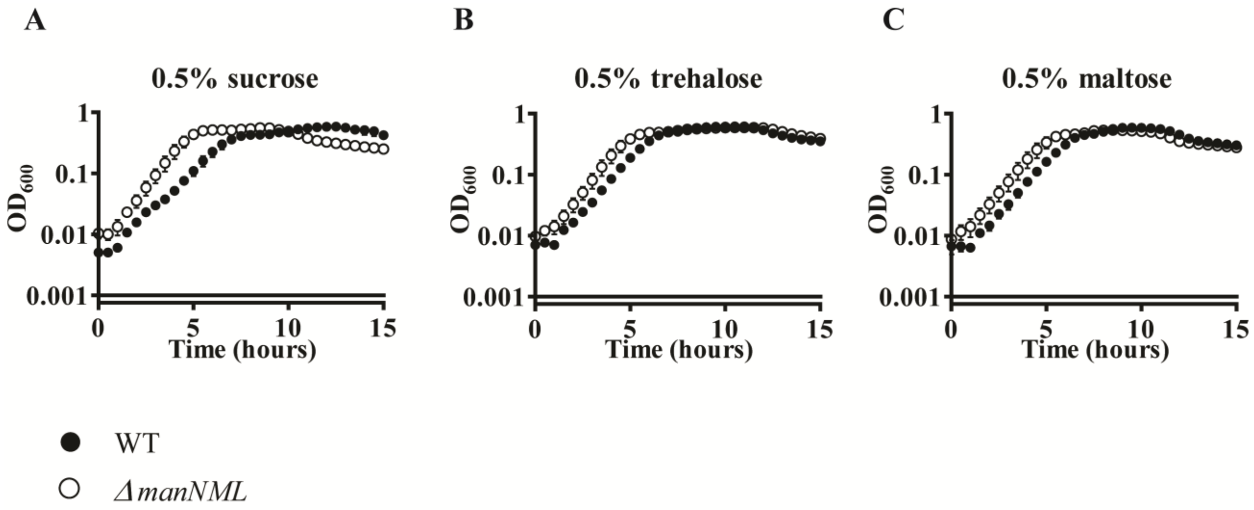
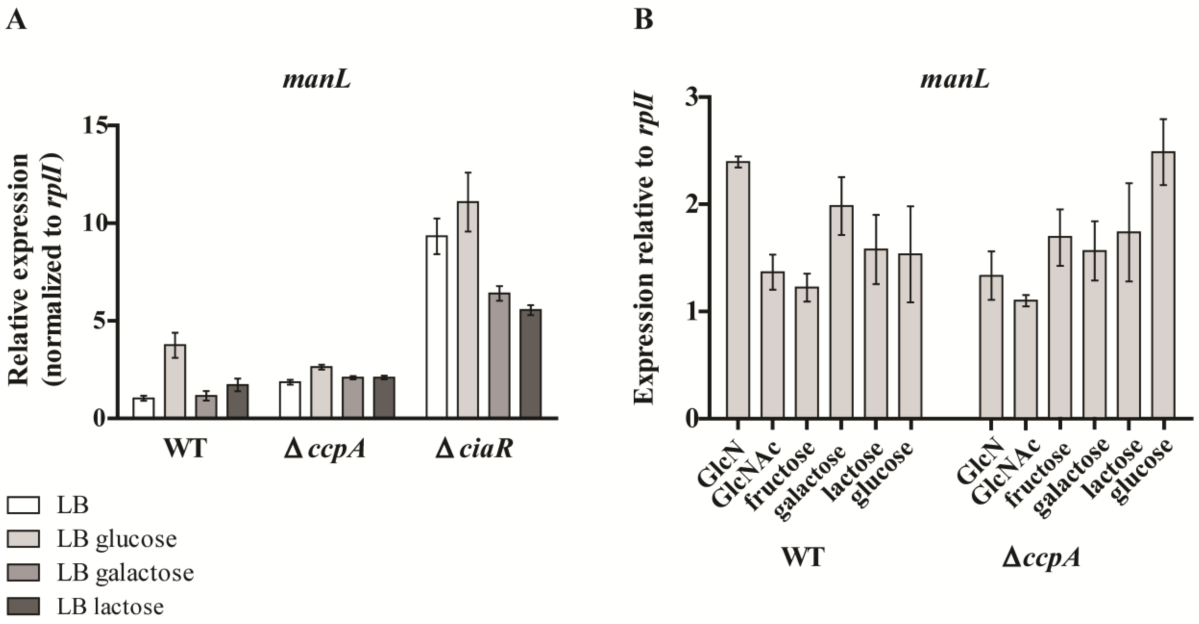
Supplemental information

ManLMN is a glucose transporter and central metabolic regulator in *Streptococcus pneumoniae*

Eleanor Fleming and Andrew Camilli*

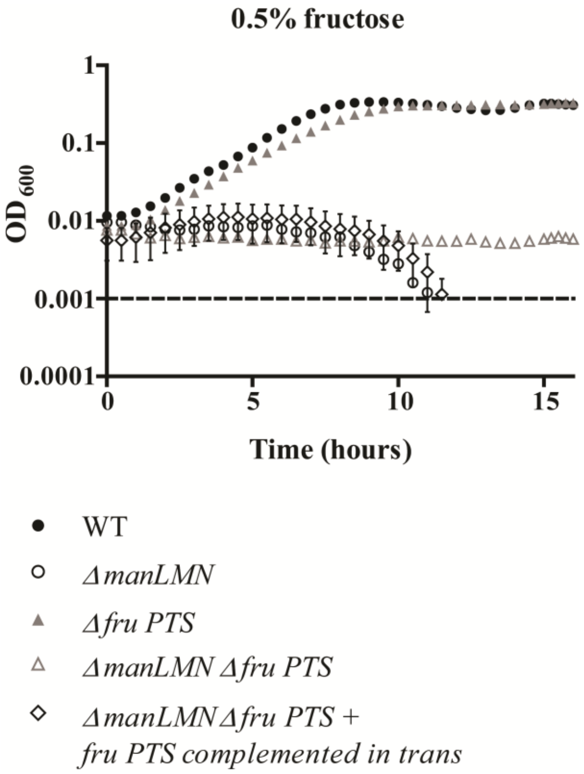


Supplemental figure 1. The Δ*manLMN* strain grows like WT on sucrose and glucose disaccharides WT (closed circles) and Δ*manLMN* (open circles) were grown in chemically defined medium (CDM) with 0.5% final concentration of one of the following carbohydrates; sucrose (**A**), trehalose (**B**), and maltose (**C**). Absorbance at 600 nm was measured every 30 minutes over the course of 15 hours of growth at 37°C. Each data point represents the average of at least six biological replicates from at least two separate days. Error bars represent the standard error of the mean (SEM).



