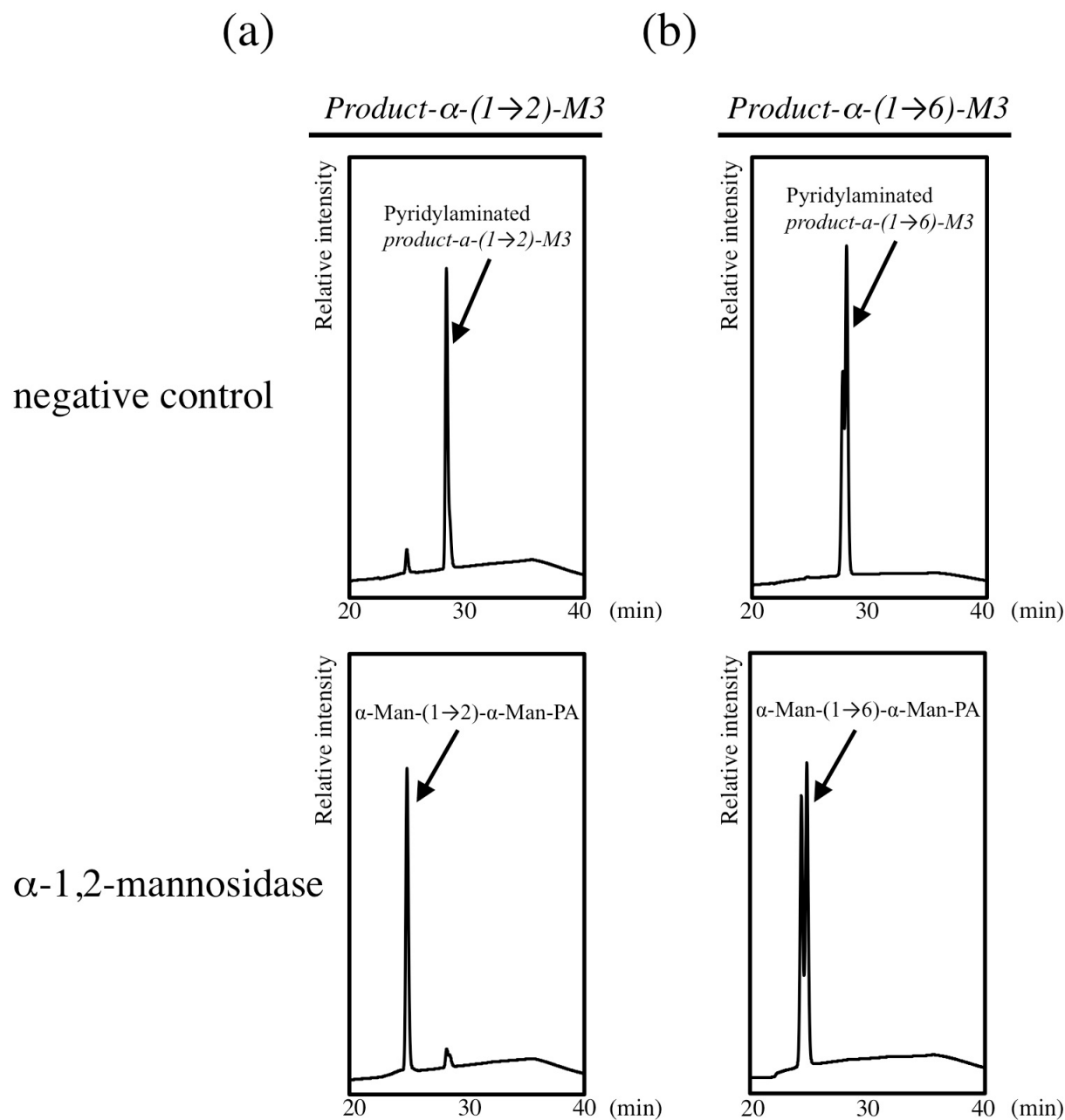


## Supplementary Materials

**Title:** Identification of Two Mannosyltransferases Contributing to Biosynthesis of the Fungal-type Galactomannan  $\alpha$ -Core-Mannan Structure in *Aspergillus fumigatus*

