Inactivation of retrotransposon Tos17Chr.7 in rice cultivar Nipponbare through CRISPR/Cas9-mediated gene editing

Yanchang Luo1,†, Dongsheng Tian1,†, Joanne Chin Yi Teo1, Kar Hui Ong1, Zhongchao Yin1,2,*

1Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604, Republic of Singapore; 2Department of Biological Sciences, 14 Science Drive, National University of Singapore, Singapore 117543, Republic of Singapore
*E-mail: yinzc@tll.org.sg Tel: +65-6872-7420 Fax: +65-6872-7007

Abstract  Retrotransposons are mobile genetic elements capable of transposition via reverse transcription of RNA intermediates. Rice cultivar Nipponbare contains two nearly identical genomic copies of Tos17, an endogenous copia-like LTR retrotransposon, on chromosomes 7 (Tos17Chr.7) and 10 (Tos17Chr.10), respectively. Previous studies demonstrated that only Tos17Chr.7 is active in transposition during tissue culture. Tos17Chr.7 has been extensively used for insertional mutagenesis as a tool for functional analysis of rice genes. However, Tos17Chr.10 transposition might generate somaclonal mutagenesis with undesirable traits during rice transformation, which would affect the evaluation or application of transgenes. In this study, we generated a Tos17Chr.10 knockout mutant D873 by using CRISPR/Cas9 gene editing system. The gene-edited allele of Tos17Chr.7 in D873, designated as Tos17Chr.7, has an 873-bp DNA deletion in the pol gene of Tos17Chr.7, which caused the deletion of the GAG-pre-integrase domain and the integrase core domain. Although the transcription of Tos17Chr.7 was activated in D873 calli, no transposition of Tos17Chr.7 was detected in the regenerated D873 plants. The results demonstrate that the GAG-pre-integrase domain and the integrase core domain are essential for Tos17Chr.7 transposition and the deletion of the two domains could be not complemented by other LTR retrotransposons in rice genome. As the Tos17Chr.7-derived somaclonal mutagenesis is blocked in the D873 plants, the generation of the Tos17Chr.7 allele will be helpful in production of transgenic rice plants for gene function study and genetic engineering. Similar approach can be used to inactivate other retrotransposons in crop breeding.

Key words: CRISPR/Cas9, gene editing, retrotransposon, rice, Tos17.

Introduction  Retrotransposons are mobile genetic elements capable of transposition via reverse transcription of RNA intermediates and are abundant in the genomes of both plants and animals (Kumar and Bennetzen 1999). They consist of two subclasses, the long terminal repeat (LTR) retrotransposons and the non-LTR retrotransposons (Kumar and Bennetzen 1999). The LTRs contain the promoters and terminators associated with the transcription of LTR retrotransposons. The LTR retrotransposons can be further divided into two major groups, Ty1-copia-like and Ty3-gypsy-like, based on the order of the internal domains (Kumar and Bennetzen 1999). Both Ty1-copia-like and Ty3-gypsy-like carry gag and pol in their coding regions. The proteins encoded by the gag and pol genes are synthesized as a polypeptide that is cleaved into functional peptides by pol-encoded protease (PR). The capsid-like protein (CP) encoded by the gag gene is involved in the maturation and packaging of the retrotransposon RNA and proteins into a form suitable for integration into the genome, whereas the integrase (INT), reverse transcriptase (RT) and RNase-H (RH) encoded by the pol gene are required for replication and transposition of the retrotransposon.

Rice (Oryza sativa L.) is the staple food for nearly half of the world’s population. Tos17 is an endogenous copia-like LTR retrotransposon present in only one to five copies in rice genome, depending on the cultivar (Cheng et al. 2006; Hirochika et al. 1996). Two nearly identical genomic copies of Tos17 reside in chromosomes 7 (Tos17Chr.7) and 10 (Tos17Chr.10) of Nipponbare, a reference cultivar whose genome has been completely sequenced and annotated. Interestingly, only Tos17Chr.7 is active in transposition during tissue culture, whereas Tos17Chr.10 is inactive possibly due to the presence of an

