SUPPLEMENTAL MATERIALS

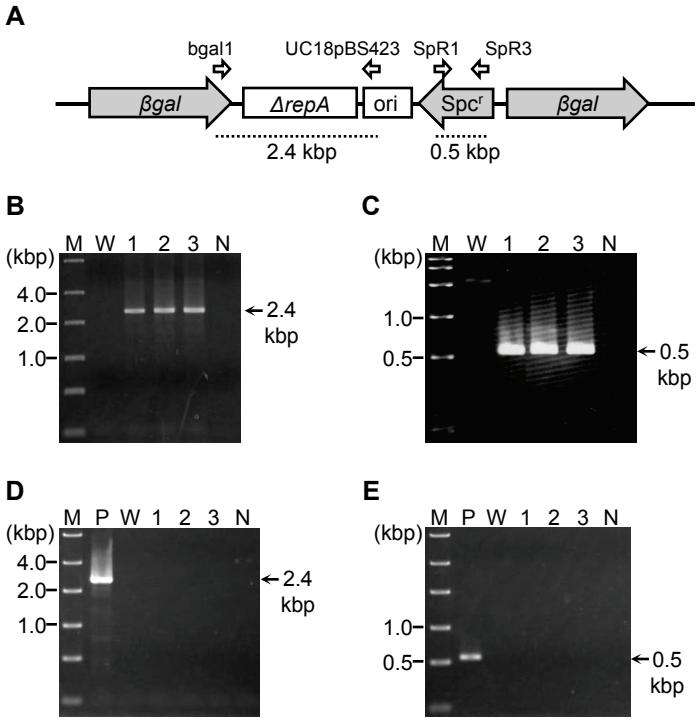

FIG. S1. (A) Predicted chromosomal structure of the *βgal* locus in the pBS423-*ΔrepAβgal* integrant after the first crossover. Primers used to check the genotype are shown as open arrows, with names above the predicted structures. The expected lengths of the amplicons generated using these primers are indicated by dashed lines below the predicted structure. (B) and (C) Genotype analysis of the pBS423-*ΔrepAβgal* transformants to confirm integration of pBS423-*ΔrepAβgal* at the *βgal* locus. Fragments amplified using (B) the bgal1/UC18pBS423 primer pair and (C) the SpR1/SpR3 primer pair were subjected to agarose gel electrophoresis. Gene Ladder Fast2 (Nippon Gene Co., Ltd., Tokyo, Japan) was used as a molecular weight marker (lane M). The sizes of representative marker fragments are shown to the left of each panel. Each lane shows amplified DNA generated from a DNA template: lane W, *B. longum* 105-A (wild-type); lanes 1-3, candidate first-crossover integrants; lane N, no template DNA (negative control for amplification). The sizes of the amplified fragments are shown to the right of each panel. (D) and (E) Genotype analysis of pTBR101-CM transformants to confirm excision and exclusion of pBS423-*ΔrepAβgal* in second-crossover recombinants. Fragments amplified using (D) the bgal1/UC18pBS423 primer pair and (E) the SpR1/SpR3 primer pair were subjected to agarose gel electrophoresis. Each lane shows amplified DNA generated from a DNA template: lane P, pBS423-*ΔrepAβgal* (positive control for amplification); lanes 1-3, candidate second-crossover recombinants. The other lanes are as in Fig. S1B and C. The sizes of the amplified fragments are shown to the right of each panel.

TABLE S1 Oligonucleotide primers used in this study

Primer name	Nucleotide sequence[a] (5' to 3')
Validation of vector integration/excision at the β-galactosidase gene locus	
bgal1	CACTCCCGCAGCGGTAAACGC
lacZFw	ACGCTGCAGGCGTTTACCGCTGCG
lacZRw	ACGCTGCAGCGGGTTGCCGCTCT
SpR1	CAGCCACTGCATTTCCCGCAA
SpR3	ATGTTTGGATCAGGAGTTGAGAGTGGA
UC18pBS423	GCTGGCCTTTTGCTCAGAATTCGAG
Construction and validation of α-galactosidase gene (*aga)* -deletion mutant	
aga1f	ACCACCGACAGAATGCCGGACACA
aga2r	CCGAGCGATTCACGCCAAGGGAAAG
aga8r	ATCCTGCAGGCACCTTCATTGGTGTCAAGG
aga11f	ATCCTGCAGCGAAACCAGTAGTGCAATGCC
aga12r	GAACAGGAGTTCGCCACCGCCGATT
aga13f	AATCGGCGGTGGCGAACTCCTGTTCGGCTGTGGCGTTAAGCTGACAGCCTAGT
SpR4	TCCACTCTCAACTCCTGATCCAAACAT
TB4_1	AACCCAACTCCACACCTTCTCGGAAGC
Construction of *aga* -complementation vector	
aga20f	TCAGTCGACCGCCCAGCGGCGATAGACAC
aga21r	CATGTCGACTTCGCTCAGGCCGTGGCTACT

[a] Underlined sequences correspond to restriction sites.