Comprehensive Analysis of CRISPR/Cas9-Mediated Mutagenesis in *Arabidopsis thaliana* by Genome-wide Sequencing

Wenjie Xu [1,2], Wei Fu [2], Pengyu Zhu [2], Zhihong Li [1], Chenguang Wang [2], Chaonan Wang [1,2], Yongjiang Zhang [2] and Shuifang Zhu [1,2,*]

[1] College of Plant Protection, China Agricultural University, Beijing 100193 China
[2] Institute of Plant Quarantine, Chinese Academy of Inspection and Quarantine, Beijing 100176, China
* Correspondence: zhusf@caiq.org.cn

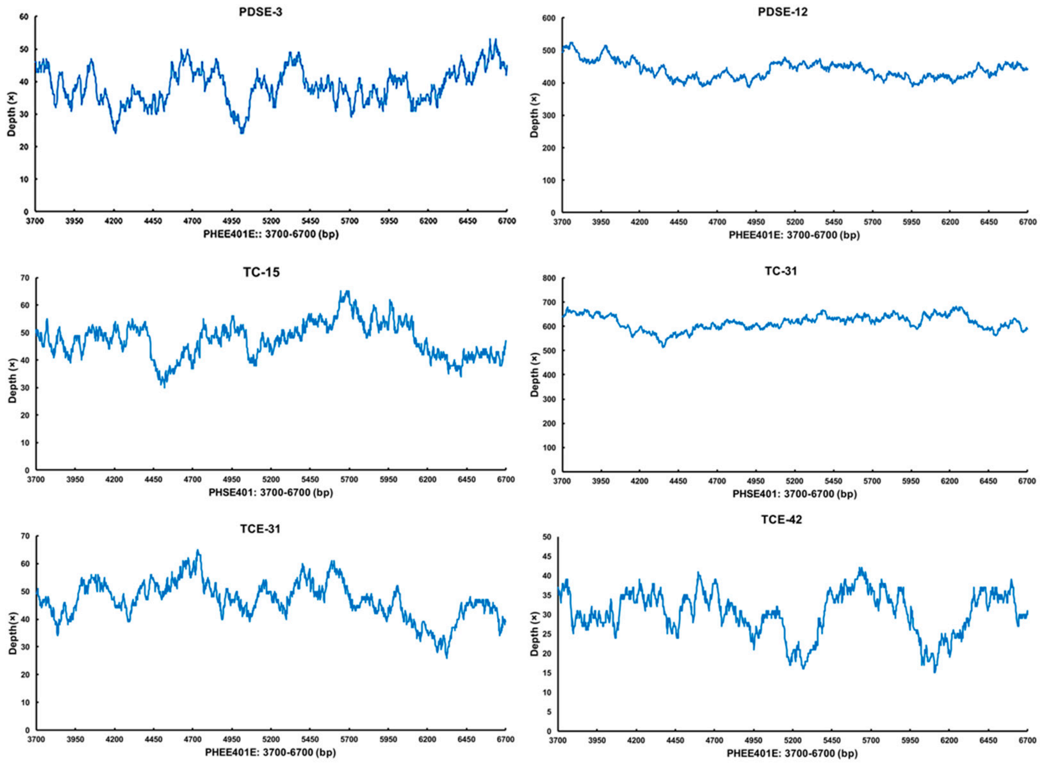

Figure S1. Sequencing depth distribution of inserted vector in the CRISPR/Cas9-edited mutants. The partially continuous specific sequences of two vectors were used for this plot.

Table S1. Sequencing depth and mapping ratio of samples for WGS.

Sample	Sequencing Depth(×)	Mapping Ratio(%)
WT1	94.43	92.61
WT2	84.67	92.86
WT3	87.94	92.74
PDSE-3	139.93	94.36
PDSE-12	132.61	94.43
TC-15	138.52	94.7
TC-31	127.79	94.06
TCE-31	126.64	93.78
TCE-42	95.85	93.67

Table S2. The number of structural variants of mutants.

Mutant	PDSE-12	PDSE-3	TC-15	TC-31	TCE-31	TCE-42
SV	63	72	77	78	80	19
SV (<10kb)	18	16	16	15	24	4

Table S3 Editing efficiency of targets for deep sequencing.

