Supplementary Data

Table S1. Gene copy number of 16s rRNA and tuf genes for the five target reference species.

Data Source	Organism	Strain Number	Copies	
			16S rRNA	*tuf*
ATCC website (genomes.atcc.org)	*B. bifidum*	ATCC 11863	3	1
ATCC website (genomes.atcc.org)	*B. breve*	ATCC 15700	3	1
NCBI, Genbank CR954253.1	*L. delbrueckii subsp. bulgaricus*	ATCC 11842	9	1
ATCC website (genomes.atcc.org)	*S. thermophilus*	ATCC 19258	5	1
NCBI, Genbank FM179322.1	*L. rhamnosus*	ATCC 53103	5	1

Table S2. Identity Matrix (%) of tuf gene sequences

Organism	Strain	Identity (%)				Overall Identity (%)	Overall Variation (%)
		ATCC 29521	ATCC 19258	ATCC 11842	ATCC 53103		
Bifidobacterium bifidum	ATCC 29521	100.00	63.83	66.29	68.24	69.31	30.7
Streptococcus thermophilus	ATCC 19258	63.83	100.00	71.31	71.26		
Lactobacillus delbrueckii subsp. bulgaricus	ATCC 11842	66.29	71.31	100.00	74.90		
Lacticaseibacillus rhamnosus	ATCC 53103	68.24	71.26	74.90	100.00		

Analysed using Multiple Sequence Comparison by Log Expectation (MUSCLE) program (https://www.ebi.ac.uk/Tools/msa/muscle/).

Table S3. Identity Matrix (%) of 16S rRNA gene sequences

Organism	Strain	Identity (%)				Overall Identity (%)	Overall Variation (%)
		ATCC 29521	ATCC 19258	ATCC 11842	ATCC 53103		
Bifidobacterium bifidum	ATCC 29521	100.00	77.20	78.28	79.16		
Streptococcus thermophilus	ATCC 19258	77.20	100.00	83.37	84.62	81.73	18.27
Lactobacillus delbrueckii subsp. bulgaricus	ATCC 11842	78.28	83.37	100.00	87.73		
Lacticaseibacillus rhamnosus	ATCC 53103	79.16	84.62	87.73	100.00		

Analysed using Multiple Sequence Comparison by Log Expectation (MUSCLE) program (https://www.ebi.ac.uk/Tools/msa/muscle/).

Table S4. Real-Time qPCR Primer Properties

Primer Name	Primer Length (bp)	Amplicon Size (bp)	Hairpin 3' end (Kcal/mol)	Primer Tm (°C)	GC (%)	Self complementarity	Self 3' Complementarity
ST-F	23	118	1.46	60.00	43.48	4.00	2.00
ST-R	23		-0.50	59.56	43.48	6.00	6.00
Bb-1F	21	149	-0.27	60.20	52.38	3.00	3.00
Bb-1R	20		-0.27	60.04	60.00	3.00	1.00
Ldb-1F	22	121	-1.26	60.22	50.00	5.00	2.00
Ldb-1R	22		0.97	59.51	50.00	2.00	2.00
Lcr-1F	22	117	0.97	59.97	50.00	6.00	0.00
Lcr-1R	22		0.97	59.76	50.00	6.00	1.00

