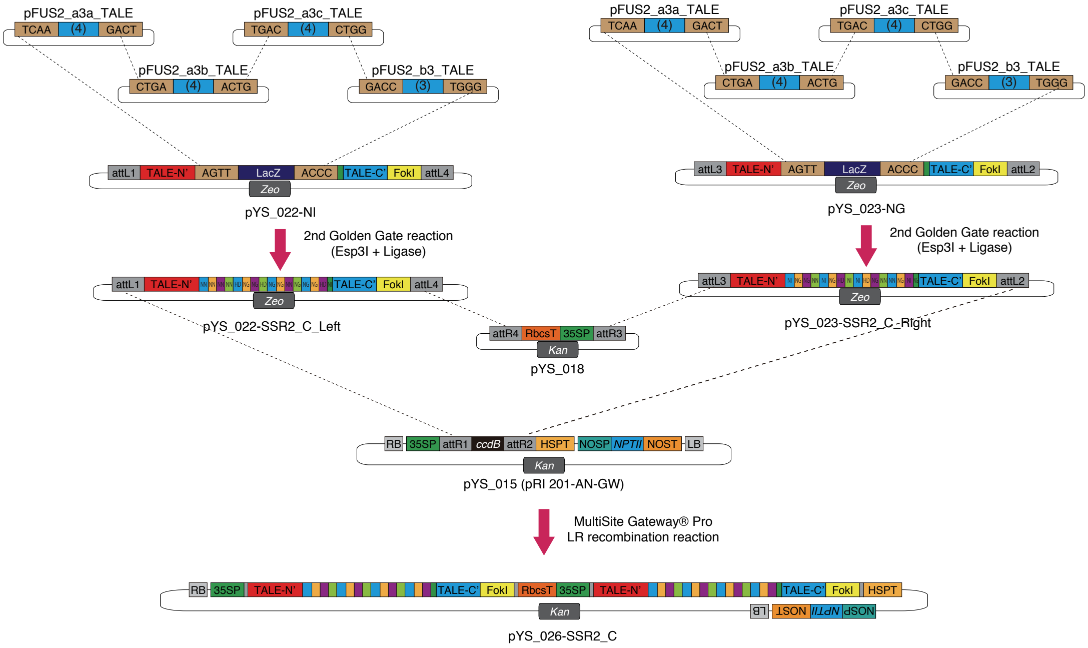
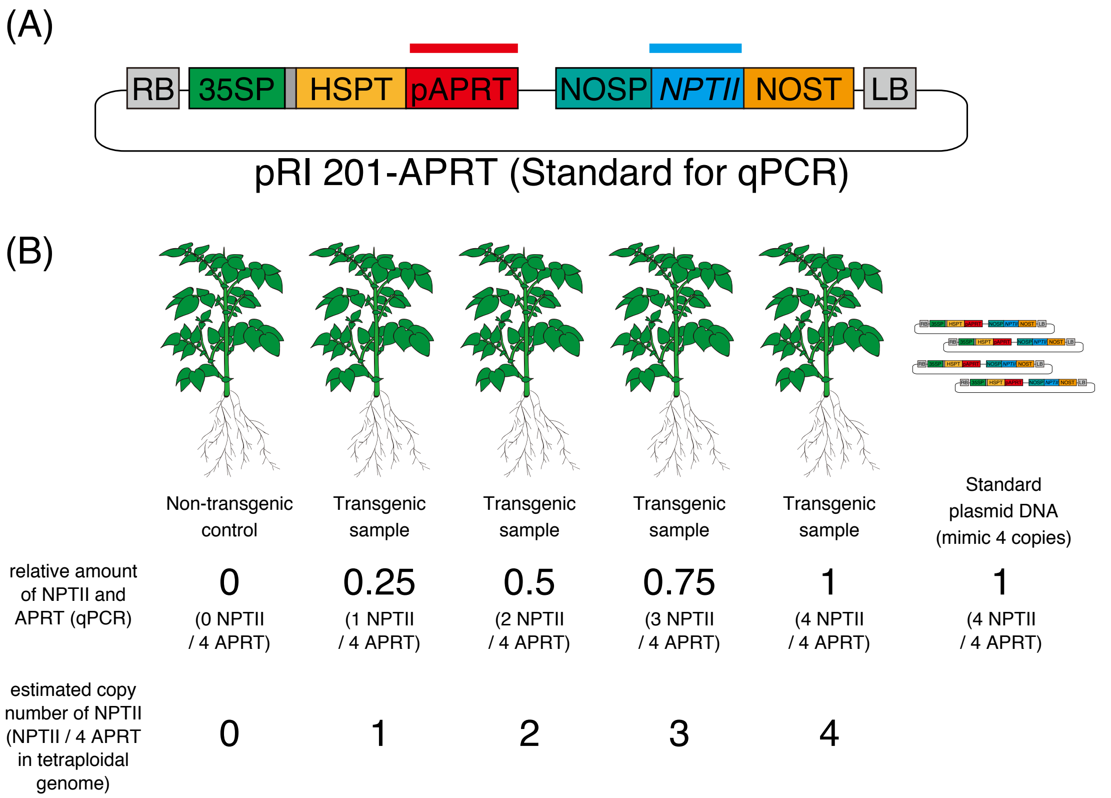