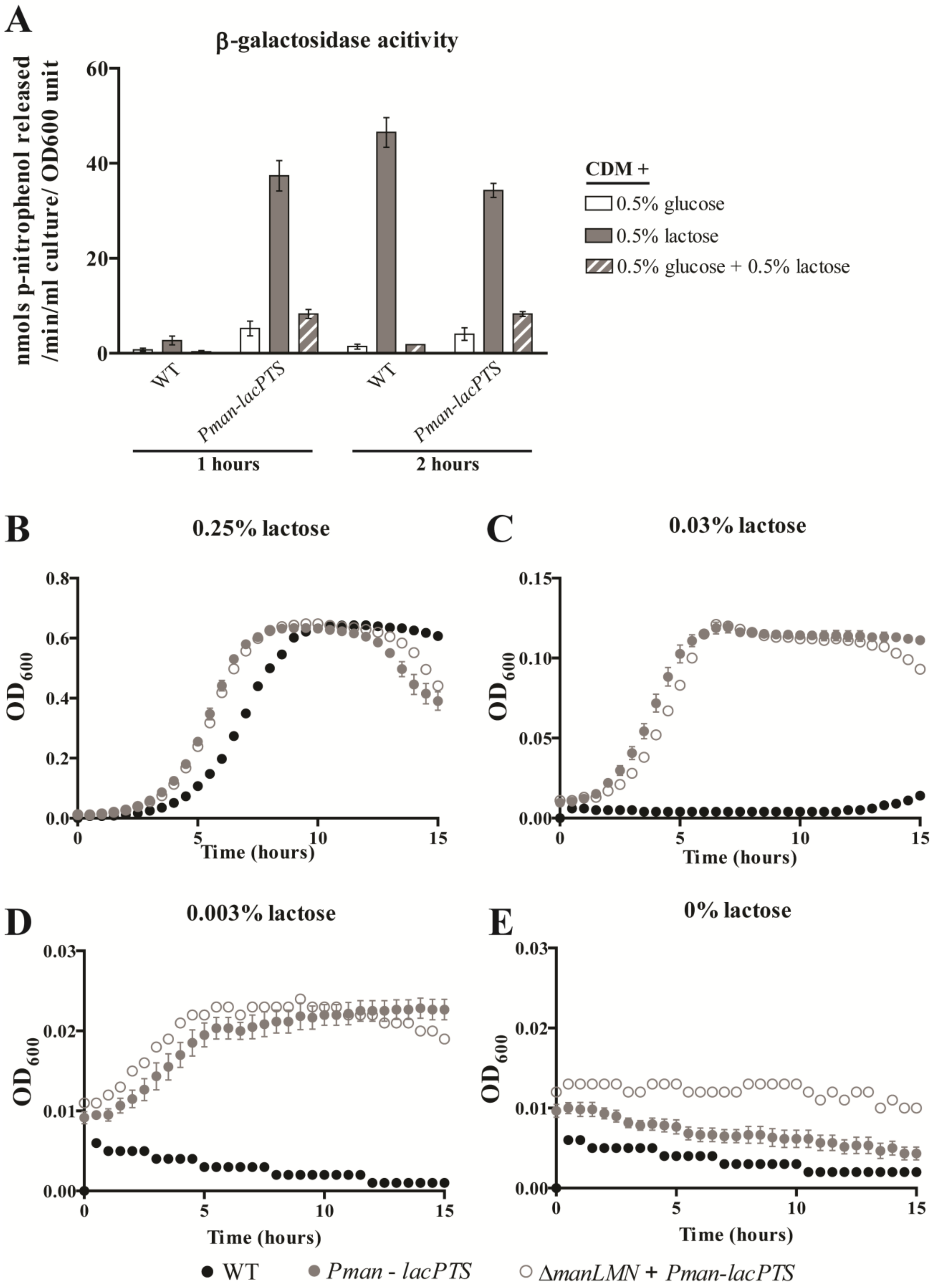
Supplemental figure 2. Additional transcriptional analysis of *manLMN*

Transcription of *manL* as a representative of the *manLMN* operon was quantified by quantitative reverse transcriptase PCR (qRT-PCR), during mid-exponential growth of WT and Δ*ccpA* in CDM with 0.5% GlcN, GlcNAc, fructose, galactose, lactose, or glucose (**A**). Transcription of *manL* was also assessed during mid-exponential growth of WT, Δ*ccpA*, and Δ*ciaR* in LB and LB with added glucose, galactose, or lactose (**B**). Each bar represents the average of at least five biological replicates collected over multiple days normalized to *rplI*. Error bars represent the SEM. In **B**, values are reported relative to WT in plain LB.



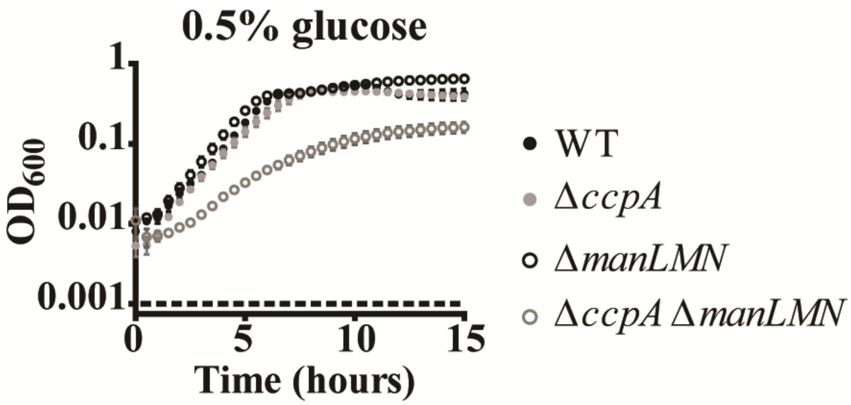
Supplemental figure 3. Constitutive expression of fructose-specific PTS system does not rescue growth of Δ*manLMN* in fructose

Growth of the ManLMN-fru PTS double deletion strain (Δ*manLMN* Δ*fru PTS*) and its corresponding complemented strain (Δ*manLMN* Δ*fru PTS+ fru PTS* complemented *in trans*) was compared to growth of WT and Δ*manLMN* in fructose. Optical density readings were taken every 30 minutes over the course of 15 hours at 37°C. Each data point represents the average of six biological replicates collected on multiple days and error bars represent the SEM, for all samples except the WT and Δ*manLMN* controls for which one representative replicate is shown.


