

# Supplementary Material

**The *fruRBA* operon is necessary for Group A Streptococcal growth in fructose and for resistance to neutrophil killing during growth in whole human blood**

Kayla M. Valdes<sup>1Ψ</sup>, Ganesh S. Sundar<sup>1Ψ</sup>, Luis A. Vega<sup>1</sup>, Ashton T. Belew<sup>1,3</sup>, Emrul Islam<sup>1</sup>, Rachel Binet<sup>2</sup>, Najib M. El-Sayed<sup>1,3</sup>, Yoann Le Breton<sup>1\*</sup>, and Kevin S. McIver<sup>1\*</sup>

<sup>1</sup>Department of Cell Biology & Molecular Genetics and Maryland Pathogen Research Institute, University of Maryland, College Park, MD 20742

<sup>2</sup>Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD 20740

<sup>3</sup>Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD 20742

\*Corresponding Authors:

Email: [lebreton@umd.edu](mailto:lebreton@umd.edu) and [kmeiver@umd.edu](mailto:kmeiver@umd.edu)

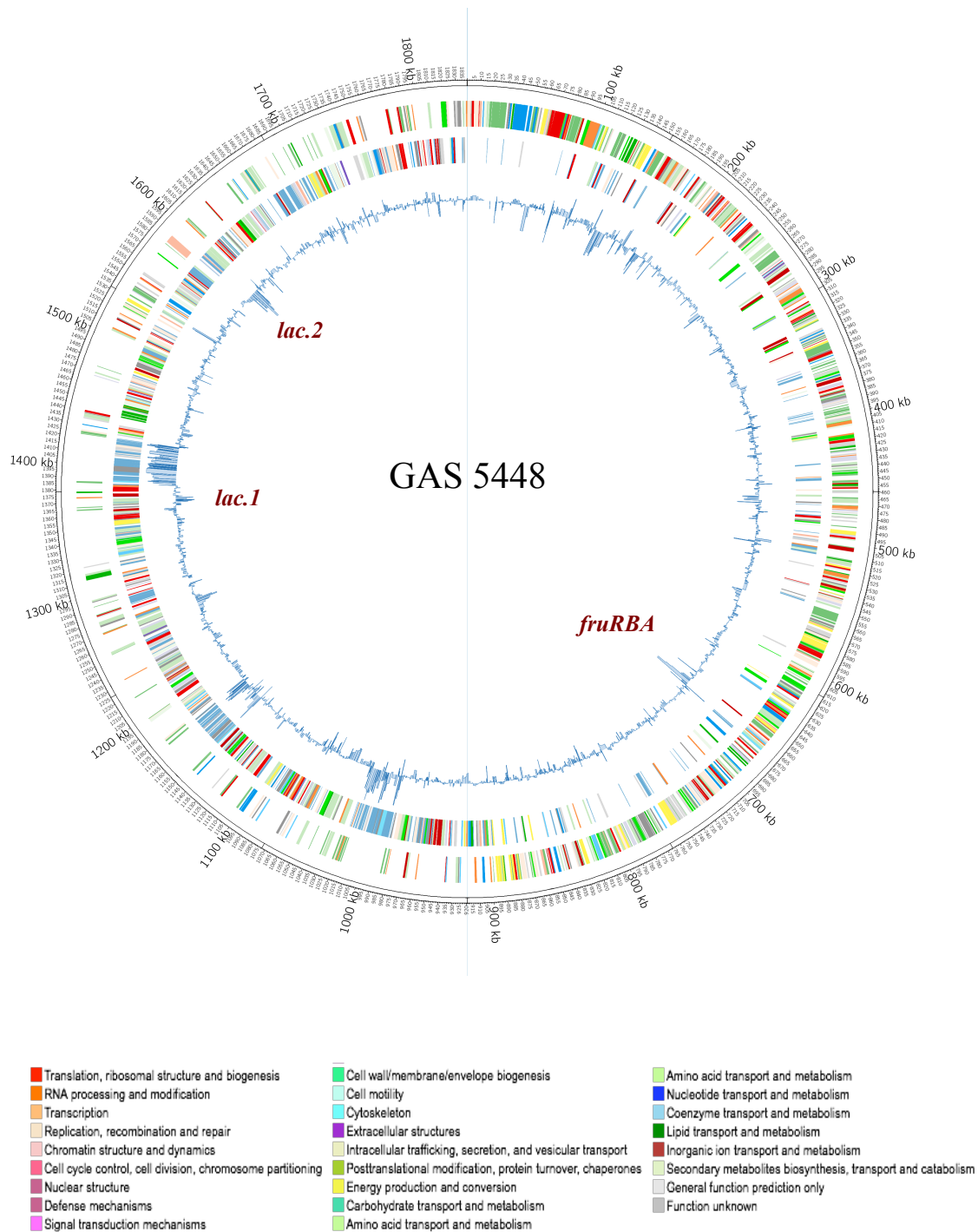
Table S1: Genes in 5448 differentially expressed in CDM-glucose compared to CDM-fructose

