Supporting Information

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SI Materials and Methods

Plant Material, Growth Conditions, and Phenotypic Analysis. An F_2 mapping population, segregating for *B2* was developed in the following way. An annual wild beet (*Beta vulgaris* subsp. *maritima*, accession 991971) homozygous at both bolting loci (*BB*/*B2B2*) was crossed with the biennial *B. vulgaris B2* mutant (accession 056822), which is homozygous for the mutated *B2* allele (*BB*/*B2′B2′*) (1). The M_3 line 056822 had been obtained after ethyl methanesulfonate (EMS) mutagenesis of the annual accession 930190 (2). Twenty-one F_1 plants (*BB*/*B2B2′*) were bag isolated to produce F_2 seeds. Later, F_3 seeds were obtained from bag isolated F_2 plants. A total of 6108 F_2 plants were grown under long day (LD) conditions (16 h light/8 h dark, 22 °C, 900 μE) in the greenhouse (sowing date April 4, 2010). Then, they were transferred into the field on May 17–18, 2010. F_2 plants were phenotyped twice a week for bolting (BBCH scale code: 51) and flowering time (BBCH scale code: 60) (3) from May 17, 2010 until October 25, 2010. Plants were classified as annual (bolting without vernalization) or biennial (bolting after vernalization). Annual plants were further classified as very early bolting, late bolting and bolting without flowering. Very early bolting plants bolted within twelve weeks after sowing (until June 24, 2010), whereas late bolting plants started bolting 13–30 wk after sowing (June 26, 2010 – October 25, 2010). Very early bolting and late bolting plants started to flower after bolting. Also late bolting plants without any visible flowers were observed. F_3 seeds were harvested from annual F_2 plants which bolted within 30 wk after sowing (before October 25, 2010). The remaining F_2 plants stayed in the field over winter, and F_3 seeds were harvested in the next year. Those plants were classified as biennial because they bolted after winter (vernalization).

Homozygous *B2B2* plants were distinguished from heterozygous *B2B2′* F_2 plants by phenotyping their F_3 progenies. Eight F_3 plants per family, derived from a single annual F_2 plant, were sown in 96mer multipot-plates (May 16, 2011) and grown under natural light conditions outside the greenhouse until October 2011. F_3 families were phenotyped for bolting (BBCH scale code: 51) (3) at three different time points (August 8, September 1, and October 12, 2011).

A second M_3 mutant line (seed code 031823) (1, 2) was used for sequencing to verify EMS mutations within the candidate genes.

For expression analysis, we used plants of the two biennial EMS mutant lines (seed code 056822 and 031823) and the annual line 001684 (selfing progeny of 930190), which had been the donor line for EMS mutagenesis. Two biennial lines (seed code 93161P and 090023), and the annual wild beet accession 991971 were used as controls. Plants were grown under LD conditions (16 h light/6 h dark) at 22 °C in the greenhouse for 7 wk. Then, plants were transferred to a climate chamber for 12 wk at 4 °C. After cold treatment, plants were grown in the greenhouse.

DNA Techniques. Two leaf samples were taken from 4-wk-old F_2 plants and freeze dried for 3 d. Extraction of genomic DNA was performed using the standard CTAB method (4) with slight modifications. DNA was used for PCR in a 10-fold dilution. Standard PCR was performed using Taq DNA Polymerase (Invitrogen). PCR fragments were separated on agarose gels (1%, 2%, or 4%). Primer sequences and PCR conditions are given in Tables S5 and S6. PCR products were Sanger sequenced at the Institute of Clinical Molecular Biology (IKMB, CAU Kiel).

We used the vector pUC18 for cDNA cloning. *BvBBX19* cDNA from the accessions 991971 and 056822 was amplified by PCR with the primers BBf and BBr (Table S6). These primers have compatible sequence ends that are recognized by the restriction enzyme *BamH*I. The resulting DNA fragments were restricted and ligated into the corresponding restriction sites of the cloning vector pUC18 and then transformed into the *Escherichia coli* strain DH5 alpha.

