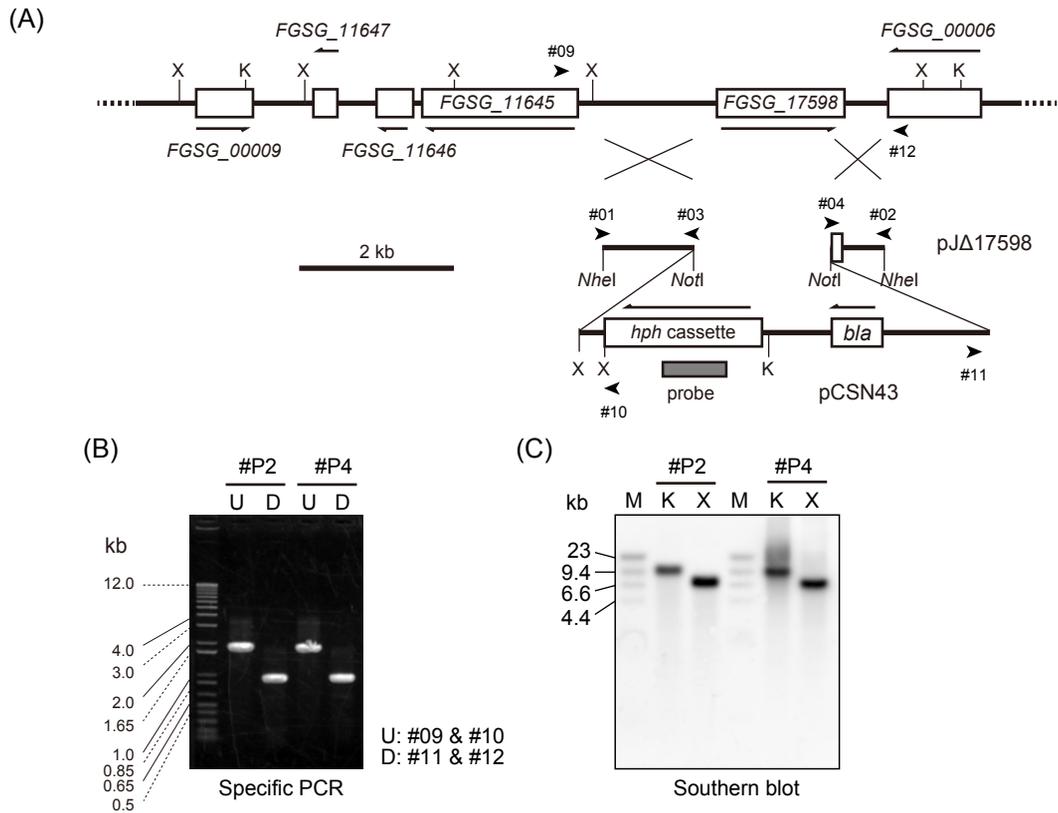


Fig. S1

Disruption of *FGSG_17598*



Disruption of *FGSG_10397*

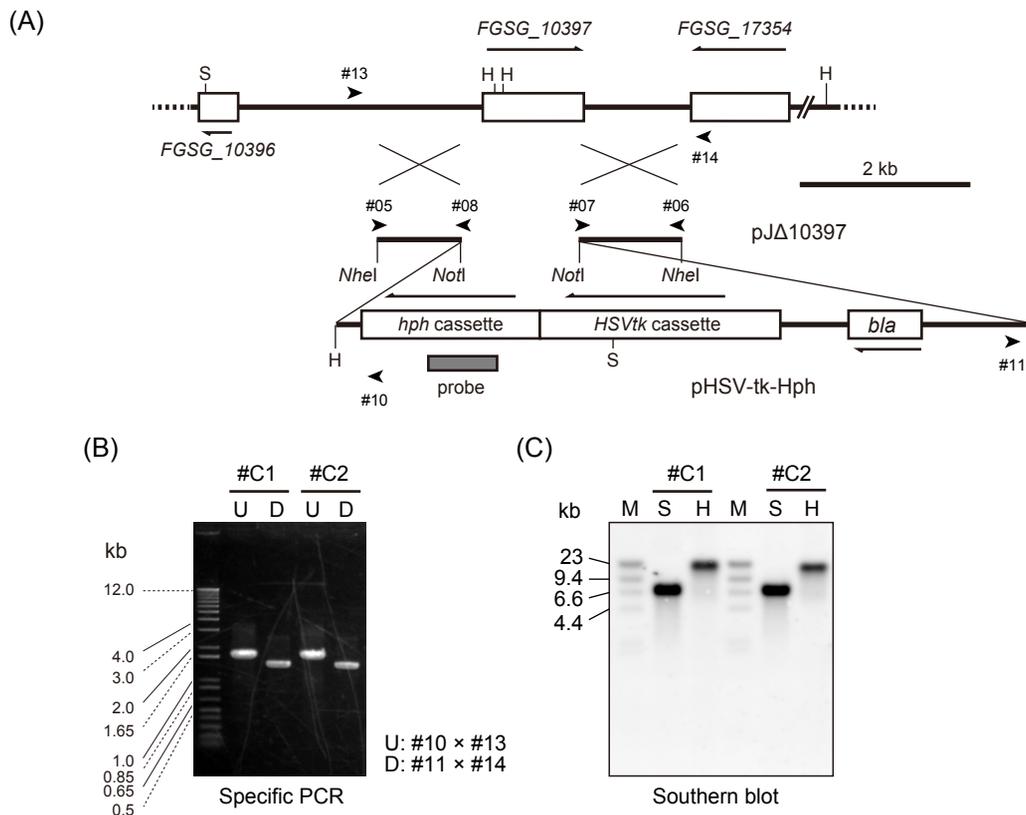


Fig. S1. Disruption of FGSG_17598 and FGSG_10397 genes. (A) Structures of the vectors used for disruption of FGSG_17598 and FGSG_10397 genes. The gene disruption vectors were constructed by replacing the coding region of FGSG_17598 and FGSG_10397 with pCSN43 or its derivative, pHSV-tk-Hph, respectively, that contain the *hph* cassette. The homologous regions upstream and downstream of the deleted region were cloned by inverse PCR (IPCR) as follows: (1) DNA fragments containing the region to be deleted were amplified by long PCR with inward primers #01 and #02 (for FGSG_17598) or #05 and #06 (for FGSG_10397) that have a *NheI* recognition site; (2) the amplified products were self-ligated after digestion with *NheI*; (3) DNA fragments flanking the region to be deleted were amplified by IPCR with the outward primers #03 and #04 (for FGSG_17598) or #07 and #08 (for FGSG_10397) that have a *NotI* recognition site; and (4) the IPCR products were cloned into the *NotI* site downstream of the *trpC* terminator in pCSN43 