

TABLE S1 Probe and primer sequences used for qPCR

Primer/Probe	Sequence
MGB Probe-map <sup>a</sup>	TTGCACTTGATTGTTTCG
F-Primer-map <sup>a</sup>	CCGATCAACAGGCTCAGTGA
R-Primer-map <sup>a</sup>	CATGTTGTGCGCTTGGAAAA
MGB Probe-spaC	CGCACGACTTCGATG
F-Primer-spaC	GCAGAGATGAACCAGGGAACA
R-Primer-spaC	AATGACCTGAACTATCGGTGTATGG

<sup>a</sup> Primers and probe for the hypothetical membrane associated protein, designated to *map*, were developed for a *L. rhamnosus* GG specific PCR.

TABLE S2 Missing genes in genomic islands LGGISL1,2 in *L. rhamnosus* GG product isolates 1 and 2A

Code	Gene	Start	End	Strand	Protein	P1	P2A
LGG_00383	is11	392315	393730	+	transposase IS5 family protein	0	1
LGG_00384	-	393742	393918	-	hypothetical protein	0	1
LGG_00385	-	394097	394282	+	hypothetical protein	0	1
LGG_00386	-	394391	395779	+	transporter major facilitator superfamily MFS_1	0	1
LGG_00387	slyA	395755	396189	+	MarR family transcriptional regulator	0	1
LGG_00388	-	396186	396746	+	hypothetical protein	0	1
LGG_00389	-	396929	397144	+	hypothetical protein	0	1
LGG_00390	aroE	397288	397575	+	hypothetical protein	0	1
LGG_00391	-	397793	398128	-	hypothetical protein	0	1
LGG_00392	-	398232	398693	-	hypothetical protein	0	1
LGG_00393 <sup>a</sup>	manR	399211	400695	+	transcription antiterminator BglG family protein	0	1
LGG_00394	-	400685	401113	+	PTS system transporter su IIA	0	1
LGG_00395	frwA1	401179	401628	+	PTS system fructose-specific transporter su IIA	0	1
LGG_00396	frwB1	401644	401961	+	PTS system fructose-specific transporter su IIB	0	1
LGG_00397	frwC1	401995	403095	+	PTS system fructose-specific transporter su IIC	0	1
LGG_00398	tal	403139	403807	+	Transaldolase	0	1
LGG_00399	rpe	403800	404489	+	allulose-6-phosphate 3-epimerase	0	1
LGG_00400	ulaA	404798	405247	+	PTS system lactose/cellobiose-specific transporter su IIA	0	1
LGG_00401	ulaB	405249	405530	+	PTS system lactose/cellobiose-specific transporter su IIB	0	1
LGG_00402	pts	405558	405971	+	PTS system transporter su IIC	0	1
LGG_00403	is12	405976	406992	-	transposase IS30 family protein	0	1
LGG_00404	ulaC	407068	408117	+	PTS system ascorbate-specific transporter su IIC	0	1
LGG_00405	tktN	408138	408995	+	Transketolase	0	1
LGG_00406	tktC	408988	410004	+	Transketolase	0	1
LGG_00407	scrK	410196	411050	+	Fructokinase	0	1
LGG_00408	lacR	411100	411858	-	lactose phosphotransferase system repressor	0	1

LGG_00409	frwA2	412099	412572	+	PTS system fructose-specific transporter su IIA	0	1
LGG_00410	frwB2	412577	412906	+	PTS system fructose-specific transporter su IIB	0	1
LGG_00411	frwC2	412931	414037	+	PTS system fructose-specific transporter su IIC	0	1
LGG_00412	is13	414201	415217	+	transposase IS30 family protein	1	1
LGG_00413	fba	415271	416113	+	fructose-bisphosphate aldolase	1	1
LGG_00414	gatY	416135	417028	+	fructose-bisphosphate aldolase	1	1
LGG_00415	pts	417573	418049	-	PTS system mannose-specific transporter su IIB	1	1
LGG_00416	pts	418021	418449	-	PTS system mannose-specific transporter su IIA	1	1
LGG_00417	manZ	418467	420155	-	PTS system mannose-specific transporter su IICD	1	1
LGG_00418	tal	420195	420905	-	putative transaldolase	1	1
LGG_00419	-	421109	422140	+	LacI family transcriptional regulator	1	1
LGG_00420	yhfZ	422379	423293	+	GntR family transcriptional regulator	1	1
LGG_00421	-	423326	423676	+	hypothetical protein	1	1
LGG_00422	-	423678	424040	+	hypothetical protein	1	1
LGG_00423	-	424101	425396	+	membrane protein	1	1
LGG_00424	php	425426	426310	+	Hydrolase	1	1
LGG_00425	yhfS	426303	427409	+	pyridoxal phosphate-dependent transferase	1	1
LGG_00426	yhfX	427400	428572	+	amino acid racemase	1	1
LGG_00427 <sup>b</sup>	YHFW	428574	429803	+	putative mutase	1	1
LGG_00428	ilvH	430131	430967	+	DNA-directed RNA polymerase su delta	1	1
LGG_00429	-	431050	431610	+	hypothetical protein	1	1
LGG_00430	cbiO	431612	433312	+	cobalt ABC transporter ATP-binding protein cbiO	1	1
LGG_00431	cbiQ	433309	434136	+	cobalt transport protein cbiQ	1	1
LGG_00432	-	434102	434266	+	hypothetical protein	1	1
LGG_00433 <sup>c</sup>	menC	434488	435621	-	N-acylamino acid racemase	1	1
LGG_00434	nagZ	436451	438265	+	beta-N-acetylhexosaminidase (GH3)	1	1
LGG_00435	is14	438735	439646	-	transposase IS30 family protein	1	1
LGG_00436	tnpR	439988	440638	+	Resolvase	1	1