A
β-galactosidase acitivity
nmols p-nitrophenol released /min/ml culture/ OD600 unit
60
40
20
0
WT
Pman-lacPTS
WT
Pman-lacPTS
1 hours
2 hours
CDM +
0.5% glucose
0.5% lactose
0.5% glucose + 0.5% lactose
B
0.25% lactose
OD600
0.8
0.6
0.4
0.2
0.0
0
5
10
15
Time (hours)
C
0.03% lactose
OD600
0.15
0.10
0.05
0.00
0
5
10
15
Time (hours)
D
0.003% lactose
OD600
0.03
0.02
0.01
0.00
0
5
10
15
Time (hours)
E
0% lactose
OD600
0.03
0.02
0.01
0.00
0
5
10
15
Time (hours)
● WT
● Pman - lacPTS
○ ΔmanLMN + Pman-lacPTS

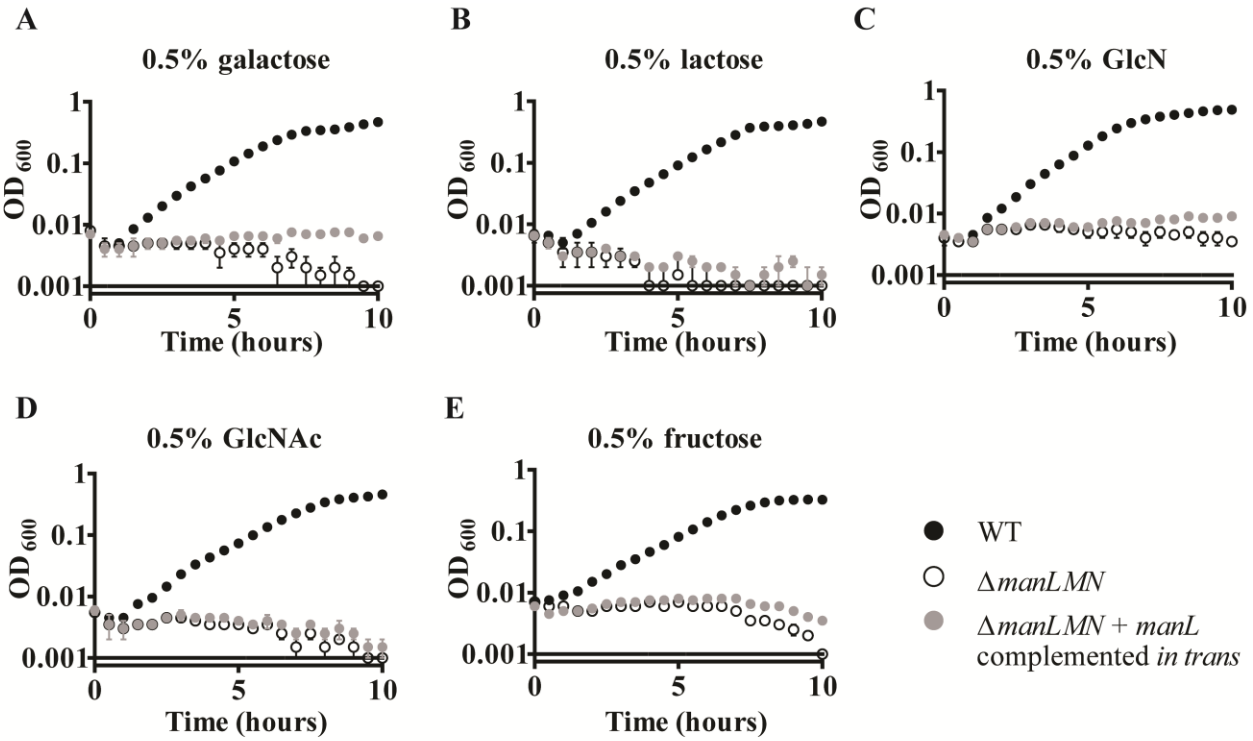
Supplemental figure 4. Constitutive expression of the lac PTS alone does not prevent CCR of β-galactosidase activity

(**A**) Mid-exponential THY cultures of WT and Δ*lac PTS + lac PTS* complemented *in trans* (P_{man} – *lac PTS*) were washed and switched to CDM containing 0.5% glucose, 0.5% lactose or 0.5% glucose + 0.5% lactose. Samples were collected one and two hours after switching to the CDM conditions. Each bar represents the average of five biological replicates collected on multiple days. Error bars represent the SEM. Growth of the P_{man} – *lac PTS* (grey closed circles) strain compared to WT (closed black circles), and Δ*manLMN* Δ*lac PTS + lacPTS* complemented *in trans* was analyzed in the following limiting lactose conditions; 0.25% lactose (**B**), 0.03% lactose (**C**), 0.003% lactose (**D**), and 0% lactose (**E**). Optical density readings were taken every 30 minutes over the course of 15 hours at 37°C. Each data point represents the average of six biological replicates collected on two separate days and error bars represent the SEM, for all samples except the WT and Δ*manLMN* Δ*lac PTS + lacPTS* complemented *in trans* controls for which one representative replicate is shown.



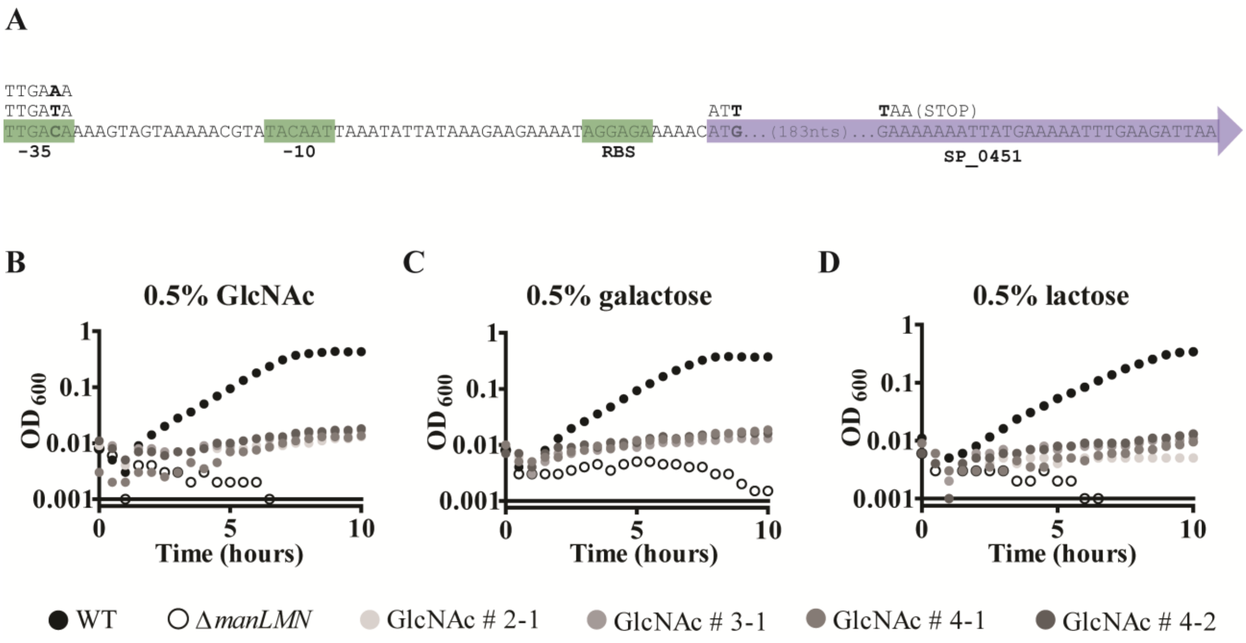
Supplemental figure 5. The Δ*manLMN* Δ*ccpA* strain has a defect for growth in glucose

Growth of WT, Δ*manLMN*, Δ*ccpA* and Δ*manLMN* Δ*ccpA* in 0.5% glucose CDM was monitored by recording the absorbance at 600 nm every 30 minutes over the course of 15 hours of growth at 37°C. Each data point represents the average of at least six biological replicates collected on multiple days and error bars represent the SEM.