Gene Expression Analysis. We measured the expression of different genes from accessions 991971, 001684, 056822 and 93161P by quantitative RT-PCR (RT-qPCR) at zeitgeber time 6. Leaf material was harvested 44 d after sowing and 2 wk after vernalization. The diurnal expression of *BvBBX19* was analyzed by RT-qPCR with leaf material from accessions 056822, 031823, 001684, 991971, 93161P, and 090023. Leaf samples were collected 44 d after sowing in 2-h intervals for 24 h. Total RNA was extracted using the peqGOLD Plant RNA kit and DNase treated on column with the peqGOLD DNase I Digest kit (PEQLAB). cDNA synthesis was done with 500 ng of total RNA using a First Strand cDNA Synthesis kit (Fermentas). cDNA was diluted 20-fold, and 2 μL were used for RT-qPCR. Three independent biological and three technical replicates of each sample were analyzed. RT-qPCR was performed with the *Power* SYBR Green PCR Master Mix (Applied Biosystems) on a CFX96 Real-Time PCR detection system (Bio-Rad) with a final reaction volume of 20 μL including a final primer concentration of 20 pM (Table S6; refs. 5 and 6). The *BvGAPDH* gene from beet was used as a reference. Resulting data were analyzed with the CFX Manager Software v2.1 (Bio-Rad). The comparative CT (ΔC_T) method was applied. Relative expression levels were calculated and normalized to the geometric mean of *BvGAPDH*.

Bioinformatic Analysis. We used the physical map (7) and two versions of the sugar beet draft genome from the doubled haploid accession KWS2320 (RefBeet-0.9), a preliminary version (RefBeet-0.4) and version which can be download (http://bvseq.molgen.mpg.de) (8) and a preliminary collection of predicted gene models (RefBeet-0.3geneModels) for which the latest version (RefBeet-1.1geneModels) is now available (8).

Ab initio gene finder FGENESH (9) was used to analyze the gene structure of *BvBBX19*. To predict and analyze the conserved domain architecture of 15 BvBBXs, peptide sequences were analyzed with the web-based domain identification and annotation tool SMART (10). Parameters of both programs were set as default.

Marker Development and Genetic Mapping. For fine mapping of the *B2* locus a genetic map of chromosome 9 was generated. Sequence scaffolds and EST sequences (11), located on chromosome 9 were used to develop molecular markers segregating in the F_2 population. Segregating SNPs were detected as cleaved amplified polymorphic sequence (CAPS) markers. All markers used in this study are listed in Table S5. Genetic distances were calculated using the Kosambi mapping function (12) of JoinMap 4.0 (13) with a LOD threshold value of 5.0.

1. Büttner B, Abou-Elwafa SF, Zhang W, Jung C, Müller AE (2010) A survey of EMS-induced biennial *Beta vulgaris* mutants reveals a novel bolting locus which is unlinked to the bolting gene *B*. *Theor Appl Genet* 121(6):1117–1131.

2. Hohmann U, Jacobs G, Jung C (2005) An EMS mutagenesis protocol for sugar beet and isolation of non-bolting mutants. *Plant Breed* 124(4):317–321.

3. Meier U (1993) *Growth Stages of Mono- and Dicotyledonous Plants. Phenological Growth Stages and BBCH-Identification Keys of Beet* (Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany).
4. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci USA* 81(24):8014–8018.
5. Pin PA, et al. (2010) An antagonistic pair of *FT* homologs mediates the control of flowering time in sugar beet. *Science* 330(6009):1397–1400.
6. Pin PA, et al. (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. *Curr Biol* 22(12):1095–1101.
7. Dohm JC, et al. (2012) Palaeohexaploid ancestry for Caryophyllales inferred from extensive gene-based physical and genetic mapping of the sugar beet genome (Beta vulgaris). *Plant J* 70(3):528–540.
8. Dohm JC, et al. (2014) The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). *Nature* 505(7484):546–549.
9. Solovyev V, Kosarev P, Seledsov I, Vorobyev D (2006) Automatic annotation of eukaryotic genes, pseudogenes and promoters. *Genome Biol* 7(Suppl1:S10):1–12.
10. Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. *Proc Natl Acad Sci USA* 95(11):5857–5864.
11. Schneider K, et al. (2007) Analysis of DNA polymorphisms in sugar beet (*Beta vulgaris* L.) and development of an SNP-based map of expressed genes. *Theor Appl Genet* 115(5):601–615.
12. Kosambi DD (1943) The estimation of map distances from recombination values. *Ann Hum Genet* 12(1):172–175.
13. Van Oijen J, Voorrips R (2001) JoinMap 3.0, software for the calculation of genetic linkage maps. (Plant Research International, Wageningen, The Netherlands).

