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Title	Bifidobacterium breve UCC2003 employs multiple transcriptional regulators to control metabolism of particular human milk oligosaccharides
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Supplemental Table S1. Locations and sizes of fragments used in electromobility shift assays, in relation to the promoter regions' respective transcription start sites.

Fragment Name	Fragment Start (Position)	Fragment End (Position)	Fragment Size (bp)	Number of identified inverted repeats	Shift Observed (+/-)
lntP1a	-174	+74	249	2	++
lntP1b	-89	+173	263	2*	+
lntP1c	+5	+258	254	0	-
lntSa	-132	+159	292	2	++
LntSb	-56	+252	309	2*	+
lntSc	+30	+337	308	0	-
nahSa	-197	+90	288	1	+
nahSb	-91	+211	303	1	+
nahSFc	+4	+289	286	0	-
nahAa	-206	+95	302	1	+
nahAb	-70	+226	297	0	-
nahAc	+11	+310	300	0	-
nagB3a	-176	+175	352	2	++
nagB3b	-84	+338	423	2*	+
nagB3c	-22	+338	361	1	+
nagB3d	+94	+338	245	0	-
nagKa	-283	+49	333	1	+
nagKb	-283	-59	225	0	-
lnpBa	-227	+93	321	1	+
lnpBb	+4	+262	259	0	-
lnpBc	-91	+262	354	1	+
gltAa	-419	+83	503	1	+
gltAb	-304	+205	510	1	+
gltAc	-59	+205	265	1	+

Binding of the amplified DNA fragment by the regulator protein, and thus formation of a DNA-protein complex is observed as a shift in band position in the EMSA gel.

A positive result for a band shift is represented by a '+'. A double-shift is represented by a '++'. No observed shift is represented by a '-'.

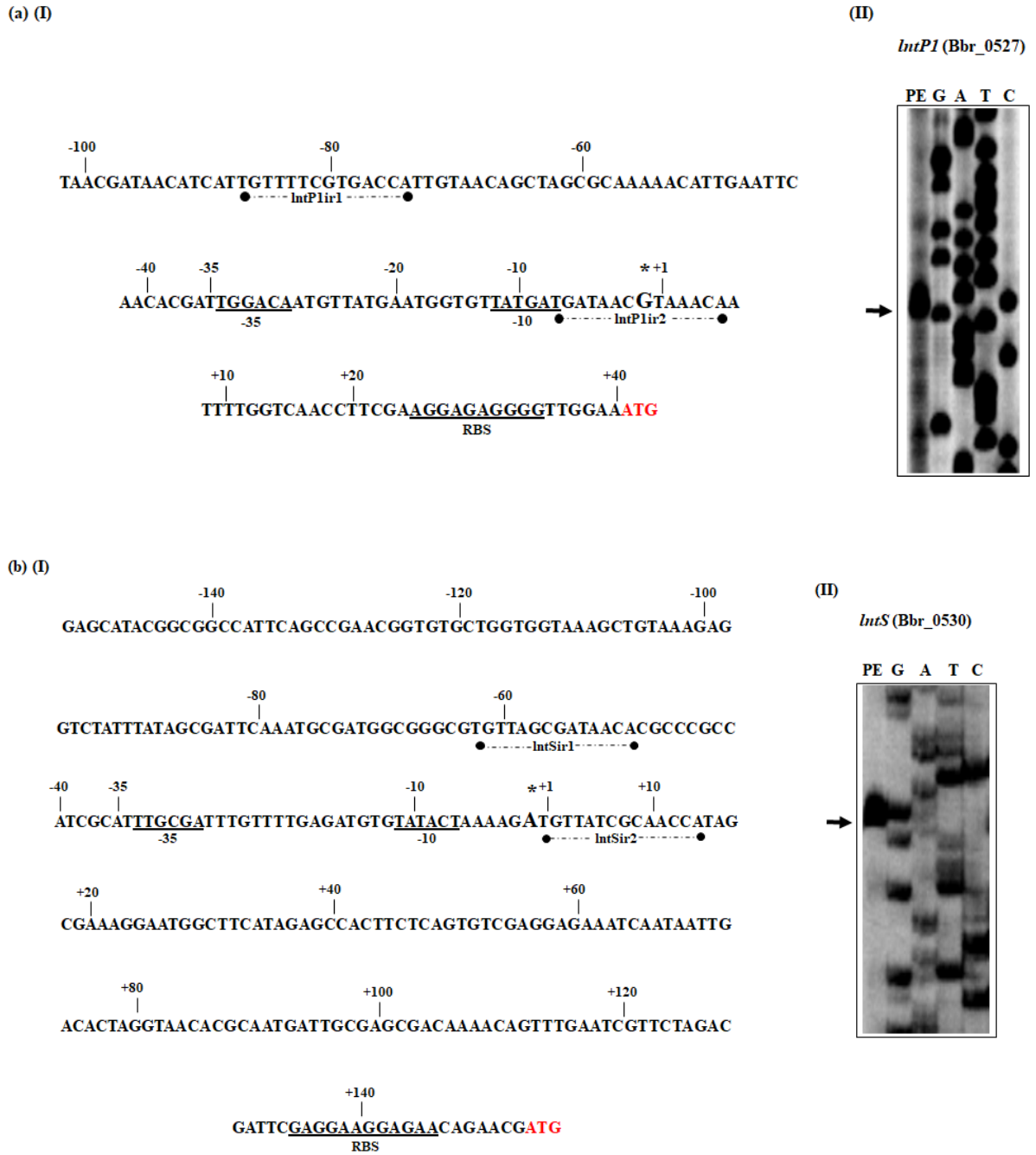
Fragment positions are given in relation to the deduced transcription start site (0).

