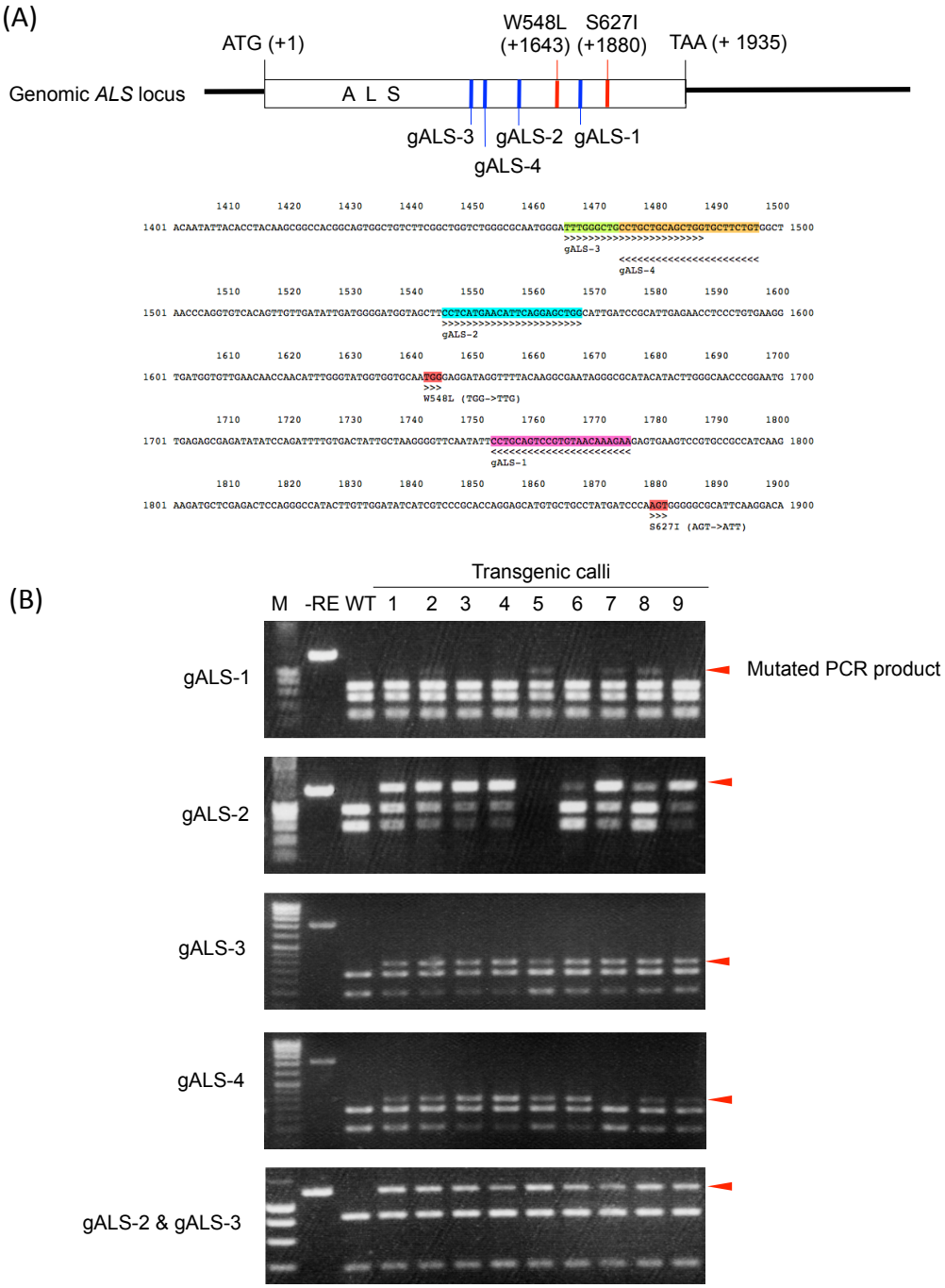
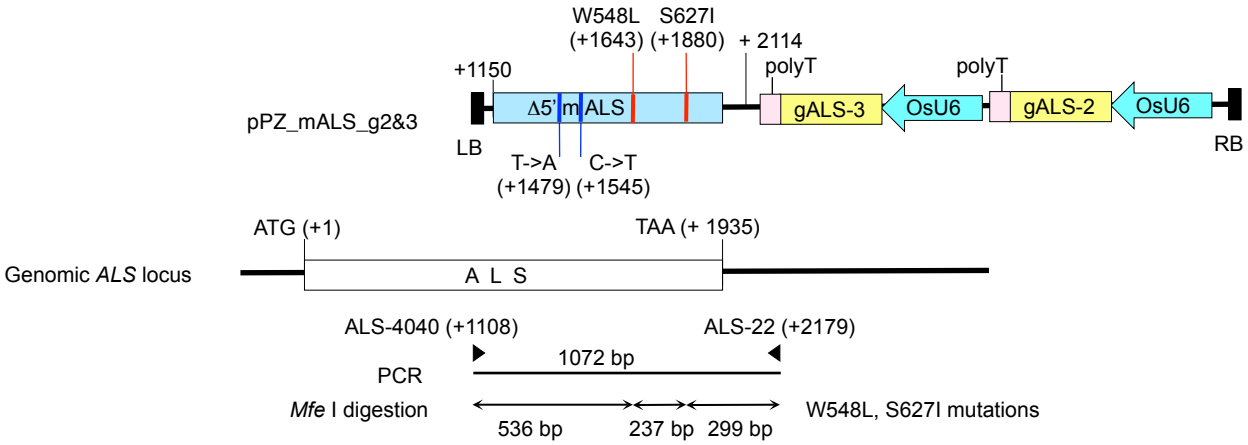
Supplementary Figure S1