†These authors contributed equally to this work.
This article can be found at http://www.jspcmb.jp/
Published online March 20, 2020
upstream promoter which may cause transcriptional interference (Cheng et al. 2006; Hirochika et al. 1996). The activity of Tos17 in rice is epigenetically regulated (Cheng et al. 2006; Liu et al. 2004; Hirochika et al. 1996). Tos17 is suppressed by heavy DNA methylation in seed plants grown under normal conditions (Cheng et al. 2006). Transcription of Tos17 is activated due to DNA demethylation in prolonged tissue culture and transposition occurs (Cheng et al. 2006; Hirochika et al. 1996). The transcription of newly transposed and original Tos17 copies is re-suppressed by de novo DNA methylation during the plant regeneration from cultured cells and the growth and development of regenerated plants (Cheng et al. 2006). To support these findings, SDG714, a histone H3K9 methyltransferase in rice, was found to be involved in Tos17 DNA methylation and its transposition suppression (Ding et al. 2007), whereas DNG701, a 5-methylcytosine DNA glycosylase/lyase in rice, was identified to be able to demethylate Tos17 DNA and promote its transposition (La et al. 2011).

Due to its favourable features, together with its preferential insertion into gene-rich regions, Tos17 has been extensively used for insertional mutagenesis as a tool for functional analysis of rice genes (Hirochika et al. 1996, 1997; Miyao et al. 2003; Piffanelli et al. 2007). However, tissue culture-induced activation of Tos17 might generate somaclonal mutagenesis with undesirable traits during rice transformation, which would affect the evaluation or application of transgenes. To prevent the interference of Tos17 in rice genome during transgenic study, we intended to generate interference of Tos17 in rice genome during transgenic evaluation or application of transgenes. To prevent the possibility of target site activation, we have designed a CRISPR/Cas9 system containing the dCas9 gene (Dellaporta et al. 1983). CRISPR/Cas9-mediated gene editing in rice has been extensively investigated and results are published previously (Dellaporta et al. 1983; Lessard 2013). Here, we report our CRISPR/Cas9-mediated gene editing of rice genome, including a gene editing tool for rice and the activity of the plasmid pYLCas9-Tos17D in D873 mutant. The three nucleotide sequences derived from gene editing in D873 are shown in italic uppercase letters. D873, gene-edited plant carrying the Tos17chr7 allele derived from T0-24. were carried out according to the method described previously (Hei et al. 1994) with slight modification. Rice callus was induced from mature rice seeds of Nipponbare on NB medium (N6 major salts, B5 minor salts and B5 vitamins, 300 mg/l casein enzymatic hydrolysate, 500 mg/l proline, 30 g/l sucrose, 3.2 g/l phytagel, pH 5.8) containing 2 mg/l 2,4-D at 25°C in darkness. Vigorously growing embryogenic callus derived from the scutellum of mature embryos was co-cultivated with A. tumefaciens strain AGL1 containing binary construct pYLCas9-Tos17D. The co-cultivated callus was washed thoroughly with sterile distilled water and cultured on NB medium containing 2 mg/l 2,4-D, 250 mg/l cefotaxime and 50 mg/l hygromycin at 25°C in darkness. After 4 to 6 weeks selection, putative hygromycin-resistant calli derived from the co-cultivated callus was excised and sub-cultured on NB medium containing 2.0 mg/l KT, 0.02 mg/l NAA and 50 mg/l hygromycin at 25°C with 16-h light and 8-h darkness. The regenerated hygromycin-resistant plantlets were transferred to half-strength MS medium (Murashige and Skoog 1962) containing 50 mg/l hygromycin for shoot and root elongation. Tissue culture plants were also regenerated from 6-month-old calli derived from mature seeds of Nipponbare or gene-edited Tos17 mutants. Transgenic plants and tissue culture plants were transplanted to soil in pots and grown in a rice greenhouse at temperatures ranging from 32°C during the daytime to 26°C at night, with average percentage of humidity at 84% and a photoperiod of 12 to 13 h.

**Materials and methods**

**Construct and A. tumefaciens strains**

Two sgRNA sequences, Target 1 (5’-ATT GGT TAA GGG TAC TCA TCA G-3’) and Target 2 (5’-GGT CCA GCT CAT TTC TGG GCA G-3’), which specifically bind to an upstream site of the GAG-pre-integrase domain (Pre-INT) and a downstream site of the integrase core domain (INT) in the coding region of Tos17, were designed for CRISPR/Cas9-mediated gene editing (Figure 1A). Two gRNA expression cassettes driven by rice U3 and U6 small nuclear RNA (snRNA) promoters, respectively, were assembled into the binary CRISPR/Cas9 vector pYLCrisPR/Cas9Pubi-H to generate binary construct pYLCas9-Tos17D, according to the method described previously (Ma et al. 2015). pYLCas9-Tos17D was introduced into Agrobacterium tumefaciens strain AGL1 by electroporation (Lessard 2013).