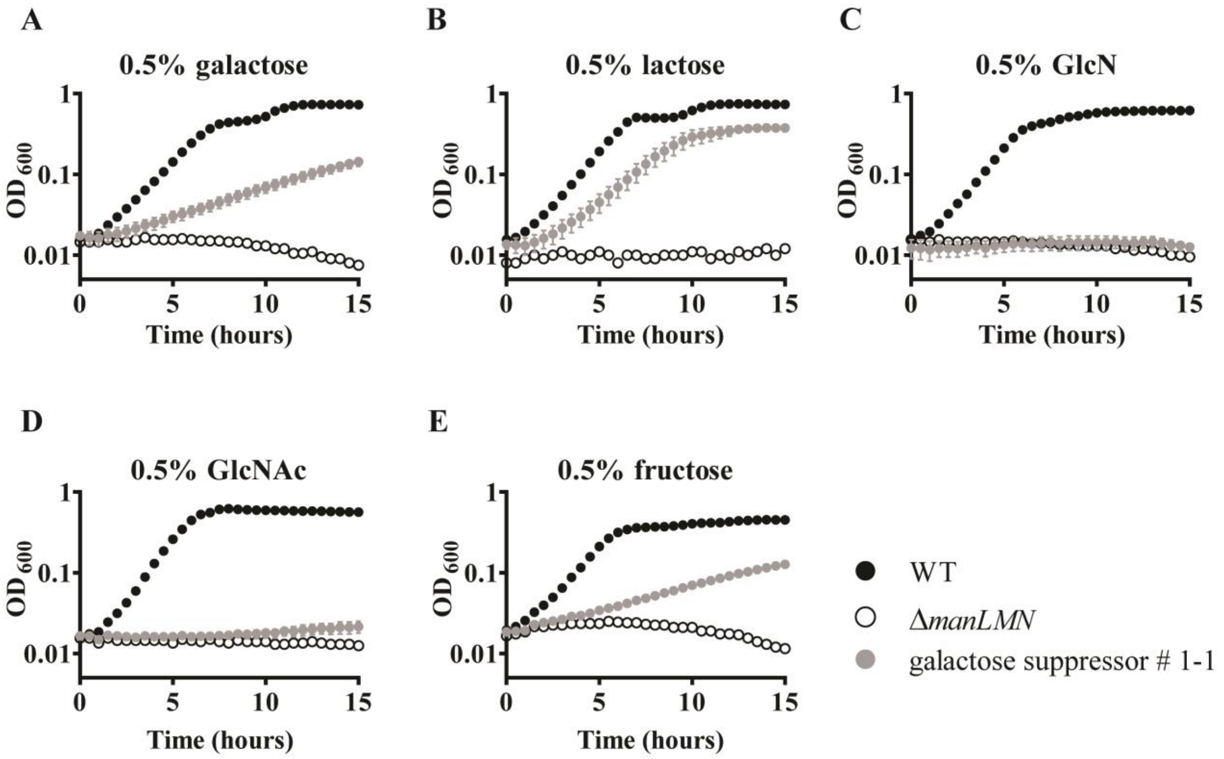
Supplemental figure 6. Complementation *in trans* with *manL* is not sufficient to restore growth to Δ*manLMN*

WT, Δ*manLMN*, the *manL* complemented strain were grown in chemically defined medium (CDM) with 0.5% final concentration of one of the following carbohydrates; galactose (**A**), lactose (**B**), GlcN (**C**), GlcNAc (**D**), or fructose (**E**). Absorbance at 600 nm was measured every 30 minutes over the course of 15 hours of growth at 37°C. Each data point represents the average of at least six biological replicates from at least two separate days. Error bars represent the standard error of the mean (SEM).



Supplemental figure 7. Suppressor mutations affecting SP_0451

The location of each suppressor mutation is shown in the SP_0451 promoter and coding region in **A.** Growth of these four suppressor isolates was compared to WT and Δ*manLMN* controls in CDM with 0.5% of GlcNAc (**B**), galactose (**C**), or lactose (**D**). Each data point represents the average of at least four biological replicates collected on two separate days with error bars representing the standard error of the mean.



Supplemental figure 8. Growth analysis of suppressor mutation affecting SP_1473

Growth of *galactose suppressor #1-1* was compared to WT and Δ*manLMN* controls in CDM with 0.5% of galactose (**A**), lactose (**B**), GlcN (**C**), GlcNAc (**D**), or fructose (**E**). Each data point represents the average of at least four biological replicates collected on two separate days with error bars representing the standard error of the mean.

Supplemental Table 1.

Promoter name	Genomic location		Operon	Notes	Reference
	Start	Stop			
P_{lacA}	1129831	1129986	Tagatose-6-phosphate pathway; *lacABCD*	includes validated LacR-2 binding site	Fleming *et al.*, 2015
P_{nagB}	1334475	1334624	glucosamine-6-phosphate deaminase; *nagB*	includes predicted NagR binding site (93.75% homologus to validated *B. subtilis* binding site)	Bertram *et al.*, 2011
P_{fruR}	822532	822706	fructose operon; *fruRCA*	includes predicted *cre* site and 4 predicted FruR binding motifs validated in *L. lactis*	Barriére *et al.*, 2005

This chart shows the promoter regions used for the α-galactosidase reporter constructs. The promoter name, genomic location in the TIGR4 genome, the gene(s) regulated by the promoter, and relevant validated or predicted transcriptional regulator binding sites are listed. P_{lacA} was used to assay for galactose and lactose-dependent induction. P_{nagB} was used to assay for GlcN and GlcNAc-dependent induction. P_{fruR} was used to assay for fructose-dependent induction.

References

Barriere, C., Veiga-da-Cunha, M., Pons, N., Guedon, E., van Hijum, S.A., Kok, J., *et al.* (2005) Fructose Utilization in *Lactococcus lactis* as a Model for Low-GC Gram-Positive Bacteria: Its Regulator, Signal, and DNA-Binding Site. *J Bacteriol* **187**: 3752–3761.

Bertram, R., Rigali, S., Wood, N., Lulko, A.T., Kuipers, O.P., and Titgemeyer, F. (2011) Regulon of the N-acetylglucosamine utilization regulator NagR in *Bacillus subtilis*. *J Bacteriol* **193**: 3525–3536.

Fleming, E., Lazinski, D.W., and Camilli, A. (2015) Carbon catabolite repression by seryl phosphorylated HPr is essential to *Streptococcus pneumoniae* in carbohydrate rich environments. *Mol Microbiol* **97**: 360–380.

