



Fig. S1. Plasmid constructions.

A: pKO403-TPCTcon, used for the *CAT* assay in a previous study [1]. **B:** pBCMAT-P_{xxx}-T_{dppA2}, used for testing promoter activities with the *CAT* gene. **C:** pBIFGLOW-P_{xxx}-T_{dppA2}, used for testing promoter activities with the *evoglow-Bs2* gene. **D:** pBCMAT-P_{gap}-T_{xxx}, used for testing terminator activities with the *CAT* gene. *Sp^r*: a resistance gene of spectinomycin. *ColE1 ori*: a replication origin for *Escherichia coli*. *pTB6 ori*: a replication origin for *Bifidobacterium* species. *CAT*: a gene-coding region of chloramphenicol acetyltransferase. *evoglow-Bs2*: a gene-coding region of evoglow-Bs2. *ori Ts*: temperature-sensitive replication origin.

Table S1. Bacterial strains and plasmids.

Type	Name	Properties
Bacterial strains	<i>Bifidobacterium longum</i> NCC2705	Transformation host
	<i>Bifidobacterium breve</i> JCM 1192	Transformation host
	<i>Bifidobacterium adolescentis</i> ATCC 15703	Transformation host
	<i>Escherichia coli</i> TOP10	Transformation host
Plasmids	pBCMAT-P _{gap} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{dppA2} , Template for PCR
	pBCMAT-P _{rpmB} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rpmB} , T _{dppA2} , Template for PCR
	pBCMAT-P _{groES} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{groES} , T _{dppA2} , Template for PCR
	pBCMAT-P _{rpmH} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rpmH} , T _{dppA2} , Template for PCR
	pBCMAT-P _{BL143} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{BL143} , T _{dppA2} , Template for PCR
	pBCMAT-P _{rplU} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rplU} , T _{dppA2} , Template for PCR
	pBCMAT-P _{tuf} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{tuf} , T _{dppA2}
	pBCMAT-P _{rplM} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rplM} , T _{dppA2} , Template for PCR
	pBCMAT-P _{hup} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{hup} , T _{dppA2}
	pBCMAT-P _{BL1230} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{BL1230} , T _{dppA2} , Template for PCR
	pBCMAT-P _{BL1769} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{BL1769} , T _{dppA2}
	pBCMAT-P _{gap-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap-imp} , T _{dppA2}
	pBCMAT-P _{groES-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{groES-imp} , T _{dppA2}
	pBCMAT-P _{rpmH-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rpmH-imp} , T _{dppA2}
	pBCMAT-P _{BL143-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{BL143-imp} , T _{dppA2}
	pBCMAT-P _{rplU-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rplU-imp} , T _{dppA2}
	pBCMAT-P _{rplM-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rplM-imp} , T _{dppA2}
	pBCMAT-P _{BL1230-imp} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{BL1230-imp} , T _{dppA2}
	pBCMAT-P _{gap-rpmB} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap-rpmB} (Chimeric), T _{dppA2}
	pBCMAT-P _{gap-rplM} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap-rplM} (Chimeric), T _{dppA2}
	pBCMAT-P _{gap-rplU} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap-rplU} (Chimeric), T _{dppA2}
	pBCMAT-P _{rpmB-gap} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rpmB-gap} (Chimeric), T _{dppA2}
	pBCMAT-P _{rplM-gap} -T _{dppA2}	<i>Sp^r</i> , <i>Cm^r</i> , P _{rplM-gap} (Chimeric), T _{dppA2}
	pBCMAT-P _{gap} -T _{rplQ}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{rplQ}
	pBCMAT-P _{gap} -T _{rplL}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{rplL}
	pBCMAT-P _{gap} -T _{BL0593}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{BL0593}
	pBCMAT-P _{gap} -T _{tuf}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{tuf}
	pBCMAT-P _{gap} -T _{tal}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{tal}
	pBCMAT-P _{gap} -T _{rpsO}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{rpsO}
	pBCMAT-P _{gap} -T _{BL0618}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{BL0618}
	pBCMAT-P _{gap} -T _{gap}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{gap}
	pBCMAT-P _{gap} -T _{ahpC}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{ahpC}
	pBCMAT-P _{gap} -T _{BL0725}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{BL0725}
	pBCMAT-P _{gap} -T _{nhaA}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{nhaA}
	pBCMAT-P _{gap} -T _{rpsO-imp}	<i>Sp^r</i> , <i>Cm^r</i> , P _{gap} , T _{rpsO-imp}
	pBIFGLOW-P _{gap} -T _{dppA2}	<i>Sp^r</i> , <i>evoglow-Bs2</i> , P _{gap} , T _{dppA2}
	pBIFGLOW-P _{rpmB} -T _{dppA2}	<i>Sp^r</i> , <i>evoglow-Bs2</i> , P _{rpmB} , T _{dppA2}
	pKKT427	Template for PCR, [2]
	pGLOW-Bs2	Template for PCR, [3]
	pKO403-TPCTcon	Template for PCR, [1]

