De novo DNA methylation of the 35S enhancer revealed by high-resolution methylation analysis of an entire T-DNA segment in transgenic gentian
[Plant Biotechnol. 28(2): 223-230 (2011)]

Table S1. Specific primers used for genome walking PCR.

Target[a]	Method[b]	Digest[c]	Direction	Length	Sequence (5′ to 3′)[d]	Anneal
genome (#3)-LB	S (1st)	*Mbo*I, *Xba*I	R	31	AACGCGCAATAATGGTTTCTGACGTATGTGC	65
	S (2nd)		R	30	CCATCCAATTCTCATGTTTGACAGCTTATC	60
genome (#15)-LB	S (1st)	*Mbo*I	R	34	AGAAATATTTGCTAGCTGATAGTGACCTTAGGCG	60
	S (2nd)		R	31	AACGCGCAATAATGGTTTCTGACGTATGTGC	65
genome (#19)-LB	S (1st)	*Mbo*I	R	31	AACGCGCAATAATGGTTTCTGACGTATGTGC	65
	S (2nd)		R	30	CCATCCAATTCTCATGTTTGACAGCTTATC	60
RB-genome (#3)	I (1st)	*Taq*I, *Eco*RI	F	34	TCCTTCAACGTTGCGGTTCTGTCAGTTCCAAACG	63
			R	27	ATCGGTGCGGGCCTCTTCGCTATTACG	
	I (2nd)		F	25	TCAGATTGTCGTTTCCCGCCTTCAG	60
			R	27	ATCGGTGCGGGCCTCTTCGCTATTACG	
RB-genome (#15)	I (1st)	*Taq*I	F	34	TCCTTCAACGTTGCGGTTCTGTCAGTTCCAAACG	63
			R	27	ATCGGTGCGGGCCTCTTCGCTATTACG	
	I (2nd)		F	25	TCAGATTGTCGTTTCCCGCCTTCAG	60
			R	27	ATCGGTGCGGGCCTCTTCGCTATTACG	
RB-genome (#19)	S (1st)	*Spe*I, *Xba*I	F	37	ATGAYGTTATTTATGAGATGGGTTTTTATGATTAGAG	56
	S (2nd)		F	30	AATGAGYTTGYATGYYGGTYGGYTGAGTGG	68

[a] LB (RB)-genome, T-DNA left (right) border with its boundary region in the gentian genome.
[b] S, Straight Walk; I, Inverse-PCR.
[c] Restriction enzyme used for genome digestion.
[d] Y=C, T.

Table S2. Primers used for bisulfite-PCR in the analysis of methylation of the pSMABR35SsGFP T-DNA region.

Target[a]	Direction	Length	Sequence (5′ to 3′)[b]	Conc.[c]	Anneal	Product[d]
NOSp-*bar*	F	26	GGGTTTYTGGAGTTTAATGAGYTAAG	1	57	497
	R	28	TCCARTCRTARRCRTTRCRTRCCTTCCA	5		
bar-rbcsT1	F	28	TGGYTYGTYGYYGAGGTGGAYGGYGAGG	5	63	431
	R	30	TTCRATRARTTCCCRTACCARCTCCAACTC	4		
bar-rbcsT2	F	28	AGATYTGAAYGGAGTGYGYGTGGYATYG	5	49	403
	R	31	TCAAAARCAARAATTATRARRATAATTTAAA	4		
rbcsT	F	31	TTTAAAYAAYATTGTGGYTYYTTTAAATTAT	4	53	397
	R	35	TAACRTAAACAAAATTTCCAAAATTTARTAACTTC	1		
rbcsT-35S	F	30	AAAGAYTGAAATTTGTYAAGYATGAAGTTA	2	52	426
	R	36	AATARTACTTCTRATCTTRARAAATATATCTTTCTC	2		
35S	F	33	AAGAAGGTTAAAGATGYAGTYAAAAGATTYAGG	2	57	462
	R	29	ACCTTCCTTTTCCACTATCTTCACAATAA	0.5		
35S-*sGFP*	F	32	AGYTATYTGTYAYTTTATTGTGAAGATAGTGG	2	57	423
	R	24	ATCRCCCTCRCCCTCRCCRRACAC	4		
sGFP	F	27	GAYGTAAAYGGYYAYAAGTTYAGYGTG	5	57	440
	R	30	ATCTTRAARTTCACCTTRATRCCRTTCTTC	4		
sGFP-NOST	F	30	AAYGTYTATATYATGGYYGAYAAGYAGAAG	5	57	448
	R	33	ACTCTAATCATAAAAACCCATCTCATAAATAAC	0.5		
NOST	F	37	ATGAYGTTATTTATGAGATGGGTTTTTATGATTAGAG	1	55	351
	R	34	AACTRACARAACCRCAACRTTRAARRARCCACTC	5		
NOST-RB	F	37	ATGAYGTTATTTATGAGATGGGTTTTTATGATTAGAG	1	55	480
	R	33	AATATATCCTRTCAAACACTRATARTTTAAACT	2		
genome (#3)-LB	F	32	AAGAAAAAATTAGAGGAATATGAGAATATATG	0.5	53	350
genome (#15)-LB	F	37	ATTAAATTAYYAAAATTGAAAAYATGAGTTTATTTGG	2	55	313
genome (#19)-LB	F	33	TAAGGAAYAYGATATTAAYYATAAAGAGAAAAG	2	53	326
genome-LB	R	30	ACTTTTRAACRCRCAATAATRRTTTCTRAC	5		
RB-genome	F	30	AATGAGYTTGYATGYYGGTYGGYTGAGTGG	5		
RB-genome (#3)	R	34	ATCCTTCTTCCATTTCCATTATTACAAARTCTAC	1	55	308
RB-genome (#15)	R	32	AAACCCTAAAACTATTTTCAATRRATTCATTT	1	55	337
RB-genome (#19)	R	31	ATCCTTCTCTCTTCTTTATCGACTCAATCAC	1	57	358

[a] LB (RB)-genome, T-DNA left (right) border with its boundary region in the gentian genome.
[b] Y=C, T; R=A, G.
[c] Primer concentration (μM) used for the reaction.
[d] Amplified product length (bp).