'*' indicates that one of the identified inverted repeat sequences is partially interrupted by or in close proximity to the terminus of the fragment.

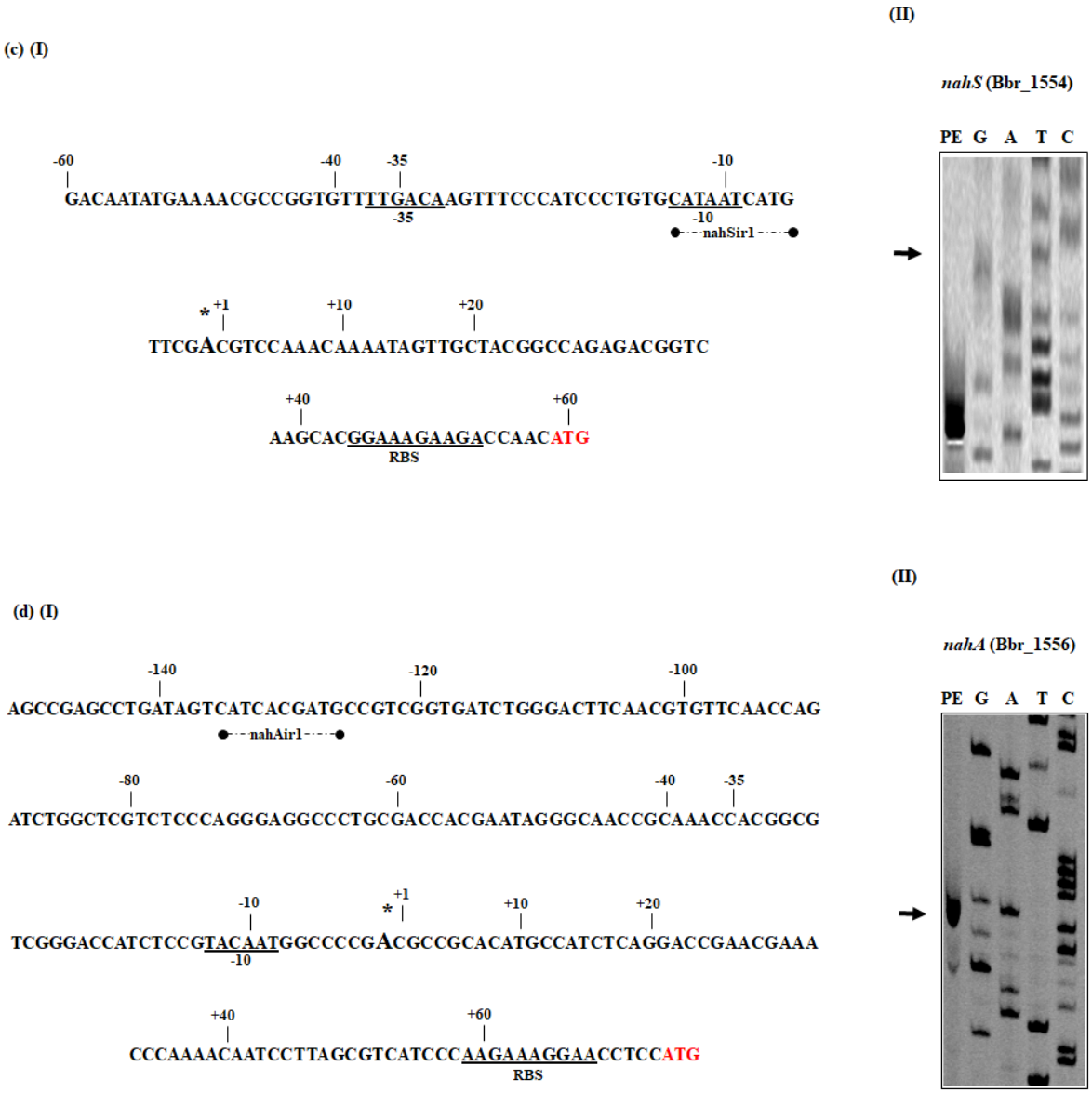
Supplemental Table S2. Predicted operator sequence used for the *in silico* generation of operator sequence motif consensuses.