<b>Spy #</b>	<b>Annotation</b>	<b>Gene Name</b>	<b>Log<sub>2</sub> FC</b>
M5005_Spy_0039	alcohol dehydrogenase/acetaldehyde dehydrogenase	<i>adh.2</i>	-2.10
M5005_Spy_0086	comG operon protein 1	<i>comYA</i>	-0.99
M5005_Spy_0089	comG operon protein 4		1.72
M5005_Spy_0120	acetate CoA-transferase alpha subunit	<i>atoD.2</i>	1.76
M5005_Spy_0123	translation initiation inhibitor		-1.82
M5005_Spy_0124	serine catabolism regulator	<i>sloR</i>	-2.32
M5005_Spy_0125	hypothetical protein		-1.09
M5005_Spy_0126	V-type sodium ATP synthase subunit I	<i>ntpI</i>	-2.01
M5005_Spy_0127	V-type sodium ATP synthase subunit K	<i>ntpK</i>	-1.97
M5005_Spy_0128	V-type sodium ATP synthase subunit E	<i>ntpE</i>	-2.22
M5005_Spy_0129	V-type ATP synthase subunit C	<i>ntpC</i>	-1.79
M5005_Spy_0130	V-type sodium ATP synthase subunit F	<i>ntpF</i>	-1.22
M5005_Spy_0131	V-type sodium ATP synthase subunit A	<i>ntpA</i>	-1.24
M5005_Spy_0132	V-type sodium ATP synthase subunit B	<i>ntpB</i>	-0.96
M5005_Spy_0133	V-type sodium ATP synthase subunit D	<i>ntpD</i>	-1.21
M5005_Spy_0145	hypothetical protein		-1.31
M5005_Spy_0150	PTS system, 3-keto-L-gulonate specific IIA	<i>ptxA</i>	-0.99
M5005_Spy_0152	L-xylulose 5-phosphate 3-epimerase		1.37
M5005_Spy_0154	hypothetical protein		1.57
M5005_Spy_0166	transposase		-1.32
M5005_Spy_0167	transposase		-1.15
M5005_Spy_0168	transposase		-1.70
M5005_Spy_0170	nicotinate-nucleotide pyrophosphorylase	<i>nadC</i>	-1.95
M5005_Spy_0184	hypothetical cytosolic protein		-1.28
M5005_Spy_0189	hypothetical protein		-1.66
M5005_Spy_0256	competence-specific sigma factor		-1.68
M5005_Spy_0297	transposase		-1.12
M5005_Spy_0395	transposase		-1.39
M5005_Spy_0451	transcriptional regulator		-1.07
M5005_Spy_0460	hypothetical protein		1.13
M5005_Spy_0474	transcription antiterminator, BglG family	<i>licT</i>	-1.36
M5005_Spy_0476	6-phospho-beta-glucosidase	<i>bglA</i>	-1.22
M5005_Spy_0479	hypothetical membrane spanning protein		-1.80
M5005_Spy_0518	oligohyaluronate lyase		1.35
M5005_Spy_0521	PTS system, N-acetylgalactosamine-specific IIB	<i>agaV</i>	-1.75
M5005_Spy_0616	ferredoxin		-1.02
M5005_Spy_0650	hypothetical protein		-1.95
M5005_Spy_0659	2-dehydropantoate 2-reductase	<i>apbA</i>	-0.95
M5005_Spy_0660	fructose repressor	<i>fruR</i>	-1.95
M5005_Spy_0661	1-phosphofructokinase	<i>fruB</i>	-2.52
M5005_Spy_0662	PTS system, Fructose-specific IIABC	<i>fruA</i>	-3.33
M5005_Spy_0666	hypothetical protein		-1.70
M5005_Spy_0667	exotoxin type C precursor		2.07
M5005_Spy_0773	hypothetical protein		0.99
M5005_Spy_0798	IFN-response binding factor 1		-1.01
M5005_Spy_0800	DNA-cytosine methyltransferase		-1.38
M5005_Spy_0853	short chain dehydrogenase		-1.25
M5005_Spy_0912	hypothetical protein		-1.41
M5005_Spy_0997	phage protein		1.20
M5005_Spy_0998	phage protein		-1.40
M5005_Spy_0999	phage protein		1.92
M5005_Spy_1000	phage protein		2.52
M5005_Spy_1010	phage protein		1.56
M5005_Spy_1013	antigen B		2.32
M5005_Spy_1017	phage protein		2.65
M5005_Spy_1023	terminase large subunit		1.52
M5005_Spy_1025	phage encoded transcriptional regulator, ArpU family		0.96