**Authors:** Takuya Onoue, Yutaka Tanaka, Daisuke Hagiwara, Keisuke Ekino, Akira Watanabe, Kazuyoshi Ohta, Katsuhiko Kamei, Nobuyuki Shibata, Masatoshi Goto and Takuji Oka

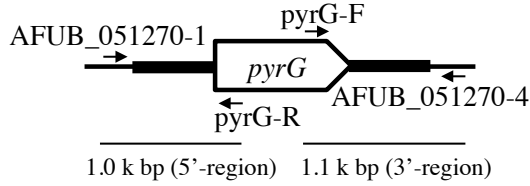
## Supplemental Figures



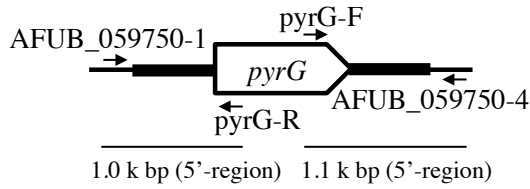
**Figure S1.** Determination of pyridylaminated *product- $\alpha$ -(1 $\rightarrow$ 2)-M3* (a) and *product- $\alpha$ -(1 $\rightarrow$ 6)-M3* (b) structures using substrate-specific  $\alpha$ -1,2-mannosidase. Upper panels display chromatographs of the purified pyridylaminated *product- $\alpha$ -(1 $\rightarrow$ 2)-M3* (a) and *product- $\alpha$ -(1 $\rightarrow$ 6)-M3* (b). These products, (a) and (b), could be digested by  $\alpha$ -1,2-mannosidase and converted to  $\alpha$ -Man-(1 $\rightarrow$ 2)- $\alpha$ -Man-PA (a) and  $\alpha$ -Man-(1 $\rightarrow$ 6)- $\alpha$ -Man-PA (b), respectively (lower panels).