Supplemental Table 2. Suppressor mutations that rescue growth of Δ *manLMN*

Strain or suppressor	**Genetic background**	**Consequence of mutation**	**Precited or verified function**	**Reference[a]**
manLMN::CHESH	*manLMN::CHESH*	SP_1002 Glu208*	uncharacterized lipoprotein	
manLMN::spec	*manLMN::spec*	SP_0647 Ala165Thr	galactose PTS EIIC	(Bidossi *et al.*, 2012)
		SP_0921 Glu286*	agmatine deiminase	
		SP_1027 Ser95Leu	hypothetical protein	
		SP_1564 synonymous mutation	hypothetical protein	
gal # 1-1	*manLMN::CHESH*	SP_0350 Gly125Asp	Cps4E, capsule biosynthesis protein	(Kolkman *et al.*, 1997)
		SP_1473 start codon mutation (ATG→ATA)	hypothetical small protein	
gal # 1-3	*manLMN::CHESH*	SP_1186 Ala53Asp	lac PTS EIIA	(Bidossi *et al.*, 2012)
gal #1-4	*manLMN::CHESH*	SP_1186 Ala31Asp	lac PTS EIIA	(Bidossi *et al.*, 2012)
gal # 3-1	*manLMN::CHESH*	G→C 85 bp upstream of SP_1187	LacT, transcriptional antiterminator	Afzal *et al.*, 2014
		SP_0927 Thr245Ile	SmrC, transcriptional regulator	Lau *et al.*, 2001
		SP_1242 Val128Ile	amino acid ABC ATPase	
		A_{11}→A_{12} 48 bp upstream of SP_1773	degenerate transposase	
gal # 3-2	*manLMN::CHESH*	T_{13}→T_{12} 48 bp upstream of SP_1331 (f=57%)	phospho-sugar binding transcriptional regulator	
		SP_1824 Gly239Val (f=53%)	ABC transporter permease	
		SP_1772 Thr2751Ala (f=52%)	PsrP, glycosylated serine rich repeat surface protein	Obert *et al.*, 2006
gal # 3-7	*manLMN::CHESH*	C→T 26bp upstream of SP_0925 (f= 66%)	hypothetical protein	
		SP_1185 Val466Gly (f = 75%),	lac PTS EIIB	Bidossi *et al.*, 2011
		A_9→A_8 79 bp upstream of SP_0138 (f=57%)	hypothetical protein	
GlcN # 1-3/ # 1-4	*manLMN::CHESH*	SP_0576 Tyr266Asp	LicT, transcriptional antiterminator	
		C→A mutation upstream of SP_0406	MutS2 family protein	
Fru # 2-3	*manLMN::CHESH*	SP_0877 Gly37Cys	fru PTS EIIABC	Bidossi *et al.*, 2011
fru #3-1	*manLMN::CHESH*	SP_0758 promoter mutation	uncharacterized PTS EIIABC	

		SP_1895 Gly48Val	raffinose ABC transporter permease	Rosenow *et al.*, 1999
fru #4-2	*manLMN::CHESH*	SP_0877 Ala484Asp	fru PTS EIIABC	Bidossi *et al.*, 2011
		SP_2112 Arg21Pro	maltose operon transcriptional repressor	Lacks, 1968, Puyet *et al.*, 1993
GlcNAc # 1-2	*manLMN::CHESH*	ΔT 47bp upstream of SP_1331	phospho-sugar binding transcriptional regulator	
		SP_0369 Ala204Pro	PBP1A (penicillin binding protein)	Martin *et al.*, 1992
		SP_1000 Ala141Ser	thioredoxin family protein	
		LacR-2 Gly156Cys	galactose operon transcriptional repressor	Fleming *et al.*, 2015
		SP_1772 Thr2751Ala	PsrP, glycosylated serine rich repeat surface protein	Obert *et al.*, 2006
GlcNAc # 1-4	*manLMN::CHESH*	C→A 43 bp upstream of SP_0060	BgaC and galactose/GlcNAc PTS operon	Jeong *et al.*, 2009
		C→T SP_1769	glycosyl transferase (already has an authentic frameshift)	
GlcNAc # 2-1	*manLMN::CHESH*	SP_0451 start codon mutation (ATG→ATT)	hypothetical small protein	
		SP_0475 Leu53Phe	cellobiose PTS EIIC	
		SP_0927 Glu20Lys	SmrC, transcriptional regulator	Lau *et al.*, 2001
GlcNAc # 3-1	*manLMN::CHESH*	SP_0451 Glu63*	hypothetical small protein	
		SP_0505 Ala51Val	HsdS, Type 1 restriction modification system S subunit	Manso *et al.*, 2014
		SP_1433 Ala182Asp	AraC family transcriptional regulator	
GlcNAc # 4-1	*manLMN::CHESH*	SP_0451 -35 mutation	hypothetical small protein	
GlcNAc # 4-2	*manLMN::CHESH*	SP_0451 -35 mutation	hypothetical small protein	
		SP_1647 Asp101Ala	PepO, endopeptidase O	Agarwal *et al.*, 2013
		SP_2208 Arg279Ser	putative helicase	
s.lac # 1-2	*manLMN::spec*	SP_0927 Thr245Ile	SmrC, transcriptional regulator	Lau *et al.*, 2001
		SP_0893 His8Tyr	putative transcriptional regulator	
		SP_1651 Arg64Cys	tpx, thiolredocine-dependent thiol peroxidase	
s.lac # 1-3b	*manLMN::spec*	SP_0927 Ser124Leu	SmrC, transcriptional regulator	Lau *et al.*, 2001
s.lac # 3-1	*manLMN::spec*	SP_0927 Glu20Lys	SmrC, transcriptional regulator	Lau *et al.*, 2001
		SP_1367 Leu224Phe	LicD3, lipopolysaccharide cholinephosphotransferase	
		SP_1989 Ile7Ser	PclR, phospholipase C transcriptional regulator	
s.lac # 3-4	*manLMN::spec*	SP_0927 Thr202Ile	SmrC, transcriptional regulator	Lau *et al.*, 2001
s.lac # 10	*manLMN::spec*	SP_0927 Glu20Lys	SmrC, transcriptional regulator	Lau *et al.*, 2001