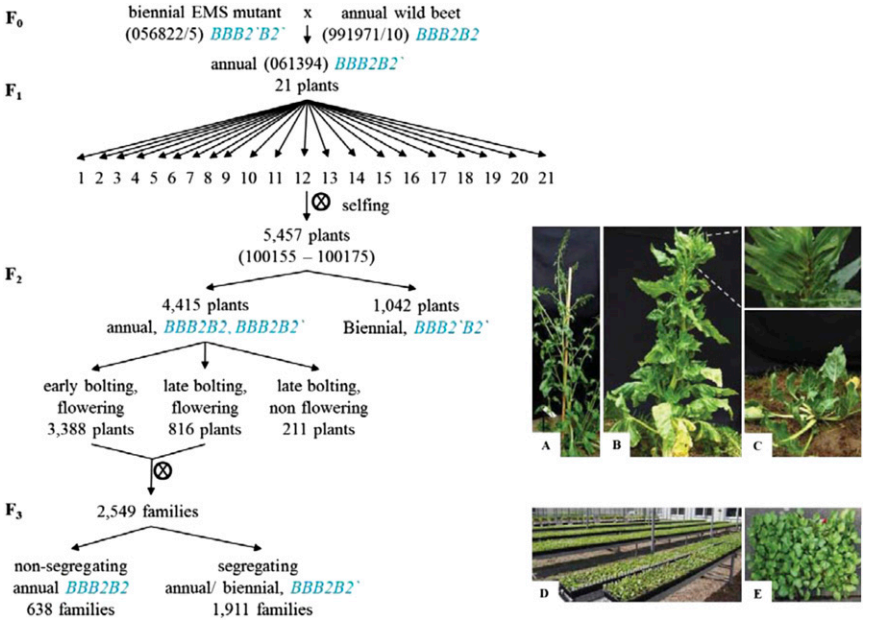

Fig. S1. Development of F_2 and F_3 populations. The crossing parents carry the dominant *B* allele and the nonmutated *B2* allele (in annuals) or the mutated *B2′* allele (biennial mutant). F_2 families were generated by selfing annual F_2 plants. (*A*) A normal flowering early bolting F_2 plant grown in the field (bolting within twelve weeks after sowing, before June 24, 2010). (*B*) A late bolting plant, which did not set flowers (bolting within 13–30 wk after sowing, between June 26 and October 25, 2010). (*C*) A nonbolting biennial plant 30 wk after sowing (until October 25, 2010). (*D*) F_3 families grown in 96mer-multipot trays outside the greenhouse under natural light conditions from May 16 until October 19, 2011. (*E*) Four-week-old F_3 plants grown in multipot trays.

