR>).

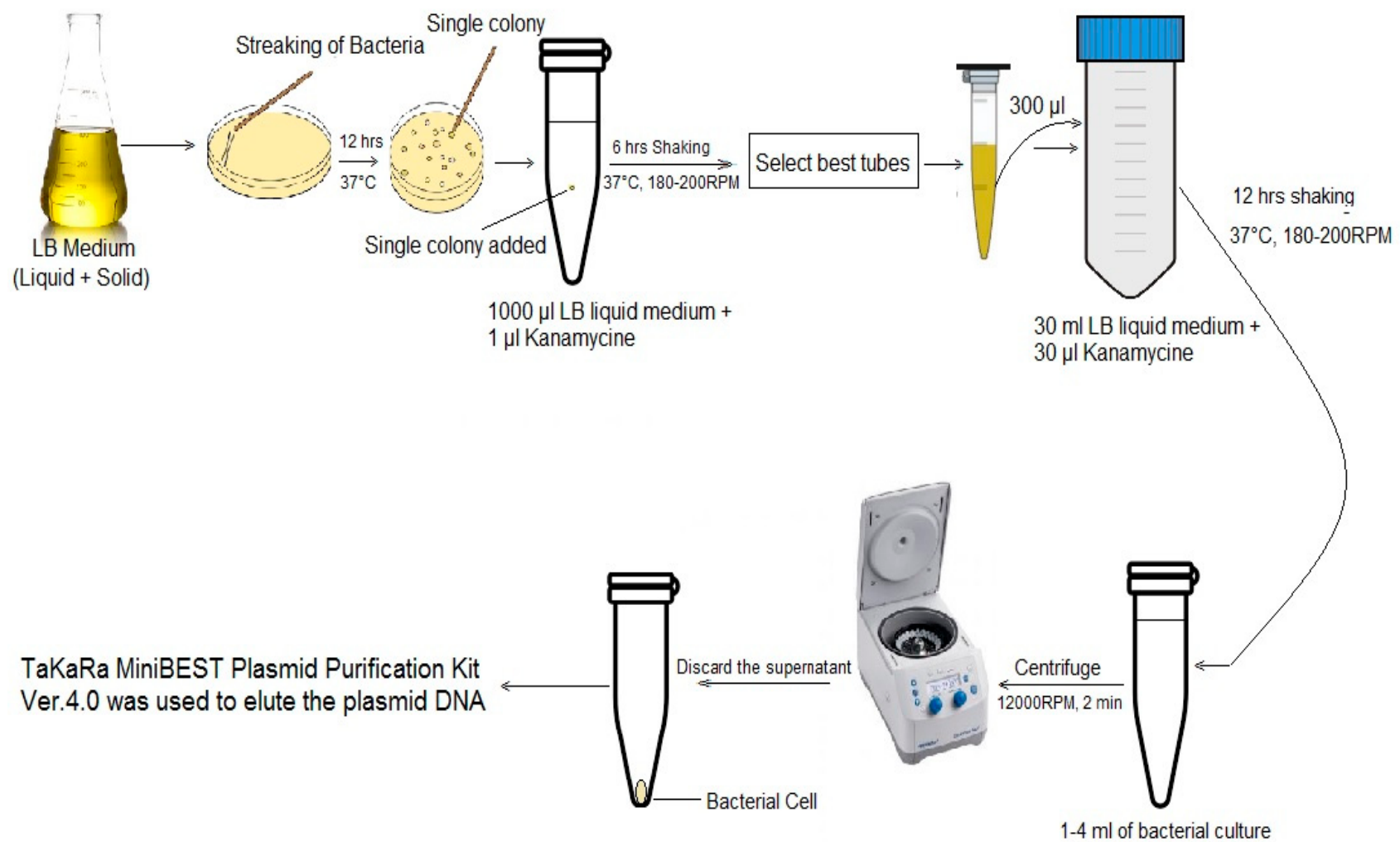


Figure S2. Isolation of the binary plasmids and sgRNA intermediate plasmids.