Regulator	Operator Name	Sequence
LntR	lntP1ir1	tgttttcgtgacca
	lntP1ir2	tgataacgtaaaca
	lntS1ir1	tgttagcgataaca
	lntS1ir2	tgttatcgcaacca
NahR	nahSir1	cataatcatg
	nahAir1	catcacgatg
NagR1	nagB3ir1	aattgtaagtagtattaacaaat
	nagB3ir2	atctgttagggaacttcactaat
	nagKir1	atttgttaagatagttgtcaata
	lnpBir1	ttatgtatatatacttaacaaat
	gltAir1	aattgttagttaggttgacaata
	gltAir2[A]	ctttgtaaggagtattaataaaa

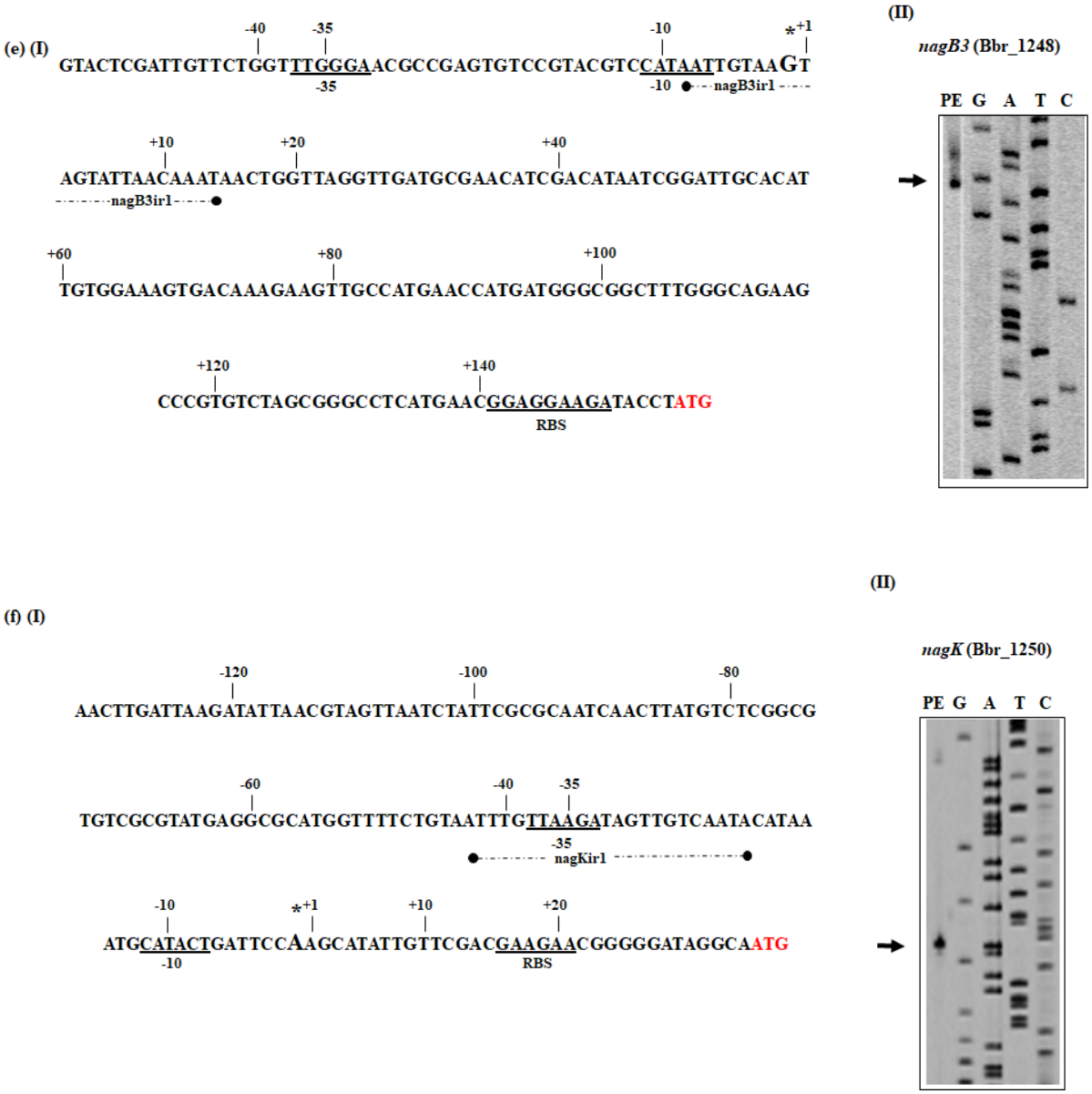
[A] Not identified by EMSA analysis.