**Rice tissue culture and Agrobacterium-mediated transformation**

Rice tissue culture and Agrobacterium-mediated transformation were performed on a PTC-100 programmable thermal controller (MJ Research Inc., Mass.). Plant genomic DNA was extracted using a protocol published previously (Dellaporta et al. 1983). The PCR reaction mixture of 20 µl consisted of 10 ng of rice genomic DNA, 0.15 mM each of dNTPs, 0.15 mM of each primer, 2 µl of 10x PCR buffer and 0.5 unit of Taq polymerase (QIAGEN, Germany). Thermal cycling was done at 94°C for 2 min followed by 35 cycles of 94°C
for 30 s, 55°C for 30 s, 72°C for 90 s. After the final cycle, the reactions were maintained at 72°C for 5 min before completion. The amplified product was resolved by electrophoresis on a 1.5% agarose gel in 1× TAE buffer. The DNA primer pair for detection of gene editing in either Tos17<sup>Chr.7</sup> or Tos17<sup>Chr.10</sup> was TosDS-F (5′ GAA CGT CTA GAT CAT AAG AGT GC/GA) and TosDS-R (5′ GAT GTC CAG AAT ACG AG/GA). The DNA primer pair for specific detection of gene editing in Tos17<sup>Chr.7</sup> was Tos17-7F2 (5′ TAA GCT TCT ATT TTT GA) and Tos17-R (5′ TCA TCT GTG GCC CCG AGC AGT C/G). The primer pair for the Cas9 gene was pYLCas9-F1 (5′ CCT CTT CGA GGA TAG GGA GTT C/G)/pYLCas9-R1 (5′ GCC CCA CTT CTT CTT CGA AGT C/G). The DNA primer pair for the Hpt gene was Hpt-F (5′ TAA GCT TCT ATT TTT GA) and Hpt-R (5′ TCA TCT GTG GCC CCG AGC AGT C/G). The DNA primer pair for the EF1α gene was EF1F2 (5′ GCC TCT CGT CCA TTG GCC) and EF1R2 (5′ AGG GAA CTT CTT CTT CAG GGT TGC). The DNA primer pair for the Tos17-10F1 (5′ TAA GCT TCT ATT TTT GA) and Tos17-R (5′ TCA TCT GTG GCC CCG AGC AGT C/G). The primer pair for the Hpt gene was Hpt-F (5′ TAA GCT TCT ATT TTT GA) and Hpt-R (5′ TCA TCT GTG GCC CCG AGC AGT C/G). The DNA primer pair for the EF1α gene was EF1F2 (5′ GCC TCT CGT CCA TTG GCC) and EF1R2 (5′ AGG GAA CTT CTT CTT CAG GGT TGC).

**Southern blot analysis**

About 2–4 μg of DNA was digested with appropriate restriction enzymes and fractionated on 0.8% agarose gel. After electrophoresis, DNA was blotted to HybondTM-N+ nylon membrane (Amersham Biosciences). The DNA blots were hybridized with DNA probes in a hybridization solution (DIG Easy Hyb Granules, Cat. No. 11796895001, Roche). DNA probes were labelled using a DIG DNA Labeling Kit (DIG Luminescent Detection Kit, Cat. No. 11363514920, Roche). The DNA probe for PstI (P17) was PCR products amplified with DNA primers Tos17-F3 (5′ GGA AAT TGG GTA TGC CAG AGG AGC 3′) and Tos17-R (5′ TAA CCA CAA AGT ACC ACC GGA G 3′). The DNA probe for the Hpt gene was PCR products amplified with DNA primers Hpt-F and Hpt-R.