Figure S3. Sequences of the sgRNA vectors and those of the expression cassettes

OsU6a-sgRNA structure in the plasmid

*Bam*H I *Bsa* I

accgggGGATCCTAGCCGGGTCTCGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC
GAGTCGGTGCTTTTTTCAAGAGCTTGGAGTGGATGGAATTTTCTCCTCGTTTTACCTGTGGAATCGGCAGCAAAGGATTTTTTCTGTAGTT
TTCCACAACCATTTTTTACCATCCGAATGATAGGATAGGAAAAATATCCAAGTGAACAGTATTCTATAAAATTCCCGTAAAAAGCCTG
CAATCCGAATGAGCCCTGAAGTCTGAACTAGCCGGTCACCTGTACAGGCTATCGAGATGCCATACAAGAGACGGTAGTAGGAACTAGGA
AGACGATGGTTGATTCGTCAGGCGAAATCGTCGTCCTGCAGTCGCATCTATGGGCCTGGACGGAATAGGGGAAAAAGTTGGCCGATAG
GAGGGAAAGGCCCAGGTGCTTACGTGCGAGGTAGGCCTGGGCTCTCAGCACTTCGATTTCGTTGGCACCAGGGGTAGGATGCAATAGAGAG
CAACGTTTAGTACCACCTCGCTTAGCTAGAGCAAACCTGGACTGCCTTATATGCGCGGGTGCTGGCTTGGCTGCCGAGAGACCTCTGAAGA
TAACATACTAAGCTTggcact(pUC18 backbone) *Bsa* I *Hind* III

PCR product of OsU6a-sgRNA (629 bp, 599 bp after *Bsa* I digestion)

TTCAGAGG**TCTCT**NNNNNNNTGGAATCGGCAGCAAAGGATTTTTTCCTGTAGTTTTCCCACAACCATTTTTTACCATCCGAATGATAGGA
TAGGAAAAATATCCAAGTGAACAGTATTCTATAAAATTCCCGTAAAAAGCCTGCAATCCGAATGAGCCCTGAAGTCTGAACTAGCCCG
TCACCTGTACAGGCTATCGAGATGCCATACAAGAGACGGTAGTAGGAACTAGGAAGACGATGGTTGATTCGTGAGGCGAAATCGTCGTC
CTGCAGTCGCATCTATGGGCCCTGGACGGAATAGGGGAAAAAGTTGGCCGGATAGGAGGGAAAGGCCCAGGTGCTTACGTGCGAGGTAG
GCCTGGGCTCTCAGCACTTCGATTCTGTTGGCACCGGGGTAGGATGCAATAGAGAGCAACGTTTAGTACCACCTCGCTTAGCTAGAGCAAA
CTGGACTGCCTTATATGCGCGGGTGCTGGCTTGGCTGCCG**GTCGTGTACGCCACCGGCGC**GTTTTAGAGCTAGAAATAGCAAGTTAAAAT
AAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAAGAGCTTGGAGTGGATGGNNNNNN**NCGAGACCC**
ACGCT

OsU6b-sgRNA structure in the plasmid

acccggGATCCTAGCCGGGTCTCGGTTTTAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC
GAGTCGGTGCTTTTTTTCAAGAGCTTGGAGTGGATGGAATTTTCTCCGTTTACCTGTGGAATCGGCAGCAAAGGATGCAAGAACGAAC
TAAGCCGGACAAAAAAGGAGCACATATACAAACCGGTTTTATTTCATGAATGGTCACGATGGATGATGGGGCTCAGACTTGAGCTA

CGAGGCCGCGAGGCGAGAGAAGCCTAGTGTGCTCTCTGCTTGTGTTGGGCCGTAACGGAGGATACGGCCACGAGCGTGTACTACCGCGCG
 GGATGCCGCTGGGCGCTGCGGGGGCCGTTGGATGGGGATCGGTGGGTCGCGGGAGCGTTGAGGGGAGACAGGTTTGTAGTACCACCTCGCC
 TACCGAACAATGAAGAACCCACCTTATAACCCCGCGCGCTGCCGCTTGTGTTGAGAGCCTCTGAAGATAACATACTAAGCTTggcact(pU
 C18 backbone)