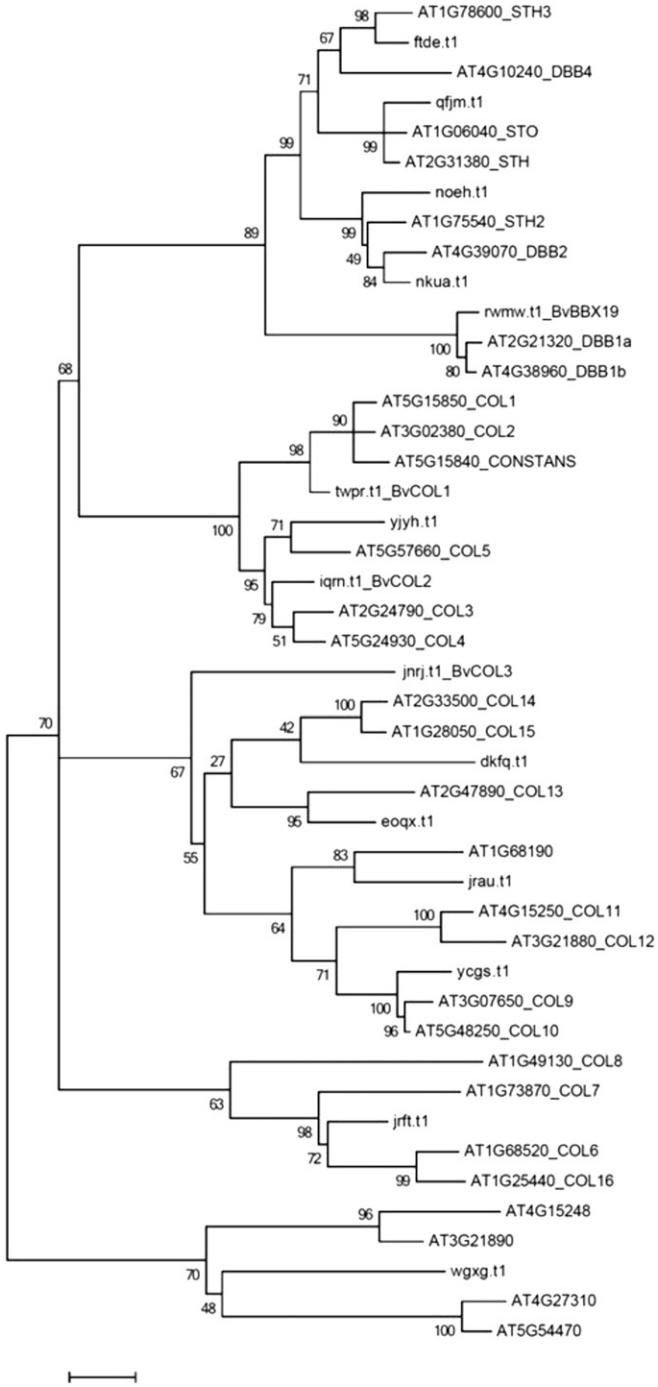

Fig. S2. Maximum likelihood phylogenetic tree of 30 AtBBX and 15 BvBBX amino acid sequences. CONSTANS is marked by an arrow. The bootstrap consensus tree inferred from 1000 replicates was constructed by Mega5.2 after aligning the predicted protein sequences from 15 BvBBXs and 30 AtBBXs by MAFFT. Bootstrap values are given at the branching points. Scale bar represents 0.2 amino acid substitutions per site.

Table S1. Cosegregation between marker alleles and the bolting time locus *B2* with 1301 F_2 plants

	Annual (*B2B2*)				Biennial (*B2′B2′*)			
B2 locus marker	M_1M_1	M_1M_2	M_2M_2	No PCR product	M_1M_1	M_1M_2	M_2M_2	No PCR product
CAU3785	338	5	0	36	2	11	645	264
CAU3786	307	3	0	69	1	12	868	41
CAU3782	342	2	0	35	2	8	810	102
CAU3784	373	0	0	6	0	0	922	0
CAU3783	341	9	0	29	0	24	856	42
CAU3787	333	25	1	20	2	53	829	38
CAU3788	335	24	0	20	0	59	791	72

F_2 plants were determined as homozygous for the annual allele (*B2B2*) or homozygous for the biennial allele (*B2′B2′*) by phenotypic analysis of their F_3 offspring. *B2* genotypes were determined by molecular marker analysis. M_1 and M_2 represent marker alleles derived from the annual parent and the biennial mutant parent, respectively.