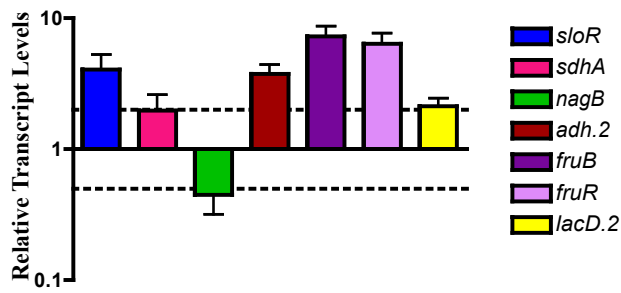
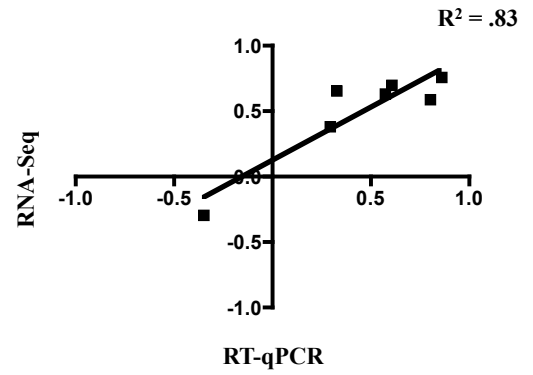
M5005_Spy_1026	phage protein		2.04
M5005_Spy_1027	phage protein		1.00
M5005_Spy_1028	phage protein		1.12
M5005_Spy_1029	phage protein		1.92
M5005_Spy_1030	phage protein		1.38
M5005_Spy_1032	phage protein		1.84
M5005_Spy_1033	phage protein		1.69
M5005_Spy_1034	phage protein		1.48
M5005_Spy_1035	phage protein		1.41
M5005_Spy_1036	phage single-strand DNA binding protein	<i>ssb2</i>	1.48
M5005_Spy_1037	phage single-strand DNA binding protein	<i>ssb1</i>	2.07
M5005_Spy_1038	phage protein		1.87
M5005_Spy_1039	phage protein		2.09
M5005_Spy_1040	phage protein		1.71
M5005_Spy_1041	phage protein		2.76
M5005_Spy_1042	phage replication protein		2.03
M5005_Spy_1043	phage protein		1.88
M5005_Spy_1045	transcriptional regulator		1.80
M5005_Spy_1062	maltodextrose utilization protein	<i>malA</i>	-1.12
M5005_Spy_1063	ABC Cyclomaltodextrin permease protein	<i>malD</i>	-1.17
M5005_Spy_1078	hypothetical protein		-1.53
M5005_Spy_1081	PTS system, Cellobiose-specific IIA component		-0.98
M5005_Spy_1089	hypothetical protein		-1.19
M5005_Spy_1090	transposase		-1.58
M5005_Spy_1139	glucosamine-6-phosphate isomerase	<i>nagB</i>	0.98
M5005_Spy_1172	holin		0.99
M5005_Spy_1173	phage protein		-1.33
M5005_Spy_1174	phage protein		2.37
M5005_Spy_1175	phage protein		1.47
M5005_Spy_1176	phage infection protein		1.34
M5005_Spy_1187	phage structural protein		1.05
M5005_Spy_1190	phage protein		-1.19
M5005_Spy_1200	phage protein		1.14
M5005_Spy_1201	phage protein		1.94
M5005_Spy_1203	phage protein		2.16
M5005_Spy_1204	recT protein		1.59
M5005_Spy_1205	phage protein		1.75
M5005_Spy_1206	phage protein		1.91
M5005_Spy_1208	phage protein		2.23
M5005_Spy_1209	DNA replication protein		1.82
M5005_Spy_1210	phage replication protein		1.45
M5005_Spy_1211	phage protein		1.42
M5005_Spy_1213	phage protein		1.72
M5005_Spy_1216	phage protein		1.57
M5005_Spy_1217	phage antirepressor protein		1.20
M5005_Spy_1218	phage protein		1.79
M5005_Spy_1307	hypothetical membrane spanning protein		-1.02
M5005_Spy_1308	ABC unknown sugar-binding protein		-1.16
M5005_Spy_1309	ABC unknown sugar permease protein		-1.82
M5005_Spy_1310	ABC unknown sugar permease protein		-1.40
M5005_Spy_1388	N-acetylglucosamine-6-phosphate deacetylase	<i>nagA</i>	0.97
M5005_Spy_1395	tagatose-bisphosphate aldolase; SpeB regulator	<i>lacD.1</i>	-1.01
M5005_Spy_1396	tagatose-6-phosphate kinase	<i>lacC.1</i>	-1.35
M5005_Spy_1397	galactose-6-phosphate isomerase lacB subunit	<i>lacB.1</i>	-1.54
M5005_Spy_1400	PTS system, Galactose-specific IIB component		-1.45
M5005_Spy_1401	PTS system, Galactose-specific IIA component		-1.14
M5005_Spy_1414	phage protein		1.42
M5005_Spy_1416	phage-associated cell wall hydrolase		1.90
M5005_Spy_1417	phage protein		1.44
M5005_Spy_1418	phage protein		2.34

