Generation of Lachnospiraceae family-specific qPCR primers.

Sequences of the 16S rRNA gene from 27 Lachnospiraceae type strains (Table S1) were downloaded from the ribosomal database project (Cole et. al. 2009) and aligned using clustal omega (Goujon et al. 2010). A consensus sequence was manually derived from the alignment and regions where ‘universal’ bacterial primers occurred were masked. Remaining regions were searched for areas of consensus at least 20 bases in length and incorporating no more than two redundant positions. These regions were flagged as potential primer sites and all possible primers were tested with the primer-BLAST tool (Ye et al. 2012). Six possible primer sets were identified as being suitable for qPCR (amplicons less than 200bp) and specific for members of the Lachnospiraceae family. All six primer sets were purchased and tested for specificity against a panel of common gut bacteria including members of 19 different bacterial families (Table S2). Two primer sets showed good specificity for members of the Lachnospiraceae and these were further tested for amplification efficiency using *Blautia producta* (DSM2950) genomic DNA as a target. The primer set chosen (see Table 1) was specific for members of the Lachnospiraceae tested and had close to 100% amplification efficiency.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM & Tiedje JM. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. 2009. Nucleic Acids Res. 37 (Database issue): D141-145; doi: 10.1093/nar/gkn879] [PMID: 19004872]

Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J & Lopez R. A. new bioinformatics analysis tools framework at EMBL-EBI. 2010. Nucleic Acids Res. 38: Issue suppl 2, W695 – W699.

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S & Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. 2012. BMC Bioinformatics. 13: 134.

Supplementary Table S1: List of species used to generate Lachnospiraceae family specific primers for qPCR

RDP link	**Species description**
AJ270487.2:1..1456	Anaerostipes caccae (T); L1-92
X73443.1:1..1524	Clostridium nexile (T); DSM 1787
FR749935.1:<1..>1522	Eubacterium fissicatena; type strain: DSM 3598; 1
AB008552.1:<1..>1508	Eubacterium ruminantium (T); GA195
AJ270482.2:1..1485	Roseburia hominis (T); type strain: A2-183
AY169419.1:<1..>1490	Ruminococcus obeum; 1-33
L34619.1:1..1480	Dorea formicigenerans (T)
L76604.1:1..1418	Ruminococcus torques (T); ATCC 27756
EF031543.1:<1..>1440	Coprococcus eutactus (T); ATCC 27759
AB571656.1:<1..>1493	Blautia coccoides (T); JCM 1395
AJ270473.3:1..1454	Roseburia inulinivorans (T); type strain: A2-194
L34627.1:1..1468	Eubacterium rectale (T)
L76595.1:1..1427	Blautia producta (T); ATCC 27340
AB439724.1:<1..>1468	Blautia glucerasea (T); HFTH-1
X85101.1:1..1457	Ruminococcus obeum (T)
X95624.1:1..1458	Blautia hydrogenotrophica (T); S5a36
AJ132842.1:30..1345	Dorea longicatena (T); III-35
FR749932.1:<1..>1616	Eubacterium hadrum; type strain: DSM 3319
AB038359.1:1..1315	Coprococcus catus (T); VPI-C6-61
AY305310.1:<1..>1456	Roseburia faecis (T); M72/1
EF031542.1:<1..>1453	Coprococcus comes (T); ATCC 27758
X94967.1:1..1481	Ruminococcus gnavus (T); ATCC 29149
M59114.2:<1..>1451	Ruminococcus hansenii (T); ATCC 27752
X94966.1:1..1482	Ruminococcus productus (T); ATCC 27340
AF445285.2:1..1525	Robinsoniella peoriensis (T); PPC31
AJ312385.1:<1..>1482	Roseburia intestinalis (T); L1-82
Y18185.1:<1..>1499	Clostridium saccharolyticum (T); DSM 2544

Supplementary Table S2: Species used to test specificity of Lachnospiraceae family-specific qPCR primer pairs