and pHSV-tk-Hph. The directions of the inserted *NotI* fragments were determined by PCR with primers #11 and #12 (for FGSG_17598) or #10 and #13 (for FGSG_10397). (B) PCR amplification of the border regions of the FGSG_17598 gene disruptants (strains #P2 and #P4) and FGSG_10397 gene disruptants (strains #C1 and #C2). Targeted integrations of vectors were demonstrated by successful amplification of amplicons with primers #09 and #10 (1,804 bp; upstream of FGSG_17598) and primers #11 and #12 (951 bp; downstream of FGSG_17598), and primers #10 and #13 (1,721 bp; upstream of FGSG_10397) and primers #11 and #14 (1,402 bp; downstream of FGSG_10397). The left lane was loaded with 1 kb Plus DNA ladders (Life Technologies, Carlsbad, CA, USA). (C) Southern blot analysis of the disruption mutants. The DNA of the disruption mutant was digested with *KpnI* or *XbaI* (FGSG_17598), and *SmaI* or *HindIII* (FGSG_10397). A DIG-labeled probe (indicated as a thick line) was prepared using a PCR DIG Probe Synthesis kit with the primers listed in [Table S1](#). DNA Molecular Marker II, DIG-labeled (Roche Diagnostics GmbH; Mannheim, Germany) was loaded on the left lane labeled “M”.