Table S2. Molecular markers used in this study

Marker Name	Application	Reference
MP_R0018	Scaffold identification	Schneider et al. (11)
KI_2783	Scaffold identification	Schneider et al. (11)
TG_E0140	Marker development, genetic mapping	Schneider et al. (11); this study
MP_E0043	Marker development, genetic mapping	Schneider et al. (11); this study
CAU3782	Marker development, genetic mapping	This study
CAU3783	Marker development, genetic mapping	This study
CAU3784	Marker development, genetic mapping	This study
CAU3785	Marker development, genetic mapping	This study
CAU3786	Marker development, genetic mapping	This study

Marker sequences were used to identify candidate scaffolds (RefBeet-0.9) by BLASTN with publically available EST sequences as queries or sequences were used to develop molecular markers for genetic fine mapping the *B2* locus.

Table S3. Predicted gene models for *B. vulgaris* located in the critical region in which *B2* is located

Gene model	Size, bp	Sugar beet EST	*Arabidopsis thaliana* homolog (At locus #)	*A. thaliana* homolog	*A. thaliana* gene: GO molecular function	*A. thaliana* gene: GO biological process
iynm.t1	3045	BQ588456, BI543524	At4g34710.2	Arginin decarboxylase 2, ADC2	Arginine decarboxylase activity, catalytic activity	Spermidine biosynthetic process, arginine catabolic process
gzdy.t1	1527	NA	At2g21270.3	Ubiquitin fusion degradation 1, UFD1	Unknown	Ubiquitin-dependent protein catabolic process
zcpq.t1	1571	BQ586826	At2g21280.1	GC1, ATULA, SULA, NAD(P)-binding Rossmann-fold superfamily protein	Coenzyme binding, nucleotide binding, catalytic activity	Cellular metabolic process
ahqj.t1	463	BF011044	At2g21290.1	Unknown protein, located in:mitochondrion	Unknown	Unknown
cpor.t1	3345	BQ592067	At2g21300.2	ATP-binding microtubule motor family protein	Microtubule motor activity, ATP binding	Microtubule-based movement
rwmw.t1	1458	BQ589556, BQ591888	At4g38960.1	B-Box type zinc finger family protein	Sequence-specific DNA binding transcription factor activity, zinc ion binding	Regulation of transcription
wffm.t3	1120	BQ487817, BQ587936, BQ489572	At4g38970.1	Fructose-bisphosphate aldolase 2, FBA2	Fructose-bisphosphate aldolase activity, catalytic activity	Response to cadmium ion, glycolysis
yyyi.t1	1142	BQ490562	At4g38980.1	Unknown protein	Unknown	Unknown
nyzd.t1	2232	NA	At4g39010.1	Glycosyl hydrolase 9B18	Hydrolase activity, hydrolyzing O-glycosyl compounds, catalytic activity	Carbohydrate metabolic process
dyiq.t2	2118	BQ590473, BQ590482	At1g75560.2	Zinc knuckle (CCHC-type) family protein	Nucleic acid binding, zinc ion binding	Unknown
ktyp.t1	4793	BQ589661	At2g16485.1	Nucleic acid binding; zinc ion binding, DNA binding	siRNA-dependent DNA methylation	Unknown
swfx.t1	1259	BQ586688	At2g16460.1	Protein of unknown function (DUF1640)	Metal ion binding	Unknown

BLASTN analysis was performed using the critical region of scaffold sc00048 (RefBeet0.9) as query against preliminary predicted gene models (RefBeet0.3_GeneModels, public available version: RefBeet1.1_GeneModels; ref. 8) (CLC Main Workbench, version 5.7.1, BLASTN thresholds: e-value: 0.0; identity: 100%). Then, the obtained gene models were used for BLASTX analysis against the TAIR protein database (BLASTX version 2.2.24, threshold e-value < 0.05).