Phylum	Class	Order	Family	Genera/species	Strain	Type strain
Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium longum subsp. longum*	ATCC 15707	Yes
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides vulgatus*	ATCC 8482	Yes
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	*Parabacteroides distasonis*	ATCC 8503	Yes
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus faecium*	DSM-20477	Yes
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus reuterii*	100-23c	No
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium butyricum*	DSM-10702	Yes
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium disporicum*	DSM-5521	Yes
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Blautia producta*	DSM-2950	Yes
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Ruminococcus obeum*	DSM-25238	Yes
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Clostridium nexile*	DSM-1787	Yes
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Dorea longicatena*	DSM-13814	Yes
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Eubacterium hadrum*	DSM-3319	Yes
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Eubacterium rectale*	DSM-17629	No
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Roseburia intestinalis*	DSM-14610	Yes
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	*Faecalibacterium prausnitzii*	DSM-17677	No
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	*Clostridium innocuum*	DSM-1286	Yes
Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	*Clostridium ramosum*	DSM-1402	Yes
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas nasdae*	DSM-14572	Yes
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Delftia acidovorans*	DSM-39	Yes
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Klebsiella oxytoca*	DSM-5175	Yes
Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	*Haemophilus parainfluenzae*	DSM-8978	Yes
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Acinetobacter guillouiae*	DSM-590	Yes

Lactobacillus-free mouse microarray control

Method

Cells were harvested from log phase (4 hours incubation) cultures of *L. reuteri* 100-23 by brief centrifugation at 12,000 x *g* and washed once with 750 µl of RNAprotect Bacteria reagent (Qiagen). *Lactobacillus* free BALB/C mice were sacrificed by cervical dislocation and the stomach contents and scrapings of the epithelial surface were washed once with RNAprotect Bacteria Reagent. Samples were centrifuged at 150 x *g* for 5 min at 5°C, then the supernatant was further centrifuged at 300 x *g* for 5 min at 5°C to remove debris. Bacterial cells were recovered by centrifugation at 5000 x *g* for 5 min at 5°C. and the cell pellets from six mice were pooled in 1 ml of TRIzol® Reagent. Cells from mouse gut and bacterial culture were disrupted by bead beating (5000 rpm, 2 x 40 seconds) and RNA was extracted with chloroform. RNA was precipitated with iso-propanol, dried and resuspended in nuclease-free water. The RNA was further purified using the RNeasy Mini Kit (Qiagen) and DNase treated using a DNA-free kit (Ambion). Contaminating mouse RNA from the stomach-derived samples of *L. reuteri* 100-23 RNA was removed with the MICROBEnrichTM Kit (Ambion, USA).

RNA (2 µg) purified from both culture and mouse stomach was used for cDNA synthesis and labeled with Cy3- or Cy5-dCTP during first strand synthesis using SuperScript II reverse transcriptase kit (Invitrogen) at 42□C for 90 min. Labeled cDNA was purified using a MinElute PCR purification kit (Qiagen) and quantified by Nanodrop spectrophotometer. The labelled samples were mixed and competitively hybridised using an Agilent Gene Expression Hybridisation Kit (part number: 5188-5242) in an Agilent hybridization oven (G2545A) at 65°C for 24 hours. Microarrays were scanned using the Agilent Microarray Scanner System (G2505B) and Agilent scan control software version 7.0 at a resolution of 5 micrometres. Generated scans were converted to data files with Agilent's Feature Extraction software (Version 9.5.3.1) and a single round of Lowess normalization performed. Microarray data were processed as described by García de la Nava et al. 2003 and van Hijum et al. 2005. Differential gene expression was determined by Cyber-T test (Long 2001).

Results

This experiment identified 155 genes, distributed throughout the *L. reuteri* 100-23 genome, that cross-hybridized with RNA from *Lactobacillus*-free mice. Of these 155 genes, twenty-six genes were annotated as phage genes (see Table below). The origin of the cross-hybridized genes must be phage that target bacteria outside of the *Lactobacillus* genus. As a result of the control hybridization, the 155 genes identified in this analysis were excluded from the results of the test hybridization comparing the *in vivo* and *in vitro* gene expression patterns of *L. reuteri* 100-23C.