A

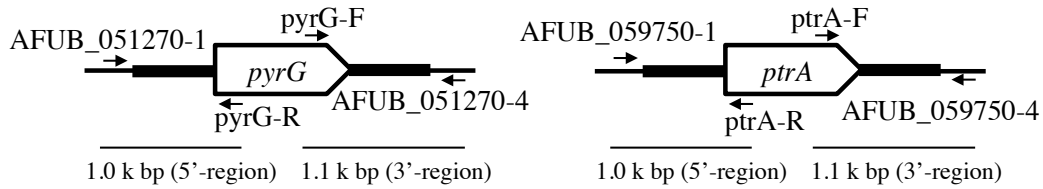
(a)  $\Delta cmsA$



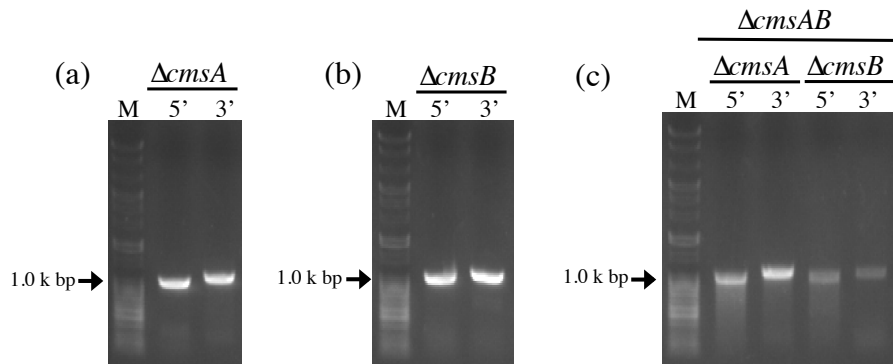
(b)  $\Delta cmsB$



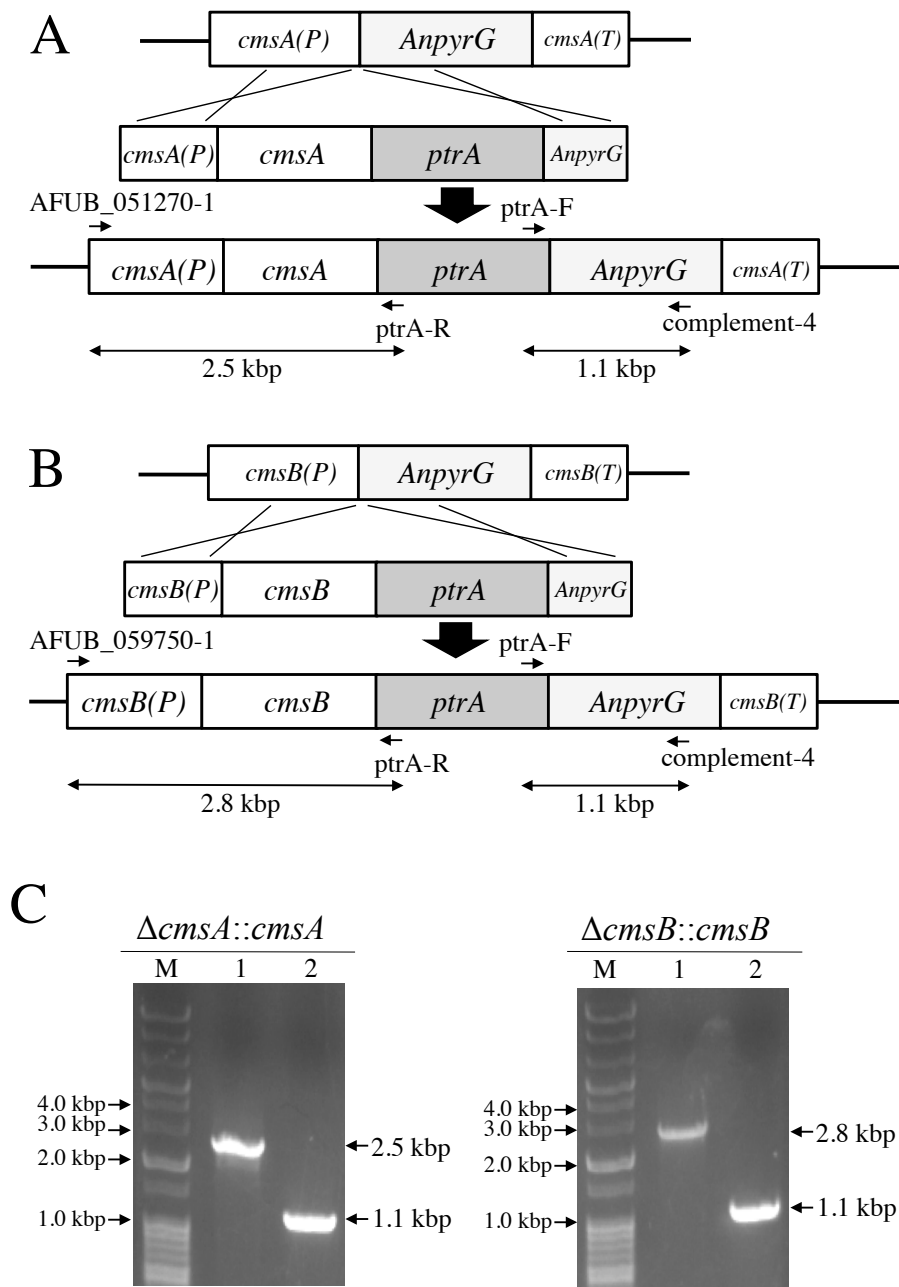
(c)  $\Delta cmsAB$



B

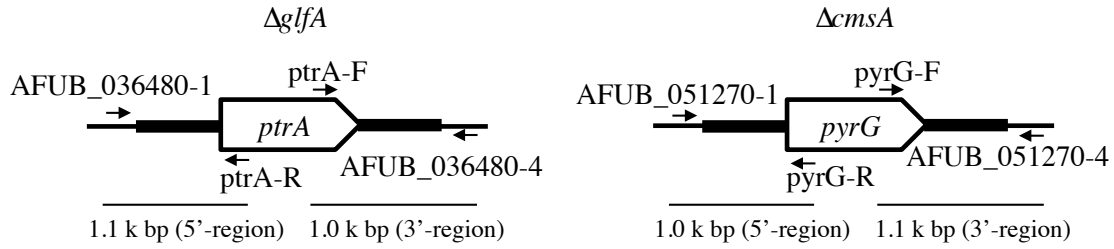


**Figure S2.** Construction of the core-mannan synthase disruptant strains  $\Delta cmsA$ ,  $\Delta cmsB$ , and  $\Delta cmsAB$ . **A.** Chromosomal maps of strains  $\Delta cmsA$  (a),  $\Delta cmsB$  (b), and  $\Delta cmsAB$  (c) and primers used for confirmation. The positions of the primers are indicated by arrows. **B.** Electrophoretic analyses of products amplified by PCR using the primer pairs AFUB\_051270-1/pyrG-R (5'-region) and pyrG-F/AFUB\_051270-4 (3'-region) for  $\Delta cmsA$  (a), AFUB\_059750-1/pyrG-R (5'-region) and pyrG-F/AFUB\_059750-4 (3'-region) for  $\Delta cmsB$  (b), and AFUB\_051270-1/pyrG-R (5'-region), pyrG-F/AFUB\_051270-4 (3'-region), AFUB\_059750-1/ptrA-R (5'-region), and ptrA-F/AFUB\_059750-4 (3'-region) for  $\Delta cmsAB$  (c). M: DNA size markers; Gene Ladder Wide 2 (Nippon Gene, Tokyo, Japan).

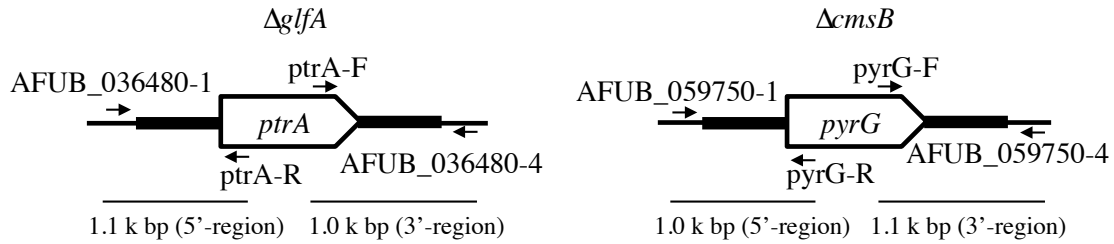