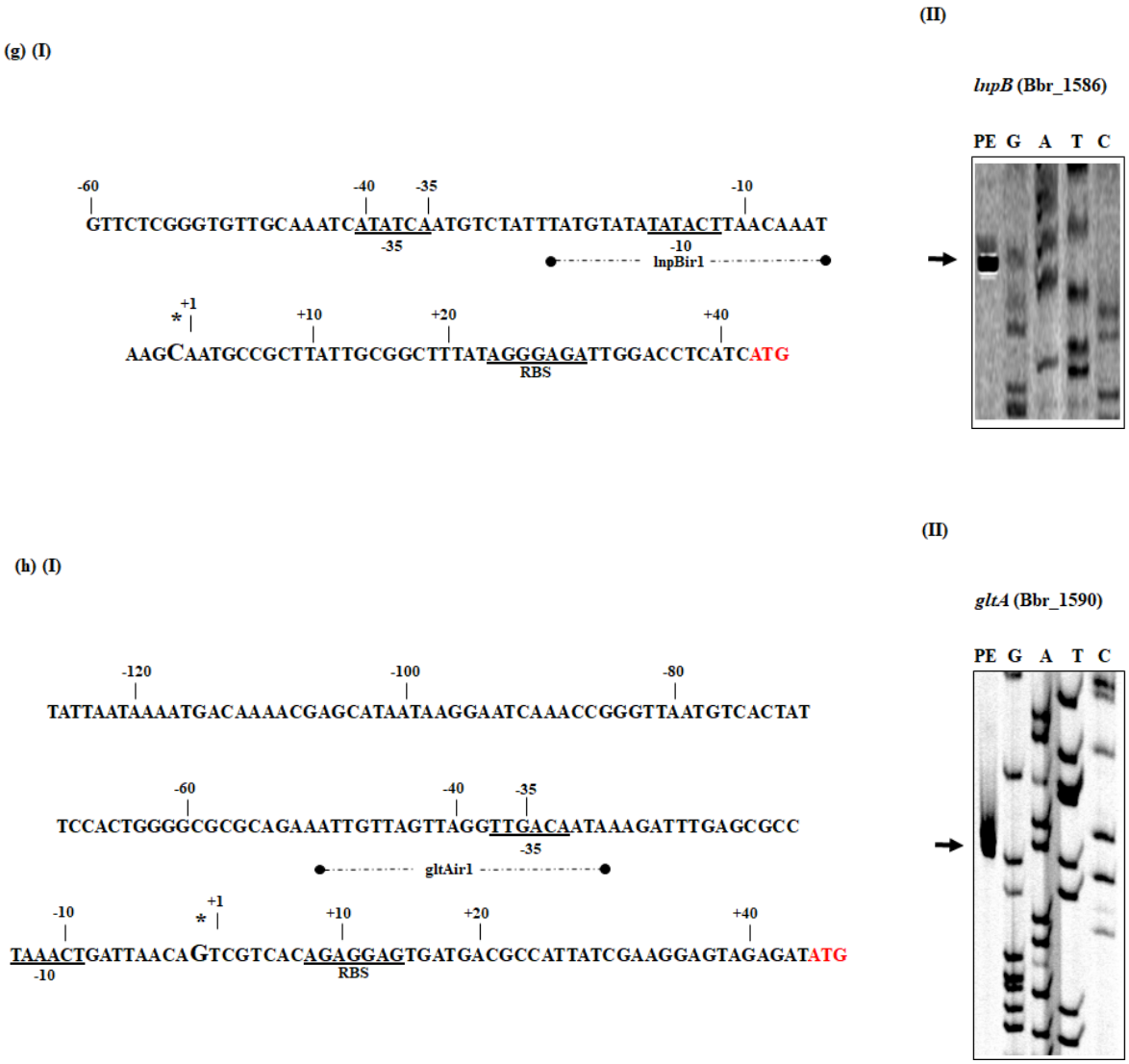
Supplemental Figure S1. Schematic representations (I) of the *lntP1* (a) and *lntS* (b) promoter regions for the binding of LntR. Underlining indicates the 10 and 35 hexamers, and ribosome binding sites deduced from the primer extension results; experimentally determined transcriptional start sites (TSS) are indicated by asterisks. The dashed lines under sequences with names in boldface indicate the inverted-repeat lntP1ir1, lntP1ir2, lntSir1 and lntSir2 binding sequences displayed above the lollipops. The arrows in the right panels (II) indicate the primer extension products.