LGG_00437	-	440644	440928	+	cytosolic protein	1	1
LGG_00438	-	440992	441147	+	hypothetical protein	1	1
LGG_00439	-	441189	441524	+	hypothetical protein	1	1
LGG_00440	-	442192	442404	+	hypothetical protein	1	1
LGG_00441	srtC1	442875	443954	-	sortase family protein	1	1
LGG_00442	spaA	444028	445032	-	pilus specific protein	1	1
LGG_00443	spaB	445035	445760	-	pilus specific protein	1	1
LGG_00444	spaC	445753	448440	-	pilus specific protein	1	1
LGG_00445	is15	448581	449597	+	transposase IS30 family protein	1	1
LGG_00446	-	450276	450833	-	hypothetical protein	0	1
LGG_00447	-	450928	451500	-	hypothetical protein	0	1
LGG_00448	-	451522	451797	-	hypothetical protein	0	1
LGG_00449	-	451872	453659	-	UvrD/REP helicase	0	1
LGG_00450 <sup>d</sup>	ybjD	453656	455746	-	hypothetical protein	0	1
LGG_00451	is16	455864	457279	-	transposase IS5 family protein	0	1
LGG_00452	is17	457654	457905	+	transposase IS3/IS911 family protein	0	1
LGG_00453	is18	457959	458750	+	transposase IS150/IS3 family protein	0	1
LGG_00454	is19	459084	459932	-	transposase IS150/IS3 family protein	0	1
LGG_00455	-	460536	460799	-	hypothetical protein	0	1
LGG_00456	pacL	460859	463681	-	cation-transporting ATPase	0	1
LGG_00457	is20	463990	464310	+	Transposase	0	1
LGG_00458	is21	464307	464777	+	transposase IS4 family protein	0	1
LGG_00459	napA	464786	465940	-	Na/H antiporter	0	1
LGG_00460	is22	466447	466917	-	transposase IS4 family protein	0	1
LGG_00461	is23	466914	467234	-	Transposase	0	1
LGG_00462	is24	467284	467958	-	Transposase	0	1
LGG_00463	eriC	468801	470366	-	chloride channel protein	0	1
LGG_00464	is25	470708	471628	+	transposase IS30 family protein	0	1

LGG_00465	-	471663	472916	-	transporter major facilitator superfamily MFS_1	0	1
LGG_00466	is26	473096	474511	+	transposase IS5 family protein	0	1

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0: gene is present; 1: missing gene; SU: subunit; P1: Product isolate 1; P2A: Product isolate 2A.

<sup>a</sup> Start of LGGISL1.

<sup>b</sup> End of LGGISL1.

<sup>c</sup> Start of LGGISL2.

<sup>d</sup> End of LGGISL2.

TABLE S3 Predicted mutations in ATCC 53103 reference strain and three *L. rhamnosus* GG product isolates