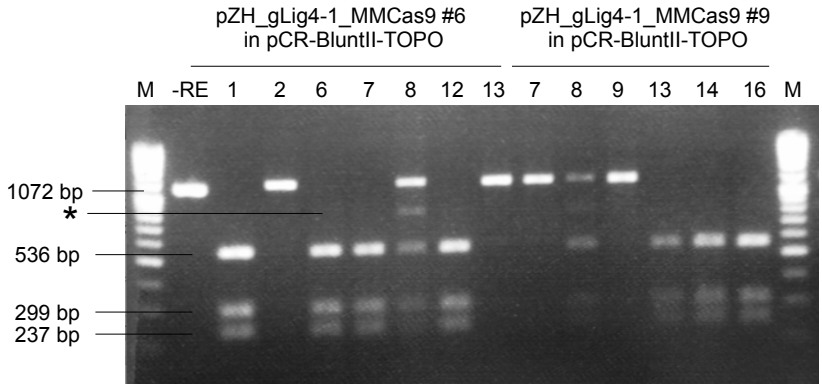


CRISPR/Cas9 mediated target mutagenesis in OsALS gene

(A) Target site of CRISPR/Cas9-mediated target mutagenesis in OsALS gene. Blue vertical lines indicate the location of target site of gALS-1~4. Red vertical lines indicate the location of W548L and S627I mutations, which confer BS resistance. Detailed sequences of target sites (20bp + 3bp, NGG) are shown in the bottom panel. Sequential arrowheads below sequence indicate the direction of gRNA. (**B**) CAPS analysis of the each target site. DNA extracted from independent pZH_gRNA (gALS-1~4)_MMCas9 transformed calli was subjected to PCR and subsequent restriction enzyme digestion. Restriction enzymes used for detecting mutations in target sites of gALS-1, 2, 3, 4 are *Pst* I, *BspH* I, *Pvu* II and *Pst* I respectively. *BspH* I were used for the CAPS analysis of gALS-2 & gALS-3 transformed calli. PCR primers used for these CAPS analysis are shown in Mikami et al. (2015a).