		SP_0293 Thr75Pro	hypothetical protein	
		SP_1206 Gly64*	XseB, exodeoxyribonuclease VII small subunit	
		SP_1196 C→T	degenerate transposase	
		SP_1905 C→T	degenerate transposase	
s.gal # 1-1	*manLMN::spec*	C→T 2 bp upstream of SP_2173	DltD, lipoteichoic acid synthesis	Kovacs *et al.*, 2006
s.gal # 2-1	*manLMN::spec*	SP_2176 Glu67*	DltA, lipoteichoic acid synthesis	Kovacs *et al.*, 2006
		SP_0293 Thr75Pro	hypothetical protein	
		SP_1206 Gly64*	XseB, exodeoxyribonuclease VII small subunit	
		SP_1196 C→T	degenerate transposase	
		SP_1905 C→T	degenerate transposase	
s.gal # 2-4	*manLMN::spec*	SP_0151 Val323fs	ABC transporter ATP binding protein	
		C→T 2 bp upstream of SP_2173	DltD, lipoteichoic acid synthesis	Kovacs *et al.*, 2006
s.GlcNAc # 1-2	*manLMN::spec*	SP_0436 C→A (synonymous)	GatB, Asn/Gln-tRNA formation	
		SP_0577 Met107Ile	β-glucoside PTS EIIABC	Bidossi *et al.*, 2011
		SP_1733 Glu92*	putative phosphatase	
s.GlcNAc # 2-4	*manLMN::spec*	SP_0058 Ile9Arg	GntR family transcriptional repressor	Fleming *et al.*, 2015
s.GlcN # 1-2	*manLMN::spec*	SP_0927 Pro103Leu	SmrC, transcriptional regulator	Lau *et al.*, 2001
		SP_0577 His534Tyr	β-glucoside PTS EIIABC	Bidossi *et al.*, 2011
		SP_0293 Thr75Pro	hypothetical protein	
		SP_1206 Gly64*	XseB, exodeoxyribonuclease VII small subunit	
		SP_1196 C→T	degenerate transposase	
		SP_1905 C→T	degenerate transposase	
s.GlcN # 2	*manLMN::spec*	SP_0293 Thr75Pro	hypothetical protein	
		SP_0577 His534Tyr	LicT, transcriptional antiterminator	
		SP_1206 Gly64*	XseB, exodeoxyribonuclease VII small subunit	
		SP_1196 C→T	degenerate transposase	
		SP_1905 C→T	degenerate transposase	
s.GlcN # 2-3	*manLMN::spec*	SP_0577 His519Asn	β-glucoside PTS EIIABC	Bidossi *et al.*, 2011

		SP_ 1356 Glu334*	chlorohydrolase	
		T→G 32bp upstream of SP_1798	ABC transporter permease	
s.GlcN # 1-1	*manLMN::spec*	SP_0927 Ser138Ile	SmrC, transcriptional regulator	Lau *et al.*, 2001
		SP_0576 Pro117Leu	LicT, transcriptional antiterminator	
s.fru # 3-1	*manLMN::spec*	SP_0577 Ser471*	β-glucoside PTS EIIABC	Bidossi *et al.*, 2011
		C→A 16bp upstream of SP_0835	purine nucleoside phosphorylase	
		SP_0877 Phe44Ser,	fru PTS EIIABC	Bidossi *et al.*, 2011
		SP_1084 synonymous C→A	methionine aminopeptidase	
s.fru # 1	*manLMN::spec*	SP_0877 Ser442*	fru PTS EIIABC	Bidossi *et al.*, 2011
		SP_0195 TER (TGA→TGG), SP_0196 Asn6Lys	hypothetical proteins	
s.fru # 1-1	*manLMN::spec*	SP_0151 Gln97His,	ABC transporter ATP binding protein	
		6bp deletion in SP_1177 (f= 65%)	HPr, PTS phosphocarrier protein	Bidossi *et al.*, 2011
		SP_1383 Val473Ille	AlaS, alanyl tRNA-synthetase	
		SP_1859 Leu142Arg	PnuC-like, ribosyl nicotinamide transporter	
		SP_2017 Gly377Cys	hypothetical protein	
		SP_2223 Thr194Ile	hypothetical protein	
s.fru # 1-3	*manLMN::spec*	SP_0152 Trp145*	ABC transporter permease	
		SP_0585 Leu90Phe	homocysteine methyltransferase	
		polymorphism 33nts 3' of SP_1177	HPr , PTS phosphocarrier protein	Bidossi *et al.*, 2011
		10 bp deletion 5' of SP_1895	raffinose ABC transporter permease	Rosenow *et al.*, 1999
s.fru # 2	*manLMN::spec*	SP_0877 Ala223Asp	fru PTS EIIABC	Bidossi *et al.*, 2011
		SP_1306 synonymous mutation	glutamate dehydrogenase	
s.fru # 2-1	*manLMN::spec*	G→T 162 bp upstream of SP_0086	mobile genetic element	
		SP_0927 Thr245Ile	SmrC, transcriptional regulator	Lau *et al.*, 2001
		SP_1177 in frame deletion of Ala70 – Asp71 (ΔCATCTG)	HPr, PTS phosphocarrier protein	Bidossi *et al.*, 2011
		SP_1897 CCTTC→GAGTΔC	raffinose ABC transporter sugar-binding protein	Rosenow *et al.*, 1999

s.fru #4	*manLMN::spec*	SP_0293 Thr75Pro	hypothetical protein	Bidossi *et al*., 2011
		SP_0877 Gly177Val	fru PTS EIIABC	
		SP_1196 C→T	degenerate transposase	
		SP_1905 C→T	degenerate transposase	

This chart shows the mutations identified by whole genome sequencing forty suppressor isolates. The predicted consequence of each mutation and the predicted/verified function of the encoded protein are listed. The suppressor names are as follows: gal; selection on galactose medium, lac; selection on lactose medium, GlcN; selected on GlcN medium, GlcNAc; selected on GlcNAc medium, and fru; selected on fructose medium. “s.” denotes suppressors in the *manLMN::spec* background. The CHESHIRE cassette used to replace the *manLMN* open reading frame is indicated by *CHESH.* Suppressor # indicates the biological replicate culture from which it arose. “f” indicates the frequency of a given mutation in the sequencing reads aligned to that genomic region. * indicates nonsense mutations.

[a] References are provided for protein functions verified *S. pneumoniae.*

References

Afzal, M., Shafeeq, S., and Kuipers, O.P. (2014) LacR is a repressor of *lacABCD* and LacT is an activator of *lacTFEG*, constituting the *lac* gene cluster in *Streptococcus pneumoniae*. *Appl Environ Microbiol* **80**: 5349–58.

Agarwal, V., Kuchipudi, A., Fulde, M., Riesbeck, K., Bergmann, S., and Blom, A.M. (2013) *Streptococcus pneumoniae* endopeptidase O (PepO) is a multifunctional plasminogen- and fibronectin-binding protein, facilitating evasion of innate immunity and invasion of host cells. *J Biol Chem* **288**: 6849–6863.

Bidossi, A., Mulas, L., Decorosi, F., Colomba, L., Ricci, S., Pozzi, G., *et al.* (2012) A Functional genomics approach to establish the complement of carbohydrate transporters in *Streptococcus pneumoniae*. *PLoS One* **7**: e33320.

Fleming, E., Lazinski, D.W., and Camilli, A. (2015) Carbon catabolite repression by seryl phosphorylated HPr is essential to *Streptococcus pneumoniae* in carbohydrate rich environments. *Mol Microbiol* **97**: 360–380.