**Figure S3.** Construction of the  $\Delta cmsA$  and  $\Delta cmsB$  complementary strains  $\Delta cmsA::cmsA$  and  $\Delta cmsB::cmsB$ . **A.** Schematic representation of *cmsA* complementation with *cmsA*. *cmsA* (*P*), *cmsA* promoter; *cmsA* (*T*), *cmsA* terminator; *cmsA*, open reading frame of *cmsA*. The positions of the primers are indicated by arrows. **B.** Schematic representation of *cmsB* complementation with *cmsB*. *cmsB* (*P*), *cmsB* promoter; *cmsB* (*T*), *cmsB* terminator; *cmsB*, open reading frame of *cmsB*. The positions of the primers are indicated by arrows. **C.** Confirmation of correct recombination of the *cmsA* gene using PCR analysis. M, DNA size markers; Gene Ladder Wide 2; lane 1, DNA fragment (2.5 kbp) amplified using PCR and the primers AFUB\_051270-1 and ptrA-R; lane 2, DNA fragment (1.1 kb) amplified using PCR and the primers ptrA-F and complement-4. **D.** Confirmation of correct recombination of the *cmsB* gene using PCR analysis. M, DNA size markers; Gene Ladder Wide 2; lane 1, DNA fragment (2.8 kbp) amplified using PCR and the primers AFUB\_059750-1 and ptrA-R; lane 2, DNA fragment (1.1 kb) amplified using PCR and the primers ptrA-F and complement-4.