**Results**

*Generation of Tos17<sup>Chr.7</sup> mutant lines using CRISPR/Cas9-mediated gene editing system*

CRISPR/Cas9-mediated gene editing system was employed to create DNA deletion in the coding region of Tos17. Two sgRNA sequences were designed, which specifically target to an upstream site of the GAG-pre-integrate domain and a downstream site of the integrase core domain in the coding region of the pol gene in Tos17<sup>Chr.7</sup> or Tos17<sup>Chr.10</sup> (Figure 1A). The physical distance between the two targets is 834 bp. Twenty-six independent transgenic T0 plants were obtained in Nipponbare background after Agrobacterium-mediated rice transformation. Primer pair TosDS-F/TosDS-R, which could detect gene editing in either Tos17<sup>Chr.7</sup> or Tos17<sup>Chr.10</sup>, was used to screen for gene editing in the Tos17 genes. Six T0 plants (T0-1, T0-2, T0-6, T0-9, T0-24 and T0-26) were identified to have undergone gene editing either on Tos17<sup>Chr.7</sup> or Tos17<sup>Chr.10</sup> or both (Figure 2A). Southern blot analysis with restriction enzyme digestion by PstI and Tos17 probe P17 demonstrated that 1-3 additional hybridization bands of Tos17 were detected in the 6 T0 plants (Figure 2B). Plants T0-6, T0-9, T0-24 and T0-26 produced PstI bands with molecular size smaller than the 4389-bp band of wild-type Tos17<sup>Chr.7</sup>, indicating that these plants might carry gene-edited Tos17<sup>Chr.7</sup> alleles (Figure 2B). T0-6, T0-24 and T0-26 were selected for further characterization, while T0-9 was infertile and abandoned.

*Identifying gene-edited Tos17<sup>Chr.7</sup> allele Tos17<sup>D873</sup>*

*through molecular and genetic characterization*

In addition to the 4389-bp PstI band of Tos17<sup>Chr.7</sup> and

![Figure 2](image-url)
the 6170-bp PstI band of \(\text{Tos17}^{\beta\text{Chr.7}}\), T0-26 produced only one extra PstI band with molecular size smaller than 4389 bp. However, both T0-6 and T0-24 produced extra PstI bands with molecular size larger than 4389 bp (Figure 2B). These extra bands could be newly transposed \(\text{Tos17}^{\beta\text{Chr.7}}\), gene-edited \(\text{Tos17}^{\beta\text{Chr.10}}\) or newly transposed \(\text{Tos17}^{\beta\text{Chr.7}}\) followed by gene editing. To eliminate these undesirable \(\text{Tos17}\) copies, T0-24 and T0-26 as well as T0-6 were used as males to cross with \text{Nipponbare} followed by backcrossing for one generation and self-pollination of BC1F1 plants. The F1, BC1F1 and BC1F2 plants derived from these crosses were screened for gene editing on \(\text{Tos17}^{\beta\text{Chr.7}}\) using \(\text{Tos17}^{\beta\text{Chr.7}}\)-specific primer pair T0-7F2/T0DS-R. The PCR products were sequenced to identify exact DNA mutation in the gene-edited \(\text{Tos17}^{\beta\text{Chr.7}}\) alleles. In the meanwhile, they were also screened for the presence or absence of T-DNA harbouring the \text{Hpt} gene and the CRISPR-Cas9 gene editing system.

The gene-edited \(\text{Tos17}^{\beta\text{Chr.7}}\) allele derived from T0-6 had 706-bp DNA deletion flanking the Target 1, which starts from the position at \(-22\) bp upstream of Target 1 to the position at \(-173\) bp upstream of Target 2. Based on the size of DNA deletion, the PstI fragment derived from this gene-edited \(\text{Tos17}^{\beta\text{Chr.7}}\) allele should be 3683 bp, which was smaller than the smallest PstI band detected in T0-6 by Southern blot analysis (Figure 2B). By comparing with another PstI band above the 4389-bp PstI band, which was most likely derived from a newly transposed \(\text{Tos17}^{\beta\text{Chr.7}}\), the \(\text{Tos17}^{\beta\text{Chr.7}}\) allele with 706-bp DNA deletion is the most likely candidate for the gene-edited \(\text{Tos17}^{\beta\text{Chr.7}}\) allele.
bp deletion in T0-6 should be derived from the newly transposed \textit{Tos17} Chr.7 rather than the original \textit{Tos17} Chr.7. Indeed, molecular and genetic analysis failed to identify a homozygous plant that contains this \textit{Tos17} Chr.7 allele with 706-bp deletion only in the absence of wild-type \textit{Tos17} Chr.7 allele (data not shown). T0-26 and its derived plants only carried a mutated \textit{Tos17} Chr.7 allele with gene editing at the Target 1 site. The gene-edited \textit{Tos17} Chr.7 allele had 435-bp DNA deletion, starting from the 14th position of Target 1 to the +421 bp position to its downstream followed by DNA insertion of 41 bp, which caused the deletion of the GAG-pre-integrate domain and frameshift of open reading frame. As T0-6 and T0-26 and its derived plants carried mutated alleles of \textit{Tos17} Chr.7 either derived from a newly transposed \textit{Tos17} Chr.7 or undergone partial gene editing, they were not included in additional study.