PCR product of OsU6b-sgRNA (515 bp, 485 bp after *Bsa* I digestion)

TTCAGAGGTCTCTNNNNNNNTGGAATCGGCAGCAAAGGATGCAAGAACGAACTAAGCCGGACAAAAAAAAAAGGAGCACATATAC
 AAACCGGTTTTATTCATGAATGGTCACGATGGATGATGGGGCTCAGACTTGAGCTACGAGGCCGAGGCGAGAGAAGCCTAGTGTGCT
 CTCTGCTTGTGTTGGGCCGTAACGGAGGATACGGCCGACGAGCGTGTACTACCGCGCGGGATGCCGCTGGGCGCTGCGGGGGCCGTTGG
 ATGGGGATCGGTGGGTCGCGGGAGCGTTGAGGGGAGACAGGTTTGTAGTACCACCTCGCCTACCGAACAATGAAGAACCCACCTTATAA
 CCCCCGCGCTGCCGCTTGTGTTG**CATCGACGGGTATGACACGC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTT
 ATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAAGAGCTTGGAGTGGATGGNNNNNNNC**GAGACCCACGCT**

OsU6c-sgRNA structure in the plasmid

acccgg**GGATCC**TAGCCG**GGTCT**GGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC
 GGTGCTTTTTTTCAAGAGCTTGGAGTGGATGGAATTTCTCCGTTTTACCTGTGGAATCGGCAGCAAAGGActcattagcggtatgcatgttgtagaagtgcgagat
 gtaataattttcattatataaaaaaggtacttcgagaaaaataatgcatacgaattaattcttttatgttttttaaaccaagtatatagaatttattgatggttaaaatttcaaaaatgatgacgagagaaggttaaactgacgcatat
 acttctgaacagagagggaatatggggtttttgtctcccaacaattcttaagcacgtaaaggaaaaagcacattatccacattgtacttcagagatatgtacagcattacgttaggtacgttttcttttctcccgagagatgatac
 aataatcatgtaaacccagaatttaaaaaatattctttactataaaaaatttaattagggaaacgtattttttacatgacaccttttgagaaagagggacttgtaatatgggacaaatgaacaatttctaagaaatgggcatatgactctc
 agtacaatggaccaattccctccagtcggccagcaatacaaaaggaaagaaatgagggggccacagggccacggccacttttccgtggtggggagatccagctagaggtccggccacaagtggcccttgcccgtggg
 acggtgggattgcagagcgcgtgggcggaacaacagtttagtaccacctgctcacgcaacgacgcgaccacttgcttataagctgctgctgaggtcag **AGAGACCTCTGAAG**
 ATAACATACT**AAGCTT**ggcact (pUC18 backbone)

PCR product of OsU6c-gRNA (924 bp, 894 bp after *Bsa* I digestion)

TTCAGAGGTCTCTNNNNNNNTGGAATCGGCAGCAAAGGActcattagcggtatgcatgttgtagaagtgcgagatgtaataattttcattatataaaaaaggtacttcgagaa
 aaataaatgcatacgaattaattcttttatgttttttaaaccaagatatagaatttattgatggttaaaatttcaaaaatgatgacgagagaaggttaaactgacgcatatacttctgaacagagagggaatatgggg
 ttttgtgtctcccaacaattcttaagcacgtaaaggaaaaaagcacattatccacattgtacttcagagatatgtacagcattacgttaggtacgttttcttttctcccgagagatgatacaataatcatgtaaaccca
 gaatttaaaaaatattctttactataaaaaatttaattagggaaacgtattttttacatgacaccttttgagaaagagggacttgtaatatgggacaaatgaacaatttctaagaaatgggcatatgactctcagtacaat
 ggaccaaatccctccagtcggccagcaatacaaaaggaaagaaatgagggggccacagggccacggccacttttccgtggtggggagatccagctagaggtccggccacaagtggcccttgcccgtg
 ggacggtgggattgcagagcgcgtgggcggaacaacagtttagtaccacctgctcacgcaacgacgcgaccacttgcttataagctgctgctgaggtca**GCGTTTCGACGGCAAAGGCC**
GGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAAGA
 GCTTGGAGTGGATGGNNNNNNNNNC**GAGACCCACGCT**