M5005_Spy_1419	phage protein		2.03
M5005_Spy_1420	phage protein		1.67
M5005_Spy_1421	phage infection protein		1.81
M5005_Spy_1422	phage protein		1.48
M5005_Spy_1423	hyaluronoglucosaminidase		1.95
M5005_Spy_1424	phage endopeptidase		1.89
M5005_Spy_1425	phage protein		2.07
M5005_Spy_1426	phage protein		2.49
M5005_Spy_1427	phage protein		2.18
M5005_Spy_1428	phage protein		2.41
M5005_Spy_1429	phage protein		2.27
M5005_Spy_1430	phage protein		1.99
M5005_Spy_1431	phage protein		1.95
M5005_Spy_1432	phage protein		2.30
M5005_Spy_1433	phage protein		2.00
M5005_Spy_1434	phage protein		2.38
M5005_Spy_1435	phage scaffold protein		2.40
M5005_Spy_1436	phage protein		2.18
M5005_Spy_1437	hypothetical protein		2.26
M5005_Spy_1438	phage protein		1.81
M5005_Spy_1439	portal protein		2.14
M5005_Spy_1440	terminase large subunit		1.79
M5005_Spy_1441	phage terminase small subunit		1.83
M5005_Spy_1442	phage transcriptional activator		2.50
M5005_Spy_1444	adenine-specific methyltransferase		1.89
M5005_Spy_1446	phage protein		1.20
M5005_Spy_1447	phage-related DNA helicase		1.49
M5005_Spy_1448	hypothetical protein		1.22
M5005_Spy_1449	DNA primase		2.37
M5005_Spy_1450	phage-encoded DNA polymerase		1.80
M5005_Spy_1451	phage protein		1.69
M5005_Spy_1452	phage protein		1.74
M5005_Spy_1453	phage protein		1.60
M5005_Spy_1454	phage protein		2.02
M5005_Spy_1455	phage protein		1.82
M5005_Spy_1457	phage protein		2.66
M5005_Spy_1458	phage protein		1.70
M5005_Spy_1459	phage protein		1.57
M5005_Spy_1460	phage protein		1.35
M5005_Spy_1461	phage protein		2.43
M5005_Spy_1463	phage protein		1.79
M5005_Spy_1509	pyruvate, phosphate dikinase		-1.36
M5005_Spy_1510	pyruvate, phosphate dikinase		-1.26
M5005_Spy_1536	transposase		-1.17
M5005_Spy_1541	hypothetical protein		-1.10
M5005_Spy_1574	universal stress protein family		2.05
M5005_Spy_1575	quinolone resistance protein	<i>norA</i>	2.33
M5005_Spy_1629	lantibiotic transport ATP-binding protein	<i>salX</i>	-1.89
M5005_Spy_1631	lantibiotic salivaricin A	<i>salA</i>	-1.67
M5005_Spy_1632	6-phospho-beta-galactosidase	<i>lacG</i>	-1.66
M5005_Spy_1633	PTS system, Lactose-specific IIBC component	<i>lacE</i>	-2.73
M5005_Spy_1634	PTS system, Lactose-specific IIA component	<i>lacF</i>	-2.22
M5005_Spy_1635	tagatose-bisphosphate aldolase	<i>lacD.2</i>	-2.18
M5005_Spy_1636	tagatose-6-phosphate kinase	<i>lacC.2</i>	-1.49
M5005_Spy_1637	galactose-6-phosphate isomerase lacB subunit	<i>lacB.2</i>	-1.67
M5005_Spy_1638	galactose-6-phosphate isomerase lacA subunit	<i>lacA.2</i>	-1.50
M5005_Spy_1643	hypothetical protein		0.99
M5005_Spy_1663	PTS system, Mannitol-specific IIB component		-1.69
M5005_Spy_1689	hypothetical protein		1.42
M5005_Spy_1744	PTS system, Cellobiose-specific IIC component	<i>celC</i>	-1.34