Fig. S2

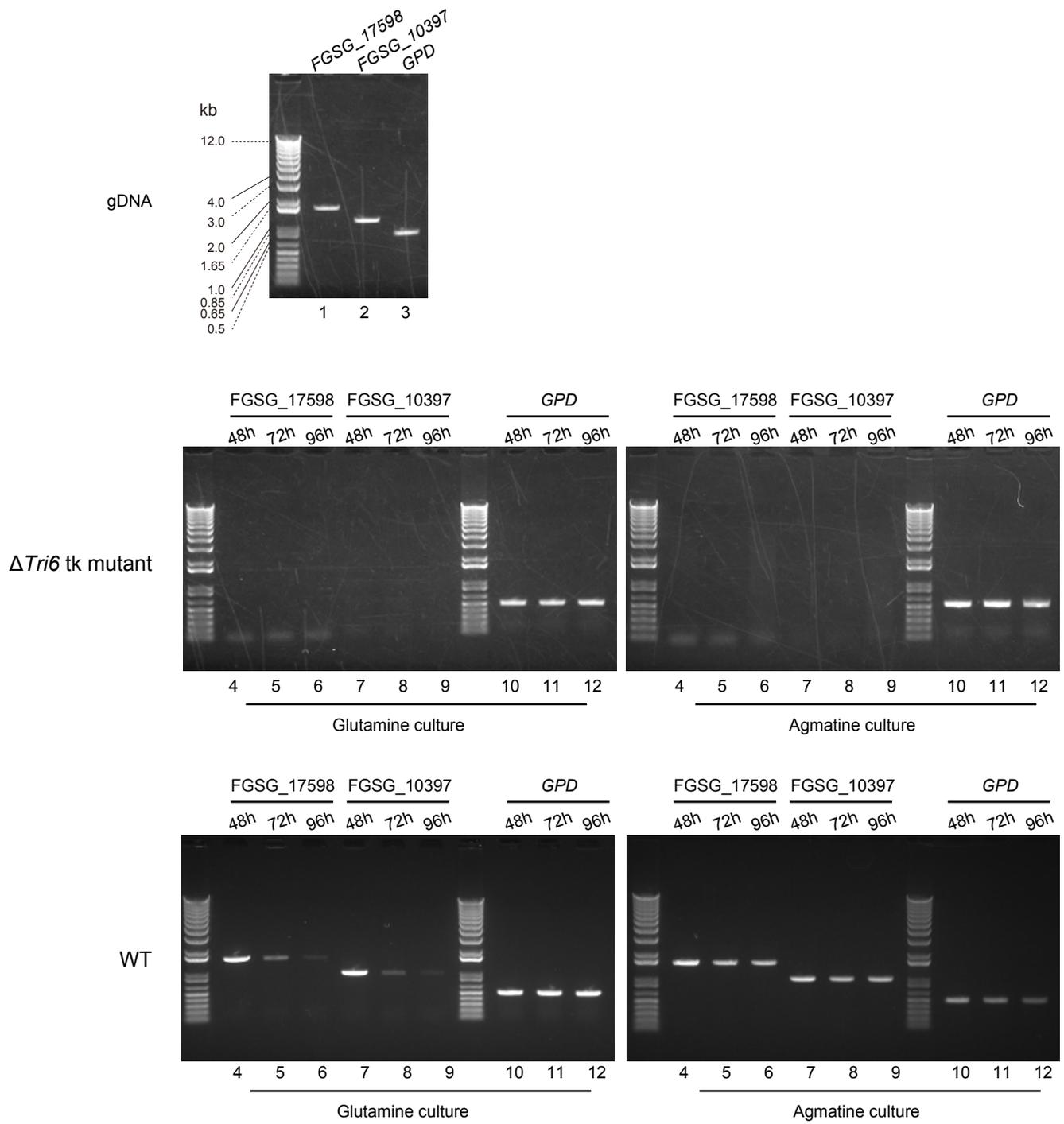


Fig. S2. RT-PCR analyses of FGSG_17598 and FGSG_10397 genes using the $\Delta Tri6$ tk mutant. Primers that allow the amplification of genomic DNA across the intron regions (lanes 1-3; upper panel) were used (Table S1). cDNA was synthesized from 1 μ g of total RNA (treated with the RNase-free DNase I) using the Superscript First-Strand Synthesis System (Life Technologies). A 0.1 aliquot was used as a template for the PCR using the primers listed in Table S1. While no amplicons were obtained for FGSG_17598 and FGSG_10397 after 35 cycles (lanes 4-9; middle panel), specific amplicons were consistently obtained for the glyceraldehyde-3-phosphate dehydrogenase gene (*GPD*) after 25 cycles (lanes 10-12). As positive controls, amplicons from the WT cDNA (25 cycles) at each time point are shown (lanes 4-9; lower panel).

Fig. S3

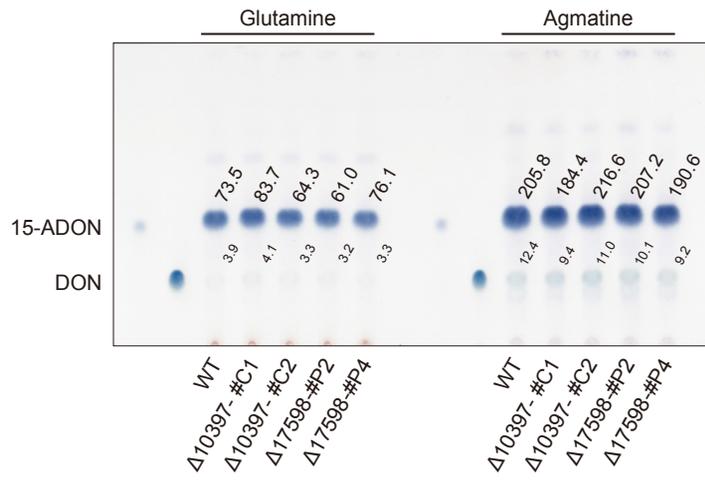


Fig. S3. TLC analysis for 15-ADON and DON in extracts of cultures of the WT, FGSG_17598 disruption mutants (Δ 17598-#P2 and Δ 17598-#P4), and FGSG_10397 disruption mutants (Δ 10397-#C1 and Δ 10397-#C2) grown in 100 μ L of synthetic liquid media distributed to a 96-well plate. The concentrations of trichothecenes (indicated as μ g/mL over the spots of 15-ADON and DON) in the culture were determined as described in the legend of [Table 1](#).