Table S3. The locus tag and function of *L. reuteri* 100-23 genes demonstrating a degree of cross hybridization to material isolated from *Lactobacillus*-free mice.

Locus Tag	Function
Lreu23DRAFT_3014	Conserved hypothetical protein
Lreu23DRAFT_3031	Binding-protein-dependent transport systems inner membrane component
Lreu23DRAFT_3053	Diacylglycerol kinase
Lreu23DRAFT_3065	DNA polymerase III, alpha subunit
Lreu23DRAFT_3083	Hypothetical protein Lreu23DRAFT_3083
Lreu23DRAFT_3141	Hypothetical protein Lreu23DRAFT_3141
Lreu23DRAFT_3142	Hypothetical protein Lreu23DRAFT_3142
Lreu23DRAFT_3144	Hypothetical protein Lreu23DRAFT_3144
Lreu23DRAFT_3149	Hypothetical protein Lreu23DRAFT_3149
Lreu23DRAFT_3152	HNH endonuclease
Lreu23DRAFT_3160	Hypothetical protein Lreu23DRAFT_3160
Lreu23DRAFT_3177	Glycoside hydrolase family 25
Lreu23DRAFT_3190	Thymidylate synthase
Lreu23DRAFT_3199	DNA protecting protein DprA
Lreu23DRAFT_3219	GCN5-related N-acetyltransferase
Lreu23DRAFT_3265	Transcriptional regulator, LacI family
Lreu23DRAFT_3277	Recombination protein U
Lreu23DRAFT_3317	Hypothetical protein Lreu23DRAFT_3317
Lreu23DRAFT_3318	Amino acid/peptide transporter
Lreu23DRAFT_3319	Metallophosphoesterase
Lreu23DRAFT_3393	Aminotransferase class I and II
Lreu23DRAFT_3400	YbaK/prolyl-tRNA synthetase associated region
Lreu23DRAFT_3429	Excinuclease ABC, B subunit
Lreu23DRAFT_3480	Sugar transporter
Lreu23DRAFT_3481	Transcriptional regulator, AraC family
Lreu23DRAFT_3493	Protein of unknown function DUF6 transmembrane
Lreu23DRAFT_3553	Xanthine/uracil/vitamin C permease
Lreu23DRAFT_3585	DNA mismatch repair protein MutS
Lreu23DRAFT_3607	Haloacid dehalogenase domain protein hydrolase type 3
Lreu23DRAFT_3616	Type II secretion system protein
Lreu23DRAFT_3618	Conserved hypothetical protein
Lreu23DRAFT_3619	Hypothetical protein Lreu23DRAFT_3619
Lreu23DRAFT_3632	6-phosphogluconolactonase
Lreu23DRAFT_3722	Phospholipid/glycerol acyltransferase
Lreu23DRAFT_3738	Hypothetical protein Lreu23DRAFT_3738
Lreu23DRAFT_3739	Hypothetical protein Lreu23DRAFT_3739
Lreu23DRAFT_3772	Nuclease SbcCD, D subunit
Lreu23DRAFT_3780	Filamentation induced by cAMP protein Fic