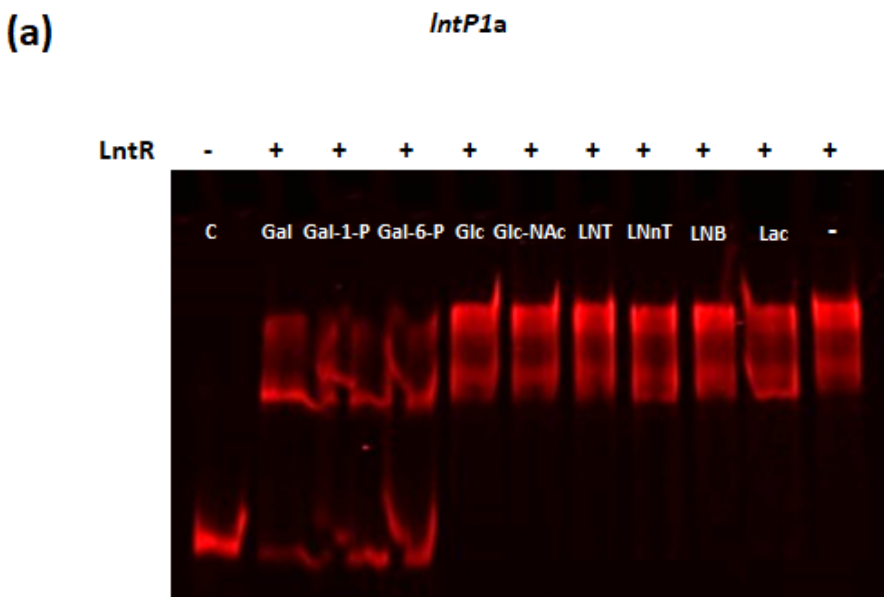
Supplemental Figure S1. Schematic representations (I) of the *nahS* (c) and *nahA* (d) promoter regions for the binding of NahR. Underlining indicates the 10 and 35 hexamers, and ribosome binding sites deduced from the primer extension results; experimentally determined transcriptional start sites (TSS) are indicated by asterisks. The dashed lines under sequences with names in boldface indicate the inverted-repeat nahSir1 and nahAir1 binding sequences displayed above the lollipops. The arrows in the right panels (II) indicate the primer extension products.



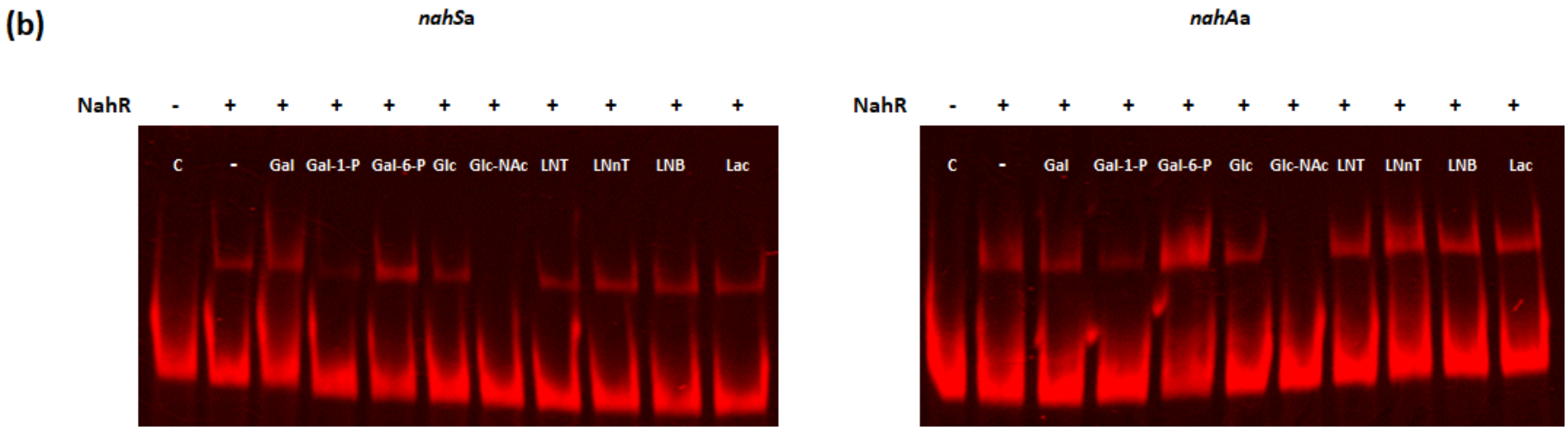
Supplemental Figure S1. Schematic representations (I) of the *nagB3* (e) and *nagK* (f) promoter regions for the binding of NagR1. Underlining indicates the 10 and 35 hexamers, and ribosome binding sites deduced from the primer extension results; experimentally determined transcriptional start sites (TSS) are indicated by asterisks. The dashed lines under sequences with names in boldface indicate the inverted-repeat nagB3ir1 and nagKir1 binding sequences displayed above the lollipops. The arrows in the right panels (II) indicate the primer extension products.



