



**FIGURE S3** Targeted gene disruption of *C. orbiculare* *FAM1* and *CoHEX1*. (A) Schematic representation of the *FAM1* gene disruption by homologous recombination. Binary plasmid pBIFAM1AH3-2 contains the AMP::HPH (ampicillin-hygromycin resistance) cassette inserted into the *FAM1* gene between the left border (LB) and right border (RB) sequences. Through double crossing over, a 3.9 kb *XhoI* fragment of wild-type is predicted to be replaced by a 6.6-kb *XhoI* fragment containing the AMP::HPH cassette. (B) DNA blot analysis of *FAM1* disrupted transformants. Genomic DNA was digested with *XhoI* and probed with an internal fragment of *FAM1* indicated in the pBIFAM1AH3-2 vector map. W, wild-type; fam1-1,1-2,1-3, *FAM1* disruption mutants; Ectopic, *FAM1* ectopic transformant; FAM1-C, *FAM1* complemented transformant of the *FAM1* mutant. (C) Schematic representation of the *CoHEX1* gene disruption by homologous recombination. Binary plasmid pBII-HEX1AH3-2-7 contains the AMP::HPH cassette inserted into the *CoHEX1* gene between the left border (LB) and right border (RB) sequences. Through double crossing over, a ~9.0 kb *BamHI* fragment of the wild-type is predicted to be replaced by a 12-kb *BamHI* fragment containing the AMP::HPH cassette. (D) DNA blot analysis of *CoHEX1* disrupted transformants. Genomic DNA was digested with *XhoI* and probed with an internal fragment of *CoHEX1* indicated in the pBII-HEX1AH3-2-7 vector map. WT, wild-type; cohex-1-1, 1-2, 1-3, *CoHEX1* disruption mutants; Ectopic, *CoHEX1* ectopic transformant.