Lreu23DRAFT_3794	Hydroxyethylthiazole kinase
Lreu23DRAFT_3800	Conserved hypothetical protein
Lreu23DRAFT_3803	Transport system permease protein
Lreu23DRAFT_3849	Glycoside hydrolase family 25
Lreu23DRAFT_3851	Hypothetical protein Lreu23DRAFT_3851
Lreu23DRAFT_3858	Peptidase M23
Lreu23DRAFT_3861	Protein of unknown function DUF1306
Lreu23DRAFT_3875	Terminase small subunit putative P27
Lreu23DRAFT_3877	N-6 DNA methylase
Lreu23DRAFT_3880	Hypothetical protein Lreu23DRAFT_3880
Lreu23DRAFT_3916	Conserved hypothetical protein
Lreu23DRAFT_3965	Exodeoxyribonuclease VII, small subunit
Lreu23DRAFT_3967	Methenyltetrahydrofolate cyclohydrolase
Lreu23DRAFT_3978	Phage uncharacterized protein, XkdX family
Lreu23DRAFT_3995	Hypothetical protein Lreu23DRAFT_3995
Lreu23DRAFT_4008	Hypothetical protein Lreu23DRAFT_4008
Lreu23DRAFT_4026	Hypothetical protein Lreu23DRAFT_4026
Lreu23DRAFT_4044	Protein of unknown function DUF464
Lreu23DRAFT_4077	Acylphosphatase
Lreu23DRAFT_4081	Amino acid permease-associated region
Lreu23DRAFT_4117	YSIRK Gram-positive signal peptide
Lreu23DRAFT_4124	Conserved hypothetical protein
Lreu23DRAFT_4133	Hypothetical protein Lreu23DRAFT_4133
Lreu23DRAFT_4150	Mannosyl-glycoprotein endo-beta-N-acetylglucosamidase
Lreu23DRAFT_4153	Carbohydrate kinase, YjeF related protein
Lreu23DRAFT_4155	Protein of unknown function UPF0150
Lreu23DRAFT_4159	DNA-3-methyladenine glycosylase I
Lreu23DRAFT_4163	VanZ family protein
Lreu23DRAFT_4197	Hypothetical protein Lreu23DRAFT_4197
Lreu23DRAFT_4198	Peptidase M23
Lreu23DRAFT_4200	Phage tape measure protein
Lreu23DRAFT_4201	Conserved hypothetical protein
Lreu23DRAFT_4203	Major tail 2 family protein
Lreu23DRAFT_4205	Phage protein, HK97 gp10 family
Lreu23DRAFT_4209	Conserved hypothetical protein
Lreu23DRAFT_4210	Hypothetical protein Lreu23DRAFT_4210
Lreu23DRAFT_4212	Phage portal protein, SPP1 family
Lreu23DRAFT_4222	Hypothetical protein Lreu23DRAFT_4222
Lreu23DRAFT_4225	Virulence-associated E family protein
Lreu23DRAFT_4226	Bifunctional DNA primase/polymerase
Lreu23DRAFT_4228	Hypothetical protein Lreu23DRAFT_4228
Lreu23DRAFT_4229	Type III restriction protein res subunit
Lreu23DRAFT_4233	Hypothetical protein Lreu23DRAFT_4233

Lreu23DRAFT_4238	Helix-turn-helix domain protein
Lreu23DRAFT_4298	Ribonuclease BN
Lreu23DRAFT_4320	N-acetylmuramoyl-L-alanine amidase family 2
Lreu23DRAFT_4323	Oxidoreductase
Lreu23DRAFT_4335	Small multidrug resistance protein
Lreu23DRAFT_4336	Hypothetical protein Lreu23DRAFT_4336
Lreu23DRAFT_4346	Transposase ISLasa1a, IS1223 family
Lreu23DRAFT_4348	Site-specific recombinase, DNA invertase Pin related protein
Lreu23DRAFT_4411	Peptidase A24A domain protein
Lreu23DRAFT_4416	Integrase catalytic region
Lreu23DRAFT_4433	UspA domain protein
Lreu23DRAFT_4451	Transcriptional regulator, LysR family
Lreu23DRAFT_4476	ABC transporter related
Lreu23DRAFT_4484	Mn2+/Fe2+ transporter, NRAMP family
Lreu23DRAFT_4487	Transcriptional regulator, XRE family
Lreu23DRAFT_4494	Glycosyl transferase family 2
Lreu23DRAFT_4498	Phosphate ABC transporter, ATPase subunit
Lreu23DRAFT_4500	Phosphate ABC transporter, inner membrane subunit PstC
Lreu23DRAFT_4501	Phosphate binding protein
Lreu23DRAFT_4502	Putative two component transcriptional regulator, winged helix family
Lreu23DRAFT_4505	Cobalamin (vitamin B12) biosynthesis CbiM protein
Lreu23DRAFT_4507	Camphor resistance CrcB protein
Lreu23DRAFT_4509	Urease accessory protein UreG
Lreu23DRAFT_4510	Urease accessory protein UreF
Lreu23DRAFT_4514	Urease, gamma subunit
Lreu23DRAFT_4539	Integrase catalytic region
Lreu23DRAFT_4584	Major facilitator superfamily MFS_1
Lreu23DRAFT_4585	Hypothetical protein Lreu23DRAFT_4585
Lreu23DRAFT_4625	Transcriptional regulator, XRE family
Lreu23DRAFT_4626	Conserved hypothetical protein
Lreu23DRAFT_4630	Lipoyltransferase and lipoate-protein ligase
Lreu23DRAFT_4673	Protein tyrosine/serine phosphatase
Lreu23DRAFT_4691	Appr-1-p processing domain protein
Lreu23DRAFT_4695	Conserved hypothetical protein
Lreu23DRAFT_4699	Conserved hypothetical small protein
Lreu23DRAFT_4701	Conserved hypothetical protein
Lreu23DRAFT_4703	Hypothetical protein Lreu23DRAFT_4703
Lreu23DRAFT_4711	Transcriptional regulator, XRE family
Lreu23DRAFT_4722	MATE efflux family protein
Lreu23DRAFT_4760	Hypothetical protein Lreu23DRAFT_4760
Lreu23DRAFT_4762	Hypothetical protein Lreu23DRAFT_4762