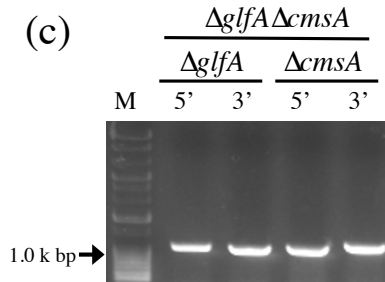
(a)  $\Delta glfA \Delta cmsA$



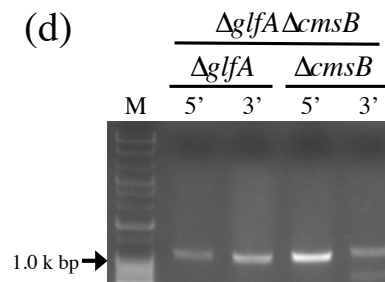
(b)  $\Delta glfA \Delta cmsB$



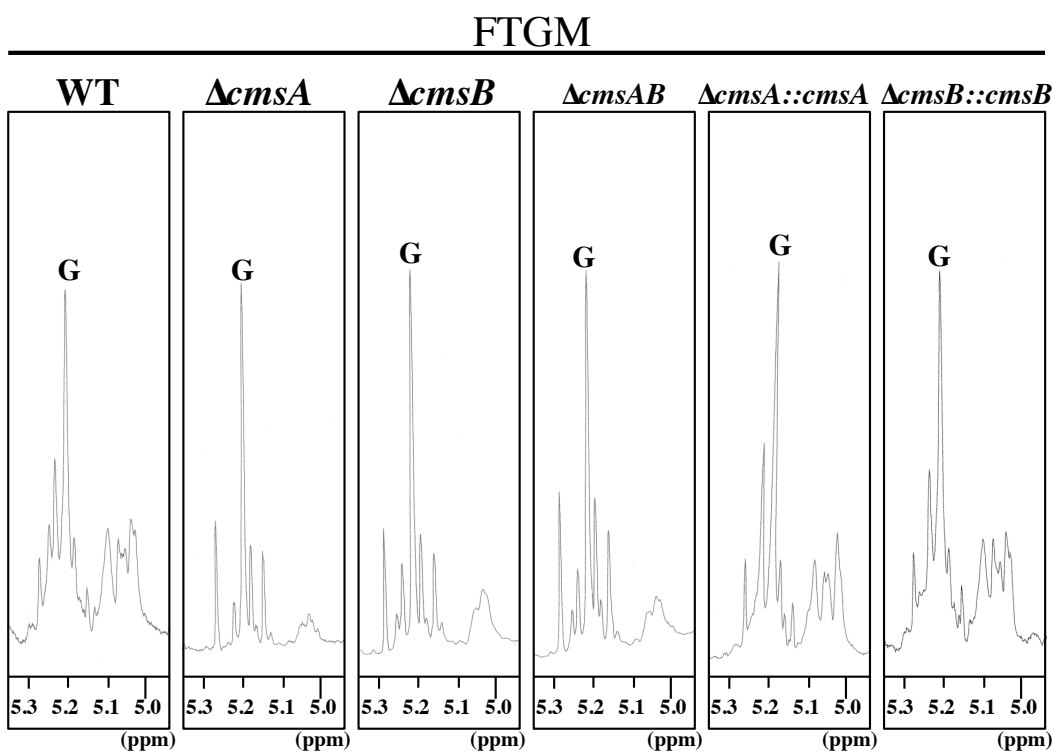
(c)



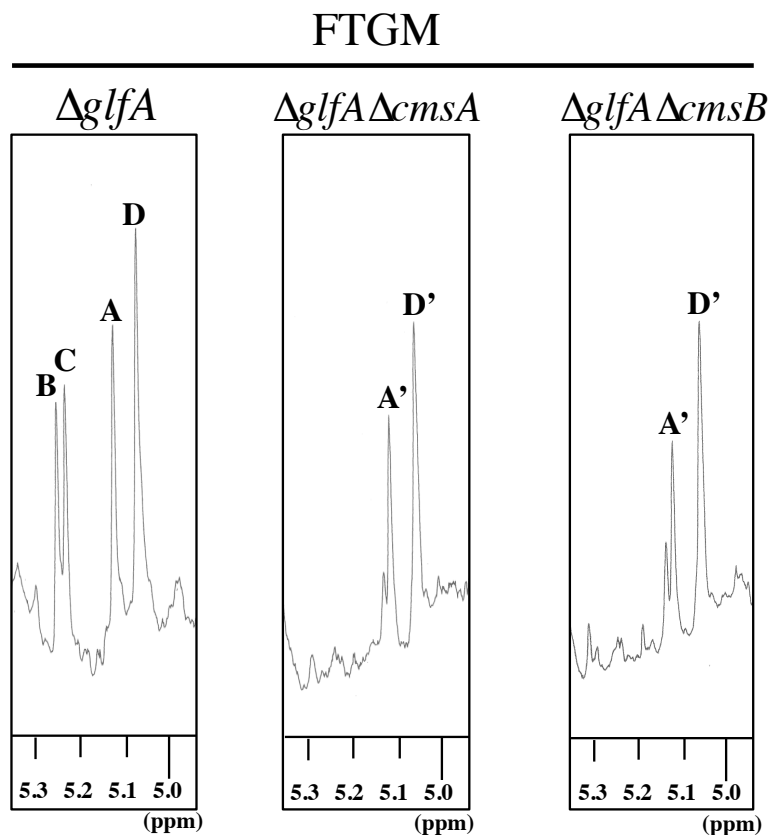
(d)