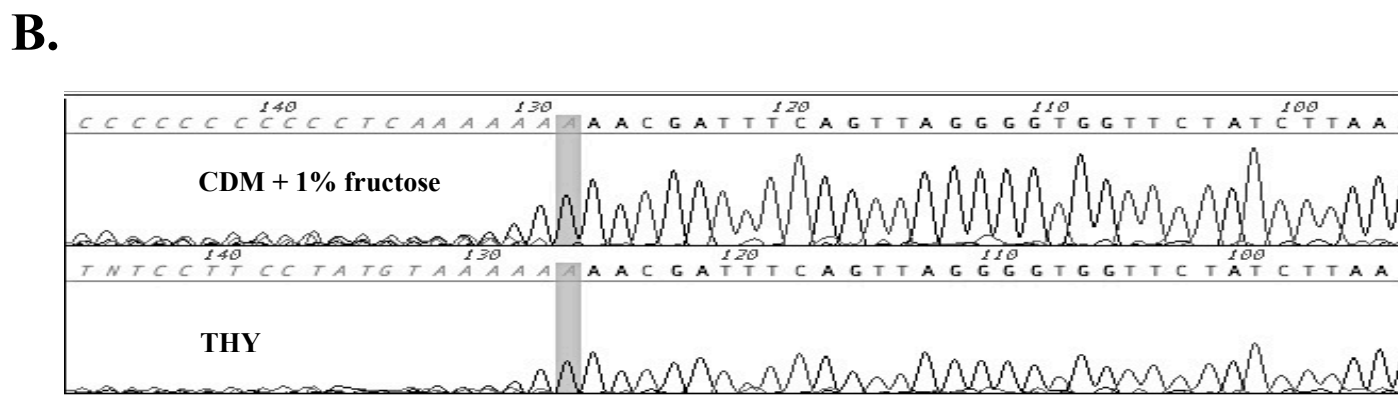
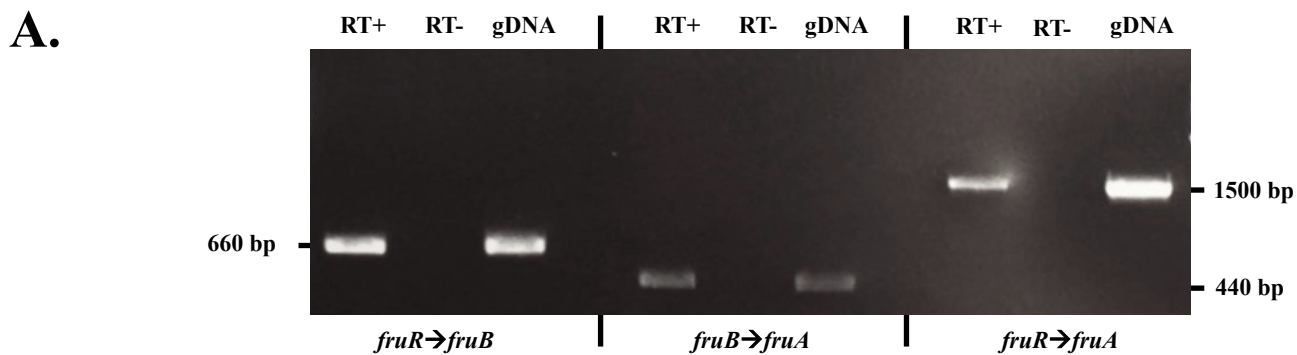
M5005_Spy_1745	PTS system, Cellobiose-specific IIB component	<i>celB</i>	-0.95
M5005_Spy_1746	PTS system, Cellobiose-specific IIA component	<i>celA</i>	1.02
M5005_Spy_1786	transcriptional regulator, MarR family		1.04
M5005_Spy_1832	hypothetical protein		-1.15
M5005_Spy_1841	L-serine dehydratase	<i>sdhB</i>	-1.06
M5005_Spy_1842	L-serine dehydratase	<i>sdhA</i>	-1.27
M5005_SpyR0011	large subunit 23S ribosomal RNA		-1.05
M5005_SpyT0020	tRNA-Pro		-1.84
M5005_SpyT0030	tRNA-Val		1.00
M5005_SpyT0031	tRNA-Gly		-1.80
M5005_SpyT0032	tRNA-Ile		-1.52
M5005_SpyT0033	tRNA-Glu		-1.35
M5005_SpyT0036	tRNA-Phe		-1.13
M5005_SpyT0040	tRNA-Gln		-2.59
M5005_SpyT0041	tRNA-Leu		-1.13
M5005_SpyT0046	tRNA-Tyr		-1.02
M5005_SpyT0052	tRNA-Thr		-1.00
M5005_SpyT0065	tRNA-Asn		-0.97