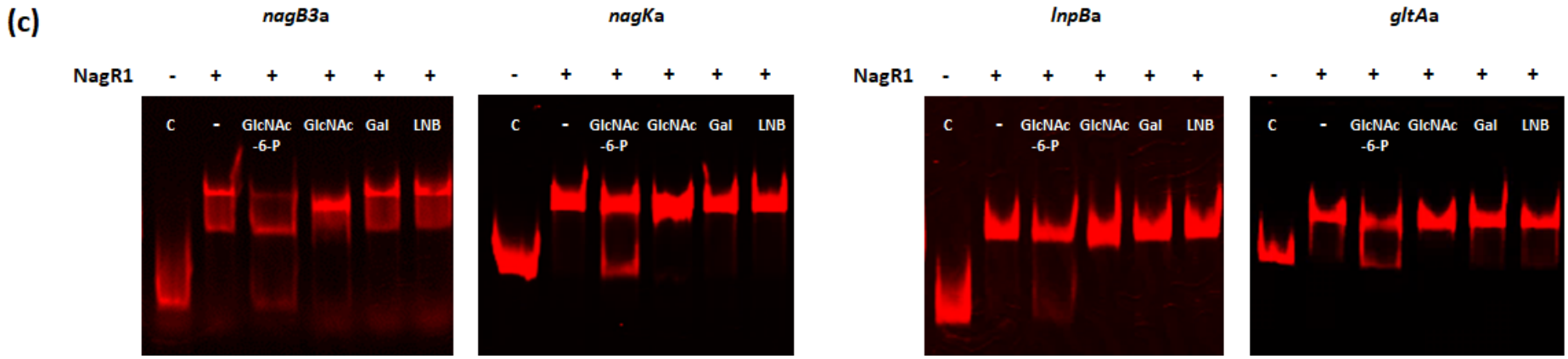
Supplemental Figure S1. Schematic representation (I) of the *lnpB* (g) and *gltA* (h) promoter regions for the binding of NagR1. Underlining indicates the 10 and 35 hexamers, and ribosome binding sites deduced from the primer extension results; experimentally determined transcriptional start sites (TSS) are indicated by asterisks. The dashed lines under sequences with names in boldface indicate the inverted-repeat lnpBir1 and gltAir1 binding sequences displayed above the lollipops. The arrows in the right panels (II) indicate the primer extension products.