OsU3-sgRNA structure in the plasmid

accgGATCCTAGCCGGGTCTCGGTTTTAGAGCTAGAAATAGCAAGTTAAATAAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGT
GCTTTTTTTTCAAGAGCTTGGAGTGGATGGAATTTTCTCCGTTTTACCTGTGGAATCGGCAGCAAAGGAAGGAATCTTTAAACATACGAACAGATCA
CTTAAAGTTCCTCTGAAGCAACTTAAAGTTATCAGGCATGCATGGATCTTGGAGGAATCAGATGTGCAGTCAGGGACCATAGCACAAGACAGGCGTCT
TCTACTGGTGCTACCAGCAAATGCTGGAAGCCGGAACACTGGGTACGTTGGAAACCACGTGTGATGTGAAGGAGTAAGATAAACTGTAGGAGAAAA
GCATTTTCGTAGTGGGCCATGAAGCCTTTCAGGACATGTATTGCAGTATGGGCCGGCCATTACGCAATTGGACGACAACAAAGACTAGTATTAGTACC
ACCTCGGCTATCCACATAGATCAAAGCTGGTTTAAAGAGTTGTGCAGATGATCCGTGGCAAAGAGACCTCTGAAGATAACATACTAAGCTTggcact(pU
C18 backbone)

PCR product of OsU3-sgRNA (603 bp, 573 bp after *Bsa* I digestion)

TTCAAGAGGCTCTNNNNNNNTGGAATCGGCAGCAAAGGACGCGTTGACATTGTAGGACTATATTGCTCTAATAAAGGAAGGAATCTT
TAAACATACGAACAGATCACTTAAAGTTCTTCTGAAGCAACTTAAAGTTATCAGGCATGCATGGATCTTGGAGGAATCAGATGTGCAG
TCAGGGACCATAGCACAAGACAGGCGTCTTCTACTGGTGCTACCAGCAAATGCTGGAAGCCGGGAACACTGGGTACGTTGGAAACCA
CGTGTGATGTGAAGGAGTAAGATAAACTGTAGGAGAAAAGCATTTCGTAGTGGGCCATGAAGCCTTTCAGGACATGTATTGCAGTATG
GGCCGGCCCATACGCAATTGGACGACAACAAAGACTAGTATTAGTACCACCTCGGCTATCCACATAGATCAAAGCTGGTTTAAAAGA
GTTGTGCAGATGATCCGTGGCA**TGGATCCGAGGCCCGAAGAC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAAGGCTAGTCCGTTAT
CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCAAGAGCTTGGAGTGGATGG NNNNNNNCG AGACCC ACGCT