Position	Mutation	Annotation <sup>b</sup>	Gene	Description	Ref	P1	P2A	P3
264278	G→A	A420T (GCC→ACC)	ioIT	transporter major facilitator superfamily MFS_1, Myo-inositol transporter	1	0	0	0
385772	Δ3 bp	intergenic (-604/+471)	is10/LGG_00377	transposase IS5 family protein/hypothetical protein	0	0	1	0
615483	T→C <sup>a</sup>	S422P (CTG→CCG)	treB	PTS system beta-glucoside-specific transporter subunit IIABC	1	1	1	1
656567	3 bp x 2	duplication	glpD	alpha-glycerophosphate oxidase	1	0	0	0
751632	2 bp→GC <sup>a</sup>	intergenic (+65/-193)	napA/gph	Na/H antiporter/haloacid dehalogenase-like family hydrolase	1	1	1	1
876404	3 bp x 2	duplication	cutC	copper homeostasis protein CutC	1	0	0	0
985365	A→G	I904I (ATT→ATC)	pacL	cation-transporting ATPase	0	1	1	0
1038046	Δ860 bp		[LGG_01023]-is36	[LGG_01023], is35, is36	1	1	1	0
1074976	G→A	E180K (GAA→AAA)	galU	UTP-glucose-1-phosphate uridylyltransferase	0	1	1	0
1118872	C→T	T67T (ACC→ACT)	LGG_01118	hypothetical protein	0	1	0	0
1154682	T→G	intergenic (-80/+353)	LGG_01157/pepD	transporter major facilitator superfamily MFS_1/dipeptidase A	1	0	0	0
1372183	T→A	L406M (TTG→ATG)	ycjM	2',3'-cyclic-nucleotide 2'-phosphodiesterase	1	0	0	0
1873058	A→G	P249P (CCT→CCC)	LGG_01843	hypothetical protein	1	1	1	0
1873063	Δ1 bp	coding (742/795 nt)	LGG_01843	hypothetical protein	1	1	1	0
1873071	+T	coding (734/795 nt)	LGG_01843	hypothetical protein	1	1	1	0
1873089	G→A	A239V (GCC→GTC)	LGG_01843	hypothetical protein	1	1	1	0
1873091	2 bp→CA	coding (713-714/795 nt)	LGG_01843	hypothetical protein	1	1	1	0
1873110	3 bp→TCC	coding (693-695/795 nt)	LGG_01843	hypothetical protein	0	1	1	0
1873122	C→T	R228H (CGC→CAC)	LGG_01843	hypothetical protein	1	1	1	0
1877309	Δ1,586 bp		LGG_01848	hypothetical protein	1	1	1	0
1877343	2 bp→AA	intergenic (-372/-78)	LGG_01847/ 01848	membrane protein/hypothetical protein	0	0	0	1
1877348	G→A	intergenic (-377/-74)	LGG_01847/ 01848	membrane protein/hypothetical protein	0	0	0	1

1877374	C→G	intergenic (-403/-48)	LGG_01847/ 01848	membrane protein/hypothetical protein	0	0	0	1
1883242	C→A <sup>a</sup>	intergenic (-292/+93)	ABC-SBP/ 01854	ABC transporter substrate-binding protein/hypothetical protein	1	1	1	1
1994717	T→A	D903V (GAT→GTT)	LGG_01953	hypothetical protein	1	0	0	0
2060386	C→A	W87L (TGG→TTG)	gmk	guanylate kinase	0	1	0	0
2150514	G→T	intergenic (-99/+336)	LGG_02090/ 02091	cytosine permease/hypothetical protein	0	1	0	0
2160010	G→A	A459T (GCG→ACG)	LGG_02095	type II restriction enzyme methylase subunit	0	1	1	0
2163622	T→C	T636A (ACG→GCG)	LGG_02097	type II restriction enzyme methyltransferase	0	1	1	0
2353548	G→A <sup>a</sup>	S767F (TCC→TTC)	LGG_02282	hypothetical protein	1	1	1	1
2452317	A→G	Q43Q (CAA→CAG)	LGG_02375	hypothetical protein	1	0	0	0

0: no mutation; 1: mutation present; Ref: Reference strain; P1, P2A, P3: Product isolates 1, 2A, 3.

<sup>a</sup> Identification of the same missing genes in all sequenced genomes, including the reference strain, suggests a sequence artifact in the published genome.

<sup>b</sup> Annotation example, first row: Replacement of the reference G at position 264,278 with a A inside the *ioIT* gene. This mutation changes the 420 codon of *ioIT* from GCC to ACC, causing an alanine (A) to threonine (T) substitution in the encoded protein. For more info see *breseq* manual.

TABLE S4 Regions missing one or more entire genes in ATCC 53103 reference strain and three *L. rhamnosus* GG product isolates

Start	End	Size	Genes <sup>b</sup>	Ref	P1	P2A	P3
414206–415277	449549–448478	33202–35344	[is13]–[is15]_34 genes <sup>a</sup>	0	1	0	0
392237–393747	474456–472946	79200–82220	is11–[is26]_84 genes <sup>a</sup>	0	0	1	0
1873127–1873845	1873871	27–872	[LGG_01843]–[LGG_01844]	1	1	1	1
2574321	2574540–2574399	76–305	[LGG_02511]– [LGG_02512]	1	1	1	1

0: genes present; 1: missing genes; Ref: Reference strain; P1, P2A, P3: Product isolates 1, 2A, 3.

<sup>a</sup> See Table S3 for annotation of missing genes.

<sup>b</sup> Identification of the same missing genes in all sequenced genomes, including the reference strain, suggests a sequence artifact in the published genome.