Supplementary Figure S2

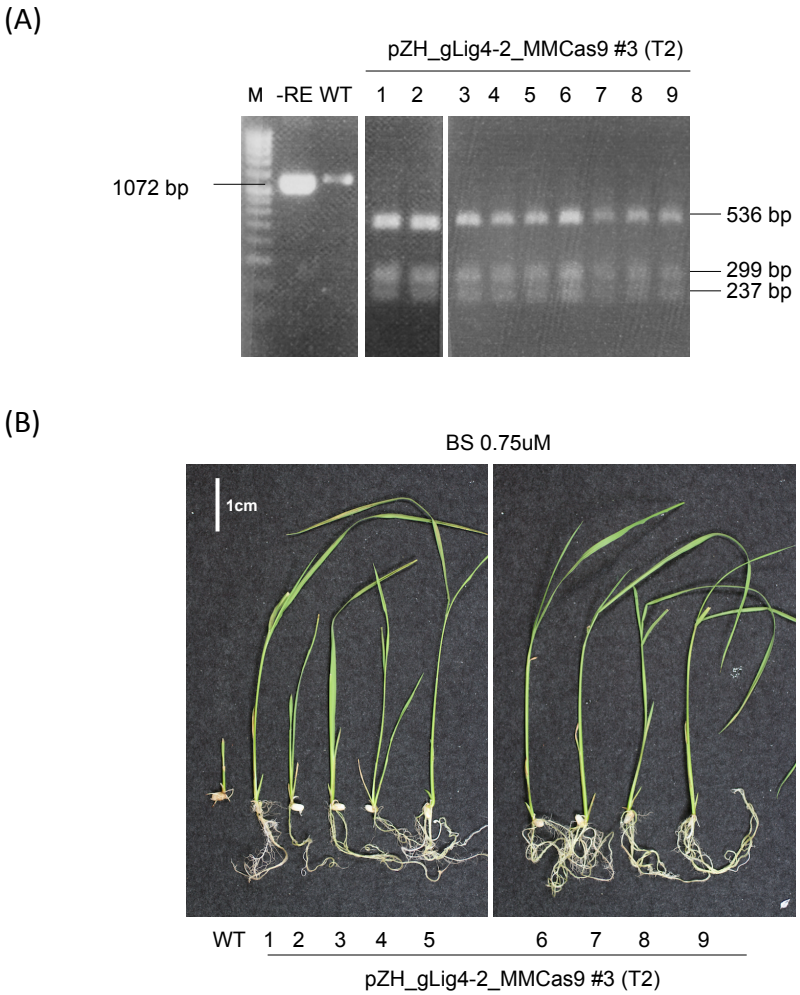




Demonstration of the fact that artificial heteroduplx of WT and mutated ALS creased by PCR is not digested by high fidelity version of *Mfe* I

PCR product (ALS-4040 ~ ALS-22) of pZH_gLig4-2_MMCas9 #6, #9 (Fig. 4B) were cloned into pCR-Blunt II-TOPO (Invitrogen) and used for the templates of same PCR reaction. PCR product of each clone were digested by *Mfe* I-HF (high fidelity version of *Mfe* I supplied by New England BioLabs). By this cloning, PCR product with/without W548L and S627I mutations are segregated. However, when mixture of wild-type *ALS* and *ALS* with W548L, S627I mutations were used as templates for this PCR (pZH_gLig4-1_MMCas9 #6 in pCR-Blunt II-TOPO #8, pZH_gLig4-1_MMCas9 #9 in pCR-Blunt II-TOPO #8), ca.700bp fragment (*) was appeared by *Mfe* I digestion again. From these results, we consider that heteroduplex of mutated and non mutated amplicons generated by PCR reaction is partially tolerant to *Mfe* I-HF and imperfect digested products at W548L appeared as ca.700bp fragment.

Supplementary Figure S3



T1 progenies of bi-allelic GT plant
(A) PCR-*Mfe* I digestion analysis of T2 progenies of pZH_gLig4-2_MMCas9 #3, in which bi-allelic GT event was observed in T1 generation. (B) Seeds were germinated on MS medium for four days and transferred seedlings to MS medium containing 0.75uM of BS.