**Figure S1. Transcriptomic landscape of 5448 during growth in fructose.** Circos plot of wild-type GAS 5448 gene global expression altered under growth in CDM + 0.5% glucose versus CDM + 1% fructose. The outer most ring represents a size ruler. The next two rings represent the the GAS open reading frames on the (+) and (-) strand of the genome, respectively. The fourth ring shows transcript levels of genes, where the height of the bars in the fourth ring represent the  $\log_2$  fold change in differential expression in glucose vs. fructose (inside is induced, outside is repressed). The *fruRBA*, and two *lacD* loci induced by fructose are indicated.

**A.****B.**

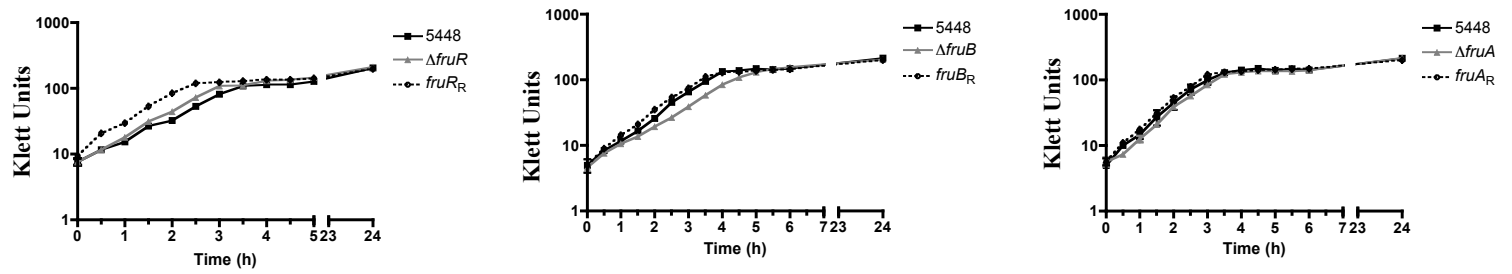
**Figure S2. Validation of RNA-Seq results using qPCR.** (A) Transcript levels were determined using qPCR on RNA taken from GAS grown in fructose compared to glucose as the sole carbon source. Error bars represent the standard error from three biological replicates. Differences greater than 2-fold in expression for fructose grown cells compared to glucose grown cells (denoted by a dashed line) are considered significant. (B) Coefficient of correlation between the RNA-Seq and RT-qPCR results. Generated a confidence in the data with an  $R^2=83\%$ .



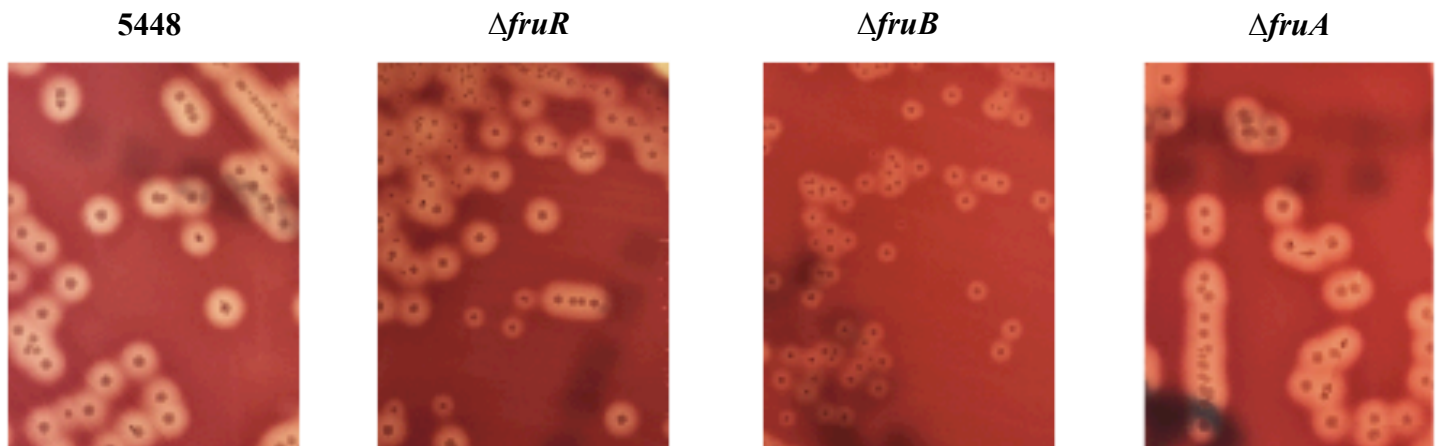
**Fig S3: *fruRBA* is an operon in GAS MIT1 5448.** (A) RT-PCR on RNA isolated from 5448 grown in CDM + 1% fructose demonstrating transcriptional linkage between *fruR/fruB*, *fruB/fruA*, and *fruR/fruA*. Reactions were run using specific primers (Table 2) either with reverse transcriptase (+RT), without reverse transcriptase (-RT) as a negative control, and using genomic DNA (gDNA) as a positive control. Expected sizes of bands are indicated. (B) The transcriptional start site (+1) was determined by 5'-RACE and subsequent DNA sequencing (gray bar) from 5448 grown in THY and CDM + 1% fructose.