Legend: Tm – Melt Temperature

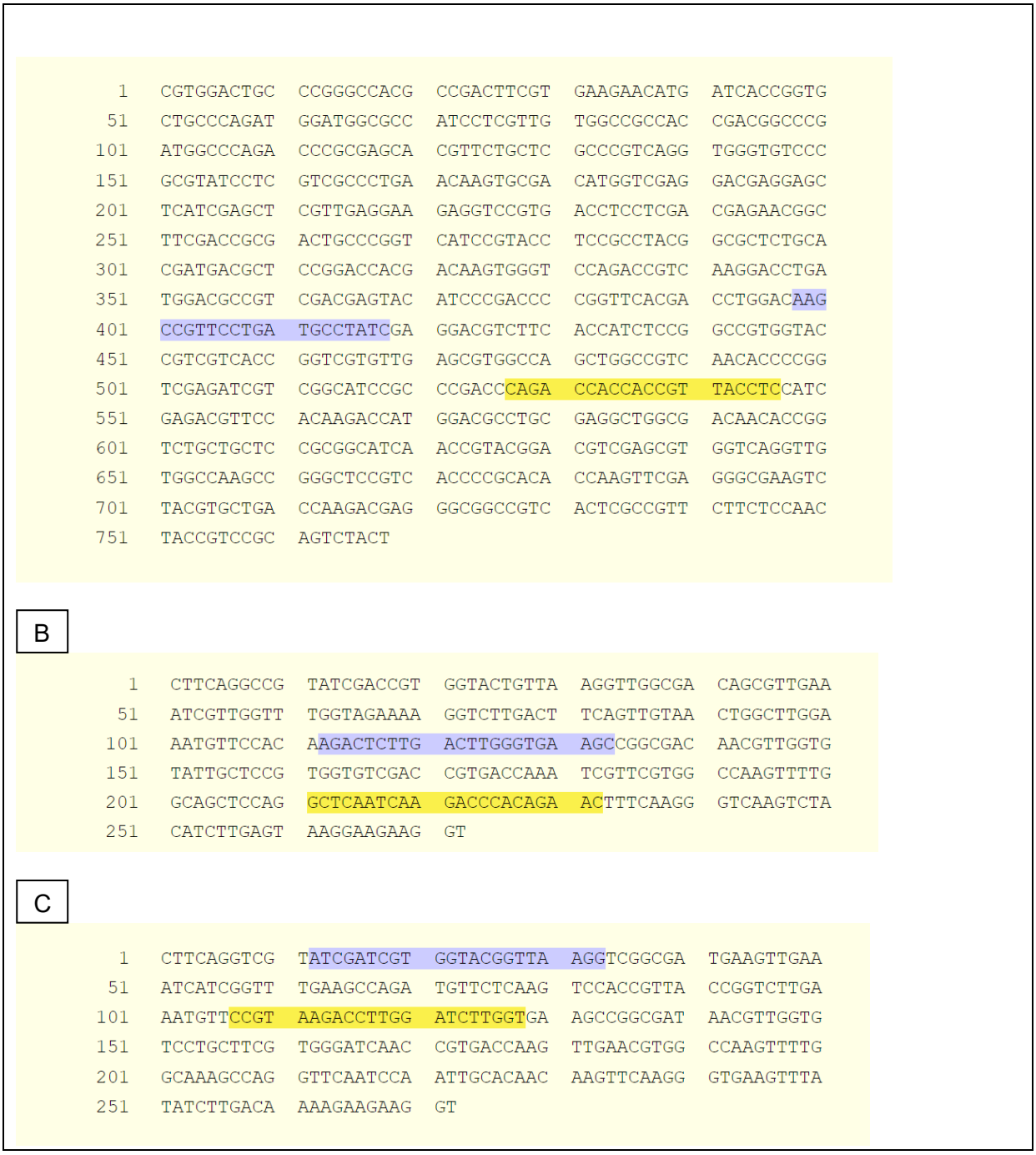

Figure S1. Designed primer sets (Yellow-Forward Primer & Purple-Reverse Primer) flanking target regions on the tuf genes of B. bifidum (A), L. delbrueckii (B) and L. rhamnosus (C). Primers were designed using the online software primer 3 plus (https://www.primer3plus.com/).