Table S2. Primers for cloning of promoters and terminators.

Name	Sequences (5' → 3')
tufpF	ccagctcttcgACACGCGCCACTGCATGAA
tufpR	ccagctcttcgCATCTGGACGTCTCGTGAGTTT
gappF	ccagctcttcgACATTCGCTGACTTGCATGCC
gappR	ccagctcttcgCATTGTAGGGTGGCCTTGGC
huppF	ccagctcttcgACACGTCTATTTTCATACCCCT
huppR	ccagctcttcgCATGTCAGGGGACAAGCACTT
BL1230pF	ccagctcttcgACACCTTACCTCTTCGGGAAA
BL1230pR	ccagctcttcgCATAAAAATTACTGACAATTA
rpmHpF	ccagctcttcgACATGGCGAAATACGTACAAC
rpmHpR	ccagctcttcgCATCTATTCGGCAACGCTTC
BLt43pF	ccagctcttcgACAGAAGAGTCGCGTGCCAC
BLt43pR	ccagctcttcgCATCCACCTCGGCATGC
groESpF	ccagctcttcgACAGCGTTGCGATTTCGACGAT
groESpR	ccagctcttcgCATCGTATCTACTTGTAGTTTGA
rpmBpF	ccagctcttcgACATCGGGTAAACGCTATGA
rpmBpR	ccagctcttcgCATGTGTCAAGTCTTTCACG
rplUpF	ccagctcttcgACAGCGGAAGAGTAACAGGTG
rplUpR	ccagctcttcgCATGTTTTGAAAGCTACCTTG
BL1769pF	ccagctcttcgACATATTGCAGCGTTTATCAG
BL1769pR	ccagctcttcgCATCTGTGAGTTTCTCCAA
rplMpF	ccagctcttcgACATAGGTGAGTCGGTTAGCC
rplMpR	ccagctcttcgCATGCTGCTTGGTGTGGCTTG
taltF	ccagctcttcgTGAGATTTGACGACTGATG
taltR	ccagctcttcgTTCGTACCACTGTAGCAAC
tuftF	ccagctcttcgTGATTTTCATCAGACAAGA
tuftR	ccagctcttcgTTCAGTCGTGGTACATATC
gaptF	ccagctcttcgTGAACCAATTGCGGTATAGG
gaptR	ccagctcttcgTTCCTTATCATGCTTGCTT
rplLtF	ccagctcttcgTGATTTGTGCGGCATAAGC
rplLtR	ccagctcttcgTTCGATCGCGTTCTCCGCC
rplQtF	ccagctcttcgTGAGCTGAATAGCGTTTCG
rplQtR	ccagctcttcgTTCGGCCGTACGTACGTTG
dppA2tF	ccagctcttcgTGAAGTACTGACTGAACGG
dppA2tR	ccagctcttcgTTCGATGGCGTGAGCAAG
BL0593tF	ccagctcttcgTGAGTTCCGGCTCGTTGCG
BL0593tR	ccagctcttcgTTC AACAGGCTAAGGCACG
BL0618tF	ccagctcttcgTGAGTAAAAGCAGTATTCC
BL0618tR	ccagctcttcgTTCAGACTCTCTATTTAC
nhaAtF	ccagctcttcgTGATGGCTTAGACGGTCCC
nhaAtR	ccagctcttcgTTC AAGGCCTCATCTCGTG
ahpCtF	ccagctcttcgTGATCGACACTGAATAGGC
ahpCtR	ccagctcttcgTTC CCCTTACATACACTGG
rpsOtF	ccagctcttcgTGATTGAAGACTTCCGCC
rpsOtR	ccagctcttcgTCATTGTTGCCGTTGGCTG
BL0725tF	ccagctcttcgTGATGACCGGGTGC GGAC
BL0725tR	ccagctcttcgTTCCTTATTGCGCGTACCG
huptF	ccagctcttcgCTTCCTTCTGCTCGTAGCGATT
huptR	ccagctcttcgTGTTGGAAGCGCTGAACTAGTC
CAT+RBS-F	ccagctcttcgATGCCCTGACCCAAGGAGAACATC
CAT-R	ccagctcttcgTCATAAAAAGCCAGTCATTAGGC
evoglow-F	ccagctcttcgATGGCGTCGTTCCAGTCG
evoglow-R	ccagctcttcgTCACTCGAGCAGCTTTTCATATT
vector-F	ccagctcttcgGAAGCCACCGTCGCCAAGG
Spectinomycin_r-R	ccagctcttcgAAGGGTTCGATTTTCGTTTCGTGAATAC
RBS+ATGcodon-R	ccagctcttcgCATGATGTTCTCCTTGGGTCA