Lreu23DRAFT_4763	Putative transposase
Lreu23DRAFT_4764	Integrase catalytic region
Lreu23DRAFT_4769	Conserved hypothetical protein
Lreu23DRAFT_4778	Transcriptional regulator, HxlR family
Lreu23DRAFT_4796	Phenolic acid decarboxylase
Lreu23DRAFT_4799	Transferase hexapeptide repeat containing protein
Lreu23DRAFT_4818	Two component transcriptional regulator, winged helix family
Lreu23DRAFT_4824	Integrase catalytic region
Lreu23DRAFT_4833	Conserved hypothetical protein
Lreu23DRAFT_4834	ABC transporter related
Lreu23DRAFT_4835	ABC-2 type transporter
Lreu23DRAFT_4851	Single-stranded nucleic acid binding R3H domain protein
Lreu23DRAFT_4904	D-isomer specific 2-hydroxyacid dehydrogenase NAD-binding
Lreu23DRAFT_4949	ABC transporter related
Lreu23DRAFT_4975	Dihydroorotate dehydrogenase family protein
Lreu23DRAFT_4976	Orotidine 5\'-phosphate decarboxylase
Lreu23DRAFT_4977	Orotate phosphoribosyltransferase
Lreu23DRAFT_4988	Phosphoribosylformylglycinamidine synthase, purS
Lreu23DRAFT_4989	Phosphoribosylformylglycinamidine synthase I
Lreu23DRAFT_4990	Phosphoribosylformylglycinamidine synthase II
Lreu23DRAFT_4992	Phosphoribosylformylglycinamidine cyclo-ligase
Lreu23DRAFT_4994	Phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
Lreu23DRAFT_4995	Phosphoribosylamine/glycine ligase
Lreu23DRAFT_5034	Binding-protein-dependent transport systems inner membrane component
Lreu23DRAFT_5050	Hypothetical protein Lreu23DRAFT_5050
Lreu23DRAFT_5054	Relaxase/mobilization nuclease family protein
Lreu23DRAFT_5078	Protein of unknown function DUF72
Lreu23DRAFT_5107	Fructose-1,6-bisphosphate aldolase, class II
Lreu23DRAFT_5124	Permease for cytosine/purines uracil thiamine allantoin
Lreu23DRAFT_5159	Hypothetical protein Lreu23DRAFT_5159
Lreu23DRAFT_5160	Hypothetical protein Lreu23DRAFT_5160
Lreu23DRAFT_5177	L-serine dehydratase, iron-sulfur-dependent, alpha subunit
Lreu23DRAFT_5180	LPXTG-motif cell wall anchor domain protein