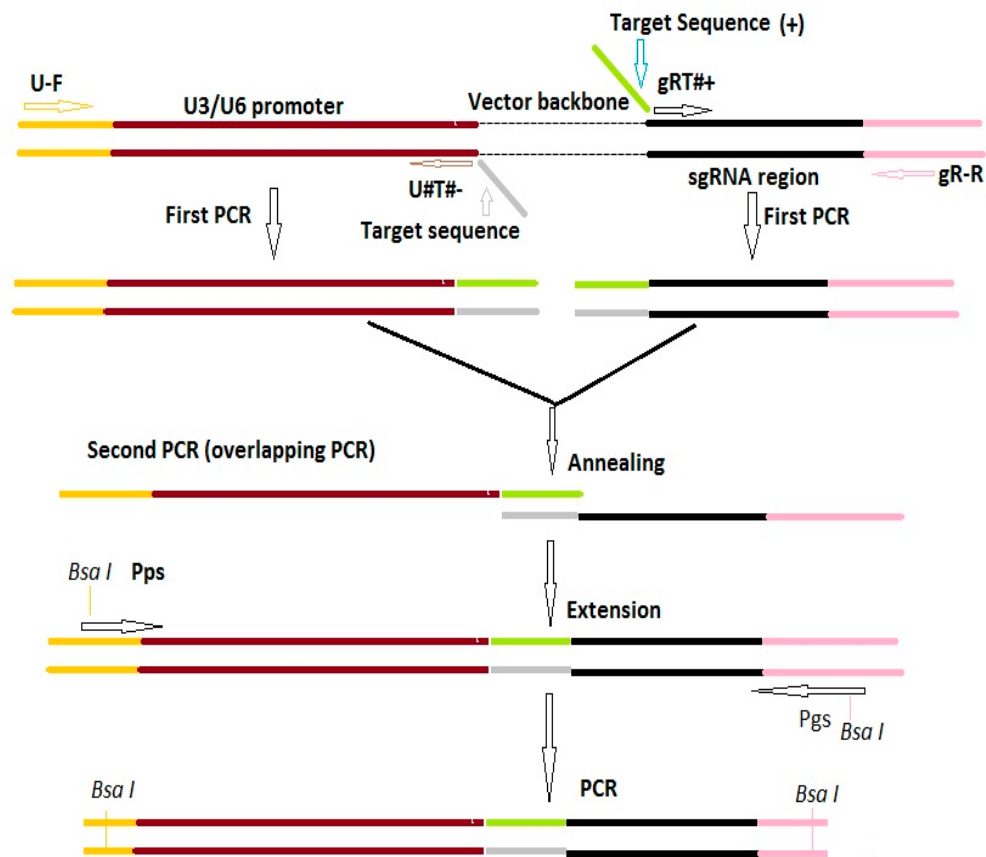


Figure S4: sgRNA expression cassette procedure by overlapping PCR containing a target sequence. The chimeric primers with target sequence strands are given in Additional file 3. The first PCR is carried out in two separated reactions with U-F/U#T#- and gRT#+/gR-R primer pair, U# indicates a given promoter, and T#+ and T#- indicate forward and reverse strands of a target sequence.