Name	WT	PDSE-3	PDSE-MIX	TC-15	TC-31	TC-MIX	TCE-MIX
PDS-1_target	3.30E-04	2.59E-04	2.21E-04	-	-	-	-
PDS-1-1	5.69E-05	9.10E-05	5.97E-05	-	-	-	-
PDS-1-2	1.74E-04	1.94E-04	1.80E-04	-	-	-	-
PDS-1-3	4.01E-04	4.43E-04	3.32E-04	-	-	-	-
PDS-1-4	1.08E-04	9.09E-05	1.06E-04	-	-	-	-
PDS-2_target	1.28E-02	4.95E-01	9.20E-01	-	-	-	-
PDS-2-1	2.35E-04	2.39E-04	2.08E-04	-	-	-	-
PDS-2-2	7.87E-05	4.94E-05	9.54E-05	-	-	-	-
*CPC*_target	1.41E-02	-	-	9.95E-01	9.95E-01	9.67E-01	8.88E-01
CPC-1	9.64E-05	-	-	1.40E-04	1.55E-04	1.13E-04	1.23E-04
CPC-2	1.08E-04	-	-	1.31E-04	1.15E-04	1.44E-04	1.58E-04
CPC-3	1.62E-04	-	-	1.54E-04	1.39E-04	2.19E-04	1.90E-04
CPC-4	4.66E-04	-	-	6.07E-04	5.92E-04	3.72E-04	3.46E-04
CPC-5	1.68E-04	-	-	1.23E-04	1.12E-04	1.54E-04	1.67E-04
*TRY*_target	2.16E-03	-	-	9.93E-01	9.96E-01	7.24E-01	9.30E-01
TRY-1	1.41E-04	-	-	5.38E-04	2.28E-04	1.85E-04	1.65E-04
TRY-2	2.73E-03	-	-	1.86E-01	9.81E-01	1.61E-02	9.24E-02
TRY-3	2.53E-04	-	-	1.41E-04	1.28E-04	1.71E-04	1.64E-04
TRY-4	4.94E-05	-	-	5.00E-05	3.93E-05	5.20E-05	4.62E-05
TRY-5	1.82E-04	-	-	-	-	-	-
TRY-6	1.40E-04	-	-	2.22E-04	1.61E-04	1.70E-04	1.66E-04
TRY-7	5.40E-04	-	-	5.16E-04	4.82E-04	6.01E-04	5.58E-04

"-" represents that the site in the sample was not detected.

Table S4 Re-analysis results for rice mutants.

Plant Name	NCBI SRA Accession	On Target	Off-target Site	Off-target Sequence
Cas9 Backbone-1	SRR6344862	-	-	-
Cas9 Backbone-2	SRR6344859	-	-	-
Cas9-E1	SRR6344819	Cas9-E	-	-
Cas9-E2	SRR6344879	Cas9-E	-	-
Cas9-J1	SRR6344848	sgRNA01	Chr9:17099504-17099516	GCAGCTCTGACATGTGGGCCCGG
			Chr5:19226195-19226217	ACAGCTCTGACATGTGGGCCCGG
			Chr2:23446231-23446253	GCAGCTCTGACATATGGGCCCGG
			Chr1:31035301-31035323	GCAGCTCTGACATGTGGGTCCGG
			Chr5:13975206-13975228	GGAGCTCTGACATGTGGGCCCGG
			Chr1:22043898-22043920	GCAGCTCTGACATGTGGGCCTGG
			Chr7:22958478-22958500	GCAGCACTGACATGTGGGCCCGG

			Chr9:17967312-17967334	GCAGCTCTGACATGTAGGCCGGG
			Chr12:8340812-8340834	GCAGTTCTGACATGTGGGCCCAG
			Chr10:8570768-8570790	GTAGCTCTGACATATGGGCCGGG
		sgRNA02	-	-
Cas9-J2	SRR6344845	sgRNA01	Chr9:17099504-17099516	GCAGCTCTGACATGTGGGCCCGG
			Chr5:19226195-19226217	ACAGCTCTGACATGTGGGCCCGG
			Chr2:23446231-23446253	GCAGCTCTGACATATGGGCCCGG
			Chr1:31035301-31035323	GCAGCTCTGACATGTGGGTCCGG
			Chr5:13975206-13975228	GGAGCTCTGACATGTGGGCCCGG
			Chr1:22043898-22043920	GCAGCTCTGACATGTGGGCCTGG
			Chr6:24673722-24673744	GCAGCTCTGACATGTGGGCCTGG
			Chr1:19854951-19854973	ACAGCTCCGACATGTGGGCCCGG
			Chr9:17967312-17967334	GCAGCTCTGACATGTAGGCCGGG
			Chr3:2628500-2628522	GCAGCTCTGACATGTGGGTCTGG
			Chr1:4302680-4302702	ATAGCTCTGACATGTGGGCTCGG
			Chr10:8570768-8570790	GTAGCTCTGACATATGGGCCGGG
		sgRNA02	-	-

"-" represents that there is no off target in the mutant for the sgRNA. New off-target sites within 3 mismatches are highlighted in yellow.

Table S5 Target sequences for Digenome-seq.

Target	Sequence
CPC	ATCCCGGGACGGACGCCGGAGG
TRY	GAAGTGAGCAGTATCGAATGGG
PDS-1	AGGGTGCTGGTAGGACATCTGG
PDS-2	GGATGGTAATCCTCCGGAAAGG
GLV1-1	CTTCTTTGGACGTCTTCAATGG
GLV6-1	GCTAGTGCACAAAGAAAGAGGG
GLV7-2	CTGAAACGAAGATGAAGAGAGG
GLV8-2	AAGACAAGCAATAAAGCTGAGG
GLV10-1	AAGGATCATTGAAGCAACATGG
GLV10-2	GATAATCTGCAAATAAGAGCGG

All target sites are listed in 5′ to 3′.