Jeong, J.K., Kwon, O., Lee, Y.M., Oh, D.-B., Lee, J.M., Kim, S., *et al.* (2009) Characterization of the *Streptococcus pneumoniae* BgaC protein as a novel surface beta-galactosidase with specific hydrolysis activity for the Galβ1-3GlcNAc moiety of oligosaccharides. *J Bacteriol* **191**: 3011–23.

Kolkman, M., Wakarchuk, W., Nuijten, P., and Zeijst, B. van der (1997) Capsular polysaccharide synthesis in *Streptococcus pneumoniae* serotype

14: molecular analysis of the complete *cps* locus and identification of genes encoding glycosyltransferases required for the biosynthesis of the tetrasaccharide subunit. *Mol Microbiol* **26**: 197–208.

Kovács, M., Halfmann, A., Fedtke, I., Heintz, M., Peschel, A., Vollmer, W., *et al.* (2006) A functional *dlt* operon, encoding proteins required for incorporation of D-alanine in teichoic acids in Gram-positive bacteria, confers resistance to cationic antimicrobial peptides in *Streptococcus pneumoniae*. *J Bacteriol* **188**: 5797–5805.

Lacks, S.A. (1968) Genetic regulation of maltosaccharide utilization in pneumococcus. *Genetics* **60**: 685–706.

Lau, G.W., Haataja, S., Lonetto, M., Kensit, S.E., Marra, A., Bryant, A.P., *et al.* (2001) A functional genomic analysis of type 3 *Streptococcus pneumoniae* virulence. *Mol Microbiol* **40**: 555–571.

Manso, A.S., Chai, M.H., Atack, J.M., Furi, L., Croix, M.D.S., Haigh, R., *et al.* (2014) A random six-phase switch regulates pneumococcal virulence via global epigenetic changes. *Nat Commun* **5**: 1–9.

Martin, C., Briese, T., and Hakenbeck, R. (1992) Nucleotide sequences of genes encoding penicillin-binding proteins from *Streptococcus pneumoniae* and *Streptococcus oralis* with high homology to *Escherichia coli* penicillin-binding proteins 1A and 1B. *J Bacteriol* **174**: 4517–4523.

Obert, C., Sublett, J., Kaushal, D., Hinojosa, E., Barton, T., Tuomanen, E.I., and Orihuela, C.J. (2006) Identification of a candidate *Streptococcus pneumoniae* core genome and regions of diversity correlated with invasive pneumococcal disease. *Infect Immun* **74**: 4766–77.

Puyet, A., Ibaiiezg, M., and Espinosa, M. (1993) Characterization of the *Streptococcus pneumoniae* maltosaccharide regulator MalR, a member of the LacI-GalR family of repressors displaying distinctive genetic features. *J Biol Chem* **268**: 25402–25408.

Rosenow, C., Maniar, M., and Trias, J. (1999) Regulation of the alpha-galactosidase activity in *Streptococcus pneumoniae*: characterization of the raffinose utilization system. *Genome Res* **9**: 1189–97.

Stülke, J., Martin-Verstraete, I., Zagorec, M., Rose, M., Klier, A., and Rapoport, G. (1997) Induction of the *Bacillus subtilis ptsGHI* operon by glucose is controlled by a novel antiterminator, GlcT. *Mol Microbiol* **25**: 65–78.

Supplemental table 3. Primers used in this study

Primer	Sequence (5' to 3')	Application
manLMN_F	TGCCGTACAGTGAAAAATGCT	SOE PCR for Δ*manLMN* mutations
manLMN_R	AGATGGCGGTCAATGGGA	
manLMNspec_Fmid	TAAATAACAGATTAAAAAAATTATAATAATCAAGCAACTAAAAAGGAACCAGGT	SOE PCR for *manLMN::spec*
manLMNspec_Rmid	GGGATCCACTAGTTCTAGAGCGCATTCCTCCTTTTTTGTTTTAGTTTTCA	
manLMNcheshire_Fmid	GGTATCGACCATGGCCCCTAATCAAGCAACTAAAAAGGAACCAG	SOE PCR for *manLMN::CHESHIRE*
manLMNcheshire_Rmid	ACGGTAAGCCAACGCGTCCCCCATTATTGTCATTCCTCCTTTTTTGTTTTAG	
manLMN_F0	GCCTTAAAGTCGGTGACCGCGT	*ΔmanLMN* strains sequence verification
manLMN_R0	CAAGCCCACACTTTCTTCTGG	
Spec5'_R0	CCACGGTACCATTTCTTGCTGAATAA	*spec*^{R} strains sequence verification
Spec3'_F0	GATGTGAGAAGAGCCATTATGGATTCG	
Cheshire_F	GGGGACGCGTTGGCTTAC	CHESHIRE cassette cloning and resolved strain sequence verification
Cheshire_R	GGGGCCATGGTCGATACC	
SP1773_F	ACACCAAGTTGCCCAGAAG	SOE PCR for complementation *in trans* strains in the SP_1773 locus
SP_1773_R	GCCAGAAAAAACAGCAGCA	
SP1773_Fmid	GGTATCGACCATGGCCCCTGAAAGAATAATTATGCATAAAAATAGGAATATAAAC	
SP1773_Rmid	GACCATGCTTATTTTAAACTAAGCAAC	
SP1773_F0	ACACCAAGTTGCCCAGAAG	complementation *in trans* strains sequence verification
SP1773_R0	ACCGAATCTACACCTTCACCTG	
manLMNcomp_R	GTAAGCCAACGCGTCCCCAAGCATAGAAAAAAGGAATCAGATTTTAGAAC	SOE PCR for *manLMN* complementation *in trans*
manLcomp_R	GTAAGCCAACGCGTCCCCTTATTTGACATTGGCTTTGTTAATCAAGTC	SOE PCR for *manL* complementation *in trans*
manL(MN)comp_F	GTTGCTTAGTTTAAAATAAGCATGGTCTCTAATAGAAACCTATTTTAAAAGTTGGAAGC	SOE PCR for *manLMN* and *manL* complementation *in trans*
SP_0061_F	GGAGTTCTGGGATGGTTGGT	SOE PCR for *SP_0061-3::cat*
SP_0061_R	ACGGGCAAGACTAGATACAACT	