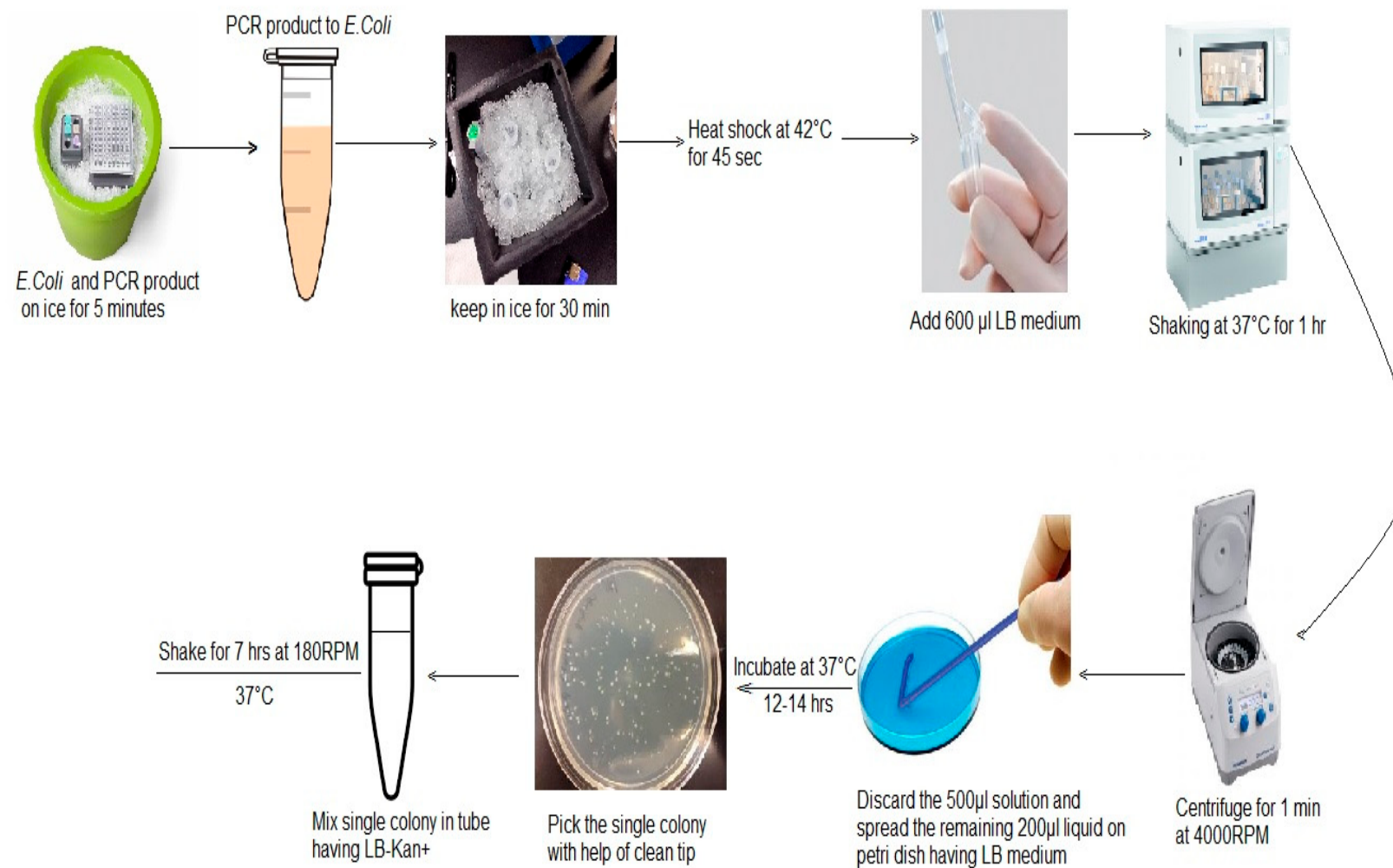


Figure S5. Illustration for transformation of *E. coli*.