Table S6 Primers used for PCR amplification and sequencing of target sites.

Target	Primer/F	Primer/R
PDS-1	TAAGCAGGATACCAGATGAA	CATGTCAAAGGCGCTAAA
PDS-2	TGTTCTTCCTGACGTCTAA	ACTCATACCCTCTCTGTTG
CPC	CGATGGAGGCTGGTAAA	CTGTCAATGACTGTGTTCAG
TRY	TCTGAAGGTACCTCTCTATTC	GCCAGCCAAGCATTTAT

Table S7 Target sequences and primers used for target amplification deep sequencing.

Name	Target sequence	Primer/F	Primer/R
PDS-1_target	AGGGTGCTGGTAGGACATCTGG	GTTCAACCACTGTTTTCATCAGATAG	GATCAATGATCGGTTGCAGTG
PDS-1-1	GCGCCGCTGGTAGGACATCAGA	GTGCACGCTCAATAGAAACAGC	CAATTACGCTAGTGTTGCCGAC
PDS-1-2	TTGATGCTGGTAGGACAACAGG	GCGAGTTGTTCTCGAGGAGC	CTCTCACAGTTGGGGTGTGCG
PDS-1-3	CTCCTACTGGTTGGACATCTGG	GAGCCCAATGCCACAGGAC	GTGATAATCTTATTGGCTGATTTCG
PDS-1-4	GAGGAGCTGGTAGGACCTTCGG	CGTCACTGGCGCCAGTTC	CATGGCCAAGAAAAAATTCCAC
PDS-2_target	GGATGGTAATCCTCCGGAAAGG	GTGCCATCGTCATTGAGCTC	GTGTGTCATAAGCTGCAACAGATG
PDS-2-1	GGATGGTATATCTCTGAAAAGG	CGACGGATACTCAAGTATGGC	GTTTGGGCCGATATTCTTGCTAG
PDS-2-2	TGTTGGATATACTCCGGAATGG	GTTAATTATCAATTTTAAGGTCACACTG	GATTTGGCATTCAATGGAACG
*CPC*_target	ATCCCGGGACGGACGCCGGAGG	CTTGTCTTGTGAATTAAGGAGAGG	GAATAAAAGGTATTTGGTTAAGTGTAAG
CPC-1	CTTCCGAGACAGACGTCGGAGG	GACTGTGATGAATCACCGACAC	GACCAGAGTGATCACGCCAAG
CPC-2	ACTTCAGGACGGACGCTGGAGG	CAAGATCGAAGACGCAATCG	CTTCACCAAATAGTCAAAAGCCTG
CPC-3	ATGGAGGAACCAACGCCGGAGG	GGAGTATTCTGGTCCGAATCAAG	CGCATATATCTCCAGAACAATCTAAG
CPC-4	AAACGGGAACGAACACCGGAGG	GCTGGGGGAAAGACTAAAAAAG	CATGTTTTTCTTATGCGGGGC
CPC-5	TTCCGGCCACGGACGCCGACGG	GTTCACGACCGACGATATCG	GAGTCACCGGTGGAGGAGAAG
*TRY*_target	GAAGTGAGCAGTATCGAATGGG	GATTAGTTTCTAGTTTTAACAAAAATTAATATC	CTTAGAAAAAGTAGAAGAAGAAGAAATTG
TRY-1	GAAGTGAGTAGCATCGAATGGG	GAATCGGACATGACAGAAGAAGAG	CAATTCATTACTCTACACTCATCCTC
TRY-2	GAGGTGAGTAGTATCGAATGGG	GATGGATCGAGAGAGAAAGAGTCTC	GCTTCTTGTTCCGAAGGTCTG
TRY-3	GAAGTGAGCAGTCTTGAGTGGG	CTCCGCTATCTCATGATCTCCATC	GAGAAAACATGAAAAACCTTTCAC
TRY-4	GAAGTGAGTAGTATGAAATGGG	TATATAAACAGAAGAAGAAAAATGTTACG	GTCTACGCTCATTCATTTGACCTG
TRY-5	GAAGTAAGTAGTATCAAAAGGT	GATCACCTAGAAGATAACGGCTATAG	CTGTGGTAAGCTCTACATTCATTGC
TRY-6	GAGATGAAGAGTATCGAATTGG	CTTAATCCATCTTCTCTGATAATCG	GAAGGAGAGTAAAAAGAGACCGTAGAG
TRY-7	GAATACAGCAGTATCAAAGGGT	CGTACTGGCAGATAGAGCAGTTG	CTGATAGGTGCAGCTTTCAAATG

Table S8. Primers used for RT-qPCR amplification.

Gene	Primer/F	Primer/R
ACTIN2	CTGTGCCAATCTACGAGGGTT	CTGTGCCAATCTACGAGGGTT
zCas9	CTACCTGTACTACCTCCAGAAT	GAATGACTGTGGAACGATATGA

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