Supplemental Figure 1. Schematic illustration of the construction of the binary plasmid DNA pYS_026-SSR2_C.

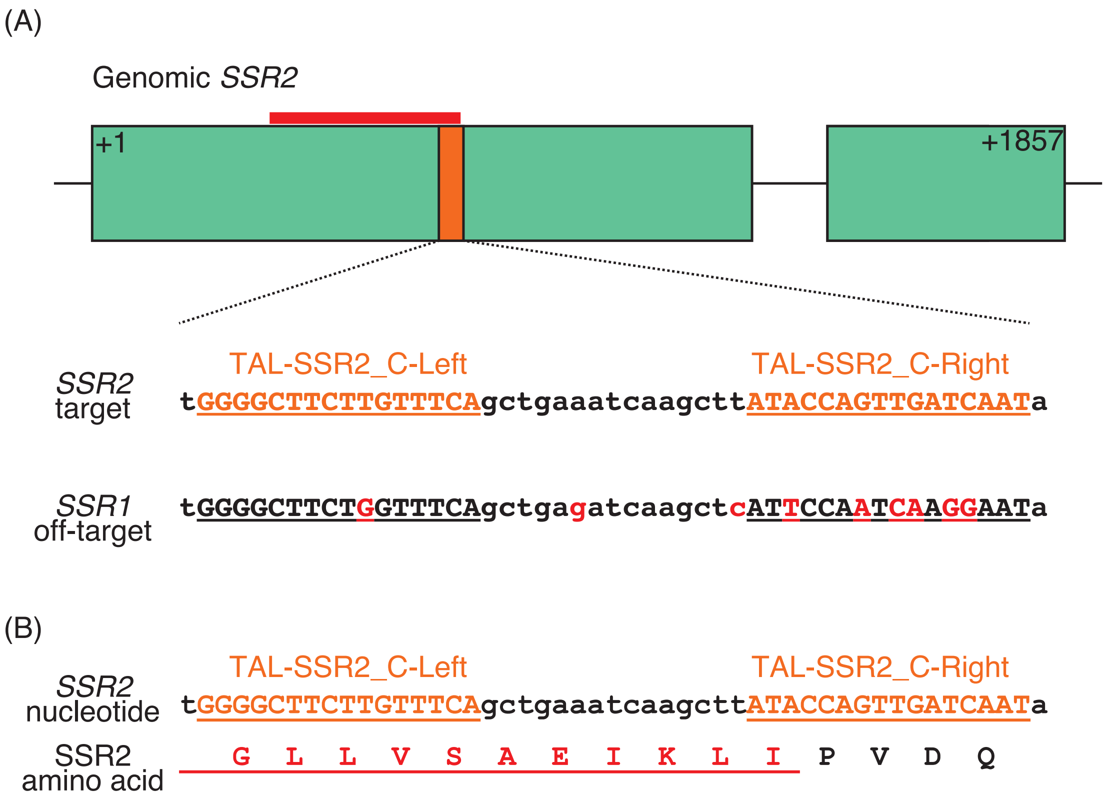
Gateway technology compatible capture vectors, pYS_022-NI and pYS_023-NG were used for second Golden Gate assemble. Three entry vectors, pYS_022-SSR2_C-Left, pYS_018, and pYS_023-SSR2_C-Right and a destination vector pYS_015 were mixed with LR Clonase II® Plus enzyme mix. The resultant binary vector, pYS_026-SSR2_C was used for *Agrobacterium* mediated potato transformation.