SP_0061_Fmid	CCTAATGACTGGCTTTTATAATAATACTCTTCGAAAATCTCTTCAAACTACG	
SP_0061_Rmid	CTTCCAATTGTCTAAATCAATTTTATTAAAGTTCATTGTCATAAGTTTTCCCCCTTTATATGTTTTAG	
SP0061_F0	GGCGAGCTACTCTGAAAGCT	*SP_0061-3::cat* strain sequence verification
SP_0061_R0	GGACCAGCGCCTAGATAGA	
SP_1185_F	AGGGAGAAGAGGTGATTGTAATTGGT	SOE PCR for *SP_1185-6::cat*
SP_1185_R	GGATGATATCTTCCAACTCAGCTGCACG	
SP_1185_Fmid	AGGCCTAATGACTGGCTTTTATAATAAGGCTAGAGACTCTGAAATAGTCTC	
SP_1185_Rmid	ATACCGTCGACCTCGAGATCCATGATAGAATTCTCCTTATATAGTTTTAAATAAATTTTTATG	
SP_1185_F0	ATTAGGCATGATCACAGGTG	*SP_1185-6::cat* strain sequence verification
SP_1185_R0	CCAAACTACCACCATTGACT	
fruA_F	AGTCACACTCAATCCATCCA	SOE PCR for *fruA::cat*
fruA_R	ACCAAATAGACCACGTTTCTCA	
fruA_Fmid	GAAGGTTTTTATATTACAGCTCCAAAAATAGAAAAATGAAAAGATTGGACCG	
fruA_Rmid	TACCGTCGACCTCGAGATCTCATCGTTTTTCTACCTCAACTTTTC	
fruA_F0	AGCTCTTGCAGGTTATTAAGG	*fruA::cat* strain sequence verification
fruA_R0	TTATGGTTTCGATCGTTTCC	
cat5'_R0	ATCCAATTTTCGTTTGTTGAACC	*cat*^{R} strains sequence verification
cat3'_F0	CCGCTATCTTTACAGGTACATC	
ciaR_F	GATTTCAGCCCTTGAGCG	SOE PCR for *ciaR::spec*
ciaR_R	CCGAAGCAATCATCTCGTAG	
ciaR_Fmid	TAAATAACAGATTAAAAAAATTATAAGGGTATCTTTTAAAAGATGTTCAGTAAAC	
ciaR_Rmid	GGGATCCACTAGTTCTAGAGAGAAACTCCTCCTTATTAAAACTATTATACC	
ciaR_F0	CGTCGTTTGTTGGCTGAG	*ciaR::spec* strain sequence verification
ciaR_R0	CAGAAGAAGCTGATCTGTGAC	
agalfusion_F	AGGATTTGGTAGACAGCTTGG	SOE PCR for α-galactosidase reporter strains
agalfusion_R	TGCCAAACCAACCATCATCTAA	
agalfusion_Rmid	TGAAGAAGGTTTTTATATTACAGCTCCATTACTTCACCTCATCACTTTATTGATTATATT	

agalfusion_Fmid	ATGGGAGTTAGGATAGAGAATAATCTATTTT	
agalfus-PlacA_F	CGATACCGTCGACCTCGAGATCAAAAGTAAAAAATAAATAACAAAAACAAACAAAAGCTGTTG	SOE PCR for *PlacA* α-galactosidase reporter strain
agalfus-PlacA_R	AAAATAGATTATTCTCTATCCTAACTCCCATATTGTATACTCCTTTTTATTTTTTCTATGCTG	
agalfus-PnagB_F	CGATACCGTCGACCTCGAGATCATATTTGTTCATTCTTTCTAAGTTCTAATCCTATCC	SOE PCR for *PnagB* α-galactosidase reporter strain
agalfus-PnagB_R	ATAGATTATTCTCTATCCTAACTCCCATCTTTTCATCCTCCATTTTTGTCTATATTTATT	
agalfus-PfruR_F	CGATACCGTCGACCTCGAGATCTTGAGTAATCTTCCAATACTTACTCAAATAGT	SOE PCR for *PfruR* α-galactosidase reporter strain
agalfus-PfruR_R	AAAATAGATTATTCTCTATCCTAACTCCCATTGAATCACCTCCTGTTATCGTT	
agalfusion_F0	GCTGTTCGGCTTAGGGAT	α-galactosidase reporter strains sequence verification
agalfusion_R0	AGTCCAAACTGCAAACCT	
SP_0061-3comp_Fmid	CTAAAACAAAAAAGGAGGAATGACAATAATGACAATTGTAGGATGCCGTATTG	SOE PCR for GlcNAc/gal PTS complementation *in trans*
SP_0061-3comp_Rmid	GTAAGCCAACGCGTCCCCTTATTCATCTTCGTCATCATCGAAGC	
SP_1185-6comp_Fmid	CTAAAACAAAAAAGGAGGAATGACAATAATGAATAGAGAAGAAGTAACATTGTTAGG	SOE PCR for lac PTS complementation *in trans*
SP_1185-6comp_Fmid	GTAAGCCAACGCGTCCCCTTAATCGAATTGCGCTTGTACG	
SP_fruAcomp_Fmid	CTAAAACAAAAAAGGAGGAATGACAATAATGAAAATTCAAGACCTATTGAGAAAAGATG	SOE PCR for fru PTS complementation *in trans*
SP_fruAcomp_Fmid	GTAAGCCAACGCGTCCCCTTATGCTTGTGGTTTGCGTAG	
Pman_R	TATTGTCATTCCTCCTTTTTTGTTTTAG	SOE PCR for PTS complementation *in trans* strains
SP_00613'_F	TGATATTGACCTTTACGCAGGG	GlcNAc/gal PTS comp. *in trans* sequence verification
SP_1186-3'_F	TGATCTTGTTATCCTTGCCCCT	lac PTS comp. *in trans* sequence verification
fruA3'_F	AGTAGCAGGTGGACTCGT	fru PTS comp. *in trans* sequence verification
SP_0451_F	GCCAGAAATGGAAGAGCG	SOE PCR for *SP_0451::cat*
SP_0451_R	CCTCACCTTCACCGTAACAC	
SP_0451_Fmid	AGGCCTAATGACTGGCTTTTATAATAATCTTTGGATAACGGCGGAT	

SP_0451_Rmid	TACCGTCGACCTCGAGATCAGCCATGTTTTTCTCCTATTTTCTTC	
SP_0451_F0	TGGTAGGTGTCGATGAGGG	*SP_0451::cat* strain sequence verification
SP_0451_R0	GTAACATCCTACCTGCAACTGG	
SP_1473_F	AGTTGACCTTGCTCGTGC	SOE PCR for *SP_1473::cat*
SP1473_R	TCACCAACGAAGGCACTG	
SP_1473_Fmid	AGGCCTAATGACTGGCTTTTATAATAATAATACTCTTCGAAAATCAAATTC AAACCAC	
SP1473_Rmid	ATACCGTCGACCTCGAGATCTTCTTCTCCTTTGTAATAAGATAAAGTCCG	
SP_1473_F0	CGTACGGGTCAAATCACTGT	*SP_1473::cat* strain sequence verification
SP1473_R0	GATGCTCCAGGTGTTAATGC	
rplI_FqRT	CGACCATCTGGACCAACTTT	For qRT-PCR of housekeeping gene *rplI* (SP_2204)
rplI_RqRT	CTAGCCAAAGAAGCGACTGC	
SP_0061_FqRT	CTCGTAAACCAGACCGCTTC	For qRT-PCR SP_0061
SP_0061_RqRT	CATTTGGAACCATCTGAGCA	
SP_1186_FqRT	GCTGCTGAAGCTGGTGATTT	For qRT-PCR of SP_1186
SP_1186_RqRT	GCCATGCATCATGGTTACAC	