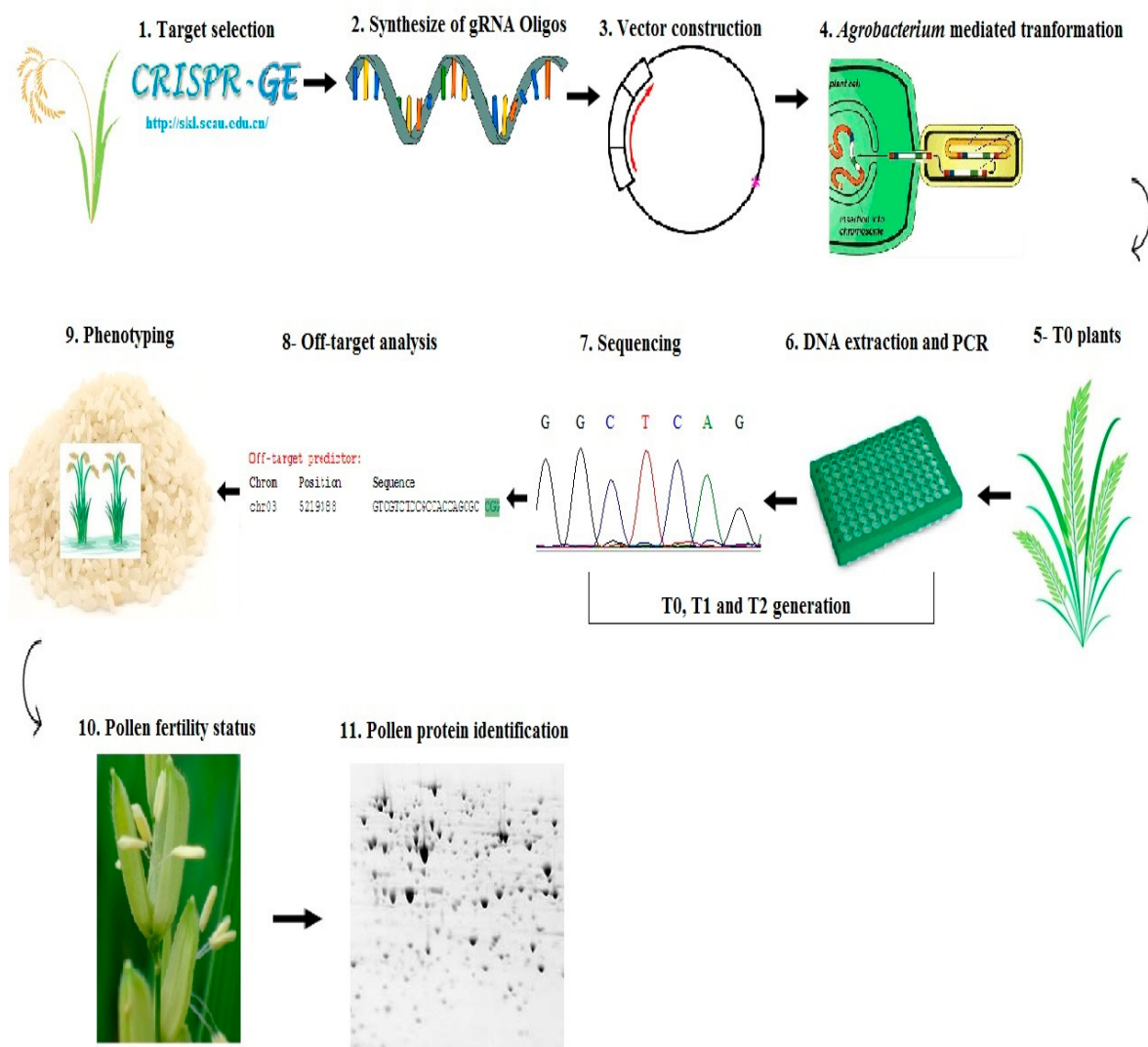


Figure S6: Schematic diagram of the procedure for CRISPR/Cas9 based generation of mutant plants and analysis of target regions. The targets were selected using CRISPR-GE online web-based tool and expression cassette was constructed by using overlapping PCR and inserted into a binary vector. Agrobacterium mediated transformation was performed and T₀ plants were regenerated and sequencing was performed, and later generations were produced by self-pollination and genotyping was performed by using target specific primers in T₁ and T₂ generations. The phenotypic data of mutant and wild type plants were recorded and further analyzed. Pollen fertility analysis and protein identification was also performed.

[illegible]