Table S4. Results from a BLASTP search using the first B-box region of the CONSTANS protein sequence as query

Conserved domains	Gene model accession ID	Gene name	Reference	Chromosome	ESTs ID
2B-box + CCT	twpr.t1	*BvCOL1*	Chia et al. (1)	2	BQ589119
					BQ588630
					BQ488270
					BQ589113
	iqrn.t1	*BvCOL2*	Chia et al. (1)	2	BQ583937
					BQ588069
	yjyh.t1	—	—	8	GT746521
					GT746522
	eoqx.t1	—	—	7	CV301775
	ycgs.t1	—	—	9	BQ593762
	dkfq.t1	—	—	6	EG551136
					EG552682
1B-box + CCT	jrft.t1	—	—	—	BQ489587
					BQ489817
	jnrj.t1	*BvCOL3*	Chia et al. (1)	6	BQ487825
					BQ487842
					BQ583972
					BQ583909
2B-box	rwmw.t1	*BvBBX19*	This paper	9	BQ589556
					BQ591888
	ftde.t1	—	—	4	BQ489825
					BQ589815
	nkua.t1	—	—	9	—
	noeh.t1	—	—	1	—
	qfjm.t1	—	—	3	BQ586969
	jrau.t1	—	—	6	GT745494
					GT745495
1B-box	wgxg.t1	—	—	7	BQ594583

Fifteen BBXs genes were identified in the sugar beet genome. Gene model IDs are from the RefBeet-1.1 (ref. 8). Chromosome localization is based on the BLASTN search result of each gene sequence against the reference sequence RefBeet-1.1.

1. Chia TY, Müller A, Jung C, Mutasa-Göttgens ES (2008) Sugar beet contains a large *CONSTANS-LIKE* gene family including a *CO* homologue that is independent of the early-bolting (*B*) gene locus. *J Exp Bot* 59(10):2735–2748.

Table S5. Nonanonymous and developed sequence based (RefBeet-0.9) molecular markers on chromosome 9 for analysis of cosegregation with bolting phenotypes in the F_2-population

Marker name	Marker type	Marker assay			Marker allele in annual parent, bp	Marker allele in mutant parent, bp
		Primers	PCR conditions for marker assay	Detection		
CAU3782	SNP/CAPS	C375 + C376	95 °C, 3′ + [(95 °C, 30″ + 52 °C, 30″ + 72 °C, 20″) × 36] + 72 °C, 10′	*Tsp*509I digest + GE* 2%	175	135 and 40
CAU3783	SNP/CAPS	C505 + C470	95 °C, 3′ + [(95 °C, 30″ + 55 °C, 45″ + 72 °C, 60″) × 36] + 72 °C, 10′	*Bse*GI digest + GE 1%	272 and 252	524
CAU3784	InDel	C507 + C508	95 °C, 3′ + [(95 °C, 30″ + 54 °C, 30″ + 72 °C, 20″) × 36] + 72 °C, 10′	GE 4%	174	152
CAU3785	SNP/CAPS	C450 + C451	95 °C, 3′ + [(95 °C, 30″ + 57 °C, 30″ + 72 °C, 30″) × 36] + 72 °C, 10′	PstI digest + GE 2%	553	346 and 207
CAU3786	SNP/CAPS	C442 + C443	95 °C, 3′ + [(95 °C, 30″ + 55 °C, 30″ + 72 °C, 30″) × 36] + 72 °C, 10′	TaqI digest + GE 1%	465	353 and 112
CAU3787[†]	InDel	C261 + C237	95 °C, 3′ + [(95 °C, 30″ + 55 °C, 30″ + 72 °C, 22″) × 36] + 72 °C, 10′	GE 2%	405	599
CAU3788[†]	InDel	C229 + C230	95 °C, 3′ + [(95 °C, 30″ + 57 °C, 30″ + 72 °C, 30″) × 36] + 72 °C, 10′	GE 2%	295	387

*GE, gel electrophoresis.
[†]Marker developed based on EST sequence derived from Schneider et al. (11).