The mutated allele of \textit{Tos17} Chr.7 derived from T0-24 has undergone relatively precise gene editing. It had 876-bp DNA deletion starting from the –14 bp position upstream of Target 1 to the 6 bp position of Target 2 followed by 3-bp DNA insertion (Figure 1B). Both GAG-pre-integrate domain and integrase core domain of \textit{Tos17} Chr.7 were deleted in the mutated allele (Figure 3). The mutated allele of \textit{Tos17} Chr.7 was designated as \textit{Tos17}\textsuperscript{D873}, while the T0-24/BC1F2 plants that contained homozygous \textit{Tos17}\textsuperscript{D873} alleles were designated as D873 plants. A 3516-bp \textit{PstI} band derived from the \textit{Tos17}\textsuperscript{D873} allele was detected in the D873 plants (Figure 4A). As the control, a 4389-bp \textit{PstI} band derived from the wild-
type \textit{Tos17}^{Chr.7} allele was only detected in Nipponbare (Figure 4A). Like Nipponbare, D873 plants produced a 6170-bp PsiI band derived from the wild-type \textit{Tos17}^{Chr.10} allele (Figure 4A). \textit{Tos17}^{Chr.10} could also be edited as it carries both Target 1 and Target 2 in its genomic sequence. Genomic DNA covering Target 1 and Target 2 on \textit{Tos17}^{Chr.10} was amplified from D873 using \textit{Tos17}^{Chr.10}-specific primer pair \textit{Tos17}-10F1/\textit{Tos1DS}-R. DNA sequencing of the PCR products indicated that \textit{Tos17}^{Chr.10} in the D873 plants was not edited (data not shown). No \textit{Hpt} or \textit{Cas9} gene was detected in the D873 plants by Southern blot hybridization (Figure 4B) and PCR analysis (Figure 4C, D), indicating that the T-DNA in T0-24 has been eliminated from the D873 plants through crossing and backcrossing with Nipponbare and/or subsequent genetic segregation. The D873 plants displayed similar morphological phenotypes at different developmental stages and had similar growth duration to that of Nipponbare (Figures 5A, C). In addition, the seed setting rate of D873 was 77.9±3.6%, similar to that of Nipponbare at 78.7±6.9%. D873, as a \textit{Tos17}^{Chr.7} knockout mutant in Nipponbare background, was selected for additional study.

**Test of \textit{Tos17}^{D873} activity through tissue culture**

Calli were induced from mature seeds of D873 and Nipponbare and sub-cultured monthly on fresh medium. Tissue culture plants were regenerated from calli that had been cultured for 6 months after callus induction. The copy number of \textit{Tos17} in the regenerated plants was detected by Southern blot analysis. In control experiments, besides for the 4389-bp \textit{PsiI} band derived from the wild-type \textit{Tos17}^{Chr.7} allele and the 6170-bp \textit{PsiI} band derived from the wild-type \textit{Tos17}^{Chr.10} allele, additional \textit{Tos17} bands were detected in 7 of the 8 regenerated plants of Nipponbare genetic background, indicating that the transposition of \textit{Tos17}^{Chr.7} had occurred after prolonged tissue culture (Figure 6). As expected, no additional \textit{Tos17} band was detected in the 10 regenerated plants in D873 genetic background tested (Figure 6). Interestingly, the deletion in the \textit{pol} gene of \textit{Tos17}^{Chr.7} did not affect the transcription of the mutated \textit{Tos17}^{Chr.7} allele during tissue culture, both \textit{Tos17}^{D873} and \textit{Tos17}^{Chr.7} transcripts were detected by RT-PCR in 6-month-old calli of D873 and Nipponbare, respectively (Figure 7). The results demonstrated that the deletion of \textit{GAG}-pre-integrase domain and integrase core domain in \textit{Tos17}^{Chr.7} had abolished its activity for transposition even though the transcription of \textit{Tos17}^{D873} was activated during tissue culture. In addition, no transposition of \textit{Tos17}^{Chr.10} was detected in the regenerated D873 plants, which confirms the previous finding that \textit{Tos17}^{Chr.10} is not activated during tissue culture (Cheng et al. 2006; Hirochika et al. 1996).