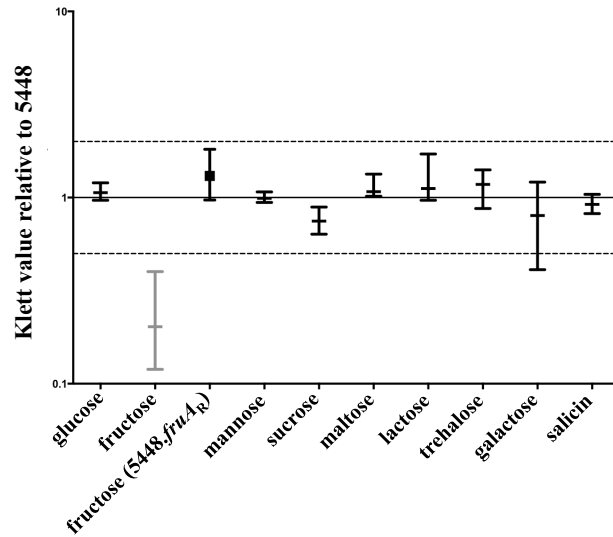
**A.**



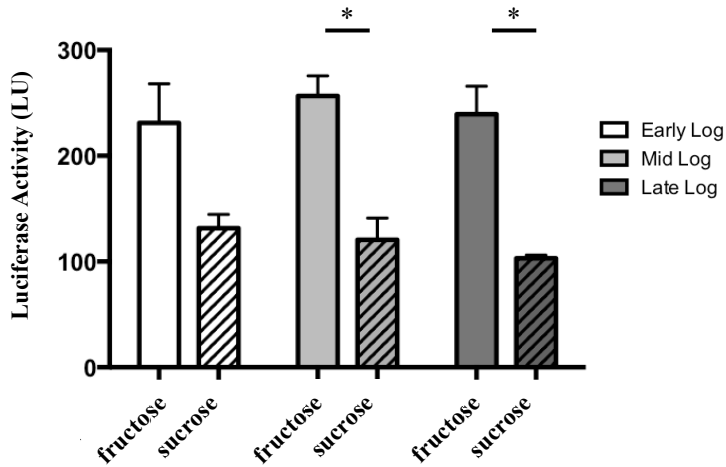
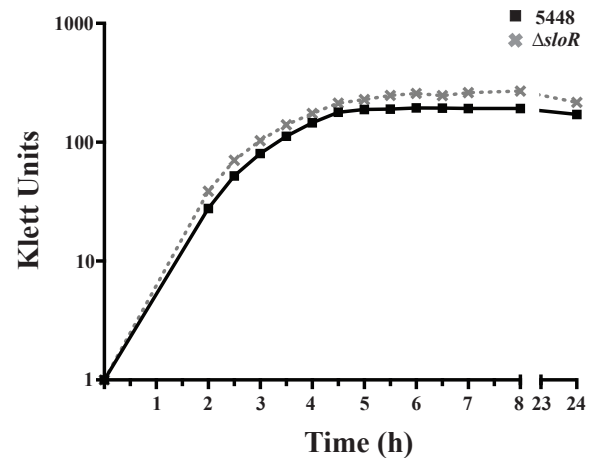
**B.**