b

Os Japonica	1	ATGAGAAGCACGGCGAGGCAAGCGGCGACCGCGCGCGGTTTCGCGCTCATTGTCTTCTCGTGCTGCTCTCGCGTCCCCTACTGCCGCCGCCACAGCCACAACGAGAATGTTCAAGACC	120
209B		ATGAGAAGCACGGCGAGGCAAGCGGCGACCGCGCGCGGTTTCGCGCTCATTGTCTTCTCGTGCTGCTCTCGCGTCCCCTACTGCCGCCGCCACAGCCACAACGAGAATGTTCAAGACC	
Os Japonica	121	ATTGACGCCCGCGGAGCCAGCATCTGGACCTCGCGGATCACTGGTCGGCCGGAGAGCGTCGCTTCGACGGCAAAGGCCGCGGCCGTACAGCGGCGTCTCCGACGGCCGATCATG	240
209B		ATTGACGCCCGCGGAGCCAGCATCTGGACCTCGCGGATCACTGGTCGGCCGGAGAGCGTCGCTTCGACGGCAAAGGCCGCGGCCGTACAGCGGCGTCTCCGACGGCCGATCATG	
Os Japonica	241	AGGTGGAACGGCGAGGCCGCTGGCTGGAGCACCTACACGTACAGCCCCAGCTACACGAAAAACAAGTGCAGGCGATCGACTCTCCCACGGTCCAGACCGAGAGCAATGCGGCCGCCCG	360
209B		AGGTGGAACGGCGAGGCCGCTGGCTGGAGCACCTACACGTACAGCCCCAGCTACACGAAAAACAAGTGCAGGCGATCGACTCTCCCACGGTCCAGACCGAGAGCAATGCGGCCGCCCG	
Os Japonica	361	TTAGGCTACGTTTCACTACAAAACGGCAACCTTACATCGCCGACGCTACATGGGATTGATGCGAGTTGGTCCAAAAGGCGGGAGGCAACCGTGTAGCCATGAAGGCTGATGGC	480
209B		TTAGGCTACGTTTCACTACAAAACGGCAACCTTACATCGCCGACGCTACATGGGATTGATGCGAGTTGGTCCAAAAGGCGGGAGGCAACCGTGTAGCCATGAAGGCTGATGGC	
Os Japonica	481	GTGCCACTTCGCTTACCAATGGGGTGGACATTGATCAGGTTACCGGAGATGTTATTTTACCAGCAGCAGCATGAACTACCAACGATCTCAGCACGAGCAAGTACGGCGACCAAGGAT	600
209B		GTGCCACTTCGCTTACCAATGGGGTGGACATTGATCAGGTTACCGGAGATGTTATTTTACCAGCAGCAGCATGAACTACCAACGATCTCAGCACGAGCAAGTACGGCGACCAAGGAT	
Os Japonica	601	TCGACCGGACGGCTCATGAAGTATGACCCACGAACCTAACAGTCACCGTTCTTCAATCCAACATAACCTACCCGAACGGTGTGCGCATGAGCGCTGACCGAACACATCTGATCGTTGCA	720
209B		TCGACCGGACGGCTCATGAAGTATGACCCACGAACCTAACAGTCACCGTTCTTCAATCCAACATAACCTACCCGAACGGTGTGCGCATGAGCGCTGACCGAACACATCTGATCGTTGCA	
Os Japonica	721	TTGACCGGGCCATGTAAGTTGATGAGGATTGGATCCGAGGCCGAAGACTGGCAAACTGAACCATTTGTTGACCTGCCAGGCTATCCTGATAATGTAGGCTGATGAAAAAGGTGGT	840
209B		TTGACCGGGCCATGTAAGTTGATGAGGATTGGATCCGAGGCCGAAGACTGGCAAACTGAACCATTTGTTGACCTGCCAGGCTATCCTGATAATGTAGGCTGATGAAAAAGGTGGT	
Os Japonica	841	TATTGGATAGCGCTTCATCGCGAGAAGTATGAGCTTCCCTTTGGTCCGATAGTCACTTGGTTGCTATGAGGGTTAGTGTGGTGGGAAGCTGTTCAACAGATGAGAGGACCAAGAGGC	960
209B		TATTGGATAGCGCTTCATCGCGAGAAGTATGAGCTTCCCTTTGGTCCGATAGTCACTTGGTTGCTATGAGGGTTAGTGTGGTGGGAAGCTGTTCAACAGATGAGAGGACCAAGAGGC	
Os Japonica	961	TTGAGGCCAACCGAAGTGTGAGAGGAAGGATGGCAAAATATACATGGGAAATGTTGAATTGCCGTATGTGCGAGTCGTCAAAGCAGCTAG	1080
209B		TTGAGGCCAACCGAAGTGTGAGAGGAAGGATGGCAAAATATACATGGGAAATGTTGAATTGCCGTATGTGCGAGTCGTCAAAGCAGCTAG	

Figure S7: Sequence alignment of the (a) Wx and (b) TGW6 gene in reference genome and 209B maintainer line. The SNPs between reference genome and 209B are indicated in red box.