Table S3. Primers for modification of promoters and terminators.

Name	Sequences (5' → 3')
gap-im-F	CAGAGTCGGCATT TATAAT AGCAAC
gap-im-R	TACACATGGCAACGTTTC
rplU-im-F	TATAAT CGAAACTCGGTGTCT
rplU-im-R	CACCACCATGCGGACTAAA
BLt43-im-F	TTATAAT AACGACTTGGCGG
BLt43-im-R	CGGCTCCGGTGGATTAT
rpmH-im-F	TAAT GGTATAGCTTGACTCAGC
rpmH-im-R	TACT CTCACCCCTACCC
groES-im-F	TAAT GTGTCTTAGCGCAA
groES-im-R	TAG CGCGATTATTAGCACT
BL1230-im-F	CATT TATAAT CATAATTGTCAG
BL1230-im-R	AATGATAATTTGAATCACAAT
rplM-im-F	ATATAAT AGTGGATTGTTGTGT
rplM-im-R	CGCCGGGCTTGACATA
rpsO_ter-im-F	GTACCTAGGATGGTGCTC
rpsO_ter-im-R	TCGGGCGT TTTT CTTCAATC
rplU-chi-F	ccagctcttcgTGTCTGACCGCAAGCTC
rpmB-chi-F	ccagctcttcgTGTGTTCCGGCATGTCGG
rplM-chi-F	ccagctcttcgTGTGTTCCCTAAGGGGTC
gap-chi-F	ccagctcttcgTGTTGGTAAACAATGGCCCG
rpmB-chi-R	ccagctcttcgACACAACCTCACAAATATATAGC
rplM-chi-R	ccagctcttcgACAACAATCCACTAGTATATCGC
gap-chi-R	ccagctcttcgACAGTTGCTACTGTAATGCCGA
gappR	ccagctcttcgCATTGTAGGGTGGCCTTGGC
rplUpR	ccagctcttcgCATGTTTTGGAAAGCTACCTTG
rpmBpR	ccagctcttcgCATGTGTCAAGTCTTTCACG
rplMpR	ccagctcttcgCATGCTGCTTGGTGTGGCTTG
RBS-F	ccagctcttcgATGCCCTGACCCAAGGAGAACAT

Table S4. Reporter genes for *Bifidobacterium*.

Gene	Length	References
β -glucuronidase	1.8 kbp	[4]
α -galactosidase	2.3 kbp	[5]
arabinofuranosidase	1.7 kbp	[6]
β -glucosidase	1.4 kbp	[7]
luciferase	5.6 kbp	[8]
Chloramphenicol acetyltransferase	0.65 kbp	This study

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