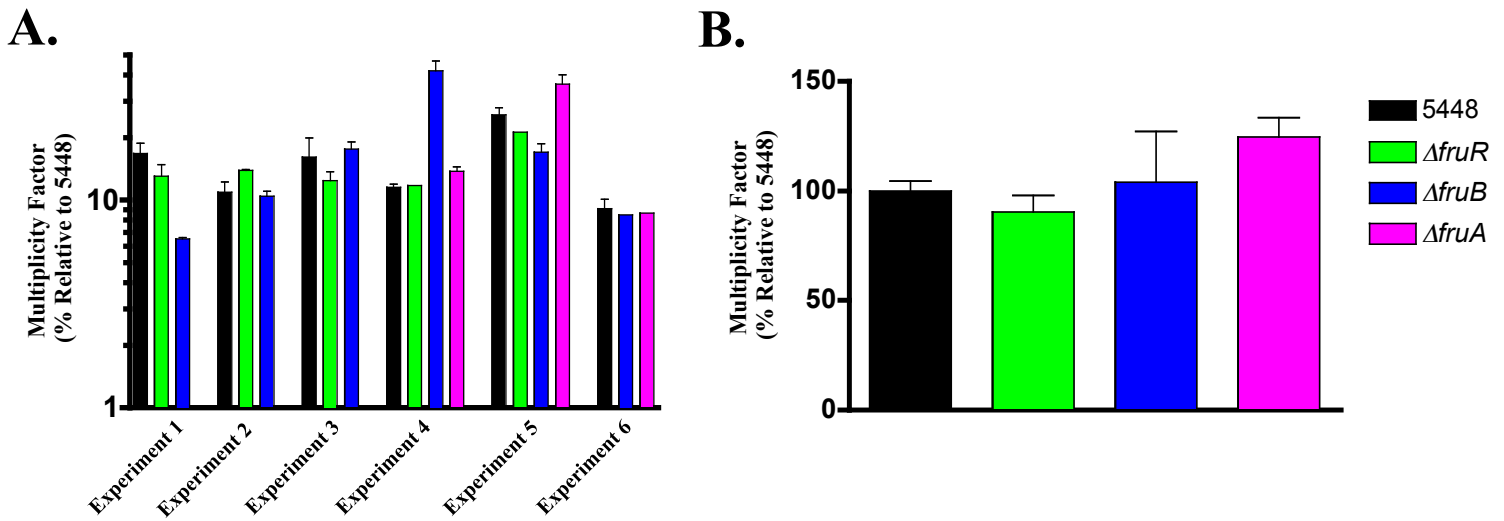
**Figure S4. Growth of individual *fruRBA* operon mutants in rich media. (A)** Each *fru* mutant was grown in THY with their respective revertant strain and the parental 5448. **(B)** Colony morphology of each *fru* mutant was streaked on blood agar (TSA + 5% sheep blood) plates, cultured overnight at 37°C + 5% CO<sub>2</sub> and imaged. Shown are the parental 5448,  $\Delta fruR$ ,  $\Delta fruB$ , and  $\Delta fruA$  as indicated.



**Figure S5: Growth curve comparison of 5448 to 5448. $\Delta fruA$  across time.** Klett unit relative to 5448 at each time point was calculated by taking the percent WT of each individual time point, and plotting the median percent WT along with the interquartile range (25-75% of the data set). Black bars represent sugars in which 5448. $\Delta fruA$  grows similarly to the parental 5448. Gray bars indicate sugars in which 5448. $\Delta fruA$  grows poorly compared to 5448. Black square represents growth comparison of 5448.fruA<sub>R</sub> to wildtype. Dashed lines represent 2-fold growth above and below 5448.

**A.****B.**

**Figure S6: The effect of fructose on *sloR*.** (A) *PsloR* promoter activity in WT 5448 containing the *PsloR-luc* luciferase reporter plasmid was grown in CDM containing either 1% fructose (solid bars) or sucrose (diagonal bars). Samples were taken at three time points in logarithmic phase of growth (early: white bars; mid: light gray bars; late: dark gray bars) and assayed for luciferase production, expressed in relative luciferase units. Error bars for represent mean  $\pm$  SD of the results from three biological replicates performed in triplicate. Significance for (A) was determined using a Student *t* test ( $p \leq 0.01$ ). (B) Growth of 5448. $\Delta sloR$  in CDM + 1% fructose compared to 5448.



**Figure S7: 5448 and  $\Delta fruRBA$  Growth in RPMI+ 20% Plasma.** Each *fru* mutant was grown along with the parental strain 5448 in RPMI + 20% plasma. Data from 6 independent experiments, with at least three replicates for each *fru* operon mutant in comparison to WT 5448 are shown in (A). The cumulative results of (A) are shown as an average of the replicates in (B).