**Discussion**

Previous studies demonstrated that Nipponbare harbours two nearly identical genomic copies of \textit{Tos17}, \textit{Tos17}^{Chr.7} and \textit{Tos17}^{Chr.10}, in its genome with only \textit{Tos17}^{Chr.7} showing transposition activity during tissue culture (Cheng et al. 2006; Hirochika et al. 1996). Our main objective in this study was to inactivate \textit{Tos17}^{Chr.7} through internal gene deletion mediated by CRISPR/Cas9 gene editing system. Two sgRNAs, Target 1 and Target 2, were designed to target to positions at the upstream of the GAG-pre-integrase domain and at the downstream of integrase core domain of \textit{Tos17}, respectively (Figure 1A). Firstly, as \textit{Tos17}^{Chr.7} and \textit{Tos17}^{Chr.10} share identical coding region for the \textit{pol} gene, both \textit{Tos17} loci could be edited in this study. Secondly, the newly transposed \textit{Tos17}^{Chr.7} generated during rice transformation could be further edited by the CRISPR/Cas9 gene editing system. Finally, gene editing might occur at only one target site that causes point mutation or partial DNA deletion. Taking together, there might be gene editing at \textit{Tos17}^{Chr.7}, \textit{Tos17}^{Chr.10} or both loci, gene editing at the transposed \textit{Tos17}^{ Chr.7}, partial gene editing either at Target 1 or Target 2 only, or the different combinations of the gene editing events. Therefore, both molecular and genetic analyses were conducted to obtain mutant plants that contain complete internal gene deletion at the \textit{Tos17}^{Chr.7} locus by eliminating undesirable gene-edited alleles and T-DNA in the gene-edited plants. In this study, twenty-six independent transgenic T0 plants were obtained, but only one T0 plant (T0-24) produced progeny that carried an expected gene edited allele of \textit{Tos17}^{ Chr.7}. The efficiency of obtaining desirable gene editing line was low, but the identification of the \textit{Tos17}^{D873} allele was sufficient for the functional study of \textit{Tos17}^{Chr.7} with knock-out mutation.

The transposition of LTR retrotransposons depend on intact \textit{cis}-acting elements, including two LTRs at both ends, a potential tRNA primer binding site (PBS) and a polypurine tract (PPT), and \textit{trans}-acting proteins encoded by the \textit{gag} and \textit{pol} genes with some having a third gene equivalent to a retroviral \textit{env} gene (Finnegan 2012). In this study, all \textit{cis}-acting elements, including the two LTRs, PBS and PPT are intact in \textit{Tos17}^{D873}. The CRISPR/Cas9-mediated gene editing in the coding region of \textit{Tos17}^{Chr.7} caused the deletion of the GAG-pre-integrase domain and the integrase core domain in \textit{Tos17}^{D873}. Although RT-PCR analysis indicated that, like \textit{Tos17}^{ Chr.7}, the transcription of \textit{Tos17}^{D873} was activated during tissue culture, the deletion of two domains completely abolished its transposition activity. Previously, bioinformatics analysis indicated that both \textit{Tos17}^{Chr.7} and \textit{Tos17}^{Chr.10} lack a \textit{gag} open reading frame and the authors claimed that \textit{Tos17} is a non-autonomous LTR retrotransposon requiring an active one in order to ensure its transposition (Sabot 2014). While it remains
to be determined experimentally whether Tos17 needs an autonomous partner to provide GAG for its transposition, our results demonstrate that the GAG-pre-integrase domain and the integrase core domain are essential for Tos17\(^{\text{Chr7}}\) transposition and the deletion of the two domains could be not complemented by trans-acting proteins encoded by other LTR retrotransposons in rice genome (Hirochika et al. 1996).

Saika et al. (2019) recently reported the production of Tos17-deficient plants by CRISPR/Cas9-mediated targeted mutagenesis. According to the report, gene editing with a sgRNA targeting to both 5' and 3' LTRs of Tos17 (Tos17\(^{\text{Chr7}}\) and Tos17\(^{\text{Chr10}}\)) could generate Tos17-deficient plants (Saika et al. 2019). In this study, Tos17\(^{\text{Chr7}}\) was mutated with deletion in the coding region of the pol gene by CRISPR/Cas9-mediated gene editing with two sgRNAs. The generation and characterization of the Tos17\(^{\text{Chr7}}\) mutant D873, confirming the previous report that only Tos17\(^{\text{Chr7}}\) is active during tissue culture (Cheng et al. 2006). As the Tos17\(^{\text{Chr7}}\)-derived somaclonal mutagenesis is blocked in the D873 plants, the generation of the Tos17\(^{\text{D873}}\) allele will be helpful in production of transgenic rice plants for gene function study and genetic engineering. Similar approach can be used to inactivate other transposons in crop breeding.