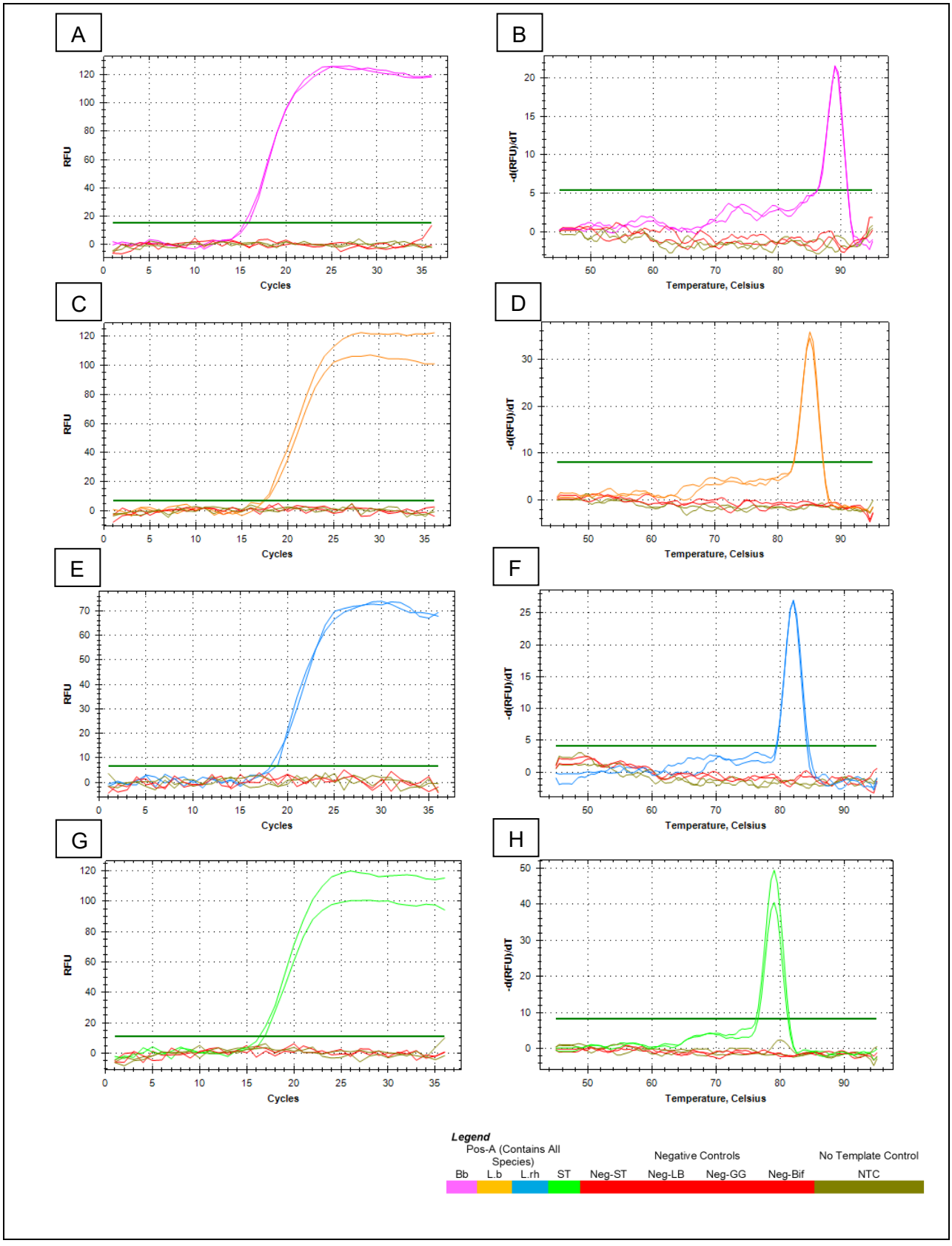

Figure S2. Amplification (left) and melt (right) curves showing the specificity of the four sets of primers in mixed species environment. Graphs A-B represented primers for B. bifidum (Bb), C-D for L. delbrueckii subsp. bulgaricus (L.b), E-F for L. rhamnosus (L.rh), and G-H for S. thermophilus (ST).

