

Supplemental Material to:

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**Tough nuts to crack: Site-directed mutagenesis of
bifidobacteria remains a challenge**

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1 Table S1: Bacterial strains, plasmids and primers used in this study

Name	Relevant feature	
Strains		
<i>E. coli</i> DH10B	Cloning host	Invitrogen
<i>E. coli</i> ET12567	Plasmid artificial modification host strain	32
<i>B. bifidum</i> S17	Fecal isolate from a breast-fed infant	24
Plasmids		
p16Slux	Chromosomal integration vector for constitutive luminescent labelling of Gram-negative bacteria	31
p16S	Backbone of p16Slux lacking <i>luxABCDE</i>	this study
pREDI	Template for amplification of <i>araC</i> and P_{araB}	30
pBAD	Intermediate vector to clone methylase genes under control of P_{araB}	this study
p16S_ <i>hsdMS</i>	Chromosomal integration vector for arabinose inducible expression of HsdMS of <i>B. bifidum</i> S17 in <i>E. coli</i> ET12567	this study
p16S_ <i>bbif0710</i>	Chromosomal integration vector for arabinose inducible expression of the type II MTase (BBIF_0710) of <i>B. bifidum</i> S17 in <i>E. coli</i> ET12567	this study
Primers for RT-PCR		
	Sequence 5'-3'	Amplicon [bp]
rt_ <i>hsdM</i> _fwd	GAC GCA GCA GAT CAT TTG CCA GAG TCT CC	1002
rt_ <i>hsdM</i> _rev	ACA CCT ACA ACC TCA CGC GCA TGA ACC	
rt_ <i>hsdS</i> _fwd	ATG ATG ACT GAA CAG GCG AAA GTT CC	1242
rt_ <i>hsdS</i> _rev	TCA CAC GAA CAT CCT GTC AAG CAA GG	
rt_ <i>hsdR</i> _fwd	GCC ACT GAT ACG GTT TCC	889
rt_ <i>hsdR</i> _rev	GCT CAT CGG CTT CTA CAC	
rt_ <i>t2mtase</i> _fwd	TCC AGA TGG CAC GGC AAT ACG AC	724
rt_ <i>t2mtase</i> _rev	CAT ACG TTC GGG CTC GAC AAG GTT C	
rt_ <i>t2rease</i> _fwd	GAG ACT GCG ATA CTC GGC ATG TAT AG	671
rt_ <i>t2rease</i> _rev	CCC GAC TCA ATC GTC GTC ATA AAC	
Cloning primers		
	Sequence 5'-3'	Restriction site
<i>hsdMS</i> _fwd	TGC GCA GGA <u>CTA</u> <u>GTA</u> TGA ATA AGC AGC AGC TCG	SpeI
<i>hsdMS</i> _rev	TCC <u>TCT</u> <u>CGA</u> <u>GTA</u> GGT GTT GGC GAA GGC GGA GTA GGT C	XhoI
<i>bbif0710</i> _fwd	TGC TGC <u>ACT</u> <u>AGT</u> ATG CCC GCC GAG CAT GGT GTT ATT TAC	SpeI
<i>bbif0710</i> _rev	CAT CAT <u>ATC</u> <u>GAT</u> CGC AGC ACG CGC CAT AGC ACT ATA C	Clal
pBAD_fwd	AAT <u>GCG</u> <u>GCC</u> <u>GCC</u> GCG TAA TAC GAC TCA C	NotI
pBAD_rev	AAT <u>GCG</u> <u>GCC</u> <u>GCG</u> CGC AAT TAA CCC TCA C	NotI

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