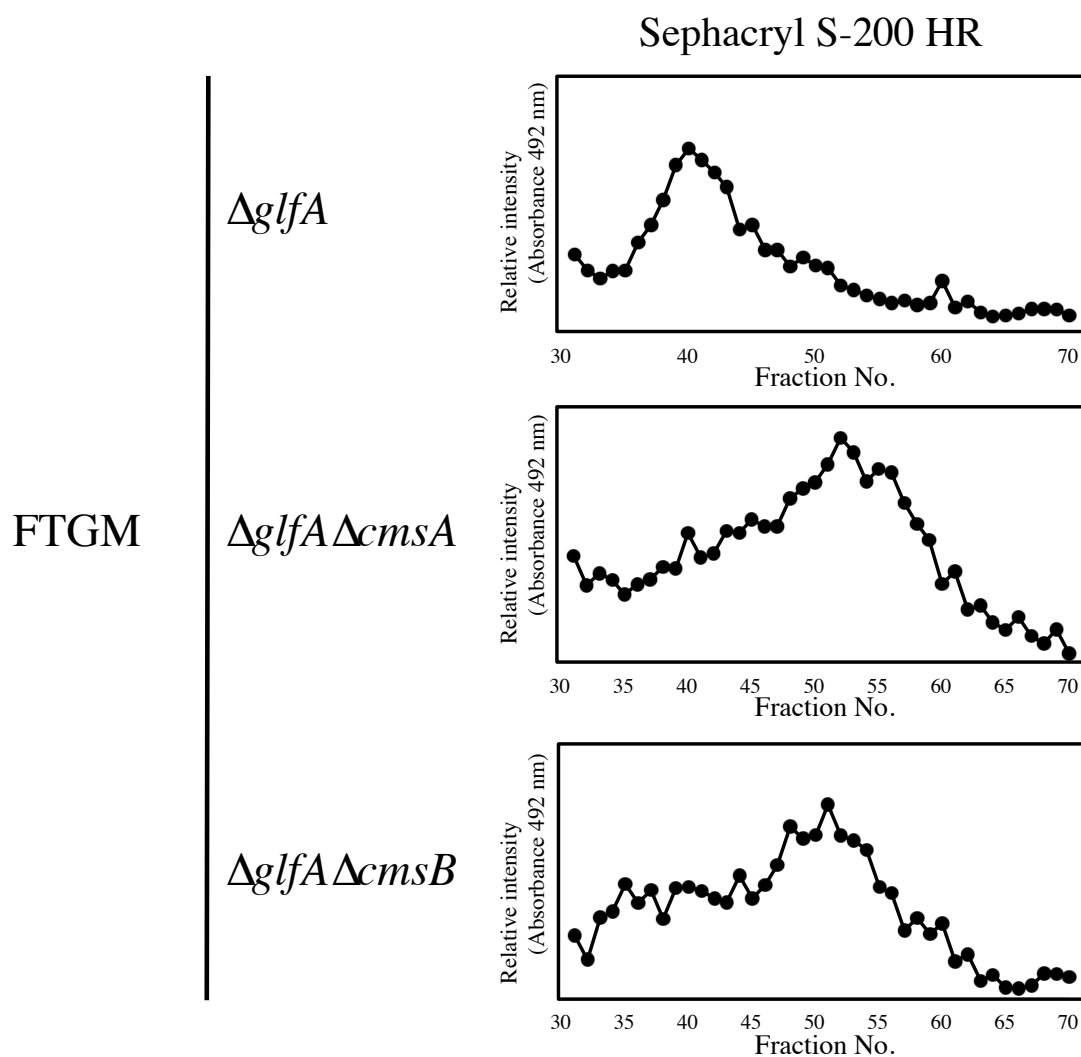
**Figure S4.** Construction of the  $\Delta glfA \Delta cmsA$  and  $\Delta glfA \Delta cmsB$  strains. **A.** Chromosomal maps of strains  $\Delta glfA \Delta cmsA$  (a) and  $\Delta glfA \Delta cmsB$  (b), and primers used for confirmation. The positions of the primers are indicated by arrows. **B.** Electrophoretic analyses of products amplified by PCR using the primer pairs AFUB\_036480-1/*ptrA*-R (5'-region), *ptrA*-F/AFUB\_036480-4 (3'-region), AFUB\_051270-1/*pyrG*-R (5'-region), and *pyrG*-F/AFUB\_051270-4 (3'-region), for  $\Delta glfA \Delta cmsA$  (c), AFUB\_036480-1/*ptrA*-R (5'-region), *ptrA*-F/AFUB\_036480-4 (3'-region) AFUB\_059750-1/*pyrG*-R (5'-region), and *pyrG*-F/AFUB\_059750-4 (3'-region), for  $\Delta glfA \Delta cmsB$  (d). M: DNA size markers; Gene Ladder Wide 2.