Standard Curves: Limit of Quantification (LOQ)

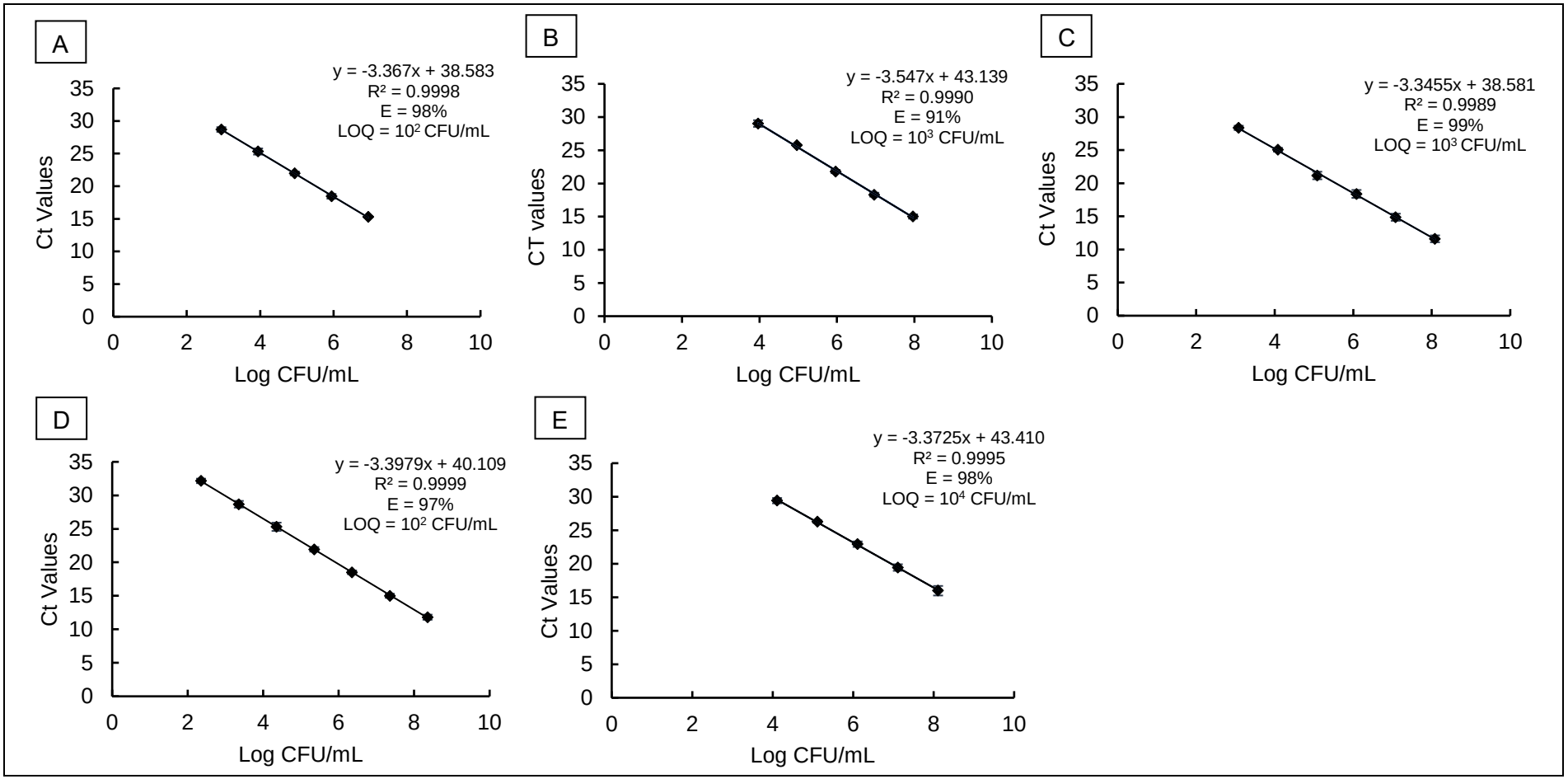

Figure S3. Standard curves of PMAxx™-qPCR assay created and used for determining the limit of quantification (LOQ) for *B. bifidum* ATCC 11863 (A), *B. breve* ATCC 15700 (B), *L. delbrueckii* subsp. *bulgaricus* (C), *S. thermophilus* (D), and *L. rhamnosus* (E). Each point represents the mean ± standard deviation of CT values of two independent runs (*n* = 4). Each run was carried out in duplicate