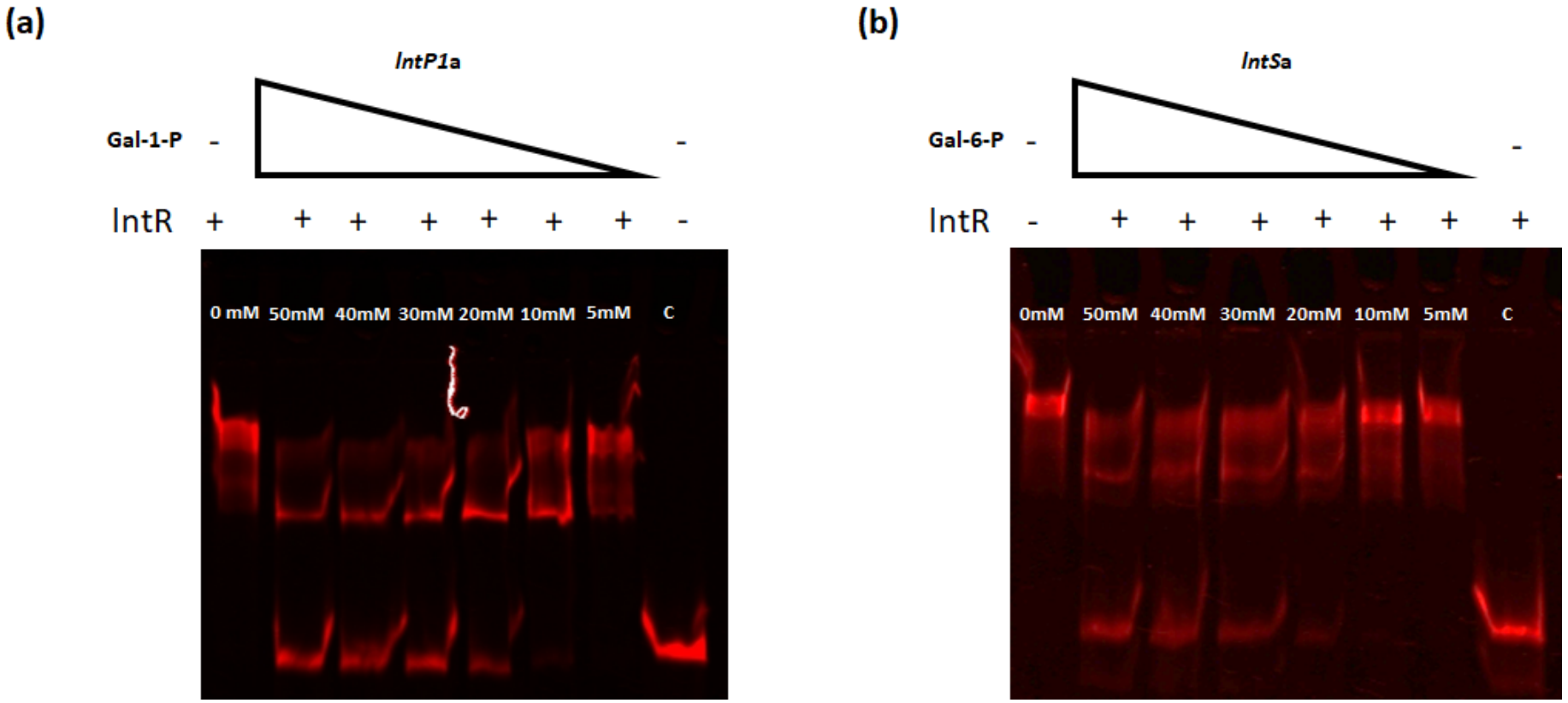
Supplemental Figure S2. EMSA images showing (a) LntR interactions with promoter-containing DNA fragment *lntP1*a, with the addition of a range of potential carbohydrate inducers, at a concentration of 20mM. In each panel, 'C' indicates a negative control, where an equivalent amount crude cell extract from NZ9000 harbouring empty pNZ8150 was added instead of crude extract from the regulator-expressing NZ9000 strain.



Supplemental Figure S2. EMSA images (b) NahR interactions with promoter-containing DNA fragments *nahS*a and *nahA*a, with the addition of a range of potential carbohydrate inducers, at a concentration of 20mM. In each panel, 'C' indicates a negative control, where an equivalent amount crude cell extract from NZ9000 harbouring empty pNZ8150 was added instead of crude extract from the regulator-expressing NZ9000 strain.



Supplemental Figure S2. EMSA images showing (c) NagR1 interactions with promoter-containing DNA fragments *nagB3*a, *nagK*a, *lnpB*a and *gltA*a, with the addition of a range of potential carbohydrate inducers, at a concentration of 20mM. In each panel, 'C' indicates a negative control, where an equivalent amount crude cell extract from NZ9000 harbouring empty pNZ8150 was added instead of crude extract from the regulator-expressing NZ9000 strain.



Supplemental Figure S3. EMSA images showing LntR interactions with promoter-containing DNA fragments, with the addition of a gradient (a) Gal-1-P and (b) Gal-6-P, ranging from 0mM-50mM. In each panel, 'C' indicates a negative control, where an equivalent amount crude cell extract from NZ9000 harbouring empty pNZ8150 was added instead of crude extract from the regulator-expressing NZ9000 strain.