**Figure S5.** <sup>1</sup>H-NMR spectra of FTGM from strains A1151 (WT),  $\Delta cmsA$ ,  $\Delta cmsB$ ,  $\Delta cmsAB$ ,  $\Delta cmsA::cmsA$ , and  $\Delta cmsB::cmsB$ . FTGM was prepared from total GM (FTGM+OMGM) by treatment with 0.5 M NaBH<sub>4</sub>/0.1 M NaOH for 24 h (β-elimination). The signal G at 5.195 ppm in the <sup>1</sup>H-NMR spectra is from H-1 at the C-1 position of the underlined Gal $\underline{f}$  residue in -β-Gal $\underline{f}$ -(1→5)-β-Gal $\underline{f}$ -(1→5)-β-Gal $\underline{f}$ -(1→5)-. The proton chemical shifts were referenced relative to internal acetone at δ 2.225 ppm.



**Figure S6.** <sup>1</sup>H-NMR spectra of FTGM from strains  $\Delta glfA$ ,  $\Delta glfA \Delta cmsA$ , and  $\Delta glfA \Delta cmsB$ . Total GM (FTGM+OMGM) was extracted and FTGM prepared by treatment with 0.5 M NaBH<sub>4</sub>/0.1 M NaOH for 24 h (β-elimination). The signals A (at 5.104 ppm), B (5.233), C (5.216), and D (5.054) in the <sup>1</sup>H-NMR spectra are from H-1 at the C-1 position of the underlined Man residue in the structures -(1→6)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→6)- (A), -(1→6)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→6)- (B), -(1→6)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→6)- (C) and -(1→6)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→6)- (D). The signals A' and D' at 5.1 and 5.05 ppm of the <sup>1</sup>H-NMR spectra are from the H-1 at the C-1 position of the underlined Man residue in t-Man-(1→6)-α-Man- and t-Man-(1→2)-α-Man-, respectively. The proton chemical shifts were referenced relative to internal acetone at δ 2.225 ppm.



**Figure S7.** Gel filtration analyses of FTGM from strains  $\Delta glfA$ ,  $\Delta glfA \Delta cmsA$ , and  $\Delta glfA \Delta cmsB$ . Total GM (FTGM+OMGM) was extracted and FTGM prepared by treatment with 0.5 M  $\text{NaBH}_4$ /0.1 M NaOH for 24 h ( $\beta$ -elimination). FTGM was analyzed by gel filtration chromatography using a Sephacryl S-200 HR (1  $\times$  75 cm) column and 0.8 M NaCl as eluent.