Table S6. Primer sequences and amplification conditions for PCR, RT-PCR, and RT-qPCR performed in this study

Gene/ marker	Forward primer	Sequence 5′→3′	Reverse primer	Sequence 5′→3′	PCR conditions
BvGAPDH	B582*	GCTTTGAACGACCACTTCGC	B583*	ACGCCGAGAGCAACTTGAAC	95 °C, 3′ + 40× (95 °C, 10″; 61 °C, 30″; 72 °C, 30″)†
BTC1	B580*	GTGAAAGCTGTGTAAGGAATGG	B581*	AAGTTCCTGCATGGATCCAG	95 °C, 3′ + 40× (95 °C, 10″; 61 °C, 30″; 72 °C, 30″)†
BvFT1	B563*	GCATCATTTGGAGAAGAGATTGTTTAC	B564*	GGCGTTGTTGTGGAGCATTTA	95 °C, 3′ + 40× (95 °C, 10″; 64.5 °C, 30″; 72 °C, 30″)†
BvFT2	B584*	GAGCCCAAGTAATCCACACTTG	B585*	GTGTTGAAGTTTTGACGCCAC	95 °C, 3′ + 40× (95 °C, 10″; 64.5 °C, 30″; 72 °C, 30″)†
BvBBX19	C565	TGAGGACTCTTTGTGATGTTTGTGAGG	C566	GGTACAGCATTAGGGGCAGCAAG	95 °C, 3′ + 40× (95 °C, 10″; 61 °C, 30″; 72 °C, 30″)†,‡
BvBBX19	BBf	ACTGTGGATCCATGAGGACTCTTTGTGATGTTTG	BBr	ACTGTGGATCCCTCATTTTTCTGGCTCGCTTTTG	95 °C, 3′ + 36× (95 °C, 3′; 57 °C, 30″; 72 °C, 40″) +72 °C, 5′‡
CAU3782	C375	TTCAGCATGCAGATCTGGG	C376	CTCGCCATCTCCTCCATC	95 °C, 3′ + [(95 °C, 30″ + 52 °C, 30″ + 72 °C, 20″) × 36] + 72 °C, 10′‡
CAU3783	C505	GTAAATAGCCCCTACCATCTC	C470	GACTTTGAGTGCCCACTATGTG	95 °C, 3′ + [(95 °C, 30″ + 55 °C, 45″ + 72 °C, 60″) × 36] + 72 °C, 10′‡
CAU3784	C507	CTACTTCCTCTGTTCACTTTTACTTG	C508	CCTTCATTCTCTTTTACTTGCCAC	95 °C, 3′ + [(95 °C, 30″ + 54 °C, 30″ + 72 °C, 20″) × 36] + 72 °C, 10′‡
CAU3785	C450	CCACTCCATCTTCGACCTCATATC	C451	CAGCTCAGGGTCAAAACCAACC	95 °C, 3′ + [(95 °C, 30″ + 57 °C, 30″ + 72 °C, 30″) × 36] + 72 °C, 10′‡
CAU3786	C442	AAAGTTTATTGGGGATGGAGGAAG	C443	CGAATAATATCTCTACGTCAGCAGATG	95 °C, 3′ + [(95 °C, 30″ + 55 °C, 30″ + 72 °C, 30″) × 36] + 72 °C, 10′‡
CAU3787	C261	GTGCACACTTTCTTGCCACAGG	C237	CTCATCAGTCCACCATATTTCAGAAG	95 °C, 3′ + [(95 °C, 30″ + 55 °C, 30″ + 72 °C, 22″) × 36] + 72 °C, 10′‡
CAU3788	C229	CCTCATCAGCACACAATCTCC	C230	CGCACCCTTGACACATTTACC	95 °C, 3′ + [(95 °C, 30″ + 57 °C, 30″ + 72 °C, 30″) × 36] + 72 °C, 10′‡

*Refs. 5 and 6.

†RT-qPCR performed with *Power* SYBR Green PCR Master Mix (Applied Biosystems).

‡PCR and/or RT-PCR performed with Taq-DNA-Polymerase (Invitrogen).