Supplemental Figure 2. Schematic illustration of the estimation of copy number of NPTII in transgenic potatoes by quantitative real time PCR.

(A) Schematic map of pRI 201-APRT was shown. pRI 201-APRT which harbors an *NPTII* (transgene) and a partial *APRT* (endogenous gene in potato) was used for standard template in quantitative real time PCR (qPCR) analysis for the estimation of copy number on NPTII gene in transgenic potatoes. The regions amplified in qPCR analysis were shown in red and blue lines.

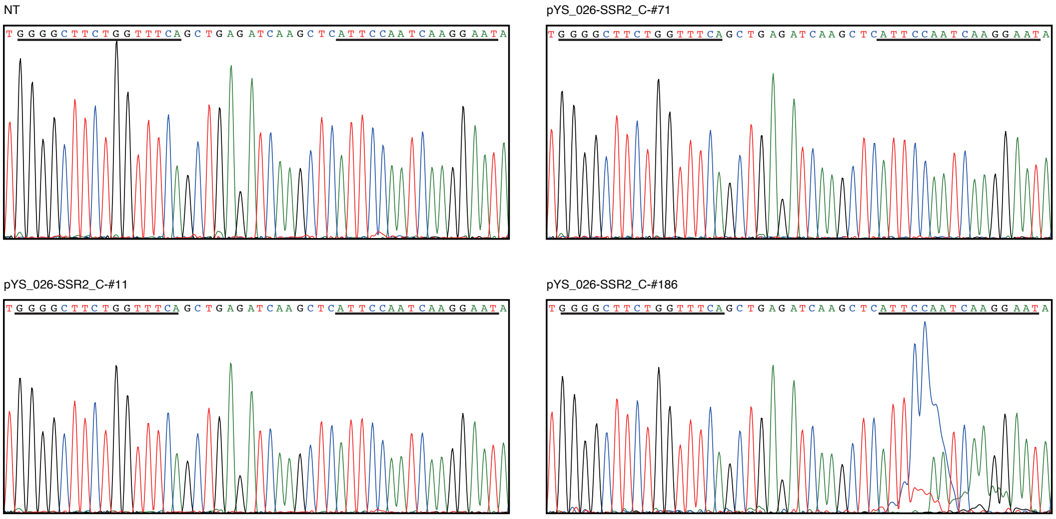
(B) The relations between the relative amount of *NPTII* and *APRT* cutulated by qPCR and the estimated copy number of NPTII gene in tetraploidal potato genome. The tetraploidal potato harbors four copy of endogenous APRT gene in the genome. The estimated copy number of NPTII gene in tetraploidal genome was calculated by quadrupling the relative copy number of NPTII and APRT genes.



Supplemental Figure 3. TALEN target DNA sequence in *SSR2* gene.

(A) The gene structure of potato SSR2 is shown. Green boxs represent exons. The correspondiong region of conserved domain of FAD/FMN-containing dehydroganase predicted by NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) is indicated by red line. The location of TALEN recognition site is indicated with a orange box. Orange underlined letters represent TALEN recognition sequence in target *SSR2* gene. The different nucleotide in *SSR1* from *SSR2* are shown in red letters.

(B) The partial SSR2 genomic nucleotide sequence and deduced amino acid sequence near the TALEN recognition site. The partial region corresponding to conserved domain of FAD/FMN-containing dehydroganase predicted by NCBI Conserved Domain Search in deduced amino acid sequence are shown in underlined red letters.



Supplemental Figure 4. Potential off-target sequences in transgenic potato plants. *SSR1* sequences of genome-edited transgenic potatoes and control plant (NT Sassy) are shown. The potential off-target TALEN-binding sequences were underlined in the sequence.