Acknowledgements
The authors thank Prof. Yao-Guang Liu for providing CRISPR/Cas9 vector pYLCRISPR/Cas9\(^{\text{ubiquitin-H}}\). Y.L., D.T. and Z.Y. designed the experiments. Y.L., D.T., J.C.Y.T. and K.H.O. conducted the experiments. D.T. and Z.Y. wrote the article. This research was initially supported by Temasek Life Sciences Laboratory and partially supported by the National Research Foundation (NRF), Prime Minister’s Office, Singapore on the project of the Disruptive & Sustainable Technology for Agricultural Precision (DiSTAP). The authors state that they have no conflict of interest.

References
Cheng C, Daigen M, Hirochika H (2006) Epigenetic regulation of the rice retrotransposon Tos17. Mol Genet Genomics 276: 378–390
Dellaporta S, Wood J, Hicks JB (1983) A plant DNA minipreparation. Version II. Plant Mol Biol Report 1: 19–21
Ding Y, Wang Y, Su L, Zhai J, Cao S, Zhang D, Liu C, Bi Y, Qian Q, Cheng Z, et al. (2007) SDG714, a histone H3K9 methyltransferase, is involved in Tos17 DNA methylation and transposition in rice. Plant Cell 19: 9–22
Finnegan DJ (2012) Retrotransposons. Curr Biol 22: R432–R437
Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (Oryza sativa L.) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. Plant J 6: 271–282
Higgins DG, Sharp PM (1988) CLUSTAL: A package for performing multiple sequence alignment on a microcomputer. Gene 73: 237–244
Hirochika H (1997) Retrotransposons of rice: Their regulation and use for genome analysis. Plant Mol Biol 35: 231–240
Hirochika H, Sugimoto K, Otsuki Y, Tsugawa H, Kanda M (1996) Retrotransposons of rice involved in mutations induced by tissue culture. Proc Natl Acad Sci USA 93: 7781–7788
Kumar A, Benetzen JL (1999) Plant retrotransposons. Annu Rev Genet 33: 479–532
La H, Ding B, Mishra GP, Zhou B, Yang H, Bellizzi MR, Chen S, Meyers BC, Feng Z, Zhu JK, et al. (2011) A 5-methylcytosine DNA glycosylase/lyase demethylates the retrotransposon Tos17 and promotes its transposition in rice. Proc Natl Acad Sci USA 108: 15498–15503
Lessard JC (2013) Transformation of E. coli via electroporation. Methods Enzymol 529: 321–327
Liu ZL, Han FP, Tan M, Shan XH, Dong YZ, Wang XZ, Fedak G, Hao S, Liu B (2004) Activation of a rice endogenous retrotransposon Tos17 in tissue culture is accompanied by cytosine demethylation and causes heritable alteration in methylation pattern of flanking genomic regions. Theor Appl Genet 109: 200–209
Ma X, Zhang Q, Zhu Q, Liu W, Chen Y, Qiu R, Wang B, Yang Z, Li H, Lin Y, et al. (2015) A Robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Mol Plant 8: 1274–1284
Miyao A, Tanaka K, Murata K, Sawaki H, Takeda S, Abe K, Shinozuka Y, Onosato K, Hirochika H (2003) Target site specificity of the Tos17 retrotransposon shows a preference for insertion within genes and against insertion in retrotransposon-rich regions of the genome. Plant Cell 15: 1771–1780
Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15: 473–497
Piffanelli P, Droc G, Mieulet D, Lanau N, Bes M, Bourgeois E, Rouviere C, Gavory F, Cruaud C, Gheshquare A, et al. (2007) Large-scale characterization of Tos17 insertion sites in a rice T-DNA mutant library. Plant Mol Biol 65: 587–601
Sabot F (2014) Tos17 rice element: incomplete but effective. Mob DNA 5: 10
Saika H, Mori A, Endo M, Toki S (2019) Targeted deletion of rice retrotransposon Tos17 via CRISPR/Cas9. Plant Cell Rep 38: 455–458